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DOI: 10.2478/eces-2021-0012 ECOL CHEM ENG S. 2021;28(2):163-172

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PASSIVE AND ACTIVE BIOMONITORING OF ATMOSPHERIC AEROSOL WITH THE USE OF MOSSES

Abstract: The aim of the carried out research was passive and active biomonitoring of woodlands in the Opole province. *Pleurozium schreberi* mosses were used during the research, in which the following heavy metals concentrations were determined: Mn, Fe, Ni, Cu, Zn, Cd and Pb. Concentrations were determined with absorption atomic spectrometry (AAS). On the basis of the carried out research, concentrations of heavy metals in moss samples used in the passive and active biomonitoring methods were compared. The obtained results indicate that *Pleurozium schreberi* mosses can be successfully used in both passive and active biomonitoring, however, these methods should not be used interchangeably in a defined study area. On the basis of carried out research it was determined that the applied biomonitoring methods can be supplementary.

Keywords: passive biomonitoring, active biomonitoring, mosses, heavy metals, atomic absorption spectrometry

Introduction

Biomonitoring is one of the biological methods for environment pollution level assessment. It allows to assess the quantity and influence of pollution on ecosystems. The method is based on suing the organisms with the ability to accumulate pollution present in the environment. Such organisms include bioindicators. The offer the possibility to obtain information on the quantitative and qualitative status of air pollution [1]. The analytes, which can be identified thanks to the use of biomonitors are, among others: heavy metals and polycyclic aromatic hydrocarbons [2, 3]. Due to the type of the carried out research, we can distinguish active biomonitoring, involving exposition of an organism in a defined area and passive biomonitoring, which bases on the samples collected from their natural habitat [4-6].

According to literature, mosses, lichens and algae are some of the most frequently used bioindicators in environment pollution level assessment [7, 8]. Thanks to their specific structure, mosses absorb all substances with water through their whole surface. Easy availability and identifiability as well as sampling are additional advantages in the conducted research [1, 9].

Determining the content of heavy metals in environment is of key importance. They are particularly dangerous due to their negative influence on living organisms. Their emission to the environment causes pollution of water, fish fodder and vegetables [10, 12].

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Mosses as a bioindicator form determining heavy metals and other pollutants present in the environment are used not only in Poland [13-16], but also in other regions of the world among others in Albania [17, 18], Kosovo [19], Georgia [20], Spain [21], Canada [22], Croatia [23], Italy [24], Serbia [25] and Turkey [26]. That fact confirms that it is an organism, which can be effectively used in biomonitoring of environment pollution, regardless of the studied region and climate conditions.

Mosses *Pleurozium schreberi* are used both in pollution level assessment in natural environment, e.g. woodlands [27-29], industrial and urban areas [6, 13, 30, 31].

The objective of the presented research was to identify correlations between heavy metals concentrations determined in mosses, which were used in passive and active biomonitoring in the selected woodland areas. The study was carried out in the woodland areas of Opole province. *Pleurozium schreberi* mosses were used; the following heavy metals were determined using the atomic absorption spectrometry method: Mn, Fe, Ni, Cu, Zn, Cd and Pb.

The research methodology

Pleurozium schreberi mosses were used in the study carried out during the period July-September 2020. In passive biomonitoring mosses were collected in the woods of Turawa commune in Opole province (south-western Poland). Moss samples were collected from the locations with 1 m x 1 m areas. The collected moss samples were taken to a laboratory and dried at room temperature, until dry mass was obtained. Green parts of gametophytes were separated from mosses and mineralized [32-34].

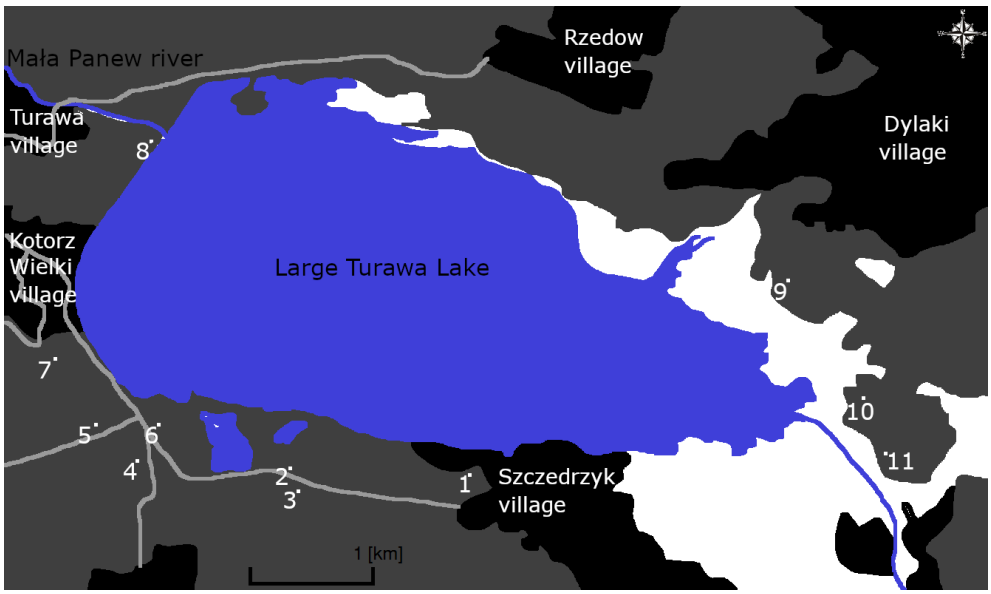

Fig. 1. Locations of biomonitoring studies (blue fields - reservoir, river; black fields - urban area; grey fields - woodlands; grey lines - roads)

Active biomonitoring was carried out by exposing mosses for 3 months (moss sample mass 3.00 g), collected from ecologically clean wood areas of Swietokrzyskie province, in 11 measuring location where mosses were collected within passive biomonitoring. Mosses were prepared prior to exposition, according to methodology [35]. The location of moss sample collecting - passive biomonitoring and sample exposition - active biomonitoring, are presented in the map in Figure 1.

Table 1 contains characteristics of the measurement locations and their GPS coordinates.

Table 1

GPS coordinates of measurement locations

Measurement location no.	GPS coordinates	Measurement location description
1	50°42'25.5"N 18°08'04.4"E	Location on the woodland border, in the vicinity of Szczedrzyk village
2	50°42'27.5"N 18°06'40.5"E	Location in the woods, approximately 150 m from the nearest road
3	50°42'20.9"N 18°06'45.1"E	Location in the woods, approximately 250 m from the nearest road
4	50°42'29.3"N 18°05'29.0"E	Location in the woods, approximately 300 m from the road
5	50°42'40.0"N 18°05'07.2"E	Location in the woods, approximately 400 m from the road
6	50°42'40.1"N 18°05'37.4"E	Location in the woods, close to bicycle paths between the large and medium Turawa lakes
7	50°42'59.5"N 18°04'44.3"E	Location in the woods, approximately 1 km from Kotorz Wielki village and 500 m away from the road
8	50°44'10.0"N 18°05'20.8"E	Location in the woods, approximately 15 km from water power plant and 500 m away from the road
9	50°43'24.8"N 18°10'39.7"E	Location in the woods, approximately 2 km away from any settlement and road
10	50°42'48.7"N 18°11'11.3"E	Location in the woods, near a bicycle path
11	50°42'32.1"N 18°11'19.7"E	Location near bicycle paths, on the eastern shore of the large Turawa lake

Equipment and reagents

The representative (averaged) moss samples with the mass of 1.000 ±0.001 g d.m. (d.m. - dry mass) were mineralized in the mixture of nitric acid(V) and hydrochloric acid (HNO_3 65 % : H_2O_2 30 % = 3:1) using a Speedwave Four Berghof, DE microwave oven. The mineralization process temperature was 180 °C. MERCK company reagents were used to prepare solutions. Heavy metals (Mn, Fe, Ni, Cu, Zn, Cd and Pb) in the mineralized samples were determined by atomic absorption spectrometry method (AAS), using the equipment iCE 3500 made by Thermo Electron Corporation (USA). The equipment was calibrated with the use of calibration standards from the company ANALYTIKA Ltd. (CZ).

Quality control

Table 2 presents the limits of detection and the limits of quantification of heavy metals for the spectrometer iCE 3500 [36].

The values of highest concentrations of the models used for calibration (2.0 mg/dm^3 for Cd, 5.0 mg/dm^3 for Cu, Zn, Ni and Pb, 7.5 mg/dm^3 for Mn and 10 mg/dm^3 for Fe) were assumed as the limit of the linear relation of the signal and concentration.

Table 3 shows heavy metals concentrations, determined in the certified reference materials as BCR-482 *lichen*, prepared by the *Institute for Reference Materials and Measurements, Belgium.*

Table 2

The instrumental detection limits (*IDL*) and instrumental quantification limits (*IQL*) for the spectrometer iCE 3500 [mg/dm^3] [36]

Metal	*IDL*	*IQL*
Mn	0.0016	0.020
Fe	0.0043	0.050
Ni	0.0043	0.050
Cu	0.0045	0.033
Zn	0.0033	0.010
Cd	0.0028	0.013
Pb	0.0130	0.070

Table 3

Comparison of measured and certified concentrations in BCR-482 *lichen*

Metal	BCR-482 *lichen*		AAS		*Dev.* **
	Concentration	± Measurement uncertainty	Average	±*SD* *	
	[mg/kg d.m.]				[%]
Mn	33.0	0.5	31.70	0.68	–3.9
Fe	804	160	771	154	–4.1
Ni	2.47	0.07	2.16	0.32	–13
Cu	7.03	0.19	6.63	0.17	–5.7
Zn	100.6	2.2	95.1	2.3	–5.5
Cd	0.56	0.02	0.53	0.03	–5.3
Pb	40.9	1.4	38.2	1.0	–6.6

* Standard deviation

** Relative difference between the measured (c_z) and certified (c_c) concentration $100\%\cdot(c_z - c_c)/c_c$

Results and analysis

The first stage of the research including assessment of heavy metals pollution in the samples of *Pleurozium schreberi* moss collected in the study area within passive biomonitoring. Table 4 presents the carried out analyses results.

Table 4

Concentrations of heavy metals determined in the mosses collected in the Turawa commune woodlands, Opole province [mg/kg d.m.]

Measurement location No.	Mn	Fe	Ni	Cu	Zn	Cd	Pb
1	160	388	< 1.25	7.35	57.5	1.09	38.7
2	241	439	< 1.25	7.42	42.2	0.96	17.6
3	261	641	< 1.25	7.82	87.0	0.94	16.9
4	124	528	< 1.25	9.68	64.5	0.53	9.71
5	1356	574	< 1.25	8.96	53.5	0.53	12.1
6	180	614	< 1.25	6.42	43.7	0.49	13.7
7	568	641	< 1.25	6.71	58.7	0.52	12.4
8	671	8148	3.67	11.4	82.6	0.50	17.8
9	88.1	403	< 1.25	8.29	38.5	< 0.33	9.23
10	130	574	< 1.25	6.96	40.7	0.49	8.68
11	281	508	< 1.25	7.95	52.8	0.54	13.3
Mean	369	1223	< 1.47	8.08	56.5	< 0.63	15.5
SD	376	2298	-	1.45	16.3	-	8.36
***CV* [%]**	102	189	-	18.0	28.8	-	54.1

- not calculated, *SD* - Standard deviation, *CV* - coefficient of variation

Pollution levels were different in the selected analytes of the mosses collected in designated locations in the study area. Concentrations of Cd, Zn and Pb were determined in the mosses collected in measurement locations 1 and 3 to be higher than the mean value defined for all analysed samples. Cu concentrations were higher than the mean value in measurement locations 4, 5, 8 and 9. Additionally, higher concentrations of Zn, Pb, Ni, Mn and Fe were determined in the samples collected from measurement location 8. Large *SD* values in reference to mean Mn and Fe concentration values signify different pollution level of mosses, their heterogeneity and indicate the possibility that the studied metals originate from several sources, among others dry deposition from the soil.

The results obtained during active biomonitoring were analysed in the second stage of the research (Table 5).

Table 5

Concentrations of heavy metals determined in the mosses exposed in the Turawa commune woodlands, Opole province [mg/kg d.m.]

Measurement location No.	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
1	256	608	< 1.25	7.83	66.5	1.08	10.4
2	211	488	< 1.25	7.48	53.0	0.89	16.4
3	318	573	< 1.25	7.00	53.1	0.88	11.3
4	219	501	< 1.25	7,48	51.0	0.90	8.67
5	203	635	< 1.25	7.28	56.7	0.92	11.5
6	241	504	< 1.25	7.98	57.7	0.94	16.1
7	314	443	< 1.25	7.47	48.6	0.89	12.8
8	230	883	< 1.25	7.86	66.5	1.09	27.6
9	278	699	< 1.25	9.01	57.1	1.00	12.4
10	193	558	< 1.25	7.48	50.9	0.93	7.92
11	210	515	< 1.25	7.41	52.7	0.93	13.5
Blind sample	154	308	< 1.25	7.52	42.1	0.58	11.1
Mean	236	583	< 1.25	7.65	54.7	0.92	13.3
SD	44	124	-	0.50	6.93	0.13	5.16
***CV* [%]**	18.5	21.3	-	6.86	10.7	7.89	39.8

- not calculated, *SD* - Standard deviation, *CV* - coefficient of variation

By analysing the results of active biomonitoring we are able to identify the locations with larger or smaller deposition of a given analyte to air, during the three-month period of moss samples exposition. The highest metals concentrations were determined in the moss samples exposed in measurement locations 1, 6, 8 and 9. The most significant pollution source in these locations was increased communication traffic, which was present during samples exposition in holiday season.

Considerably lower SD values (9 times lower for Mn and 19 times lower for Fe) in reference to the research results of passive method signify larger homogeneity of the mosses exposed in the study area, which results from their preparation methodology [35], showing at the same time restricted influence of the dry deposit from soil on the result of active biomonitoring study.

The maps in Figures 2-7 present spatial distribution of analytes determined in moss samples, both in passive biomonitoring (metal concentrations in [mg/kg d.m.] determined in mosses collected from the study area) and active (absolute concentrations of analytes $c = c_z - c_0$; where: c_z - concentration measured in [mg/kg d.m.], c_0 - concentration in blind sample in [mg/kg d.m.]) in the research area.

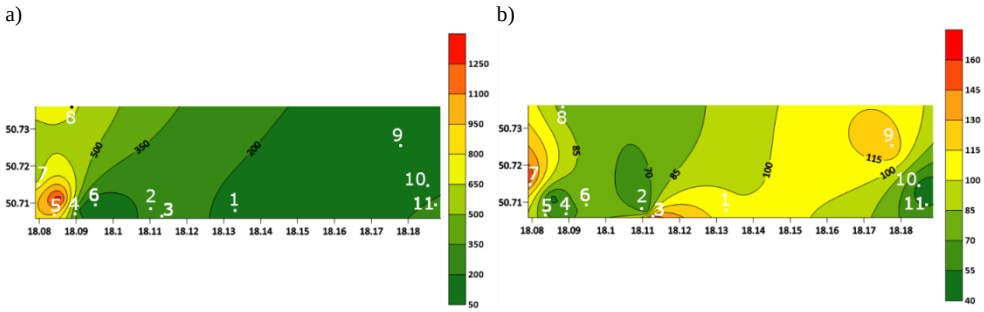

Fig. 2. Spatial distribution of Mn in the research area determined on the basis of concentration of the analyte in moss samples [mg/kg d.m.]: a) collected by passive method, b) exposed in active biomonitoring

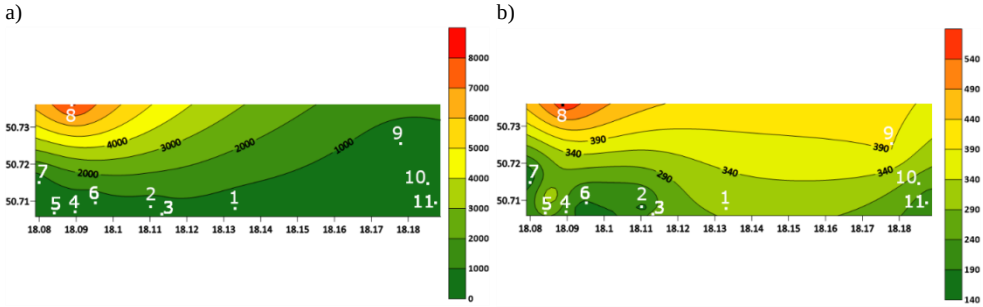

Fig. 3. Spatial distribution of Fe in the research area determined on the basis of concentration of the analyte in moss samples [mg/kg d.m.]: a) collected by passive method, b) exposed in active biomonitoring

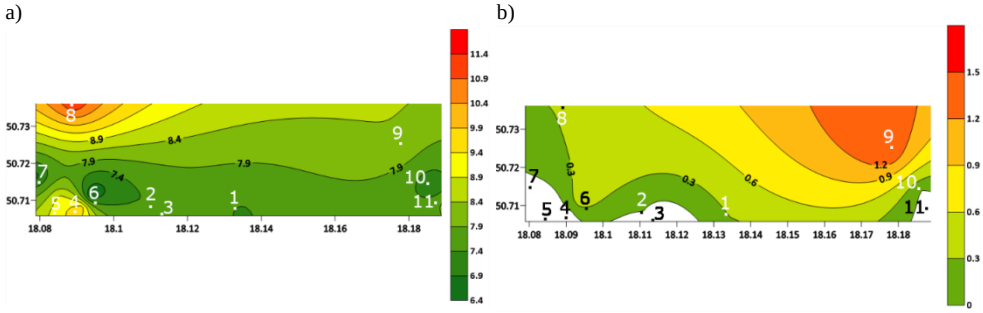

Fig. 4. Spatial distribution of Cu in the research area determined on the basis of concentration of the analyte in moss samples [mg/kg d.m.]: a) collected by passive method, b) exposed in active biomonitoring

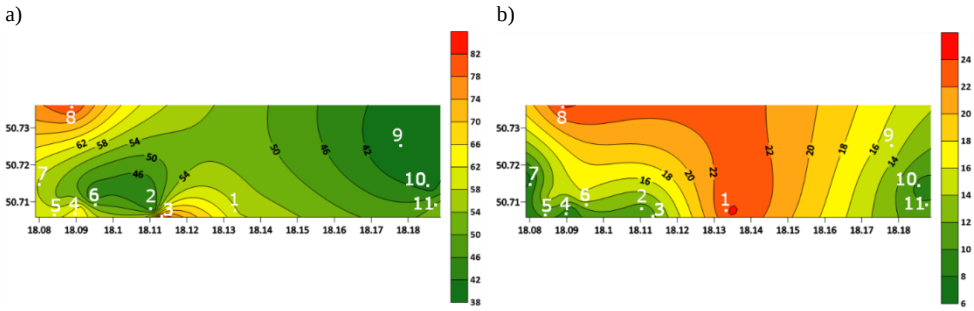

Fig. 5. Spatial distribution of Zn in the research area determined on the basis of concentration of the analyte in moss samples [mg/kg d.m.]: a) collected by passive method, b) exposed in active biomonitoring

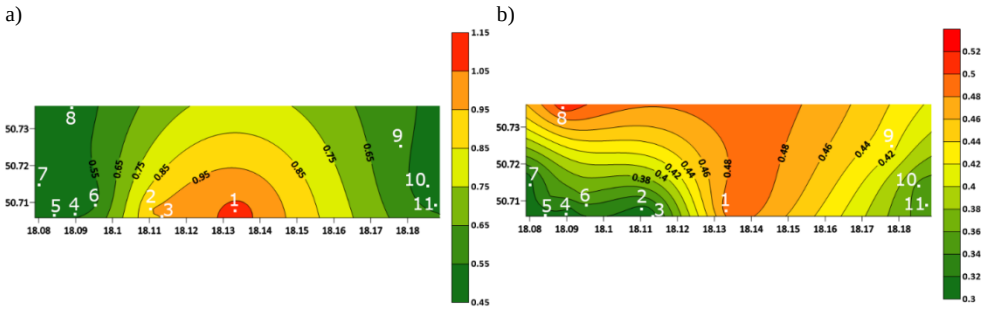

Fig. 6. Spatial distribution of Cd in the research area determined on the basis of concentration of the analyte in moss samples [mg/kg d.m.]: a) collected by passive method, b) exposed in active biomonitoring

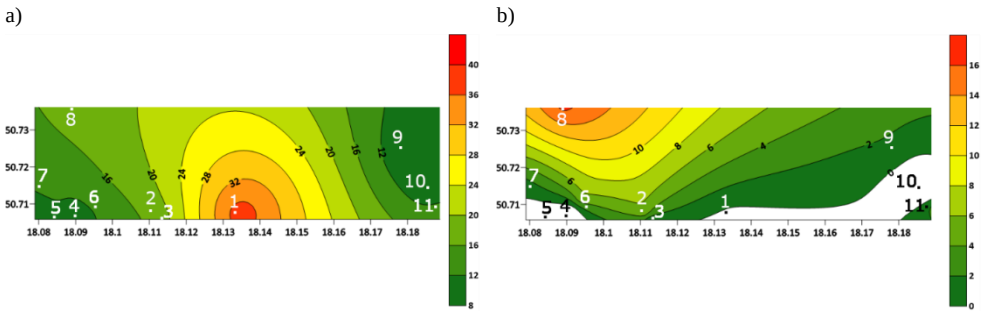

Fig. 7. Spatial distribution of Pb in the research area determined on the basis of concentration of the analyte in moss samples [mg/kg d.m.]: a) collected by passive method, b) exposed in active biomonitoring

Comparison of spatial distribution of metals in the research area, determined on the basis of the determined concentration of analytes in mosses one can state that passive and active biomonitoring methods cannot be used alternatively but they can be complementary. This results from too many variables which influence the research results obtained by both

methods, among others mosses lifetime/exposition, climate, influencer of secondary deposition from soil, selection of measurement locations. Simultaneous application of both methods may help detect, for example, concentrated sources of pollution or emission from distant sources occurring during mosses exposition period. Analysis of the carried out research results proved that in mosses collected by passive method in location 9, cadmium concentration was below detection limit of the applied analytical method. However, the active method detected in this location concentrations above the mean value set for all measurement locations. The pollution level of atmospheric aerosol with Cd in this measurement location can be influenced by emission from distant sources, e.g. burning of communal waste during the three-month exposition period of mosses [37].

In order to determine uncertainty of the measurement method, moss samples were analysed three times, maintaining the whole cycle of the research method. For the moss samples collected/exposed in measurement locations, the value of coefficient of variation *CV* determined on the basis of standard deviation value *SD* (CV [%] = $(SD_i / c_{x,aver,i}) \cdot 100$ %, where: $SD_i / c_{x,aver,i}$ is the standard deviation calculated for the *i*-series (i = 11), referring to the mean value from that series ($c_{x,aver,i}$)) is within the range 18-189 % for the passive method and 6.86-39.8 % for the active method. The active method, considering the proper moss samples exposition preparation method, exposition at the height of approximately 1.5 m (which limits the influence of secondary deposition from soil) seems to be more reliable and illustrate the actual heavy metals pollution levels in atmospheric aerosol during the three-month exposition period of mosses.

Conclusion

Mosses are perceived as one of the major bioindicators of heavy metal pollution in air biomonitoring. Analysis of, among others, heavy metals concentrations captured in mosses provides much information regarding the pollution introduced to atmospheric aerosol, allows to asses changes in air quality and to identify the sources of pollution.

Currently, two methods of atmospheric aerosol biomonitoring with the use of mosses are used: passive and active. The research results obtained thanks to the use of these methods have not been compared so far. This is the result of heterogeneity of the research material and many factors, which influence the final result.

On the basis of carried out research, the authors suggest that passive and active biomonitoring methods should not be used alternatively in the same study area, but they can be complementary. Simultaneously it was proved that active biomonitoring method produces more reliable results (lower *CV* values) in comparison to passive biomonitoring.

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