Wojciech Dmuchowski*, Dariusz Gozdowski, Aneta H. Baczewska***, Paulina Brągoszewska*****

EVALUATION OF THE ARSENIC ENVIRONMENTAL POLLUTION ON THE BASIS OF DIFFERENT BIOINDICATIVE METHODS

OCENA ZANIECZYSZCZENIA ŚRODOWISKA ARSENEM ZA POMOCĄ RÓŻNYCH METOD BIOINDYKACYJNYCH

Słowa kluczowe: biomonitoring, zanieczyszczenie środowiska, arsen, mech, brzoza, sosna.
Keywords: biomonitoring, environmental pollution, arsenic, moss, birch, Scots pine, moss bag.

Celem pracy było porównanie czterech metod bioindykacyjnych w ocenie zanieczyszczenia środowiska arsenem. Teren badań obejmował rejon Olkusza, bardzo silnie zanieczyszczony metalami ciężkimi, o tradycjach przemysłu hutniczego sięgających XIII wieku. Obecnie na tym obszarze znajduje się m.in. zakład wydobycia i przetwórstwa rud cynkowo-ołowiowych oraz hałda odpadów hutniczych wysokości 25–30 m i powierzchni 109 ha.

Badania polegały na określeniu akumulacji arsenu: w eksponowanym mchu Sphagnum fallax – metoda transplantacyjna moss-bag, w rosnącym mchu Pleurozium schreberi, w liściach brzozy brodawkowatej i w igłach sosny zwyczajnej.

Wyniki badań przedstawiono w formie map z izoliniami, różnicującymi strefy zanieczyszczenia proporcjonalnie do skali zawartości arsenu w poszczególnych bioindykatorach. Czyn-

* Dr hab. Wojciech Dmuchowski, prof. nadzw. – Pracownia Ekologii Roślin, Polska Akademia Nauk Ogród Botaniczny – CZRB, ul. Prawdziwka 2, 02-973 Warszawa; tel: 22 754 14 26; e-mail: w.dmuchowski@obpan.pl oraz Szkoła Główna Gospodarstwa Wiejskiego, Wydział Rolnictwa i Biologii, ul. Nowoursynowska 159, 02-776 Warszawa; tel: 22 593 26 89; e-mail: wojciech_dmuchowski@sggw.pl

** Dr inż. Dariusz Gozdowski – Wydział Rolnictwa i Biologii, Szkoła Główna Gospodarstwa Wiejskiego, ul. Nowoursynowska 159, 02-776 Warszawa; tel: 22 593 27 30; e-mail: darek12345@gfmail.com

***Mgr Aneta Helena Baczewska, mgr Paulina Brągoszewska – Pracownia Ekologii Roślin, Polska Akademia Nauk Ogród Botaniczny – CZRB, ul. Prawdziwka 2, 02-973 Warszawa; tel: 22 648 38 56; e-mail: a.h.baczewska@wp.pl, p_jablonska1@wp.pl

nikiem różnicującym bezwzględny wynik stopnia zanieczyszczenia środowiska jest przede wszystkim czas ekspozycji, wynikający ze specyfiki zastosowanej metody bioindykacyjnej, źródło zanieczyszczenia (powietrze, gleba) oraz cechy (budowa morfologiczna) rośliny wskaźnikowej.

Poziom akumulacji arsenu we wszystkich roślinach wskaźnikowych w rejonie Olkusza był wysoki i bardzo zróżnicowany. Analiza statystyczna wyników wykazała zależność między zastosowanymi metodami bioindykacyjnymi. Zależność ta, wyrażona współczynnikiem korelacji prostej Pearsona, była dodatnia i istotna dla wszystkich zastosowanych metod bioindykacyjnych. Wartość współczynnika determinacji we wszystkich przypadkach była większa niż 0,5, co świadczy o znacznym podobieństwie obrazów imisji zanieczyszczenia. Wykazano, że rejon Olkusza, a szczególnie okolice huty ołowiu i cynku, należy uznać za ekstremalnie skażone arsenem. Zostały tam przekroczone progi toksyczności dla roślin, które i tak są mniej wrażliwe niż ludzie.

1. INTRODUCTION

Small quantities of arsenic can have a stimulating influence on human, animal and plant organisms, but has not been considered an indispensable element. Toxic quantities of arsenic in plants cause stifling of leaves and change of their colour, as well as damages to the root system and retardation of growth. In human organism arsenic compounds of may block the action of many enzymes and may cause, in particular, the disturbance of the Krebs cycle. Acute poisonings with arsenic bring about first of all damage to the digestive tract, while the chronic ones – anemia, weakening of nails and hair, as well as different skin disorders, such as callusing and colour changes. Besides, even after a long period of latency the cancerous symptoms may appear, because arsenic is a carcinogenic and theratogenic factor [Kabata-Pendias and Pendias 1999].

Main source of arsenic emission are burning of coal and foundries of non-ferrous metals It is emitted, as well, from the natural sources, such a volcanoes or low temperature volatilisation (biological methylation) [Peterson and Girling 1981, Chilvers and Peterson 1987].

The goal of this work was to evaluate various bioindication methods for providing biologically–based complementary information to the mandatory environmental monitoring with arsenic. This has been done based on environmental monitoring in extremely high levels of pollution near Olkusz, in which four various bioindication methods have been used.

2. STUDY AREA

The study area is a region around the town of Olkusz that is one of the oldest European centers for the metallurgic industry, with industrial tradition reaching back, as mentioned, to the 13th century. The largest industrial plant in the area is the Mining and Metallurgic Complex “Bolesław” in Bukowno. 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]. In addition, dust emissions from the stacks of the “Bolesław” Complex can be transported over much longer distances, and this applies also to other industrial plants, located nearby outside of the study area, such as the Katowice Steel Works.

The study (Fig. 1) area is situated to the east of Olkusz, its western border reaches the suburbs of the town (Fig. 1), and it is 14 km x 14 km, divided into 49 smaller squares of 2 km x 2 km. Four additional monitoring sites were selected near Bolesław complex.

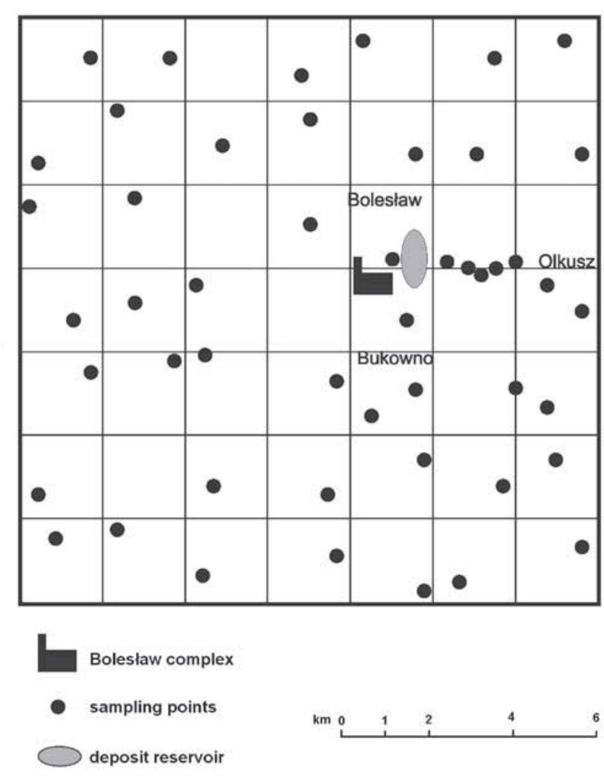

Fig. 1. Locations of the measurement points in the surroundings of Olkusz
Rys. 1 Lokalizacja punktów pomiarowych w okolicach Olkusza

3. MATERIALS AND METHODS

The study was conducted using four common methods for biomonitoring environmental pollution due to metals:

1) Determination of accumulation in the exposed *Sphagnum fallax* (H. Klinggr.) moss – the moss-bag transplant method. The method is based on exposing bags with moss collected from a relatively unpolluted territory, in the study area. After exposure the level of accumulation of heavy metals in moss is determined. The method was first applied by Goodman and Roberts [1971] and adapted to the Polish conditions by Chmielewski et al. [1993].
2) Determination of accumulation in growing moss *Pleurozium schreberi* (Brid.) Mitt. The procedure of sample collection and their preparation to the analyses was carried out conform to the European Program [Grodzińska et al. 1997]. Moss was collected from the forest openings at a certain distance from the trees, in order to avoid the filtering influence of the tree canopies, which could significantly impact upon the results [Rühling et al. 1987]. From the surface of approximately 200 m^2 only plant parts of gametophyte were collected without bedding, and only its green parts, representing usually the last three years were used for monitoring purposes.
3) Determination of accumulation in leaves of *Betula pendula* Roth. The leaves of silver birch were picked from the upper part of the canopy at the height of about 3 m on four sides of the tree circumference [Dmuchowski 2005].
4) Determination of accumulation in the needles o*f Pinus silvestris* L. The needles were picked from the 2^{nd} to 4^{th} whorl from the top, by cutting branches from the outer parts of the canopy, from the possibly most uncovered places. Analysis was carried out only for the needles of the previous year growth, according to the methodology presented by Dmuchowski and Bytnerowicz [1995]. This method was recommended by UNEP [Environmental Data Report 1989].

The material for the analyses (moss, needles and leaves) was collected in the second half of July. Samples were collected from eight trees, with a single mixed sample made of the equal amount of biomass from each tree. In the same locations bags with peat moss were placed for the period of 12 weeks later. In each of the 49 squares an area distanced at least 300 m from the main roads with heavy traffic. the appropriate pines, birches and moss (within the distance of at most 100 m). Four additional monitoring sites were selected near Bolesław complex.

Reference samples consisted of pine needles, silver birch leaves and *Pleurozium schreberi* and *Sphagnum fallax* mosses collected in the Augustów Primeval Forest, considered to be in relative terms the least polluted area in Poland [Dmuchowski and Bytnerowicz 1995; Grodzińska et al. 1997].

The material collected was placed in linen bags, dried at 70°C and ground to a powder. Needles and leaves were washed for one minute in distilled water before being ground. The powdered samples were dry-mineralized in a muffle oven. The concentration of arsenic was determined with the atomic absorption spectrophotometry with generation of hydrides using the FIAS 200 countershaft [Perkin Elmer 1990].

Relationships between contents of the arsenic in various bioindicators were evaluated using Pearson' correlation coefficient and simple linear regression [Sokal and Rohlf 1995].

Cluster analysis using nearest neighbour method was used for evaluation of similarity of four bioindication methods [Everitt et al. 2001]. For all analyses significance level was set at 0.05. The statistical analyses were performed in Statgraphics 4.1 statistical package and the figures were prepared using MS Excel.

The results of this work are presented as maps of arsenic pollution deposition. All of the maps were drawn digitally with the use of the specialized MapInfo software [Daniel et al. 2002].

4. RESULTS AND DISCUSSION

The results of environmental arsenic pollution are presented in the form of maps (Fig. 2) with respective isoquants. These isoquants differentiate the zones of pollution in proportion to the scale of arsenic concentration, accumulated in particular bioindicators: Table 1 presents the basic descriptive statistics of the results obtained.

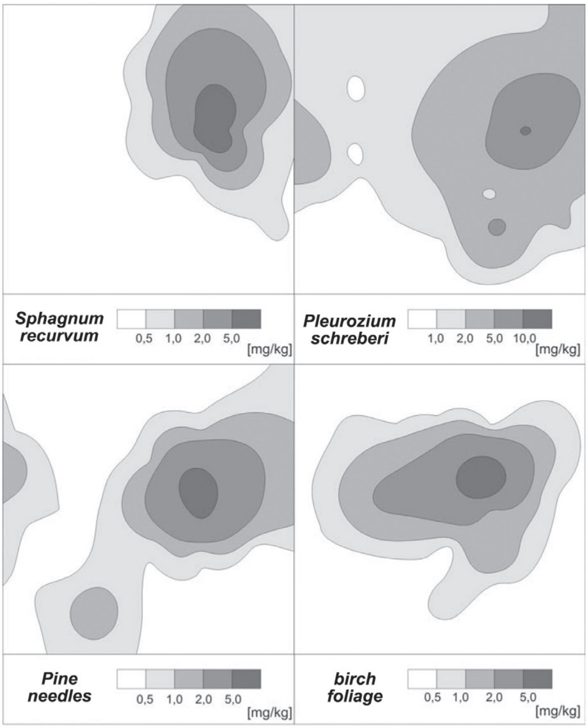

Fig. 2 Contamination of environment with arsenic on the basis of accumulation in concentration of this element in the bioindicators.

Rys. 2. Zanieczyszczenie środowiska arsenem na podstawie kumulacji stężenia tego pierwiastka w biowskaźnikach.

Table 1. Medians and range of arsenic content in examined bioindicators
Tabela 1. Mediany i średnie zawartości arsenu w badanych biowskaźnikach

Value	As
Moss bag	
Median	0.46
Minimum	0.15
Maximum	17.1
Control	0.11
Pl. schreberi	
Median	1.47
Minimum	0.23
Maximum	11.0
Control	0.21
P. sylvestris	
Median	0.45
Minimum	0.13
Maximum	6.44
Control	0.11
B. pendula	
Median	0.37
Minimum	0.11
Maximum	9.05
Control	0.10

In the moss-bag metod arsenic concentration i moss were quite variable, and ranged between 0.15 and 17.11 mg/kg. Chmielewski and Dmuchowski [1999] applied a similar methodology in the area of the Bielany quarter in Warsaw, in 1999 recorded the highest measurement in the vicinity of the Lucchini Steel Mill, i.e. 0.26 mg/kg.

The arsenic concentration in moss *Pleurozium schreberi* ranged from 0.23 to 10.6 mg/kg, and the reference level of 0.23 mg/kg. The literature data concerning the content of arsenic in mosses are relatively sparse. In Poland, in 1998, in the province of Podlasie , the the least polluted region, had arsenic concentrations in mosses ranging between 0.12 and 1.16 mg/kg, while the strongly contaminated regions, the Upper Silesian – Cracovian Industrial region had these concentrations between 0.03 and 2.8 mg/kg, and the Legnica-Głogów Copper Basin between 0.25 and 6.0 mg/kg [Grodzińska et al. 2003]. In France, the maximum arsenic concentration measured in mosses was 1.0 ppm [Galsomiès et al. 2003], while in the Czech Republic – to 1.4 mg/kg [Sucharová and Suchara 1998].

Arsenic concentrations in the pine needles were between 0.12 and 6.44 mg/kg, and these values were higher in all the collected needle samples than the reference sample value (0.09 mg/kg). The maximum values recorded are similar to the one provided by Steinnes et al. [2000] for the contaminated areas close to the non-ferrous metal works on the Kola peninsula, equal to 5.1 mg/kg. Lower concentrations were measured in the Polish industrial regions: in the centre of Upper Silesia the measured concentrations of arsenic pine needles ranged from 1.42 to 1.50 mg/kg [Dmuchowski and Bytnerowicz 1995]. Molski and Dmuchowski [1990] measured in northern Finland, in Lappland, on the average 0.49 mg/kg of arsenic. This is a higher level of concentration than in the non-polluted areas of Poland, but Lappland is under the influence of emissions from the non-ferrous metal works located in Russia on the Kola Peninsula.

The arsenic concentration in the birch foliage ranged between 0.12 and 9.05 mg/kg, and the background reference sample of 0.10 mg/kg. Only limited literature information on arsenic content in birch leaves is available. The mountain birch foliage from the vicinity of the nickel and copper works in Monchegorsk on the Kola Peninsula contains 1.5 mg/kg of nickel, according to Steinnes et al. [2000], while according to Reimann et al. [2001] – 0.71 mg/kg. In the polluted area of the Katowice Steel Mill neighbourhood, the concentration of arsenic in the silver birch leaves was 0.92 mg/kg [Dmuchowski, 2000].

Despite the application of various bioindication methods with different monitoring plants, the spatial distributions of arsenic contamination as revealed in the maps were similar. Although 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. Region of Olkusz, should be considered as extremely strongly contaminated with arsenic.

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 arsenic in the bioindicator plants is the time of exposure (Tab. 2). 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 2. Properties of the bio-indication methods applied

Tabela 2. Właściwości biowskaźników w zastosowanej metodzie

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

Correlations between arsenic content in all bioindicators were significant (Fig. 3, Tab. 3). The strongest correlation was observed between arsenic content in *B. pendula* and *P. sylvestris* (r=0.73). The relationship is presented graphically in dendrogram (Fig. 4), branches of these two bioindicators join at the lowest distance. Medians and regression functions indicate that content of arsenic was about 25% lower for *P. silvestris* in comparison with *B. pendula,* but pattern of arsenic accumulation was very similar for both these species. Very strong correlation was observed between *P. sylvestris* and *Pl. schreberi* (r=0.72), but the arsenic content for *Pl. schreberi* was much higher than in *P. sylvestris* especially in less polluted areas where was about 3 times higher. Relationships between moss bag method with other three bioindication methods were moderate. Correlation coefficients were from 0.51 to 0.59. In the

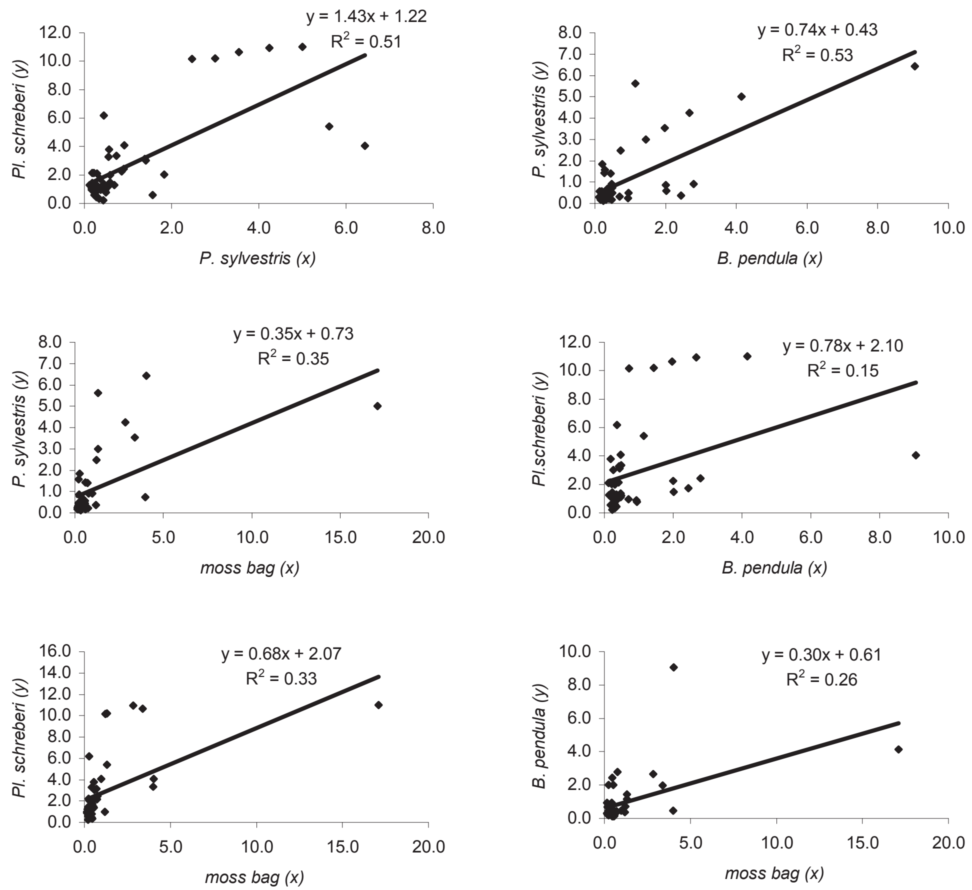

Fig. 3. Relationships between arsenic content in examined bioindicators (linear regression functions and coefficients of determination – R^2)

Fig. 3. Zależność między zawartością arsenu w poszczególnych biowskaźnikach (funkcja regresji liniowej i współczynnik determinacji – R^2)

Table 3. Correlation coefficients between content of arsenic in various bioindicators.
Tabela 3. Wartość współczynnika korelacji między zawartością arsenu w różnych biowskaźnikach

	Moss bag	*B. pendula*	*P. sylvestris*
B. pendula	0.51		
P. sylvestris	0.59	0.73	
Pl. schreberi	0.58	0.39	0.72

All correlations are significant at $P<0.05$

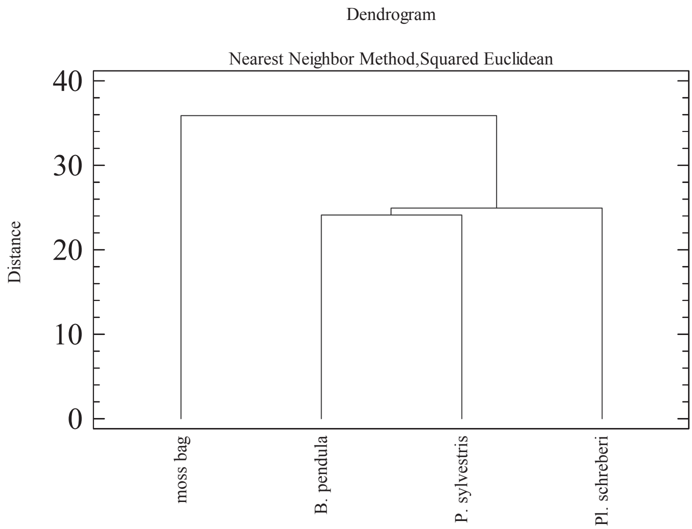

Fig. 4. Dendrogram based on cluster analysis for arsenic content in various bioindicators
Rys. 4. Dendrogram na podstawie analizy skupień zawartości arsenu w różnych biowskaźnikach

dendrogram presenting results of cluster analysis the branch for moss bag method joins with other branches at the highest distance. Arsenic content in moss bag was lower than in *Pl. schreberi* but higher than in *B. pendula* and *P. silvestris*, but the regression functions indicate that in more polluted areas moss bag method content of arsenic is much higher than in other methods, while in less polluted areas the differences are low (Fig. 3).

5. CONCLUSIONS

1. The region of Olkusz, and especially the surroundings of the lead and zinc foundry in Bukowno, should be considered as extremely strongly contaminated with arsenic.
2. Despite application of various methods and the resulting diversity of the specific exposure periods of different bioindicators, the spatial distribution of environmental ar-

senic contamination, shown on the maps, was similar, as confirmed by the statistical analysis of results.

3. Mean levels of arsenic content in four bioindicators were rather different but correlations between the arsenic contents were quite strong. The strongest correlation was between content of arsenic in *B. pendula* and *P. sylvestris*.
4. Regression equations make possible the comparison of various data of arsenic contamination as evaluated by one or more of the examined bioindication methods.
5. The bioindication methods, because of the possibility of collecting data at a large number of points, enable presentation of results in the form of maps of heavy metals depositions, with the isoquants illustrating environmental pollution, in a manner that is intuitive and understandable for everyone.

Acknowledgement.: The work was supported by grant MNiSW agreement 3689/B/P01/2010/38.

REFERENCES

ALLEN S.E., GRISHAW H.M., PARKINSON J.A., QUARMBY C. 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Osney Mead, Oxford.

CHILVERS D.C., PETERSON P.J. 1987. Global Cycling of Arsenic. In: Hutchinson T.C., MEEMA K.M. (eds) Lead, mercury, cadmium and arsenic in the environment. John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore: 279–301.

CHMIELEWSKI W., DMUCHOWSKI W., GWOREK B. 1993. Wykorzystanie metody moss-bag w lokalnym biomonitoringu zanieczyszczenia powietrza metalami ciężkimi. Prądnik, Prace i materiały Muzeum im. Prof. Władysława Szafera 7–8: 181–185.

CHMIELEWSKI W., DMUCHOWSKI W. 1999. Ocena rozprzestrzeniania się zanieczyszczenia powietrza metalami ciężkimi na obszarze gminy Warszawa-Bielany. Sprawozdanie z badań. Ogród Botaniczny-CZRB PAN (manuscript).

DANIEL L., LOREE P., WHITENER A. 2002. Inside Mapinfo Professional. On World Press, Albany.

DMOWSKI K, BADUREK M. 2002. Thallium contamination of selected plants and fungi in the vicinity of the Bolesław zinc smelter in Bukowno (S. Poland). Preliminary Study. Acta Biologica Cracoviensia Series Botanica 44: 57–61.

DMUCHOWSKI W., BYTNEROWICZ A. 1995. Biomonitoring of the environmental pollution in Poland using chemical analysis of Scots pine (*Pinus silvestris* L.) needles. Environ. Pollut. 87: 87–104.

DMUCHOWSKI W. 2000. Zanieczyszczenie środowiska w województwie katowickim określone na podstawie analizy chemicznej liści brzozy brodawkowatej. In: Kurczyńska E. (ed.) Zanieczyszczenie środowiska a anatomiczne i komórkowe podstawy zrzucania organów u drzew. Sprawozdanie z badań – grant KBN (manuscript).

DMUCHOWSKI W. 2005. Use of plant bioindicators in assessment of environmental contamination with heavy metals. Reports of The Botanical Garden of The Polish Academy of Sciences, Series: Monographs and treatises 7: 1–116.

ENVIRONMENTAL DATA REPORT 1989/90. United Nation Environment Programme. Blackwell, Oxford.

EVERITT B.S., LANDAU S., LEESE M. 2001. Cluster analysis. Arnold, London.

GALSOMIÈS L., AYRAULT S., CARROT F., DESCHAMPS C., LETROUIT-GALINOU M.A. 2003. Interspecies calibration in mosses at regional scale-heavy metal and trace elements results from Ile-de France. Atmos. Environ. 37: 241–251.

GODZIK B. 1993. Heavy metals content in plants from zinc dumps and reference areas. Polish Bot. Stud. 5: 113–132.

GOODMAN G.T., ROBERTS T.M. 1971. Plants and soil as indicators of metals in the air. Nature 231(4): 287–292.

GRODZIŃSKA K., SZAREK-ŁUKASZEWSKA G., GODZIK B., BRANIEWSKI ST., BUDZIAKOWSKA E., CHRZANOWSKA E., PAWŁOWSKA B., ZIELONKA T. 1997. Ocena skażenia środowiska Polski metalami ciężkimi przy użyciu mchów jako biowskaźników. Państwowa Inspekcja Ochrony Środowiska, Biblioteka Monitoringu Środowiska, Warszawa.

GRODZIŃSKA K., FRONTASYEVA M., SZAREK-ŁUKASZEWSKA G., KLICH M., KUCHARSKA-FABIŚ A., GUNDORINA S.F., OSTROVNAYA T.M. 2003. Trace element contamination in industrial regions of Poland studied by moss monitoring. Environmental Monitoring and Assessment 87: 255–270.

KABATA–PENDIAS A., PENDIAS H. 1999. Biogeochemia pierwiastków śladowych. Wydawnictwo Naukowe PWN, Warszawa

MOLSKI B, DMUCHOWSKI W. 1990. The Comparison of Environmental Pollution in Northern Finland near Kevo and in Poland with the use of *Pinus sylvestris* L. as bioindicator. Rep. Kevo Subarctic Res. Stat. 21: 27–30.

PERKIN ELMER. 1990. Analytical methods for atomic absorption spectrophotometry. Bodenseewerk, Pub. B 353, 233

PETERSON P.J., GIRLING C.A. 1981. Rother trace metals. In: Lepp N. W. (ed.) Effect of heavy metal pollution on plants. Effect of trace metals on plant function. Department of Biology, Liverpool Polytechnic, Liverpool: 213–278.

REIMANN C., KOLLER F., KASHULINA G., NISKAVAARA H., ENGLMAIER P. 2001. Influence of extreme pollution on the inorganic chemical composition of some plants. Environ. Pollut. 115: 239–252.

RÜHLING Å., RASMUSSEN, PILEGAARD K., MÄKINEN A., STEINNES E. 1987. Survey of atmospheric heavy metal deposition in the Nordic countries in 1985 -monitored by moss analyses. NORD: 21.

SOKAL R.R., ROHLF F.J. 1995. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and company, New York: 887.

STEINNES E., LUKINA N., NIKONOV V., AAMLID D., ROYSET O. 2000. A gradient study of 34 elements in the vicinity of a copper-nickel smelter in the Kola Peninsula. Environ. Moni. Assess. 60: 71–88.

SUCHAROVÁ J., SUCHARA I. 1998. Biomonitoring of the atmospheric deposition of metals and sulphur compounds using moss analysis in the Czech Republic. Result of the international biomonitoring programme 1995. Res. Inst. of Ornamental Gardering, Průhonice: 1–183.