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Preliminary study of the use of terrestrial moss (*Pleurozium schreberi*) for passive biomonitoring related to Hg deposition

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Abstract: This study aimed to assess the possibility of using mosses as part of passive biomonitoring of mercury deposition from anthropogenic sources in the municipality of Murów (Opole Voivodeship). Hg concentrations were analyzed using the AMA254 mercury analyzer in the *Pleurozium schreberi* species mosses. The results obtained made it possible to identify potential sources of contamination and to indicate the direction of environmental spread of mercury in the study area. The study confirmed that the mosses *P. schreberi* accumulate mercury from atmospheric aerosol and its concentration is proportional to its concentration in the air. The direction of winds is crucial in the spread of pollution from anthropogenic sources. In the study area, mercury polluting sources of atmospheric aerosol such as glass processing plants, sawmills and traffic were identified.

Keywords: mosses, environment quality, pollution, mercury

Introduction

Mercury has been used in industry for many years. However, despite this, it also poses serious risks. Like other heavy metals, mercury released into the environment is incorporated into its biological cycle (Chmielewski et al., 2020). Furthermore, mercury is called a persistent environmental pollutant because it is not converted to harmless forms (Kot et al., 2016). Although mercury is released into the environment by both anthropogenic and natural sources, it is recognized that the main source is human activity. The burning of fossil fuels, the mining and processing of gold and non-ferrous metals and the paper and cement industries are

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considered to be the main sources. It is estimated that the amount of mercury released from natural, anthropogenic sources and re-emissions is 6000-11000 tons/year. The literature reports that about 80% comes from anthropogenic sources (Chmielewski et al., 2020; Yang et al., 2020).

In the environment, mercury exists in three forms: elemental, inorganic and organic. Each form of mercury is toxic and differs in its transport mechanisms and effects on the human body (Yang et al., 2020). Humans are exposed to mercury through inhalation and ingestion. Nails, skin, hair and kidneys are extremely vulnerable to mercury exposure. Inhalation of fumes from elemental mercury leads to pneumonia and, in cases of very high concentrations, necrotizing bronchitis (Maqbool et al., 2017). Methylmercury (CH_3Hg^+), is considered to be the most toxic form of mercury; it is an organic mercury that acts primarily on the central nervous system. It is a potent neurotoxin that also has nephrotoxic and hepatotoxic effects and the ability to bioaccumulate and biomagnify (Chmielewski et al., 2020; Díez, 2009; Kot et al., 2016). Damage caused by methylmercury can manifest itself in the form of tingling, a numb feeling in the fingers and toes, difficulty walking, general weakness, loss of vision and hearing and, ultimately, unconsciousness leading to death (Díez, 2009). All these risks make it extremely important to monitor the concentration of this element in the environment.

Mercury levels in the environment can be monitored using several different methods. Mercury can be determined in air samples, using sorption sensors in which mercury is sorbed onto a gold surface (Król and Kukulska-Zając, 2020). Other methods are biological methods for assessing environmental quality, which can be divided into biomonitoring and biotesting (Jakubus and Tatuśko, 2015). Biotests are routine toxicological and pharmacological procedures rarely performed in the field, mostly in the laboratory. There are three types of toxicity bioassays: laboratory-based, where the toxic compound is introduced artificially, laboratory-based tests conducted based on collected samples, and using acute toxicity tests (Kozak and Włodarczyk-Makuła, 2016). Biomonitoring uses bioindicators as test material on which observations are made. The collected data enable qualitative and quantitative assessment of the state of the environment. We can divide bioindicators into two groups. These are organisms that appear under the influence of a given pollutant and those that cease to appear in their natural environment under the influence of this factor. Organisms that accumulate pollutants in their tissues are also used in biomonitoring. Such bio-indicators are used in passive and active biomonitoring. Active biomonitoring involves the use of organisms grown in unpolluted conditions or collected from unpolluted areas, while passive biomonitoring uses organisms that occur naturally in the environment under study (Jakubus and Tatuśko, 2015; Świsłowski P et al., 2019)

Many types of organisms can be used in biomonitoring. Commonly used organisms are mosses, which can accumulate metals in quantities that exceed their physiological needs because they do not have cuticles and because of the high cation exchange capacity of their cell walls (Macedo-Miranda et al., 2016; Markert et al., 1999). In addition, they are common plants in the environment, which favours the collection of a large number of samples in a selected area (Macedo-Miranda et al., 2016). One of the most widely used moss species is *P. schreberi*, which was used, among others, by Świsłowski et al. (2019) in a study on the impact of traffic on pollution deposition along a motorway (Świsłowski P et al., 2019). This species was also used by Migaszewski et al. (2010) in a study that aimed to identify sources of Hg (Migaszewski et al., 2010) and Zawadzki et al. (2016), who analyzed the metal accumulation capacity of this species compared to *Polytrichum commune* (Zawadzki et al., 2016). Other moss species that are also used in biomonitoring include *Hylocomium splendens* (Migaszewski et al., 2010; Świsłowski P et al., 2019), *Scleropodium purum* (Fernández et al., 2000), *Hypnum cupressiforme* (Fernández et al., 2000; Świsłowski P et al., 2019). Organisms that are also commonly used in biomonitoring are lichens, for example, *Anaptychia setifera* used to assess heavy metal pollution in urbanized areas (Abdollahi et al., 2025). Algae are also used in this type of research, an example being *Palmaria palmata* used by Zielińska et al. (2013) during active biomonitoring of the Oder River (Zielińska et al., 2013). Organisms of other genera can also be used in biomonitoring, for example, *Echinogammarus veneris*, belonging to the amphipod crustaceans used by Ronci et al. (2016) when comparing the use of active and passive biomonitoring in assessing the genotoxicity and bioaccumulation of metals in this organism (Ronci et al., 2016). Human hair, which was used in a study by Brodzka et al. (2009), can also be considered as an indicator of environmental metal pollution (Brodzka and Trzcinka-Ochocka, 2009).

The aim of the study was a preliminary assessment of the possibility of using mosses of the *P. schreberi* species (passive biomonitoring) to identify sources of mercury and directions of its dispersal in the area of the municipality of Murów (Opole Province). The research hypothesis, which was verified in the course of the study, was that the mosses *P. schreberi* accumulate mercury from atmospheric aerosol, and its concentration is proportional to its concentration in the air.

Materials and methods

Passive biomonitoring covered an area of 25 km^2 in the municipality of Murów, located in the Opolskie Voivodeship (PL). The study was conducted from 23 October to 31 December 2024. To delimit the study area, a grid was superimposed on the map, consisting of 25 squares measuring 1 km x 1 km (Fig 1a). The squares were then numbered (Fig. 1b). Three samples of *P. schreberi* mosses of approximately 2 g fresh weight were collected from each square and placed in paper bags. We collected only the green parts of moss gametophyte (i.e. including live and active tissues) (Boquete et al., 2014). Table 1 provides a brief characterization of the areas contained within each square. The collected material was pre-cleaned in the field (e.g. soil particles) and then transported to the MCBR laboratory (International Research and Development Center of the University of Opole).

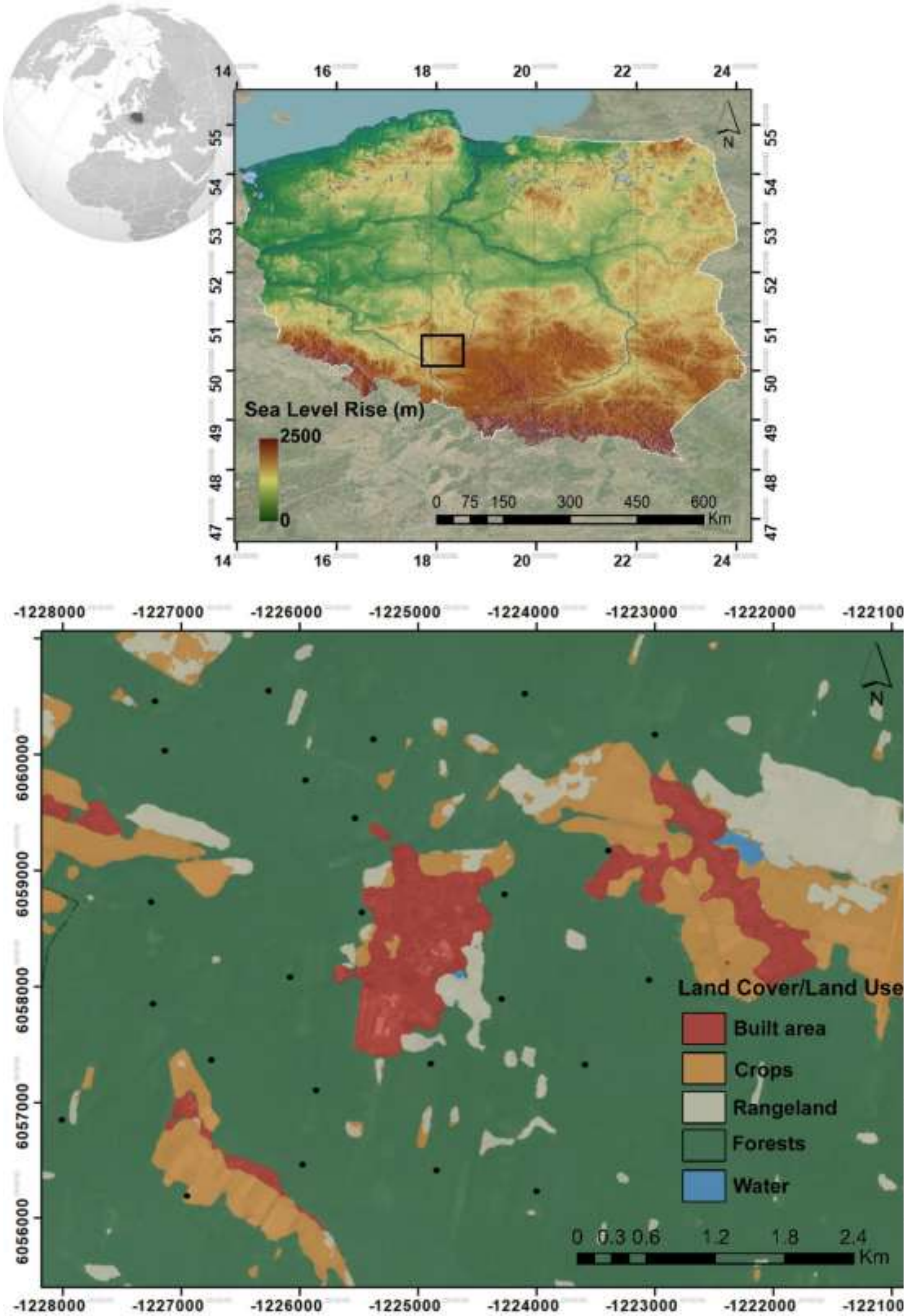

Fig 1a: Map of the study area against Poland with land cover

Source: ArcGIS software

Fig. 1b: Map of the study area with superimposed 1x1 km grid (Google Earth Pro)

Source: Google Earth Pro

Table 1. Characteristics of the areas falling within each of the 25 quadrants

Sample No.	Location description	GPS data
1	Samples collected in the forest, next to the village of Czarna Woda and a little-used road.	50° 52' 35.7226" N, 17° 54' 36.1937" E
2	Samples collected in the forest, next to a little-used road.	50° 52' 43.8701" N, 17° 55' 22.4972" E
3	Samples collected in the forest, there were no villages or major roads in the immediate area.	50° 52' 36.1556" N, 17° 56' 9.6799" E
4	Samples collected in the woods, near the village of Zagwiździe, no major roads in the area.	50° 52' 55.6702" N, 17° 57' 8.7696" E
5	Sampled in the forest on the outskirts of the village of Zagwiździe, along a very little-used road.	50° 52' 50.9502" N, 17° 58' 6.01" E
6	Samples were taken in a forest near Okoły, with a little-used road and the Budkowiczanka river in the area.	50° 52' 22.8821" N, 17° 54' 43.9546" E
7	Sampling took place in the forest between Okoły and Murów; the area included a little-used road, the Budkowiczanka river and a sewage treatment plant.	50° 52' 21.7463" N, 17° 55' 44.6804" E

Sample No.	Location description	GPS data
8	Samples were taken on the outskirts of the village of Murów, near a forest clearing area, by a little-used road and the Budkowiczanka river, close to the sewage treatment plant.	50° 52' 14.1413" N, 17° 56' 8.1856" E
9	Samples collected in the forest between the villages of Murów and Zagwiździe, next to a moderately used road.	50° 52' 0.9005" N, 17° 57' 15.8922" E
10	Sampled on the outskirts of the village of Zagwiździe, near a moderately used road.	50° 52' 17.7931" N, 17° 57' 55.7748" E
11	Samples collected in the forest near the village of Okoły, by the Budkowiczanka river.	50° 51' 41.7784" N, 17° 54' 50.4925" E
12	Samples collected in the forest next to the village of Murów, next to a frequently used road.	50° 51' 28.3428" N, 17° 55' 53.6992" E
13	Samples collected in the forest on the outskirts of Murów and from the center of the village, close to the sawmill and production plant.	50° 51' 49.2977" N, 17° 56' 18.4549" E
14	Samples collected in the forest near the village of Murów, the production plant was located nearby, no busy roads.	50° 51' 33.0" N, 17° 57' 23.1" E
15	Samples collected in the forest, no busy roads in the area.	50° 51' 45.1238" N, 17° 58' 22.7053" E
16	Samples collected in the forest, close to a busy road.	50° 51' 14.5379" N, 17° 54' 59.049" E
17	Samples collected in the forest between the villages of Murów and Grabczok, close to a busy road.	50° 51' 2.5837" N, 17° 55' 27.493" E
18	Samples collected in the forest in the vicinity of a sawmill, near a moderately used road.	50° 50' 59.4197" N, 17° 56' 13.4448" E
19	Samples collected in the forest near the village of Murów and the sawmill, no busy roads in the area.	50° 51' 12.2" N, 17° 56' 58.7" E
20	Samples collected in the forest, no busy roads in the area.	50° 51' 19.4306" N, 17° 58' 2.663" E
21	Samples collected in the forest near Grabczok, next to a busy road.	50° 50' 39.1085" N, 17° 54' 30.5928" E
22	Samples collected in the village of Grabczok, close to a moderately used road.	50° 50' 24.8575" N, 17° 55' 28.4113" E
23	Sampled in the forest near Grabczok, next to a moderately used road.	50° 50' 38.9609" N, 17° 56' 13.7803" E
24	Samples collected in the forest, there were no busy roads in the area.	50° 50' 43.9102" N, 17° 57' 9.3222" E
25	Samples collected in the forest in the area there were no busy roads.	50° 50' 43.2438" N, 17° 57' 52.3325" E

The mosses *P. schreberi*, after collection in the field and transport to the laboratory, were cleaned of mechanical impurities, e.g. needles, insects, and dried at room temperature to dry mass (d.m.). Averaged moss samples of 0.0400 ± 0.001 g d.m. were analyzed in an AMA 254 mercury analyzer from Altec Ltd, CZ. For mercury, the instrument's limits of detection (*IDL*) and quantification (*IQL*) are 0.003 ng (0.03 μg Hg/dm^3) and 0.01 ng (0.1 μg Hg/dm^3) in the test sample, respectively. To calibrate the apparatus, standards from ANALYTIKA Ltd. (CZ).

Spatial distribution map was created using ArcGIS software to spatially understand the accumulation of Hg in mosses to relate them to land use (Isinkaralar et al., 2023).

Statistica (ver 13.3), and Microsoft Excel 2021 software were used to process and present the data. For descriptive analysis, the minimal and maximal values, mean with standard deviation, were calculated for each analyzed element. Shapiro-Wilk's test was used to check data normality due to sample sizes (Mishra et al., 2019). Therefore, differences between the points in terms of Hg concentrations in the mosses were evaluated by the Student t test and nonparametric Wilcoxon test. A difference was considered to be statistically significant when $p < 0.05$.

Results and discussion

Table 2 presents the basic statistical parameters of mercury determinations in moss samples collected from the Murów commune in the Opole Voivodeship. In turn, Figs. 2-3 shows the variability of mercury concentrations at each measurement point.

Table 2. Results of Hg determinations (mg/kg d.m.) in moss samples collected from the Opolskie Voivodeship (PL)

Sample No.	Min.	Max.	Mean	±SD
1	0.02343	0.03725	0.03080	0.006956
2	0.02877	0.05722	0.04023	0.015010
3	0.03195	0.04233	0.03683	0.005215
4	0.03913	0.06445	0.05064	0.012815
5	0.02985	0.04883	0.03901	0.009507
6	0.02594	0.04022	0.03294	0.007144
7	0.03559	0.04710	0.03965	0.006465
8	0.02371	0.02612	0.02513	0.001261
9	0.02774	0.03876	0.03306	0.005520
10	0.02653	0.03511	0.03081	0.004291
11	0.02751	0.04045	0.03559	0.007045
12	0.01896	0.02368	0.02199	0.002632
13	0.02806	0.03064	0.02963	0.001377
14	0.02402	0.03111	0.02641	0.004077

Sample No.	Min.	Max.	Mean	±SD
15	0.03002	0.03755	0.03314	0.003931
16	0.03522	0.06181	0.04452	0.014985
17	0.02659	0.03079	0.02884	0.002120
18	0.03122	0.03914	0.03630	0.004414
19	0.02710	0.03891	0.03281	0.005915
20	0.02742	0.04755	0.03775	0.010502
21	0.02229	0.02373	0.02299	0.000722
22	0.02216	0.03018	0.02522	0.004329
23	0.02886	0.04223	0.03424	0.007062
24	0.02249	0.03011	0.02753	0.004366
25	0.02398	0.02785	0.02624	0.002013

Min. – minimal; Max. – maximum; ±SD – standard deviation

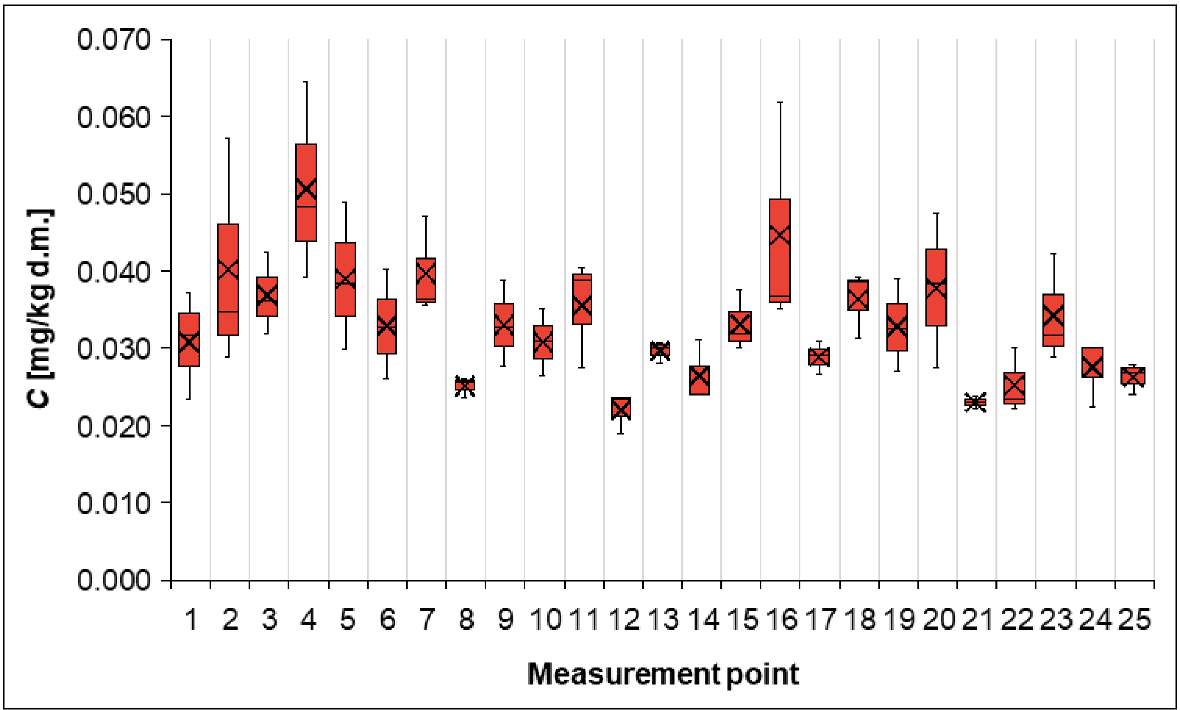

Fig. 2. Distribution of mercury concentrations at individual measurement points. (X) indicates the mean value, and the horizontal dash (–) is the median value. Chart whiskers from bottom to top indicate min and max data, respectively

Source: Microsoft Excel 2021 software

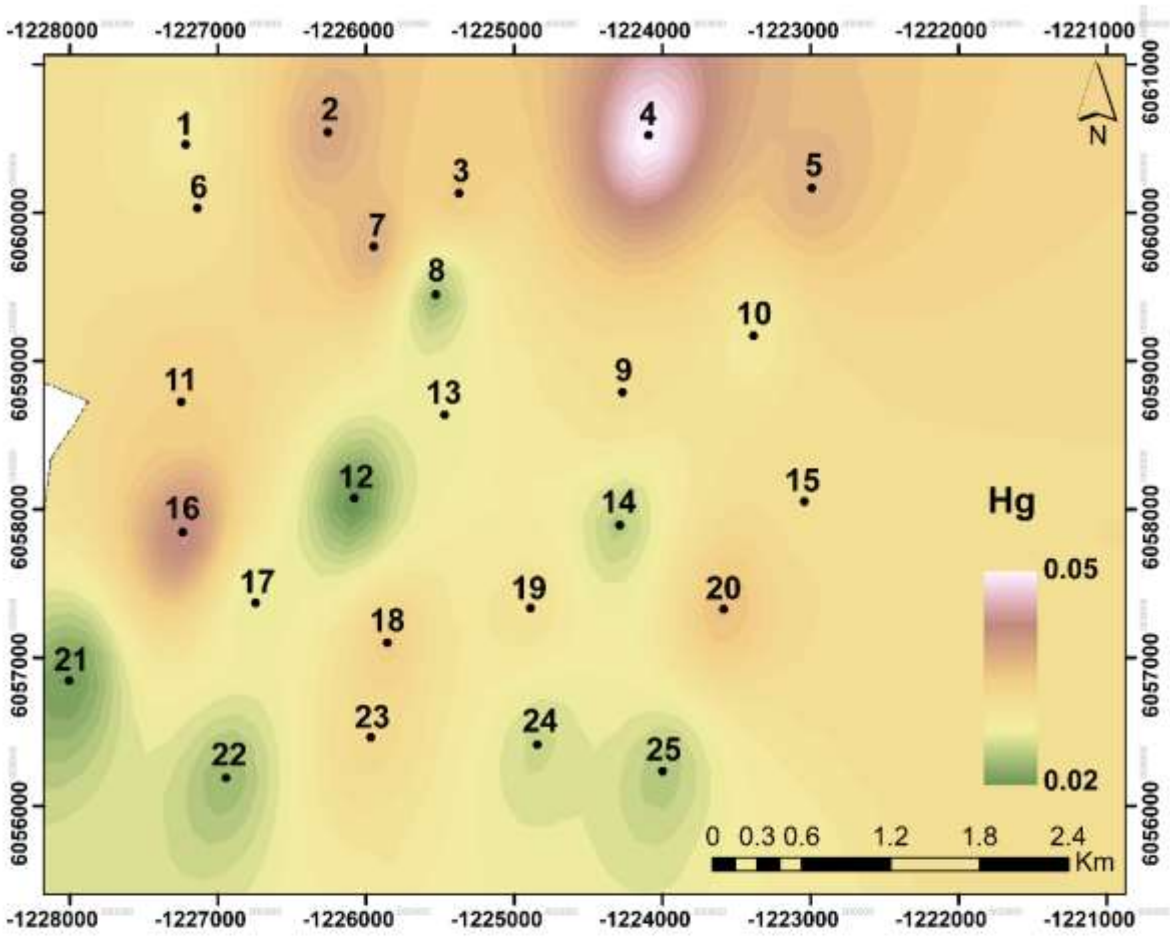

Fig. 3. Spatial distribution of Hg in the moss samples (mean values). Black dots represent the sampling sites

Source: ArcGIS software

To illustrate statistically significant differences, the previously mentioned tests were used to assess differences in concentrations for individual measurement points (Table 3).

Table 3. Student t test comparing statistical significance of concentrations between points

Group 1	Group 2 (Points)	T	*P*
Point 3 vs	8	3.78	*
	12	4.40	*
	21	4.55	*
	22	2.97	*
	25	3.28	*
Point 4 vs	8	3.43	*
	12	3.79	*
	13	2.82	*
	17	2.91	*

Group 1	Group 2 (Points)	T	*P*
	21	3.73	*
	22	3.25	*
	25	3.26	*
Point 5 vs	12	2.99	*
	21	2.91	*
Point 7 vs	8	3.82	*
	12	4.38	*
	21	4.44	*
	22	3.21	*
	25	3.43	*
Point 8 vs	13	-4.17	*
	15	-3.36	*
	18	-4.22	*
Point 9 vs	12	3.13	*
	21	3.13	*
Point 10 vs	12	3.03	*
	21	3.11	*
Point 11 vs	12	3.13	*
	21	3.08	*
Point 12 vs	13	-4.45	*
	15	-4.08	*
	17	-3.51	*
	18	-4.82	**
	19	-2.90	*
	23	-2.81	*
Point 13 vs	21	7.39	**
Point 15 vs	21	4.40	*
Point 17 vs	21	4.53	*
Point 18 vs	21	5.15	**
	22	3.10	*
	25	3.59	*
Point 19 vs	21	2.86	*

values are statistically significant at the level: [*] $p < 0.05$, [**] $p < 0.01$

Analysis of the above results indicates that the highest mean mercury concentration (0.05064 mg/kg d.m.) was found in the sample in quadrant number 4, and the sample in quadrant number 16 (0.04452 mg/kg d.m.). Samples from areas to the north were generally characterized by higher concentrations than samples further south (see Fig. 3.). According to www.WeatherOnline.pl, the prevailing wind direction during the study period was south, and to a lesser extent, west (Weatheronline.pl, 2024). It can therefore be concluded that the source of the elevated mercury in the samples is to the north of them. To the south of these points were several plants: sawmills and a glass manufacturer. According to the literature, both sawmills (Kwasigroch et al., 2021) and glass processing plants (Kondej M, 2006) are potential sources of mercury contamination, so they could be the reason for the elevated levels in the areas to the north of the study area. Point 16, on the other hand, was located to the south-west of these plants, so it should not be so affected by them. On the other hand, it was in the vicinity of a busy road, including by delivery vehicles for sawmills and other plants. These roads also extend to the north of this point. By this, it can be inferred that they influenced the elevated mercury levels in the area (Cai and Li, 2019). The results of the statistical analysis show that 72% of the points were statistically different due to mercury concentration (see Table 3 for comparison). Points 1, 2, 6, 14, 16, 20, 24 were not statistically significant relative to the others in terms of analyte concentration. The points that differed most from the others (see Table 3) were points numbered 12 and 21, where the lowest mercury concentrations were determined (compare with Fig. 2). *P. schreberi* is considered a good indicator of mercury pollution (Bargagli, 2016) as evidenced by its cumulative concentrations of 0.44 mg/kg in the vicinity of the chlor-alkali industry (Zawadzki et al., 2014). Studies by other authors, also confirm that the source of mercury contamination by *P. schreberi* is industrial enterprises, is considered the primary source (Gatina et al., 2024). National studies also confirm that heavily industrialized areas (influenced by heavy industry and transport) have significantly higher concentrations for native samples of *P. schreberi* at 0.45 mg/kg, influenced of the complex of two power stations (Samecka-Cymerman et al., 2013).

Conclusions

In modern environmental quality testing methods, classical instrumental monitoring methods are increasingly being supplemented or replaced by biomonitoring methods. Biomonitoring can compete effectively with classical methods of monitoring atmospheric aerosol quality, as its main advantage is the low-cost and no special training required for sampling. Passive biomonitoring using mosses has a very high potential in monitoring environmental pollution by heavy metals such as mercury. It is a method that, in combination with other means of assessing the environment, allows for a very thorough investigation of its condition.

The study confirmed the effectiveness of a passive biomonitoring method using mosses of the *P. schreberi* species. They make it possible to assess qualitatively and quantitatively the level of environmental mercury pollution. They also provide an opportunity to determine the direction from which these contaminants originate and the potential source of their deposition.

The results from the study, indicate that biomonitoring can be widely used to assess atmospheric aerosol pollution in selected areas, e.g. it can be used for short-term (smog period) or long-term air quality monitoring, which will be simple to carry out, economically viable and reliable.

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Not applicable.

Consent to publish

Not applicable.

Data availability statement

All data generated and analyzed during this study are available from the corresponding author on reasonable request.

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