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Polish Botanical Journal 59(1): 137–144, 2014
DOI: 10.2478/pbj-2014-0019

PAHs CONCENTRATIONS IN POLAND USING MOSS *PLEUROZIUM SCHREBERI* AS BIOINDICATOR

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Abstract. Concentrations of PAHs in the moss *Pleurozium schreberi* (Brid.) Mitt. were compared in three regions differing in degree of industrialization and urbanization: the Silesia–Cracow region (heavily polluted), Mazovia (moderately polluted) and Podlasie (northeast Poland, control area). Ten moss samples of moss were taken from each area. PAH concentrations were determined by HPLC. Total PAHs levels in *P. schreberi* varied depending on the region: mean 7350 (± 4075) ng kg^{-1} d. wt. for the Silesia–Cracow region, mean 2127 (± 1686) ng kg^{-1} d. wt. for Mazovia, and mean 838 (± 943) ng kg^{-1} d. wt. for the control area. PAHs concentrations differed significantly between the three regions. The number of individual PAH compounds detected depended on the region. The following PAHs tended to occur in pairs: phenanthrene and benzo(k)fluoranthene, pyrene and chrysene, pyrene and dibenz(ah)anthracene, benz(a)anthracene and benzo(b)fluoranthene, benz(a)anthracene and benzo(ghi)perylene, chrysene and dibenz(ah)anthracene, benzo(b)fluoranthene and benzo(ghi)perylene, and benzo(b)fluoranthene and indeno(123cd)pyrene. The results of PAHs analysis in *Pleurozium schreberi* indicate the usefulness of mosses for monitoring these compounds in the environment.

Key words: PAHs, biomonitoring, *Pleurozium schreberii*, Poland

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are compounds commonly found in the environment. They are formed through natural processes (e.g., forest fires, volcanic emissions) and through human activity. They are synthesized during pyrolysis of organic substances during many industrial processes and in incomplete combustion of organic substances (Maliszewska-Kordybach 1999; Sapota 2002).

PAHs are a large group of compounds containing two or more aromatic rings in the molecule. They have similar physicochemical properties. In the air they occur as vapors and aerosols, where they form multicomponent mixtures related to suspended particulates in the air (especially those < 10 μm in diameter) (Sapota 2002). PAHs emitted by natural and anthropogenic combustion processes enter the atmosphere, which is their manner of transportation rather than storage. Most PAHs are deposited in soils (94.4%). Some compounds also occur in freshwater sediments (5.4%) (Maliszewska-Kordybach 1999). In the air, PAHs may undergo transformation and degradation. In soils they may volatilize, abiotically degrade (photolysis and oxidation), biodegrade (as a result of microbial activity) or accumulate in plants. The rate and extent of PAHs biodegradation in soil depends on environmental factors such as organic matter content, soil structure and particle size, microbial activity, the presence of other pollutants, and also on the physical and chemical properties of PAHs (Anonymous 1995).

As some PAHs have carcinogenic and mutagenic effects, they are subject to statutory monitoring of their environmental levels. The United States Environmental Protection Agency (U.S.

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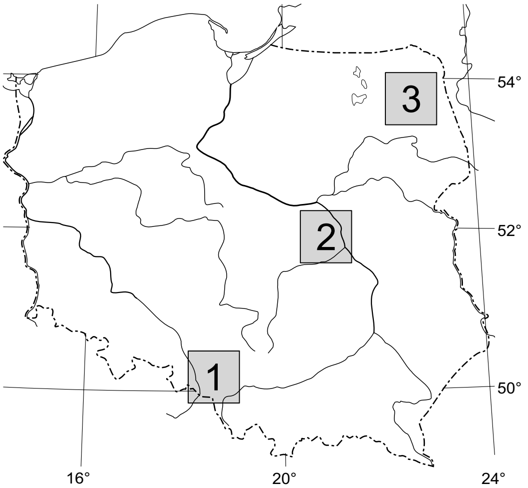

Fig. 1. Locations of *Pleurozium schreberi* (Brid.) Mitt. sampling plots for PAHs analysis in three regions of Poland: 1 – Silesia–Cracow region, 2 – Mazovia region, 3 – Podlasie region.

EPA) has classified 16 PAHs as priority pollutants on the basis of their toxicity, potential exposure to humans, and incidence in waste depositories. Six of the 16 PAHs have been recognized by the U.S. EPA as probably carcinogenic to humans: benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(ah)anthracene and indeno(123cd)pyrene. Carcinogenic potency is determined as the toxic equivalency factor, TEF. Benzo(a)pyrene was taken as the reference compound (TEF = 1) and the carcinogenicity of the other compounds was calculated accordingly (Sapota 2002). Under 'Directive 2004/107/EC of the European Parliament and of the Council of 15 December 2004' (Anonymous 2005) the target level in ambient air for benzo(a)pyrene is 1 ng m^{-3} for total content in the PM_{10} fraction averaged over a calendar year.

In Europe, *ca* 14,000 tons of PAHs are introduced to the atmosphere annually. Power plants and individual household stoves are the source of 70% of all emissions (Maliszewska-Kordybach 1999). In Poland, it was estimated that in 2005 more than 149 tons of PAHs were emitted to the atmosphere, the majority (85.6%) from combustion in the municipal sector and in housing. Approximately 11% of the total national emissions of PAHs comes from manufacturing processes and less than 2% from road transport (Anonymous 2013).

Most PAHs enter plant tissue through leaves from the air by the deposition of dust particles carrying these compounds. Absorption speed depends on the concentration, solubility and molecular weight of PAHs, and on the plant species. The properties of the plant's epidermis or periderm (e.g., roughness) affects the molecules' adhesion on the leaf surface and cuticular waxes play an important role in sorption of lipophilic compounds (De Nicola *et al*. 2008).

Environmental pollution is assessed by technical methods or using indicator organisms. Mosses and lichens are the plants most frequently used in bioindication, and less frequently vascular plants (Onianwa 2001; Markert *et al*. 2003; Boquete *et al*. 2011). Among mosses, *Pleurozium schreberi* (Brid.) Mitt. is considered the best indicator of the level of air pollution by heavy metals. From many years this moss has been used in European environmental monitoring (Rühling 1994; Rühling *et al*. 1997; Buse *et al*. 2003; Suchara *et al*. 2007; Harmens *et al*. 2008, 2010, 2013). It has also been used as an indicator of sulfur pollution (Szarek & Chrzanowska 1991), and nitrogen (Harmens *et al*. 2011; Kapusta *et al*. 2014). *Pleurozium schreberi* usually grows in pine and spruce forests but can also be found in mixed forests and on peatlands or moorlands. It develops in habitats ranging from dry to very wet. It prefers acidic soils. It occurs in both lowlands and mountain areas (Grodzińska *et al*. 1997; Klich & Szarek-Łukaszewska 2001).

Various plant species are used to assess environmental levels of PAHs, such as *Quercus ilex* L. (Alifani *et al*. 2001), *Brassica oleracea* L. (Franzaring *et al*. 1992, *Populus* sp. (Kuhn *et al*. 1998), *Laurus nobilis* L. (Lodovici 1994, 1998), *Picea abies* (L.) H. Karst. (Niu *et al*. 2003) and *Pinus sylvestris* L. (Tremolada *et al*. 1996). Generally, lichens such as *Hypogymnia physodes* L. are used for this type of research in Poland (Migaszewski 1999; Migaszewski *et al*. 2002).

The aim of this study was to evaluate the usefulness of *Pleurozium schreberi* moss as an indicator of environmental pollution by PAHs. In this study we compared the level of air pollution by PAHs and the shares of individual compounds in moss tissues in three regions differing in the extent of industrialization and urbanization, the density of the road network, population density, and consequently the level of air pollution.

Study area, material and methods

During the last sampling of mosses in 2010, done within the framework of European monitoring (European International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops, ICP Vegetation), material from selected sites was collected in order to test the feasibility of using mosses as indicators of PAHs and nitrogen in a number of countries, including Poland. The research was done in three regions on a northeast–southwest transect extending through Silesia and Cracow (southwestern Poland; heavily polluted), Mazovia (central Poland; moderately polluted) and Podlasie (northeastern Poland, control area) (Fig. 1).

In each of the three regions, samples of *Pleurozium schreberii* moss were collected at 10 sites for PAHs content analysis. All sites were in forest areas, usually pine forest or mixed forest with predominance of pine. Geographical location was determined with a GPS transmitter. Plant materials were collected in the field in late July and August 2010. Combined samples (*ca* 1 liter) from 5–10 spots in a 2500 m^2 area (50 × 50 m plots) were placed in sealed polythene bags. They were transported in a portable cooler to the laboratory where they were kept frozen until used for chemical analyses. The living (photosynthetic) parts of the mosses were used for those analyses.

Chemical analyses were performed in the laboratory of the Department of Ecology, Institute of Botany, Polish Academy of Sciences. PAHs were extracted from the moss samples with an ASE-200 extractor (Dionex) in accordance with the procedures described in Application Note 313 DIONEX ‘Extraction of PAHs from Environmental Samples by Accelerated Solvent Extarction (ASE)’. Extraction was done with a mixture of dichloromethane and acetone (1:1) at 100°C at 14 MPa nitrogen pressure. Fresh diatomaceous earth was added to each of the analyzed test portions (*ca* 5.0 g) of plant samples in order to remove water. Further extract purification steps employed semipermeable membranes (SPM, 80 microns). The receiving solvent was n-hexane. The described procedure was repeated three times. The extracts were additionally concentrated under a stream of air, then dissolved in 1 ml acetonitrile and injected to a Vydac 201TP5415 column. The individual compounds were separated by HPLC in a Dx-500 system. The mobile phase was an acetonitrile-water system dispensed in a gradient of 50–100% acetonitrile for 45 min at a flow rate of 1 ml/min. Using a UV-VIS detector (wavelength 254 nm, temperature 30°C) 11 PAHs compounds were quantified: phenanthrene, fluoranthene, pyrene, benzo(a)atracen, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, dibenz(ah)anthracene and indeno(123cd)pyrene, 10 of which (not phenanthrene) are classified as heavy polycyclic aromatic hydrocarbons. Certified reference material CR104-100 was used to control the quantitative analysis. The concentrations of PAHs in mosses are given in ng kg^{-1} dry weight.

To find differences between regions and determine the relationships between individual PAHs, the data were square-root-transformed to normalize the data distributions. Differences in the concentrations and number of particular PAHs between regions were tested for significance by one-way ANOVA followed by Tukey’s post hoc test. Correlation analysis was used to determine the relationship between the number of detected PAHs and their total concentration in the samples (Spearman correlation), and the interdependence of concentrations for each pair of PAHs (Pearson correlation).

Results

The concentration of all identified PAHs in *Pleurozium schreberi* depended on the region: mean 7350 (± 4075) ng kg^{-1} d. wt. for the Silesia–Cracow region, 2127 (± 1686) ng kg^{-1} d. wt. for Mazovia, and 838 (± 943) ng kg^{-1} d. wt. for Podlasie, the control (Table 1).

The PAHs concentrations were highest in the Silesia–Cracow region. At three of ten sites (Miasteczko Śląskie, Kobiór, Libiąż) the amount of PAHs compounds identified in moss tissues exceeded 10,000 ng kg^{-1} (Table 1). Mazovia showed moderate values for total PAHs in moss tissue. The highest concentration was found in material from the vicinity of Okuniew, a village west of Warsaw (5623 ng kg^{-1} d. wt.), and the lowest in material from Izabelin (102 ng kg^{-1} d. wt.). In northeastern Poland, total PAHs ranged from 0.28 ng kg^{-1} d. wt. (Augustów) to 3079 ng kg^{-1} d. wt. (Monkinie);

Table 1. PAHs concentrations (ng kg^{-1} d. wt.) in moss *Pleurozium schreberii* (Brid.) Mitt. collected in three regions of Poland. [a], [b] – means differing significantly by a Tukey's test. PHE – Phenanthrene, FLU – fluoranthene, PYR – Pyrene, B(a)A – Benzo(a)anthracene, CHR – Chryzene, B(b)F – Benzo(b)fluoranthene, B(a)P – Benzo(a) pyrene, B(ghi)P – Benzo(ghi)perylene, DIB(ah)A – Dibenzo(ah)anthracene, IND(123cd)P – Indeno(123cd)pyrene.

Site	PHE	FLU	PYR	B(a)A	CHR	B(b)F	B(k)F	B(a)P	B(ghi)P	DIB(ah)A	IND(123cd)P	Sum PAHs
Silesia–Cracow region (south–west Poland)												**7350[b] (±4075)**
Rudziniec			150.54	0.15	4.71	0.15	15.36	3.19	231	4420	1103	5927
Hutki			0.33	0.67	2.90	0.78	3.45	4.23	769	1862	1950	4594
Zawadzkie	1.49			0.88	6.83	0.41	6.15	1.49	4717		113	4847
Miasteczko Śląskie				12.51	0.44	2.47	1.02	0.15	12632	488	770	13905
Rudy (Sośniowice)				5.37	1.01	0.54	1.24	3.03	6400		923	7334
Żory	1.54			10.01		0.44	9.35	3.41	7869	344	475	8712
Wola Filipowska		9.20	0.50			0.17	13.56	12.30	1956		281	2272
Kobiór				0.96	1.82	1.04	9.64	5.56	10720	422	647	11807
Libiąż			8.77	7.05		6.54	8.77	12.55	8407	7.2	3227	11685
Chruszczobród-Piaski (Sikorka)			0.25		0.17	1.00	2.24	6.06	1977		432	2419
Mazovia region (central Poland)												**2127[a] (±1686)**
Wyszków									1521			1521
Julinek				2.10		0.16	3.55	1.45		223	1833	2064
Famułki Królewskie				2.46				1.57	1692	73		1769
Arkadia-Nieborów				3.35		3.35	3.89	4.97	1913			1928
Okuniew	66.23			7.14	0.14		46.06	0.98	5398	105		5623
Popowo Kościelne				2.15					1274			1276
Beniaminów				1.29	1.61	1.93	4.19	2.90		160	2003	2175
Cyganówka			13.98	2.77	1.45	1.19	3.30	5.41	3759	603		4390
Magdalenka				0.92				0.49	425			427
Izabelin (Laski)						1.44				101		102
Podlasie region (north–east Poland)												**838[a] (±943)**
Jelonka								0.55				0.55
Monkinie	8.45	1.76			5.69		0.60	1.40	2989	73		3079
Krzywe	1.02			1.61				0.92	599	136		738
Hajnówka									328			328
Augustów								0.28				0.28
Białowieża			2.51	0.36	0.60		3.58	3.34	586		447	1043
Studzieńczyna				0.06	2.48		1.54	26.87	475		168	673
Ostrów Płn.									942			942
Biele (Sochcin)				3.51				0.81	1564			1568
Żerczyce					0.27			0.99				1.26

it was higher than 1000 ng kg^{-1} in material from only three localities (Table 1).

One-way ANOVA showed statistically significant differences between regions ($p < 0.05$). The values for total PAHs for Silesia–Cracow and for Mazovia differed significantly from the value from the control area, Podlasie, in northeastern Poland. Silesia–Cracow and Mazovia also differed significantly (Table 1).

The number of detected individual PAHs depended on the region and substantially varied within region. The number of compounds detected was higher in the moss material from Silesia–Cracow, ranging from 7 to 9 (Table 1). In Mazovia the mean number of detected PAHs was *ca* 5 (1–8). Mosses from northeastern Poland had fewer PAHs, on average 3 or 4 (1–7); 7 compounds were detected in material from only two sites (Table 1). These values differed significantly between Silesia–Cracow and the other regions, and not between Mazovia and Podlasie ($p < 0.05$). All the samples showed clear predominance of one or two compounds and very low shares of the others. The dominant compound in most of the samples was benzo(ghi)perylene. Generally, the heavier compounds were detected in most of the samples, and the lighter ones only in some of them (Table 1). Correlation analysis showed a significant association between the number of detected compounds and the concentration of total PAHs in the samples (Spearman correlation coefficient = 0.87, $p < 0.05$).

Correlation analysis showed a significant correlation between the concentration of benzo(a)pyrene and the total of other detected concentrations (Pearson correlation coefficient = 0.50, $p < 0.05$).

Correlation analysis carried out for each pair of compounds showed interdependence between a number of PAHs compounds. We found statistically significant correlations ($p < 0.05$) for the following pairs: phenanthrene and benzo(k)fluoranthene, pyrene and chrysene, pyrene and dibenzo(ah)anthracene, benzo(a)anthracene and benzo(b)fluoranthene, benzo(a)anthracene and benzo(ghi)perylene, chrysene and dibenzo(ah)anthracene, benzo(b)fluoranthene and benzo(ghi)perylene, and benzo(b)fluoranthene and indeno(123cd)pyrene (Table 2).

DISCUSSION

In the past two decades the environmental levels of heavy metals and thus the threats posed by them have declined; attention has shifted to the presence of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), in the environment. PAHs are ubiquitous. They occur in the air, water and soil (Maliszewska-Kordybach 1999). Most PAHs are introduced as a result of combustion processes in the municipal sector and housing, and they persist in the environment (Anonymous 2009). Some of these compounds adversely affect animals, particularly humans. EU regulations set a norm for the permitted concentration of benzo(a)pyrene as an indicator of PAHs (toxic equivalency factor TEF = 1) at 1 ng m^{-3} (Anonymous 2008). This standard is often exceeded in heavily industrialized and urbanized areas (Anonymous 2009). In the analyzed mosses from the three regions (Silesia–Cracow, Mazovia, Podlasie) benzo(a)pyrene was present in most samples but it was a dominant compound in material from only 3 sites. Other compounds with higher toxicity coefficients, such as dibenzo(ah)anthracene, benzo(ghi)perylene and indeno(123cd)pyrene, had significant shares in the mixture of PAHs and occurred more frequently and at higher concentrations than benzo(a)pyrene.

The study showed statistically significant differences in PAHs levels between Silesia–Cracow and the other two regions. The former contains a large number of industrial plants that are especially harmful to the environment and which emit particulates into the air. It is also one of the country's most densely populated regions. Particulate emissions (associated with PAHs) from pollution-generating plants reached 20,000 tons in 2010, and the population density of the area was 234 persons per km^2 (Anonymous 2012). In the other regions both particulate emissions and population density were lower than in Silesia–Cracow: 9400 tons and 143 persons per km^2 in Mazovia, and 6300 tons and 59 persons per km^2 in Podlasie (Anonymous 2012).

Since the main share of PAHs emissions to the atmosphere comes from combustion processes in the municipal sector, housing and manufacturing, it is no surprise that the mosses collected from

Table 2. Pearson correlation coefficients for each pair of compounds. Statistically significant ($p < 0.05$) correlations are in boldface and italics. PHE – Phenanthrene, FLU – fluoranthene, PYR – Pyrene, B(a)A – Benzo(a)anthracene, CHR – Chryzene, B(b)F – Benzo(b)fluoranthene, B(a)P – Benzo(a)pyrene, B(ghi)P – Benzo(ghi)perylene, DIB(ah)A – Dibenzo(ah)anthracene, IND(123cd)P – Indeno(123cd)pyrene.

PAHs	PHE	FLU	PYR	B(a)A	CHR	B(b)F	B(k)F	B(a)P	B(ghi)P	DIB(ah)A
FLU	–0.023									
PYR	–0.048	–0.044								
B(a)A	0.284	–0.151	–0.102							
CHR	–0.018	–0.011	***0.384***	–0.217						
B(b)F	–0.114	–0.094	–0.027	***0.441***	–0.102					
B(k)F	***0.869***	0.172	0.231	0.314	0.071	0.037				
B(a)P	–0.098	0.286	0.015	–0.0414	0.125	0.262	0.089			
B(ghi)P	0.163	–0.033	–0.111	***0.765***	0.071	***0.462***	0.282	0.078		
DIB(ah)A	–0.051	–0.075	***0.909***	–0.053	***0.438***	–0.038	0.222	–0.010	–0.055	
IND(123cd)P	–0.131	–0.068	0.175	0.264	0.086	***0.639***	0.102	0.244	0.247	0.290

sites in Silesia–Cracow registered significantly higher PAHs levels than those from the other studied areas. The air load of PAHs is increased further by transport, representing approximately 1% of the total PAHs emissions to the atmosphere (Anonymous 2008a). Silesia–Cracow is one of Poland's high-traffic areas: 1.35 cars per person or 329 cars per km^2 (Anonymous 2012). Although there were no statistically significant differences between the Podlasie region (northeastern Poland, control region) and Mazovia, the mosses from Podlasie clearly registered lower concentrations of PAHs and fewer PAHs.

The properties of individual PAHs determine their persistence in the atmosphere. Light PAHs (2–3 benzene rings) are less stable, and heavy PAHs (more than 4 rings), with higher molecular weight (above 200 g mol^{-1}) (Bojes & Pope 2007), are mostly adsorbed by particulate matter. They are therefore less mobile and are rapidly deposited near emission sources (Maliszewska-Kordybach 1999). The proximity of measurement sites to the emission source in the Katowice conurbation also affected our results. Most of the compounds detected in mosses were heavy PAHs. The number of individual PAHs identified in the moss samples from Silesia–Cracow differed significantly from the number detected in samples from the other regions, reflecting lower contamination.

This study of three regions of Poland showed mainly the presence of heavy polycyclic aromatic hydrocarbons (4 or more benzene rings). In most of the samples the dominant compounds were benzo(ghi)perylene, indeno(123cd)pyrene, dibenzo(ah)anthracene and benzo(a)pyrene. Of these four compounds, only benzo(ghi)perylene is not recognized by the U.S. EPA as carcinogenic (Bojes & Pope 2007). These results differ from those found for the Świętokrzyskie Mts, where fluoranthene and pyrene had the largest shares (Gałuszka 2006). Other relationships between various PAHs were demonstrated in studies using mosses in Austria (Krommer *et al*. 2007). The differences in results may indicate differences in type of air pollution source between the studied areas, and differences related to the bioindicators themselves. Moss species differ in their accumulation of PAHs (Gałuszka 2006).

Correlation analyses for pairs of compounds showed positive relationships for eight pairs of PAHs. Studies conducted in the Świętokrzyskie Mountains and Spain demonstrated positive correlations for a number of compounds but no correlations for those eight pairs (Gałuszka 2006; Foan *et al*. 2010).

Plants (especially those with waxy leaves) are good biomonitors of persistent organic pollutants (Holoubek *et al*. 2000). Mosses are efficient accumulators of heavy metals (Markert *et al*. 2003). For years they have been used in Poland and other European countries to determine environmental pollution (Rühling 1994; Rühling *et al*. 1997; Buse *et al.* 2003; Suchara *et al.* 2007; Harmens *et al.* 2008, 2010, 2013). Wegener *et al.* (1992) were among the first to propose the use of this group of plants to register environmental levels of PAHs. Our study employing mosses showed differences in environmental pollution levels which corresponded to the diverse levels of industrialization and urbanization in the three studied regions. Such pilot studies of the use of the moss *Pleurozium schreberi* as PAHs bioindicator indicate the need for more detailed research.

ACKNOWLEDGEMENTS. We are grateful to the anonymous reviewers for helpful remarks on the manuscript. This study was financially supported by the National Science Centre (NCN, project No. N 304 356138) and by the W. Szafer Institute of Botany, Polish Academy of Sciences, from its statutory funds.

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Received 4 April 2014