DOI: 10.2478/v10216-011-0041-8 ECOL CHEM ENG S. 2012;19(4):585-595

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APPLICATION OF MOSSES TO IDENTIFICATION OF EMISSION SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS

WYKORZYSTANIE MCHÓW DO IDENTYFIKACJI ŹRÓDEŁ EMISJI WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH

Abstract: The moss *Pleurozium schreberi* was used to evaluate the emission of polycyclic aromatic hydrocarbons (PAHs) at Polish cemeteries on the All Saints' Day, when Poles traditionally light candles and candle lamps in memory of the deceased. Moss samples were exposed for 7 days at 4 cemeteries and, for comparison, in a city centre and in a rural area. During exposition, the mean content of 16 monitored PAHs in the samples increased by 455 ng/g at the cemeteries and by 689 ng g^{-1} in the city centre. In the rural area, the samples showed no statistically significant changes. PAHs whose content increased only in the moss samples exposed at the cemeteries included naphthalene, pyrene, benzo[b]fluoranthene together with benzo[k]fluoranthene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene. The concentrations of other PAHs increased in samples exposed in the city centre and at two cemeteries located in the suburban areas. The results presented confirm the possibility of using mosses in biomonitoring of PAHs.

Keywords: PAH, environmental pollution, biomonitoring, moss-bag method

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are numerous compounds that contain from two (naphthalene) up to more than ten aromatic rings in their molecular structures. They occur naturally or are produced in the environment due to volcanic eruptions and forest fires. However, the main sources of PAHs, which are purely anthropogenic, include incomplete combustion of fuels, and industrial processing of coal and oil. PAHs of low molecular masses (128÷178 g/mol) occur in the atmosphere in the gaseous form and can be transported over long distances. On the other hand, heavier PAHs (228÷278 g/mol) are solids adsorbed on particles, *eg* on soot particles, and are transported over relatively smaller distances [1-3]. In the environment, PAHs usually occur in multicomponent mixtures, the

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exact qualitative and quantitative composition of which depend on their sources. PAHs are toxic and carcinogenic to higher organisms, including humans.

Already for many years, the biomonitoring methods support the instrumental monitoring techniques [4-7]. Mosses and lichens are usually applied to evaluate the contamination of atmospheric aerosol. The biomonitoring studies include, for instance, the analyses of chemical composition of lichens and/or mosses collected in their natural environment [8-10]. Often, the exposition techniques are used, which involve the relocation of biological materials from little contaminated areas to the urbanised or industrialised ones [11-13]. The moss-bag method was introduced by Goodman and Roberts in 1971 [14].

The research on using mosses to evaluate the environmental pollution with PAH compounds started in the last decades of the 20th century. One of the first internationally circulated papers was published in 1992 and described a study in which six PAH compounds were determined in peat-mosses *Sphagnum* sp. exposed for 1 month in the vicinity of aluminium works in Rotterdam (NL) [15].

The moss exposition techniques were often used to assess PAHs in traffic emissions. For instance, mosses were exposed in the Laaer-Berg tunnel close to Vienna [16], close to highways [17, 18], and along local roads [18]. Active moss-based biomonitoring was carried out in urban areas [19-21] and within households, *eg* in the Makwampur district of Nepal [22].

PAH pollution was also assessed using mosses naturally growing in the monitored areas - a technique that belongs to passive biomonitoring. Several studies were carried out close to industrial facilities in Czech Republic [23], in Swietokrzyskie province of Poland [24-27], in Hungary [28], in Spanish provinces La Corunia and Lugo [21], and in China [29].

Another interesting study revealed the historical changes of PAH concentrations in atmospheric aerosol based on the analyses of moss samples stored in a herbarium of the University of Navarra in Spain [30].

Our research aimed to evaluate the influence of the qualitative and quantitative content of PAHs in atmospheric aerosol on the qualitative content of PAHs absorbed by mosses exposed to the aerosol for 7-day periods. The relation observed justified the application of mosses in monitoring the PAH pollution of the atmosphere, especially in the vicinity of emitters with characteristic PAH profiles.

Materials and methods

Main sources of PAH emission in the area selected for the present study included fuel combustion in municipal and household facilities, and road traffic. The area was influenced by emissions from a coke plant located 40 km away from Opole (Fig. 1) and by emissions from the Upper Silesia region. The air quality research carried out in 2010 in urban centres of the Opole province showed the year-avearge concentration of benzo[a]pyrene in air exceeded the target threshold of 1 ng/m^3, and reached 7.5 ng/m^3 in the city of Opole [31].

Collection and exposure of mosses

Samples of the moss *Pleurozium schreberi* used for exposition had been collected in forests located 20 km north to Opole. Green parts of the moss were detached, cleaned from mechanical impurities, mixed together and used to prepare 25 packets, each weighing 8 g,

which were wrapped in nylon net bags. The bagged samples were exposed for 7 days, from 29th November to 4th December, at the heights ranging from 1.8 to 2 m above the ground level. Before exposition, the samples were sprinkled with demineralised water. Figure 1 shows the localization of the exposition sites.

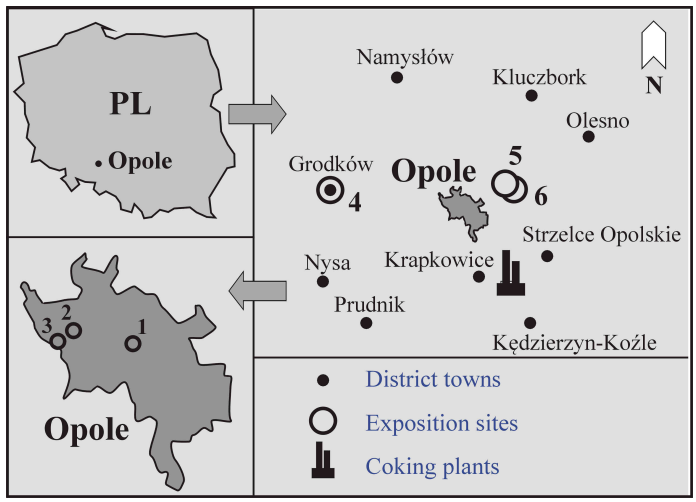

Fig. 1. Localisation of the exposition sites

Moss samples were exposed in: (1) an area in the centre of the Opole city covered with low buildings only; (2) an old forested part of a municipal cemetery located 4 km away from the Opole centre; (3) a new forested part of the latter cemetery; (4) a cemetery in a district town Grodkow, 40 km away from Opole; and (5) a cemetery in Kotorz village 15 km away from the Opole centre. Control moss samples were exposed on a site located 2 km away from the Kotorz village. A blank moss sample was stored in the laboratory in a tight plastic container. The samples were exposed at 5 points on each cemetery site, and at 3 points on the city and the village sites.

Analysis

In order to isolate the PAHs, moss samples were placed for 5 hours into a fexIKA® vario control extractor. The extraction solvent contained hexane and dichloromethane (Loba-Chemie) in a volumetric proportion of 1:1, as recommended in the literature. For the chromatographic analysis, reagents of the chromatographic purity were used.

The extract obtained was inspissated with a stream of neutral gas, and purified with aluminium oxide (Al_2O_3, Fluka) on a glass column 12 cm high and 0.5 cm in diameter. The column was stoppered at the bottom with a glass wool packing and filled with dichloromethane. Then, the column was packed with a layer of dry sodium sulphate (POCH) 0.5 cm high, followed by a layer of 5 g of neutral aluminium oxide preactivated at 130ºC, and another 0.5 cm layer of dry sodium sulphate.

After packing, dichloromethane was drained and the column was flushed three times with 1 cm^3 aliquots of hexane. Then, the column was filled with hexane up to the level of

2-3 mm above the upper sodium sulphate bed. A sample of extract was put on the column so prepared. The column was flushed four times with 1 cm^3 aliquots of hexane, the eluates being discarded. Then, a fraction containing the PAHs was eluted with 40% solution of dichloromethane in hexane.

The eluate obtained was inspissated to 340÷400 cm^3 and subjected to the gas chromatographic analysis. The latter was carried out using a Varian GC 3800 instrument equipped with an autosampler and a Varian VF 5ms column (30 m x 0.25 mm x 0.25 mm^3). The flow of a carrier gas He was set to 1 cm^3/min. The GC injector and the FID detector were operated at 300 and 310ºC, respectively. The temperature programme was set as follows: an isothermal hold at 105ºC for 2 min, followed by a temperature ramp of 4ºC per minute up to 300ºC, and an isothermal hold at 300ºC for 10 min. Sixteen polycyclic aromatic hydrocarbons were determined in the samples, according to US EPA recommendation. Table 1 shows names and acronyms of these compounds as well as corresponding *quantification limits* (*LOQ*), *recovery rates* (*RR*) and *standard deviations* (SD_{RR}).

Table 1
Names and acronyms of analysed PAHs as well as corresponding quantification limits (*LOQ*), recovery rates (*RR*) and standard deviations (SD_{RR})

PAH	Acronym	*LOQ* [ng/g]	*RR* [%]	$\pm SD_{RR}$ [%]
Naphthalene	NAP	0.22	82.5	13.2
Acenaphtylene	ACY	0.24	83.2	11.7
Acenaphtene	ACE	0.21	86.7	9.6
Fluorene	FLU	0.21	92.1	12.3
Phenanthrene	PHE	0.17	88.6	15.4
Anthracene	ANT	0.18	87.9	14.8
Fluoranthene	FLA	0.23	93.4	13.6
Pyrene	PYR	0.26	90.6	16.1
Benzo[a]anthracene	BAA	0.97	91.1	11.2
Chrysene	CHR	0.98	97.5	12.5
Benzo[b]fluoranthene	BBF	0.99	86.8	14.8
Benzo[k]fluoranthene	BKF	1.00	88.5	17.5
Benzo[a]pyrene	BAP	1.24	92.4	10.4
Benzo[g,h,i]perylene	BGP	1.72	94.2	12.3
Indeno[1,2,3-cd]pyrene	INP	1.75	91.6	11.6
Dibenzo[a,h]anthracene	DAH	1.79	88.3	13.2

Statistical analysis

For statistical computations, the R language (R Development Core Team 2009) was utilized. R is a free software environment for statistical computing and graphics. The capabilities of R can be extended through packages, which allow specialised statistical techniques, graphical devices, programming interfaces and import/export capabilities to many external data formats.

Results and discussion

Table 2 shows the increase of the total PAH concentration in the exposed moss samples, corrected basing on data in Table 1.

Table 2

The increase of the total PAH concentration in the exposed moss samples [ng/g]

Exposition site	1	2	3	4	5	6
ΣPAH	$6.9 \cdot 10^2$	$6.5 \cdot 10^2$	$2.9 \cdot 10^2$	$4.7 \cdot 10^2$	412	99
±*SD*	$1.9 \cdot 10^2$	$2.9 \cdot 10^2$	$1.6 \cdot 10^2$	$1.5 \cdot 10^2$	35	17

The increase of the total PAH concentration, ΣPAH, in moss samples exposed for 7 days in Opole (site 1) was comparable with the mean ΣPAH of 793 ng/g determined in the moss *Pseudoscleropodium purum* exposed for 2 month in the Santa Cruz city on the Tenerife island [21], and to the mean ΣPAH of 827 ng/g determined in the moss *Hylocomium splendens* exposed for 3 weeks in Trieste [20]. Much higher mean values of ΣPAH were determined in the moss *Hylocomium splendens* exposed for 4 weeks in the Laaer Berg tunnel on the A23 road close to Vienna (3990 ng/g) and by the international roads A10 and A13 (1840 ng/g) [16]; as well as in the moss exposed for 4 weeks in Warsaw (2130 ng/g) [19]. For the cemetery sites, the smallest mean ΣPAH was observed in mosses exposed in the forested part of the cemetery in Opole - 290 ng/g. The aforementioned data were obtained for different moss species which had different properties and sorption preferences [25]. Moreover, the moss-bag methods applied differed in the time of exposition, shape and packing density of the bags, and in the placement of samples. Therefore, the comparison presented is only approximate.

Figure 2 shows the average PAH composition of control and blank samples.

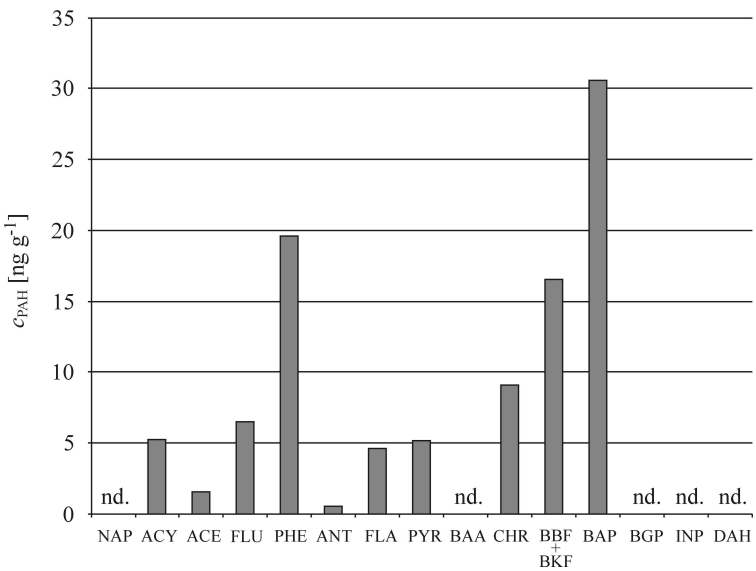

Fig. 2. Mean PAH composition in control and blank samples; nd. means 'below the detection limit'

Average total concentrations of PAHs in the control samples (6) and in the blank sample are comparable with those in blank samples of mosses collected for research in several little polluted areas: 120 ng/g in mosses from pine forests close to Untertauern in

Austrian Alps [16]; 139 ng/g in mosses collected in the vicinity of Kuopio in Eastern Finland [17]; and 172 ng/g in mosses from the *Senorío de Bertiz* preserve in Spain [30].

Comparison of quantitative content of individual PAH pollutants in the samples revealed the information on their sources. A plot in Figure 3 shows the percent increase of PAHs in samples exposed in the Opole centre (site 1). The percentage values are concentration increases of individual PAHs in relation to the total concentration increase of PAHs, ΣPAH (Table 2).

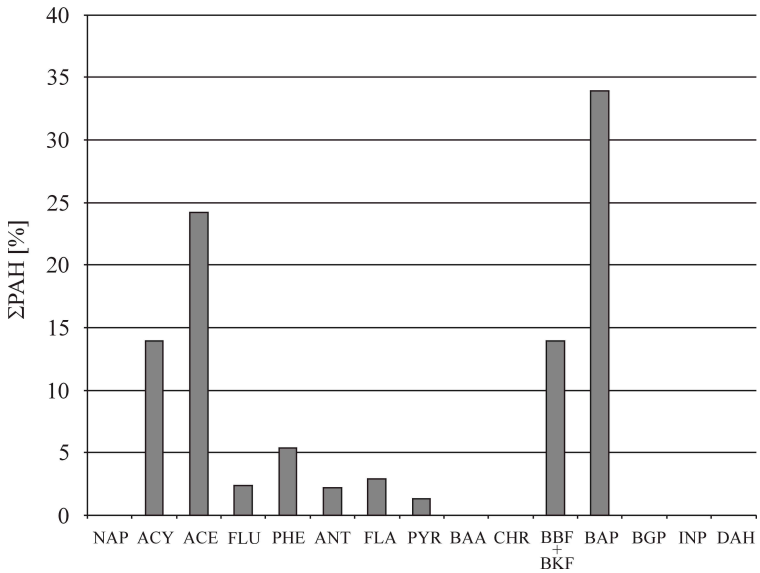

Fig. 3. Percent increase of PAH concentrations in samples exposed in the Opole centre (site 1)

The ratios PHE/ANT < 10 [27] and ANT/(ANT + PHE) > 0.1 [32] indicated the combustion of solid fuels. Coal combustion was also indicated by FLA/PYR > 1 [32] and by the ratio of 3- and 4-ring PAHs to total PAHs (3+4)/ΣPAH < 0.7 [26]. For moss samples exposed in the centre of Opole, where most of the buildings were family houses heated by coal-fired systems, the values of the indicators were: PHE/ANT = 2.4; ANT/(ANT+PHE) = 0.29; FLA/PYR = 2.2; and (3+4)/ΣPAH = 0.52.

Figure 4 shows the percent increase of PAHs in samples exposed at the cemeteries (sites 2-5).

Unlike the samples exposed in the city centre, those exposed at the cemeteries absorbed more NAP, BGP, INP and DAH; relatively more PYR and significantly more BBF+BKF (samples from the city centre contained less than 15% BBF+BKF). Box plots in Figure 5 show the distribution of the absolute concentration increments of NAP, PYR, BGP, INP, DAH and (BBF+BKF) accumulated in mosses exposed at the 4 cemeteries. The distribution of variables was described by the maximal and minimal values, upper and lower quartiles, Q3 and Q1, and medians [33].

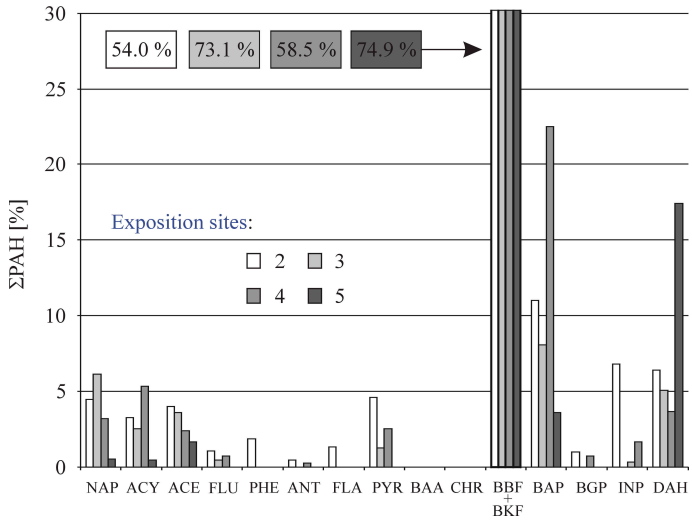

Fig. 4. Percent increase of PAH concentrations in samples exposed at the cemeteries (sites 2-5)

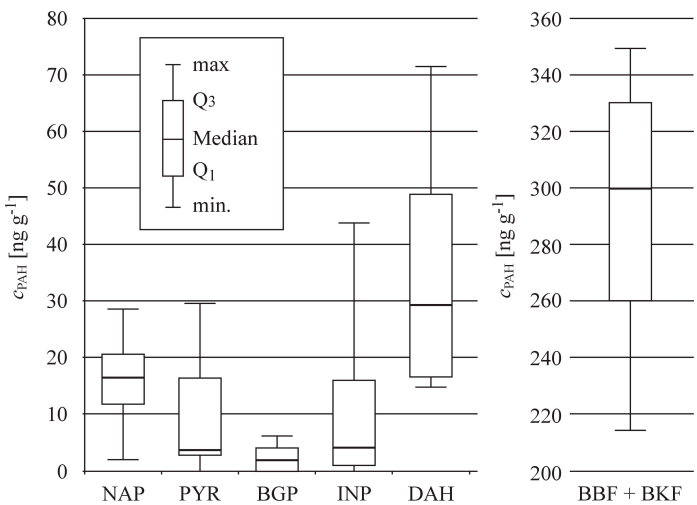

Fig. 5. Statistical parameters describing the distribution of PAH concentrations in moss samples exposed at the cemeteries

Comparably large concentrations of BBF+BKF were found in moss samples collected in the vicinity of chemical works DEZA a.s. in Valasske Mezirící - mean value 564 ng/g [23]; and in moss samples exposed for 4 weeks in the Laaer Berg tunnel close to Vienna - mean value 400 ng/g [16]. In both cases, the share of BBF+BKF in ΣPAH was less than 20%. Large values of absolute concentration increments of BBF+BKF (Fig. 5) along with BBF+BKF share exceeding 50% determined in samples exposed at the cemeteries can be characteristic of emission from burning candles and candle lamps. This was confirmed by a laboratory experiment in which moss samples were exposed for 12 hours to smoke from

randomly selected candles and candle lamps. The total concentration of PAHs in mosses, ΣPAH, increased by 5430 ng/g, and 73% of this amount was BBF+BKF.

One should keep in mind that the qualitative and quantitative composition of PAHs emitted from burning candles and candle lamps depend on the type of paraffin used by various makers, impurities thereof, additives such as dyes, and material of flammable plastic containers, if used. This is evidenced by the results obtained for samples exposed in the randomly chosen areas of the cemeteries (Figs 4 and 5).

An interesting conclusion stems from the comparison of PAH increments in samples exposed in the Opole centre (site 1) and at the suburban cemeteries (sites 2 and 3). The only PAHs considered were those whose concentrations increased in samples exposed in the city but not in samples exposed at the cemeteries (these PAHs were not included in Fig. 5). Figure 6 shows the correlations obtained.

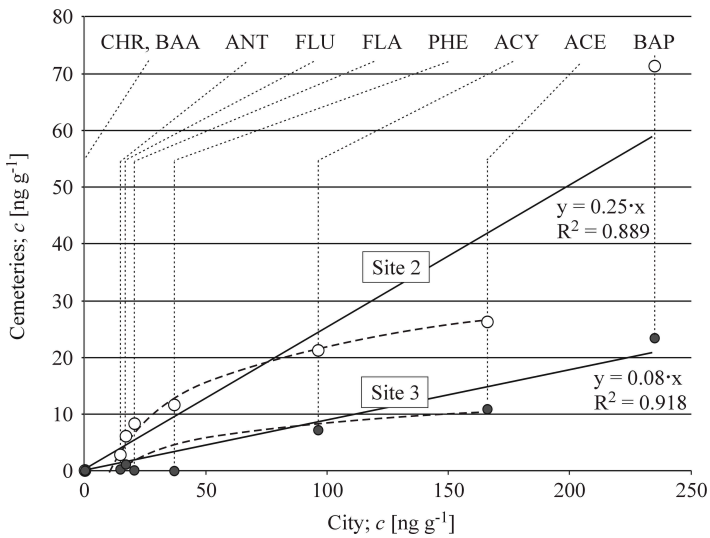

Fig. 6. Increments of PAH concentrations in mosses exposed in the centre of Opole (site 1) and at the cemeteries (sites 2 and 3)

The correlations in Figure 6 indicate one common source of PAHs absorbed in all samples. The PAH concentrations determined decreased in the following order: the city centre (site 1); the non-forested cemetery (site 2); and the forested cemetery (site 3). Apparently, the pollutants originated in the city centre only and dispersed to the city suburbs. A forest cover on site 3 reduced their deposition. Similar role of a forest canopy was demonstrated in a study on sorption of stable C, N and S isotopes in mosses [34].

Data for gaseous PAHs in Figure 6, the null values disregarded, could be approximated with logarithmic functions (dashed lines). The correlation coefficient R^2 was: 0.978 for ANT, FLU, FLA (which can also occur in the solid phase), PHE, ACY and ACE, on sites 1 and 2; and 0.987 for FLU, ACY and ACE on sites 1 and 3.

At the present state of knowledge, the proposed interpretation of results presented in Figure 6 is only a supposition. Further studies are required to fully explain the correlations obtained.

Conclusions

The results presented indicate that the *moss-bag* method can be an effective and inexpensive tool for evaluating the sources of PAH emissions. Up to now, the moss-based studies of the environmental pollution with PAH compounds were rather small by scale, compared with similar studies of heavy-metal pollution. The proper research procedures have not been developed yet. Researchers utilised various moss species which had different sorption properties. The particular moss-bag methods applied differed in the exposition time, shape and packing density of the bags, and the way the samples were placed. All the differences given, the results obtained provided much information on PAHs in the environment. For instance, the percent composition of PAHs determined was characteristic of a particular type of emission involved, and indicated the respective sources of the PAHs. Our results showed that burning candles and candle lamps resulted in emission of BBF+BKF, DAH, NAP, PYR, INP and BGP (the order corresponds to decreasing percent share in the total emission), while PAHs normally emitted during combustion of coal stayed at the background level.

For the sake of further research, the relations between absolute concentrations of PAHs contained in atmospheric aerosols and accumulated in mosses still remain unknown.

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WYKORZYSTANIE MCHÓW DO IDENTYFIKACJI ŹRÓDEŁ EMISJI WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH

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Abstrakt: Mchy *Pleurozium schreberi* wykorzystano do oceny emisji WWA na cmentarzach podczas Święta Wszystkich Świętych. W Polsce jest to tradycyjny okres pamięci o zmarłych, dla których uczczenia spalane są duże ilości świec i zniczy. Mchy eksponowano przez 7 dni na 4 cmentarzach oraz, dla porównania, w centrum miasta i na terenie wiejskim. W próbkach mchów oznaczano 16 związków z grupy WWA. Po okresie ekspozycji średni przyrost ΣWWA wyniósł: na cmentarzach 455 ng/g i w centrum miasta 689 ng/g. Nie stwierdzono statystycznie istotnych zmian w próbkach eksponowanych na terenie wiejskim. W próbkach eksponowanych na cmentarzach wykazano charakterystyczne przyrosty stężeń: naftalenu, pirenu, sumy benzo[b]fluorantrenu i benzo[k]fluorantrenu, benzo[g,h,i]perylenu, indeno[1,2,3-cd]pirenu i dibenzo[a,h]antracenu. Stwierdzono także proporcjonalne przyrosty stężeń pozostałych WWA w próbkach eksponowanych w centrum miasta oraz na 2 cmentarzach położonych na jego peryferiach. Zaprezentowane wyniki badań potwierdzają opinię o możliwościach zastosowania mchów w biomonitoringu emisji WWA.

Słowa kluczowe: WWA, zanieczyszczenie środowiska, metoda woreczkowa