



## Article

# Active Biomonitoring of Heavy Metal Concentrations in Aquatic Environment Using Mosses and Algae

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**Abstract:** In this paper, we present an analysis of the pollution of surface water and air by the heavy metals: Mn, Fe, Ni, Cu, Zn, Cd and Pb in the area of the smelter in Ozimek (Opolskie Voivodeship, Poland). The marine algae *Palmaria palmata* was exposed in the Mała Panew River, and three species of forest mosses: *Pleurozium schreberi*, *Sphagnum fallax* and *Dicranum polysetum* were also applied. Active biomonitoring was also carried out using *P. schreberi* mosses to assess air pollution, since the area and the river are influenced by the smelter's activities. Analytes were determined by atomic absorption spectrometry. Student's *T*-test and Mann–Whitney U test were used to assess the significance of differences in elemental concentrations between algae and mosses. Forest mosses were found to have comparable sorption properties to algae under laboratory conditions. During exposure in the river, statistical significance was found between these matrices. The results of water biomonitoring studies using forest mosses offer the possibility of their inclusion in aquatic ecosystem monitoring as an alternative to existing classical surface water biomonitors. Perspectives, attention should be paid to comparing the sorption properties of aquatic species with forest mosses in order to optimize the water biomonitoring system using mosses.

**Keywords:** biomonitoring; water; algae; mosses; heavy metals; industry



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## 1. Introduction

Heavy metal pollution is a serious problem for plants, animals and humans because of the negative effects they cause around the world. Analytes primarily enter the air, surface water and soil due to rapidly expanding agriculture, metallurgy and inadequate disposal of waste and fertilizers or pesticides [1,2] but primarily come from anthropogenic activities such as metallurgy, foundry, mining and road transport [3]. They also come from naturally occurring sources in the environment: volcanic eruptions, metal corrosion, soil erosion or rock weathering [4]. Considering the role of trace elements in biological systems, they can be divided into those that are essential for living organisms, such as manganese, copper, iron and zinc, but only in low concentrations, and those that are toxic, such as cadmium, lead or mercury [5].

Organic matter, suspended solids, nitrogen, phosphorus, heavy metals and oils, as well as other contaminants, go directly into rivers and lakes, thanks to rainwater inflows, which are one of the main causes of water pollution [6]. Technological progress and industrial development have led to the poisoning of aquatic and terrestrial biotopes so that a deterioration in environmental quality is perceived [7,8]. Heavy metal contamination of waters causes adverse effects on the ecological balance of the aquatic ecosystem, which can lead to a reduction in the number of individuals living in them [9,10]. Sources of water pollution also include the weathering of sedimentary rocks, but also domestic wastewater, and the burning of solid waste and coal, oil or metallurgy [2,11]. There is a significant impact of industrial activities on ambient water pollution [12–15]. Various biological

technologies, such as effective microorganisms (EM) technology [16], assessment of the ecological status of waters based on macrophytes [17] or environmental monitoring using biomonitors [18], represent the prospect of biological monitoring of pollution and quality in a water environment. Some of the advantages of using biomonitoring include: ease of application, effective, natural and environmentally safe cleanup, no need to wear protective clothing and own highly specialized equipment, low cost of conducting research and the possibility of implementing long-term solutions for a given ecosystem [19,20].

The biomonitoring survey is an innovative way of analyzing air pollution. The method is widely used in Europe to monitor environmental contamination with heavy metals, PAHs or radionuclides [21]. Various species of algae [22] and aquatic plants [23], as well as mosses, are the most commonly used in surface water pollution monitoring in aquatic ecosystems [24,25]. Prior to exposure, algae exist naturally in low-concentration ecosystems, from where they are then transported and exposed to a highly polluted environment (active biomonitoring) [26]. Due to their high ability to accumulate trace elements, they are very good bioindicators and can also be useful in the phytoremediation of water and wastewater treatment method [27]. Likewise, aquatic plants also work well as passive biomonitors for assessing river pollution [28] or phytoremediators for reducing the level of pollutants discharged into a lake [29]. In the case of mosses, to determine contaminant concentrations, the length of the shoot segment can affect monitoring results. Heavy metals most often accumulate in the older parts, near the base of the plant [30,31]. The water moss *Fontinalis antipyretica*, which is common in the northern hemisphere and easy to identify, is most commonly used to monitor water quality [32–34]. To date, however, no attempt has been made to use species of forest mosses to assess surface water (rivers, lakes) pollution by comparing it with a typical biomonitor of aquatic ecosystems (e.g., algae), which was considered a gap to be experimentally tested.

The purpose of this study was to find out a variation in heavy metal concentration (Mn, Fe, Ni, Cu, Zn, Cd and Pb) during active biomonitoring (moss-bag technique) in aquatic environments using three species of mosses: *Pleurozium schreberi*, *Sphagnum fallax* and *Dicranum polysetum* and the marine alga *Palmaria palmata*. Active biomonitoring (moss-bag technique) of atmospheric aerosol was also carried out using the moss *Pleurozium schreberi* to demonstrate the potential for polluting effects of emissions from the smelter on air quality and, indirectly, the Mała Panew River flowing in its vicinity.

## 2. Materials and Methods

### 2.1. Characteristics of the Study Area

Ozimek is located in the Opolskie Voivodeship at a distance of about 21 km east of Opole (southwestern Poland). The study area is located on the Opole Plain, in Upper Silesia, at a distance of about 10 km from the Turawa Lakes complex. The Mała Panew River, which is a right tributary of the Oder River, flows through the town. Some of the potential sources of pollution in the study area include traffic, low emissions, runoff from fields and the smelter, which has been in operation for years. The steel mill has been in existence for more than 250 years. It is currently the oldest steel foundry in Poland. Despite limitations (production volume, exhaust emissions and industrial wastewater), the Ozimek steel mill still produces waste, albeit only to a small extent. Some of it is recovered and stored, but a certain amount is exposed to the environment. The main sources of heavy metal emissions at this plant are: (a) electric arc furnaces, the dust from which during their operation in steel smelting contains iron, nickel, manganese, cadmium and zinc; (b) extraction systems in the production halls, transmitting pollutants to the atmosphere from the molding masses, which have chromium, manganese, cadmium and lead; (c) industrial water for hardening steel in the form of effluent into the Mała Panew River; and (d) partly waste molding and core masses dumped on the heap, holding the components in question, which can be washed out by the rains or carried as dust by the wind from the landfill [35].

The biomonitoring study covered the Mała Panew River flowing through Ozimek (Opolskie Voivodeship, Poland) along a stretch of about 5 km and assessed atmospheric aerosol pollution in the study area. The study was conducted from June to August 2022.

## 2.2. Method of Conducting Laboratory Experiment

Biomonitoring studies were preceded by laboratory analyses to compare the sorption properties of selected moss and algal species. The studies were performed on live mosses *P. schreberi*, *S. fallax* and *D. polysetum* and dried algae *P. palmata* [36]. Mosses for analysis were collected from areas uncontaminated with heavy metals—forests in Swietokrzyskie Voivodeship, southeastern PL. The initial concentration of copper determined in moss and algae samples were below the limit of quantification of the analytical method used ( $c_{Cu} < 1.03$  mg/kg d.m.). Samples of mosses and algae weighing  $0.200 \pm 0.001$  g were placed in a perforated container of about 15 cm<sup>3</sup> and immersed with it in a CuSO<sub>4</sub> solution of 200 cm<sup>3</sup>. The solution was stirred using a magnetic stirrer (VELP Scientifica Srl, Usmate, IT). Periodically, the solution was drawn directly from the vessel in which the experiment was conducted to determine (AAS) the concentration of the metal. The process was carried out for 60 min. Measurements were also taken of changes in the conductivity and pH of the solution during the copper accumulation process in the biota.

### Apparatus and Reagents

For the determination of Cu, an iCE 3500 atomic absorption spectrometer from Thermo Electron Corporation (Grand Island, NY, USA) was used. To test the conductivity and pH of the solutions in which the mosses were immersed, equipment, was used: pH meter CP551 (Elmetron Sp. j., Zabrze, Poland) and conductivity meter CC551 (Elmetron Sp. j., Zabrze, Poland), whose absolute errors of indication was  $\Delta pH = 0.02$  and  $\Delta \kappa = 0.1$   $\mu S/cm$ , respectively. CuSO<sub>4</sub> solutions were prepared using MERCK reagents. The uncertainty of indication of the laboratory balance used was  $\pm 0.001$  g.

## 2.3. Method of Conducting Biomonitoring Study in the Field

Biomonitoring studies of the Mała Panew River and assessment of atmospheric aerosol pollution in the study area were carried out using the mosses: *P. schreberi*, *S. fallax* and *D. polysetum* and the dried algae *P. palmata* [36]. The location of the measurement points is shown on the map in Figure 1.

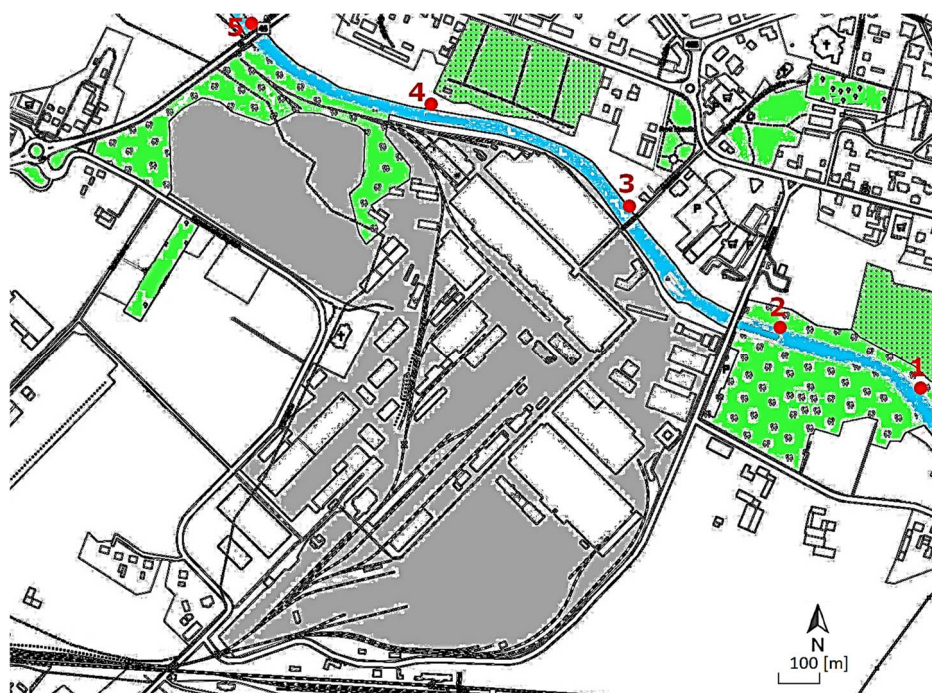
Representative (averaged) samples of mosses and algae, each weighing  $2.000 \pm 0.001$  g, were placed in pouches (mosses) and perforated polyethylene containers (algae) and exposed in the river for 60 min at locations marked on the map in Figure 1. Samples were submerged at a distance of about 1 m from the shoreline. After exposure, the algae and moss samples were rinsed with deionized water, dried and then mineralized in a Speedwave Four microwave mineralizer (Berghof GmbH, Eningen, Germany). A flame atomic absorption spectrometer was used to determine heavy metals (Mn, Fe, Ni, Cu, Zn, Cd and Pb).

To assess atmospheric aerosol pollution near the river, *P. schreberi* mosses were exposed using the moss-bag technique at an altitude of 1.5–2.0 m for a period of 3 months [37,38]. After exposure, moss samples were dried and then mineralized in a microwave mineralizer. An atomic absorption spectrometer was again used to determine heavy metals.

### Apparatus and Reagents

Representative (averaged) samples of algae and mosses after exposure weighing  $0.800 \pm 0.001$  g d.m. (d.m.—dry weight) were mineralized in a mixture of 10 cm<sup>3</sup> nitric acid (V) and 6 cm<sup>3</sup> perhydrol (HNO<sub>3</sub> 65%, H<sub>2</sub>O<sub>2</sub> 30%) in a Speedwave Four microwave mineralizer from Berghof GmbH, Eningen, Germany. The mineralization process was carried out at 180 °C. Solutions were prepared using reagents from MERCK (Darmstadt, Germany). Solutions after mineralization and dilution were filtered into volumetric flasks of 25 cm<sup>3</sup>. For the determination of heavy metals (Mn, Fe, Ni, Cu, Zn, Cd and Pb), an atomic absorption spectrometer (Thermo Electron Corporation (Grand Island, NY, USA)

was used. For calibration of the instrument, standards from ANALYTIKA Ltd. (Prague, Czech Republic) were used.



**Figure 1.** Exposure sites of moss and algae samples; red color indicates measurement points; green color symbolizes green areas, parks, forests, etc., gray color indicates the area around the smelter.

#### 2.4. Quality Control

Table 1 lists the limits of detection and limits of quantification of heavy metals, characterizing the iCE 3500 spectrometer [39]. The values of the highest concentrations of standards (manufactured by: ANALYTIKA Ltd. (Prague, Czech Republic)) used for calibration ( $2.0 \text{ mg/dm}^3$  for Cd,  $5.0 \text{ mg/dm}^3$  for Cu, Zn, Ni and Pb,  $7.5 \text{ mg/dm}^3$  for Mn and  $10.0 \text{ mg/dm}^3$  for Fe) were taken as the limit of the linear dependence of the signal on concentration.

**Table 1.** The instrumental detection limits (IDL) and instrumental quantification limits (IQL) for the spectrometer iCE 3500 [ $\text{mg/dm}^3$ ] [39].

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 2 shows the concentrations of heavy metals determined in certified reference materials BCR-414 *plankton* and BCR-482 *lichen*, produced by the Institute for Reference Materials and Measurements, Belgium.

Microsoft Excel 2016 and Statistica (ver. 13.3) software were used to process and present the data. Shapiro–Wilk’s test was used to check data normality. Student’s *T*-test and Mann–Whitney U test were used to assess the significance of differences in elemental concentrations between algae and moss species.

**Table 2.** Comparison of measured and certified concentrations in BCR-414 *plankton* and BCR-482 *lichen* [40].

Metal	BCR-482 Lichen		AAS		Dev. **
	Concentration	±Uncertainty	Mean	±SD *	
	[mg/kg d.m.]				[%]
Mn	33.0	0.5	31.7	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
BCR-414 Plankton					
Mn	299	12	284	13	−5.0
Fe	1.85	0.19	1.79	0.20	−3.2
Ni	18.8	0.8	18.2	0.9	−3.2
Cu	29.5	1.3	28.4	1.6	−3.7
Zn	112	3.0	107	3	−4.5
Cd	0.383	0.014	0.371	0.018	−3.1
Pb	3.97	0.19	3.75	0.21	−5.5

Note: \* standard deviation. \*\* relative difference between the measured ( $c_m$ ) and certified ( $c_c$ ) concentration 100% \*  $(c_m - c_c)/c_c$ .

### 3. Results and Discussion

The first stage of the study compared the sorption properties of the three selected species of mosses: *P. schreberi*, *S. fallax* and *D. polysetum*, classically used in air biomonitoring, with the accumulation properties of marine algae *P. palmata*. Table 3 shows the initial concentrations of copper in the  $c_{s,Cu(0)}$  solution and Cu concentrations after sorption in  $c_{s,Cu(1)}$  solution and accumulated in moss/algae (M/A) at equilibrium  $c_{M/A,1}$  (stable indications of measuring instruments). Concentrations of sorbed metals per unit mass of moss/algae were determined from the relationship:

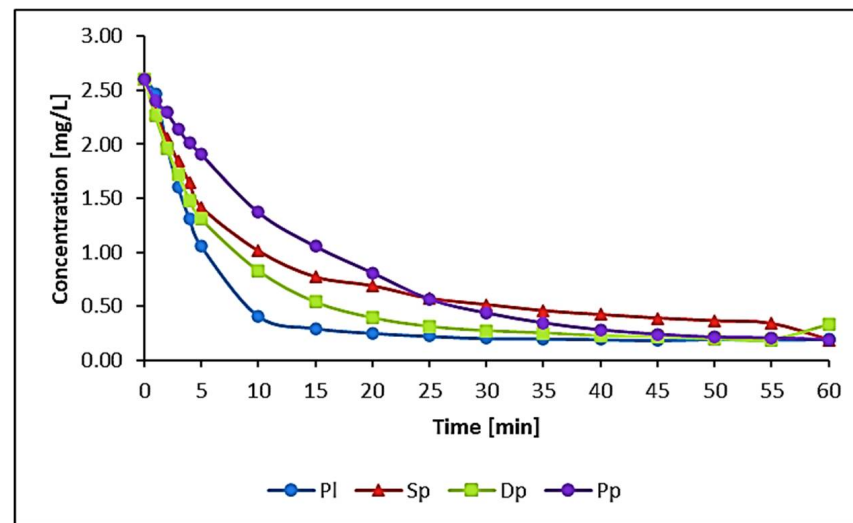
$$c_{M,1} = \frac{(c_{s,0} - c_{s,1}) * V}{m}$$

where:  $V$ —the volume of solution from which sorption was carried out and  $m$ —a mass of mosses/algae

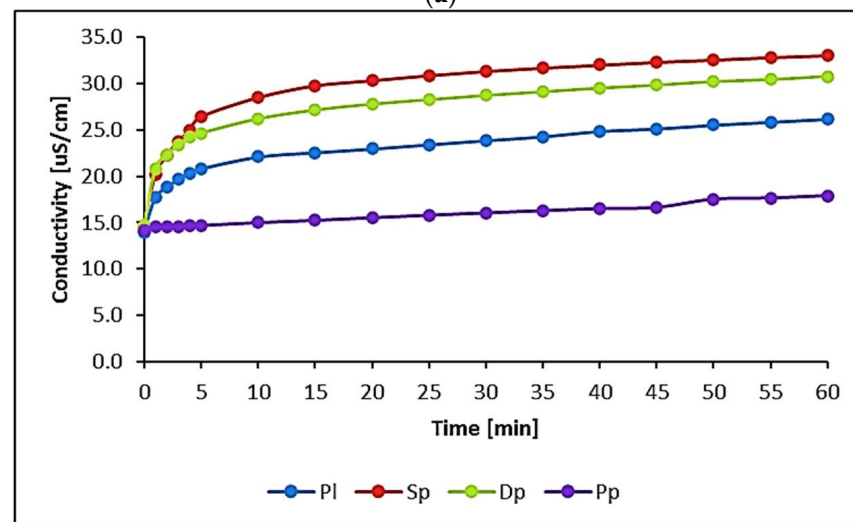
**Table 3.** Changes in Cu concentrations in solutions (mg/dm<sup>3</sup>) and in mosses and algae (mg/g d.m.) during the sorption process ( $n = 3$ ).

<i>P. schreberi</i>			<i>S. fallax</i>			<i>D. polysetum</i>			<i>P. palmata</i>		
$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{M,1}$	$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{M,1}$	$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{M,1}$	$c_{s,Cu(0)}$	$c_{s,Cu(1)}$	$c_{A,1}$
2.60	0.20	2.40	2.60	0.33	2.30	2.60	0.19	2.40	2.60	0.19	2.41

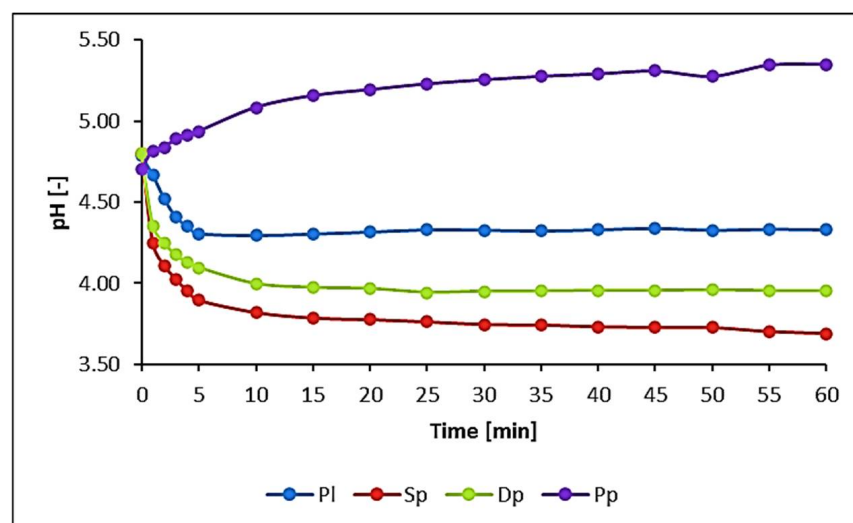
The graphs in Figure 2 show the changes in copper concentrations, conductivity and pH in solutions of salts of the analyzed metal after immersing selected species of mosses and algae in it and conducting the sorption process.



(a)



(b)



(c)

**Figure 2.** Physicochemical changes in Cu solution during the accumulation process on moss gametophytes and algae: (a) its concentration; (b) conductivity; (c) pH (PI—*P. schreberi*, Sp—*S. fallax*, Dp—*D. polysetum* and Pp—*P. palmata*).

In the biosorbent solution system, regardless of the species, a state of equilibrium is reached after the 60 min process, as indicated by stable conductivity and solution pH readings and no change in Cu concentrations in the solutions. In the mosses *P. schreberi* and *D. polysetum*, about 92.3% of Cu ions found in the initial solution accumulated during the 60 min process (in *S. fallax*—about 88.5% and in *P. palmata* about 92.7%) (Table 3). In the first 10 min of the process, about 84.2% of copper ions are sorbed from the solution into the moss gametophytes by *P. schreberi* (68.1% by *D. polysetum*, 61.0% by *S. fallax* and 47.3% by *P. palmata*), with respect to the concentration of this analyte accumulated in the biosorbent at equilibrium (Figure 2). The intensity of metal accumulation in mosses depends, among other things, on their affinity for the functional groups that make up the compounds that form the cell wall, as well as the structure and development of the surface of the gametophyte leaves or algal thallus [41]. Under the conditions of the experiment carried out, copper accumulation depending on the species of moss/algae increases in number: *S. fallax*, *D. polysetum*, *P. schreberi* and *P. palmata*. An increase in conductivity was also observed during the process (Figure 2b). The increase in the conductivity of the solution after the introduction of mosses/algae into it is caused, among other things, by the dissolution of salts naturally accumulated on the surface of the biota and the progressive irreversible changes in the structure of cell membranes over time, which causes the leakage of ionic substances from moss/algae cells into the solution [42–44]. The process of copper sorption on mosses, regardless of species, is accompanied by sorption of H<sup>+</sup> ions, while in the case of algae, we observed a desorption process (Figure 2c). This different nature of changes is due to, among other things, different habitat conditions and mechanisms of micro- and macronutrient accumulation [45,46].

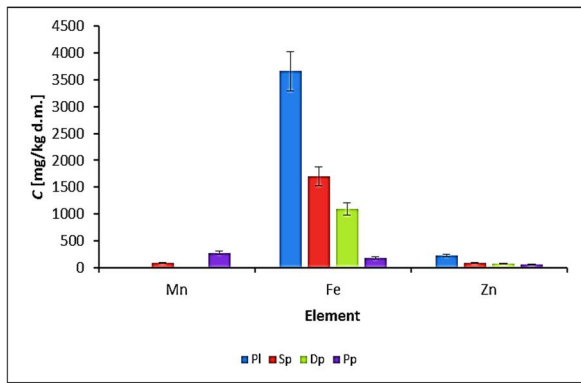
Assessment of sorption properties is particularly important when using different biota species in biomonitoring studies and then analyzing and comparing the results.

### 3.1. Water Biomonitoring Using Mosses and Algae

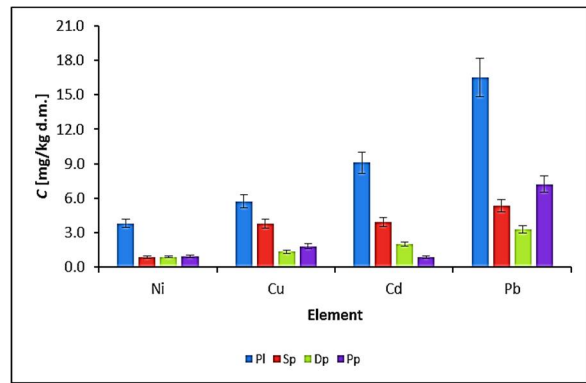
In the second stage of analysis, biomonitoring of the Mała Panew River and assessment of atmospheric aerosol pollution in the study area were carried out. For the first time, three species of forest mosses used in air quality biomonitoring—*P. schreberi*, *S. fallax* and *D. polysetum*—were used for exposure in surface waters.

The graphs in Figure 3 show the changes in concentrations of individual analytes determined in four biological matrices exposed at five measurement points on the Mała Panew River.

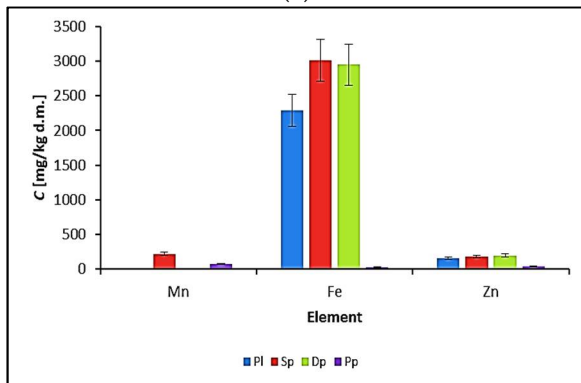
As shown in Figure 3., elemental concentrations vary at points depending on the exposed matrix. For manganese, no increases were recorded in the mosses *P. schreberi* and *D. polysetum*. The highest concentrations (for all elements) were recorded at point 1 (the area around the allotments) for marine algae. Similarly, for the moss *P. schreberi*, where the highest concentrations of analytes were also recorded at this point. For the other two species of mosses exposed in the Mała Panew River, the highest increases were observed at points 2 (a forested area outside the smelter) and 4, which is a green area but also located near a still-active section of the smelter dump. The results of the study indicate that the use of several species at once provides better opportunities for analyzing the results due to the different sensitivity of a given biomonitor to individual elements [47]. The differences in elemental concentrations in mosses are due to their different morphology and different responses to analytes [48]. Therefore, in order to obtain accurate results in biomonitoring studies, different species should be used as bioaccumulators for individual elements [33,49].



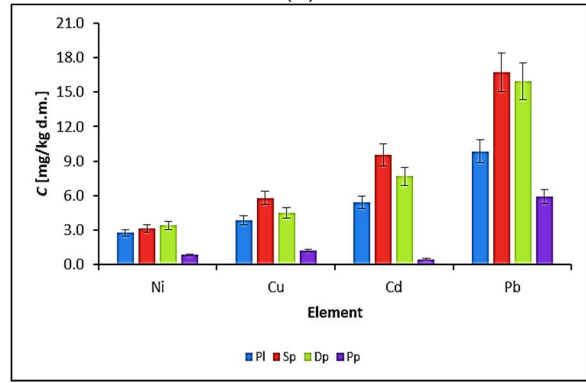
(a)



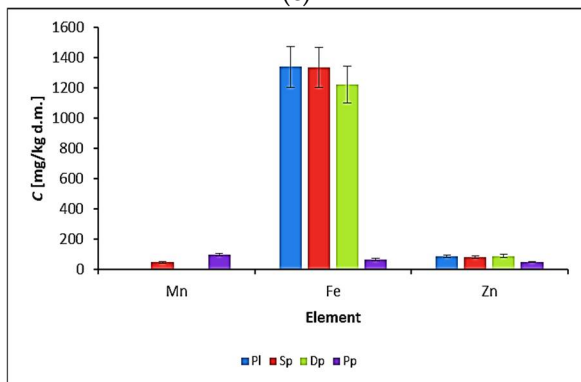
(b)



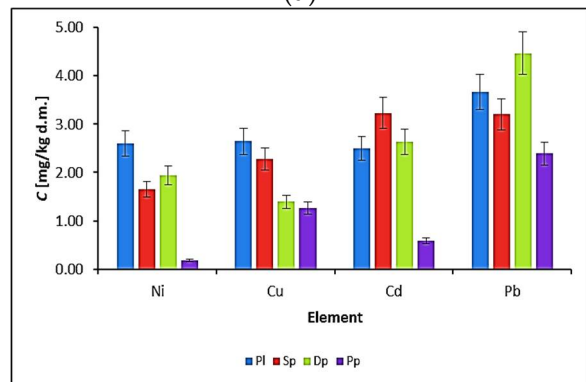
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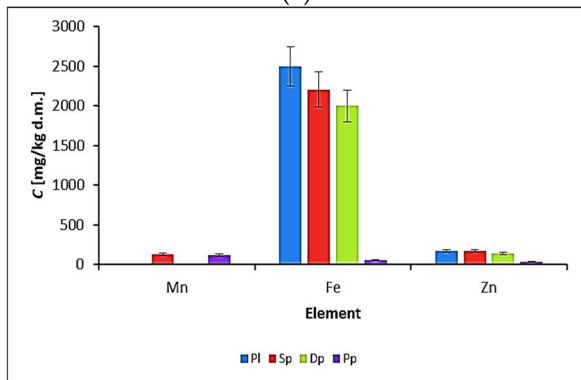
(d)



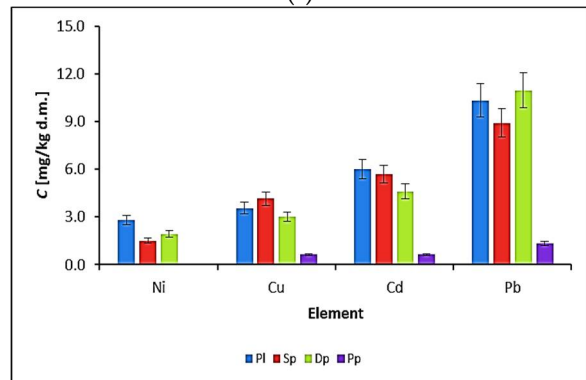
(e)



(f)



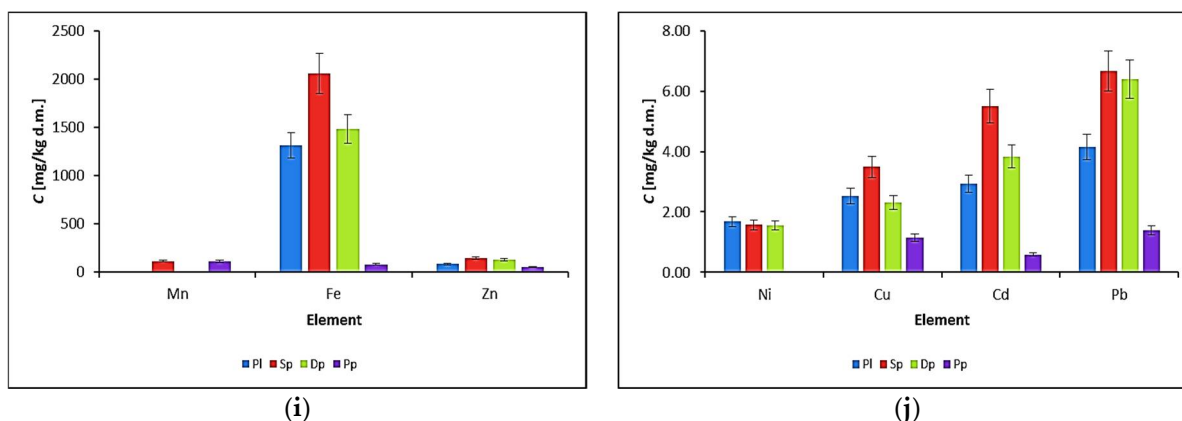
(g)



(h)

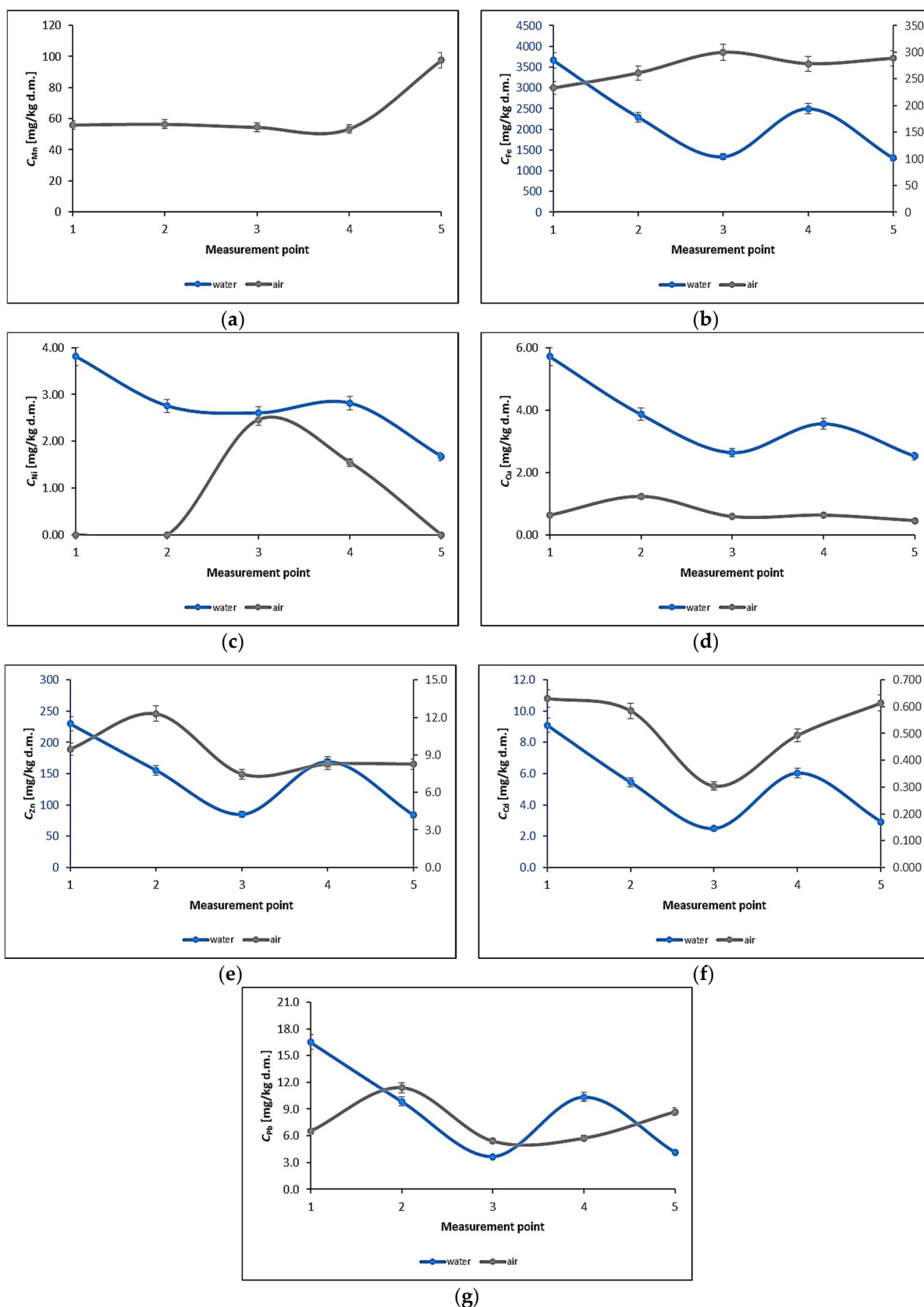
Figure 3. Cont.





**Figure 3.** Changes in concentrations of heavy metals determined in mosses and algae exposed in the Mała Panew River; at point 1: (a,b); at point 2: (c,d); at point 3: (e,f); at point 4: (g,h); at point 5: (i,j).

An attempt was made to identify the best matrix, among the four tested, in terms of its ability to accumulate heavy metals in aquatic ecosystems. Statistical analyses indicate that for the cases of manganese and lead, the exposed matrix does not matter, as there are no statistically significant differences between algae and mosses in terms of the accumulation of these analytes. For the other elements, on the other hand, there are statistically significant differences between algae and mosses, but the forest moss species used does not matter—the complete results are presented in Supplementary Materials, Table S1. So far, typical aquatic moss species such as *F. antipyretica* have been used in water quality biomonitoring [50,51]. Comparing our own results with the medians of this work [52] (especially with samples exposed in Poland), we found that only in the case of cadmium, in our forest, mosses exposed determined concentrations in the samples that were more than two times higher than in the cited work. For other elements, median comparisons indicated better sorption properties of the water moss *F. antipyretica* compared with our species. In contrast, comparing other literature data (passive biomonitoring), for example, the median value of iron determined in our mosses was 2010 mg/kg compared with about 1750 mg/kg in the cited authors (Figure 4 in their paper) [31]. We also determined zinc concentrations three times higher, and cadmium and lead were also characterized by values higher in our mosses than those cited here [31]. On the other hand, comparing the terrestrial mosses *Sphagnum denticulatum* (which are not suitable for use in aquatic environments according to the authors) [53] with our peat moss *S. fallax*, it appears that in our river this species was able to accumulate less in relation to the previously cited *S. denticulatum*, which, when juxtaposed with *F. antipyretica*, is not suitable for water biomonitoring [53]. We, on the other hand, can conclude, confirmed by statistical analysis, that terrestrial mosses are an alternative for surface water quality monitoring to the marine algae used so far. Terrestrial mosses relative to aquatic species are certainly a less reliable source of information on the state of contamination of the aquatic ecosystem, but in the natural absence of such species in a given area, they should be allowed to be included in active water biomonitoring as an alternative to other matrices (such as algae) for use in the aquatic environment.



**Figure 4.** Changes in heavy metal concentrations of (a) manganese; (b) iron; (c) nickel; (d) copper; (e) zinc; (f) cadmium; (g) lead determined in *P. schreberi* mosses—water and atmospheric aerosol biomonitors.

### 3.2. Biomonitoring of Atmospheric Aerosol Using *P. schreberi* Mosses

At the present time, the main air pollutants in the urbanized environment include fine dust components (particulate matter). This dust is the result of complex chemical reactions of heavy metal compounds such as sulfur dioxide and nitrogen oxides, among others. They are mainly transmitted by power plants, industry or cars, but can also come from district heating plants as well as households. The dustiest areas in atmospheric aerosol in Europe include Poland, the Czech Republic, Italy, Bulgaria, Greece, Macedonia and Turkey [54]. Air pollutants are currently considered the fifth greatest threat to human health. European directives define permissible and sufficient values in the observation and subsequent verification of pollutants [55]. According to statistics, it follows that the predominant sources of lead (about 58%) and zinc (about 45%) emissions in Poland are production processes (Selected Nomenclature for sources of Air Pollution- SNAP 04) such as iron and steel metallurgy, ferrous and non-ferrous metal manufacturing; cadmium (about 40%) industrial combustion processes (SNAP 03), mainly coal; for Cu (approx. 38%) and other factors, of which about 34% are road transport (SNAP 07), such as the abrasion of car brakes and tires; combustion processes outside the industry (SNAP 02), such as coal consumption in residential buildings, for Ni (about 35%); and combustion processes in the production and energy transformation sector (SNAP 01), such as coal and fuel oil firing [56]. Therefore, during the biomonitoring studies conducted, atmospheric aerosol pollution in the study area was also analyzed. The graphs in Figure 4 show a comparison of the concentrations of metals determined in the moss *P. schreberi*, used as a biomonitor in the waters of the Mała Panew River, as well as the atmospheric aerosol biomonitor in the study area.

The results of the exposure of the moss *P. schreberi* within the framework of active air and water biomonitoring indicate differences in the obtained concentrations of heavy metals in these two environments. For atmospheric aerosol monitoring under the moss-bag technique, the mosses showed higher concentrations of manganese (no increase in water) and comparable concentrations of nickel and lead at each point in relation to their exposure to the river at these measurement sites. Emissions, for example Mn, are due to fuel combustion or tire wear [49], which was confirmed by our results (point no. 5 was located next to a traffic road). Air pollution may come from dust emissions from the smelter, which mainly introduces iron and lead into the environment, and nickel concentrations were just the highest at measurement point no. 3 (right next to the smelter). In turn, increasing lead concentrations from the smelter may be due to the transfer of pollutants with the direction of the wind to further distances [57,58]. For *P. schreberi* mosses exposed in the river, on the other hand, a decreasing trend in elemental concentrations can be observed at points 1–3, followed by an increase at point 4 and a decrease at the next measurement site. Similar trends have been observed before in the algae *Spirogyra* sp., which are related to the characteristic point sources of pollution [59], which we consider here to be the impact of the smelter activity and the leaching of pollutants with distance from the pollution source [60,61]. The differences in sorption are due to the different forms of availability of individual elements in the different environments in which the mosses were exposed, as well as various additional sources of emissions, including transport and low emissions [62].

## 4. Conclusions

For the first time, active biomonitoring of a river was carried out using three species of forest mosses under the moss-bag technique. Increments in analyte concentrations in mosses exposed in water were statistically significant relative to elemental concentrations in marine algae. This indicates that they can be used as an additional water biomonitor in addition to the previously used moss *F. antipyretica*. The analysis of heavy metal concentrations in *P. schreberi* mosses exposed in water and in the air indicates the influence of smelter operations as a source of pollution in the area. So far, elemental concentrations in terrestrial mosses have confirmed the effect of industrial pollution on the deposition of these metals in their tissues, but without their exposure in water. The differences in

the concentrations of analytes in mosses exposed in water and air are due to the different nature of the availability of these elements in each of these ecosystems and the possibility of additional anthropogenic sources of pollution. In the future, in order to optimize water biomonitoring using mosses, attention should be paid to comparing the sorption properties of forest mosses and typical aquatic species, as well as looking for other indicator organisms with comparable or better sorption properties.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14203335/s1>, Table S1: Statistical significance between algae and moss species in relation to the elements analyzed.

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## References

1. Lawniczak, A.E.; Zbierska, J.; Nowak, B.; Achtenberg, K.; Grześkowiak, A.; Kanas, K. Impact of Agriculture and Land Use on Nitrate Contamination in Groundwater and Running Waters in Central-West Poland. *Environ. Monit. Assess.* **2016**, *188*, 1–17. [[CrossRef](#)] [[PubMed](#)]
2. Izydorczyk, G.; Mikula, K.; Skrzypczak, D.; Moustakas, K.; Witek-Krowiak, A.; Chojnacka, K. Potential Environmental Pollution from Copper Metallurgy and Methods of Management. *Environ. Res.* **2021**, *197*, 111050. [[CrossRef](#)] [[PubMed](#)]
3. Bohdálková, L.; Bohdál, P.; Břizová, E.; Pacherová, P.; Kuběna, A.A. Atmospheric Metal Pollution Records in the Kovářská Bog (Czech Republic) as an Indicator of Anthropogenic Activities over the Last Three Millennia. *Sci. Total Environ.* **2018**, *633*, 857–874. [[CrossRef](#)]
4. Briffa, J.; Sinagra, E.; Blundell, R. Heavy Metal Pollution in the Environment and Their Toxicological Effects on Humans. *Heliyon* **2020**, *6*, e04691. [[CrossRef](#)] [[PubMed](#)]
5. Ali, H.; Khan, E.; Ilahi, I. Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *J. Chem.* **2019**, *2019*, 6730305. [[CrossRef](#)]
6. Yang, F.; Fu, D.; Liu, S.; Zevenbergen, C.; Singh, R.P. Hydrologic and Pollutant Removal Performance of Media Layers in Bioretention. *Water* **2020**, *12*, 921. [[CrossRef](#)]
7. De Castro, O.; Gianguzzi, L.; Colombo, P.; De Luca, P.; Marino, G.; Guida, M. Multivariate Analysis of Sites Using Water Invertebrates and Land Use as Indicators of the Quality of Biotopes of Mediterranean Relic Plant (*Petagnaea Gussonei*, Apiaceae). *Environ. Bioindic.* **2007**, *2*, 161–171. [[CrossRef](#)]
8. Carter, J.L.; Resh, V.H.; Hannaford, M.J. *Macroinvertebrates as Biotic Indicators of Environmental Quality*; Elsevier Inc.: Amsterdam, The Netherlands, 2017; Volume 2, ISBN 9780128132692.
9. Baby, J.; Raj, J.; Biby, E.; Sankarganesh, P.; Jeevitha, M.; Ajisha, S.; Rajan, S. Toxic Effect of Heavy Metals on Aquatic Environment. *Int. J. Biol. Chem. Sci.* **2011**, *4*, 939–952. [[CrossRef](#)]
10. Riani, E.; Cordova, M.R.; Arifin, Z. Heavy Metal Pollution and Its Relation to the Malformation of Green Mussels Cultured in Muara Kamal Waters, Jakarta Bay, Indonesia. *Mar. Pollut. Bull.* **2018**, *133*, 664–670. [[CrossRef](#)]
11. Singh, A.; Sharma, A.; Verma, R.K.; Chopade, R.L.; Pandit, P.P.; Nagar, V.; Aseri, V.; Choudhary, S.K.; Awasthi, G.; Awasthi, K.K.; et al. Heavy Metal Contamination of Water and Their Toxic Effect on Living Organisms. In *The Toxicity of Environmental Pollutants*; Dorta, D.D., De Oliveira, P.D.P., Eds.; IntechOpen: Rijeka, Croatia, 2022.
12. Xu, H.; Gao, Q.; Yuan, B. Analysis and Identification of Pollution Sources of Comprehensive River Water Quality: Evidence from Two River Basins in China. *Ecol. Indic.* **2022**, *135*, 108561. [[CrossRef](#)]
13. Chen, L.; Caro, F.; Corbett, C.J.; Ding, X. Estimating the Environmental and Economic Impacts of Widespread Adoption of Potential Technology Solutions to Reduce Water Use and Pollution: Application to China's Textile Industry. *Environ. Impact Assess. Rev.* **2019**, *79*, 106293. [[CrossRef](#)]
14. Gunkel, G.; Kosmol, J.; Sobral, M.; Rohn, H.; Montenegro, S.; Aureliano, J. Sugar Cane Industry as a Source of Water Pollution—Case Study on the Situation in Ipojuca River, Pernambuco, Brazil. *Water. Air. Soil Pollut.* **2007**, *180*, 261–269. [[CrossRef](#)]
15. Jabłońska-Czapla, M.; Nocoń, K.; Szopa, S.; Lyko, A. Impact of the Pb and Zn Ore Mining Industry on the Pollution of the Biała Przemsza River, Poland. *Environ. Monit. Assess.* **2016**, *188*, 1–16. [[CrossRef](#)] [[PubMed](#)]

16. Dobrzyński, J.; Kulkova, I.; Wierzchowski, P.S.; Wróbel, B. Response of Physicochemical and Microbiological Properties to the Application of Effective Microorganisms in the Water of the Turawa Reservoir. *Water* **2022**, *14*, 12. [[CrossRef](#)]
17. Tomczyk, P.; Wiatkowski, M.; Gruss, Ł. Application of Macrophytes to the Assessment and Classification of Ecological Status above and below the Barrage with Hydroelectric Buildings. *Water* **2019**, *11*, 1028. [[CrossRef](#)]
18. Bezuidenhout, J.; Nekhoroshkov, P.; Zinicovscaia, I.; Yushin, N.; Frontasyeva, M. Accumulation Features of Micro and Macroelements in Indigenous and Alien Molluscs in Saldanha Bay, South Africa. *Ecol. Chem. Eng. S* **2020**, *27*, 495–508. [[CrossRef](#)]
19. Zhou, Q.; Zhang, J.; Fu, J.; Shi, J.; Jiang, G. Biomonitoring: An Appealing Tool for Assessment of Metal Pollution in the Aquatic Ecosystem. *Anal. Chim. Acta* **2008**, *606*, 135–150. [[CrossRef](#)]
20. Li, L.; Zheng, B.; Liu, L. Biomonitoring and Bioindicators Used for River Ecosystems: Definitions, Approaches and Trends. *Procedia Environ. Sci.* **2010**, *2*, 1510–1524. [[CrossRef](#)]
21. Iodice, P.; Adamo, P.; Capozzi, F.; Di Palma, A.; Senatorea, A.; Spagnuolo, V.; Giordano, S. Air Pollution Monitoring Using Emission Inventories Combined with the Moss Bag Approach. *Sci. Total Environ.* **2016**, *541*, 1410–1419. [[CrossRef](#)]
22. Rajfur, M. Algae as a Source of Information on Surface Waters Contamination with Heavy Metals. *Ecol. Chem. Eng. A* **2013**, *20*, 1089–1101. [[CrossRef](#)]
23. Krems, P.; Rajfur, M.; Waclawek, M.; Klos, A. The Use of Water Plants in Biomonitoring and Phytoremediation of Waters Polluted with Heavy Metals. *Ecol. Chem. Eng. S* **2013**, *20*, 353–370. [[CrossRef](#)]
24. De Traubenberg, C.R.; Ah-Peng, C. A Procedure to Purify and Culture A Clonal Strain of the Aquatic Moss *Fontinalis Antipyretica* for Use As A Bioindicator of Heavy Metals. *Arch. Environ. Contam. Toxicol.* **2004**, *46*, 289–295. [[CrossRef](#)] [[PubMed](#)]
25. Favas, P.J.C.; Pratas, J.; Rodrigues, N.; D'Souza, R.; Varun, M.; Paul, M.S. Metal(Loid) Accumulation in Aquatic Plants of a Mining Area: Potential for Water Quality Biomonitoring and Biogeochemical Prospecting. *Chemosphere* **2018**, *194*, 158–170. [[CrossRef](#)] [[PubMed](#)]
26. Rajfur, M.; Klos, A. Use of Algae in Active Biomonitoring of Surface Waters. *Ecol. Chem. Eng. S* **2014**, *21*, 561–576. [[CrossRef](#)]
27. Rajfur, M.; Krems, P.; Klos, A.; Kozłowski, R.; Józwiak, M.A.; Kříž, J.; Waclawek, M. Application of Algae in Active Biomonitoring of the Selected Holding Reservoirs in Swietokrzyskie Province. *Ecol. Chem. Eng. S* **2016**, *23*, 237–247. [[CrossRef](#)]
28. Baldantoni, D.; Bellino, A.; Lofrano, G.; Libralato, G.; Pucci, L.; Carotenuto, M. Biomonitoring of Nutrient and Toxic Element Concentrations in the Sarno River through Aquatic Plants. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 520–527. [[CrossRef](#)]
29. Eid, E.M.; Galal, T.M.; Shaltout, K.H.; El-Sheikh, M.A.; Asaeda, T.; Alatar, A.A.; Alfarhan, A.H.; Alharthi, A.; Alshehri, A.M.A.; Picó, Y.; et al. Biomonitoring Potential of the Native Aquatic Plant *Typha Domingensis* by Predicting Trace Metals Accumulation in the Egyptian Lake Burullus. *Sci. Total Environ.* **2020**, *714*, 136603. [[CrossRef](#)]
30. Vázquez-Arias, A.; Debén, S.; Casanova, A.; Pacín, C.; Aboal, J.R.; Fernández, J.A. Effect of Current Velocity on Cd Accumulation in the Aquatic Moss *Fontinalis Antipyretica*. *Water* **2021**, *13*, 157. [[CrossRef](#)]
31. Vázquez, M.D.; Real, C.; Villares, R. Optimization of the Biomonitoring Technique with the Aquatic Moss *Fontinalis Antipyretica* Hedw.: Selection of Shoot Segment Length for Determining Trace Element Concentrations. *Water* **2020**, *12*, 2389. [[CrossRef](#)]
32. Gecheva, G.; Mollov, I.; Yahubyan, G.; Gozmanova, M.; Apostolova, E.; Nikolova, M.; Dimitrova-Dyulgerova, I.; Radoukova, T.; Vasileva, T. Can Biomarkers Respond upon Freshwater Pollution?—A Moss-Bag Approach. *Biology* **2021**, *10*, 3. [[CrossRef](#)]
33. Gecheva, G.; Yancheva, V.; Velcheva, I.; Georgieva, E.; Stoyanova, S.; Arnaudova, D.; Stefanova, V.; Georgieva, D.; Genina, V.; Todorova, B.; et al. Integrated Monitoring with Moss-Bag and Mussel Transplants in Reservoirs. *Water* **2020**, *12*, 1800. [[CrossRef](#)]
34. Debén, S.; Aboal, J.R.; Giráldez, P.; Varela, Z.; Fernández, J.A. Developing a Biotechnological Tool for Monitoring Water Quality: In Vitro Clone Culture of the Aquatic Moss *Fontinalis Antipyretica*. *Water* **2019**, *11*, 145. [[CrossRef](#)]
35. Stojanowska, A.; Mach, T.; Olszowski, T.; Białowicz, J.S.; Górka, M.; Rybak, J.; Rajfur, M.; Świsłowski, P. Air Pollution Research Based on Spider Web and Parallel Continuous Particulate Monitoring—A Comparison Study Coupled with Identification of Sources. *Minerals* **2021**, *11*, 812. [[CrossRef](#)]
36. Klos, A.; Rajfur, M. Influence of Hydrogen Cations on Kinetics and Equilibria of Heavy-Metal Sorption by Algae—Sorption of Copper Cations by the Alga *Palmaria Palmata* (Linnaeus) Weber & Mohr (Rhodophyta). *J. Appl. Phycol.* **2013**, *25*, 1387–1394. [[CrossRef](#)] [[PubMed](#)]
37. Demková, L.; Bobul'ská, L.; Árvay, J.; Jezný, T.; Ducsay, L. Biomonitoring of Heavy Metals Contamination by Mosses and Lichens around Slovinky Tailing Pond (Slovakia). *J. Environ. Sci. Health—Part A Toxic/Hazard. Subst. Environ. Eng.* **2017**, *52*, 30–36. [[CrossRef](#)]
38. Dmuchowski, W.; Gozdowski, D.; Baczevska, A.H.; Bragoszewska, P. Evaluation of Various Bioindication Methods of Measuring Zinc Environmental Pollution. *Int. J. Environ. Pollut.* **2013**, *51*, 238–254. [[CrossRef](#)]
39. Thermo Fisher Scientific Inc. *ICE 3000 Series AA Spectrometers Operator's Manual*; Thermo Fisher Scientific Inc.: Waltham, MA, USA, 2011; pp. 1, 7–18.
40. Świsłowski, P.; Kosior, G.; Rajfur, M. The Influence of Preparation Methodology on the Concentrations of Heavy Metals in *Pleurozium Schreberi* Moss Samples Prior to Use in Active Biomonitoring Studies. *Environ. Sci. Pollut. Res.* **2021**, *28*, 10068–10076. [[CrossRef](#)]
41. Stanković, J.D.; Sabovljević, A.D.; Sabovljević, M.S. Bryophytes and Heavy Metals: A Review. *Acta Bot. Croat.* **2018**, *77*, 109–118. [[CrossRef](#)]

42. Cruz de Carvalho, R.; Bernardes da Silva, A.; Branquinho, C.; Marques da Silva, J. Influence of Dehydration Rate on Cell Sucrose and Water Relations Parameters in an Inducible Desiccation Tolerant Aquatic Bryophyte. *Environ. Exp. Bot.* **2015**, *120*, 18–22. [[CrossRef](#)]
43. Koster, K.L.; Balsamo, R.A.; Espinoza, C.; Oliver, M.J. Desiccation Sensitivity and Tolerance in the Moss *Physcomitrella Patens*: Assessing Limits and Damage. *Plant Growth Regul.* **2010**, *62*, 293–302. [[CrossRef](#)]
44. Ćosić, M.; Vujičić, M.M.; Sabovljević, M.S.; Sabovljević, A.D. What Do We Know about Salt Stress in Bryophytes? *Plant Biosyst.* **2019**, *153*, 478–489. [[CrossRef](#)]
45. Klos, A.; Rajfur, M.; Waclawek, M.; Waclawek, W. Ion Equilibrium in Lichen Surrounding. *Bioelectrochemistry* **2005**, *66*, 95–103. [[CrossRef](#)]
46. Lee, Y.C.; Chang, S.P. The Biosorption of Heavy Metals from Aqueous Solution by *Spirogyra* and *Cladophora* Filamentous Macroalgae. *Bioresour. Technol.* **2011**, *102*, 5297–5304. [[CrossRef](#)]
47. Zawadzki, K.; Samecka-Cymerman, A.; Kolon, K.; Wojtuń, B.; Mróz, L.; Kempers, A.J. Metals in *Pleurozium Schreberi* and *Polytrichum Commune* from Areas with Various Levels of Pollution. *Environ. Sci. Pollut. Res.* **2016**, *23*, 11100–11108. [[CrossRef](#)]
48. Sergeeva, A.; Zinicovscaia, I.; Vergel, K.; Yushin, N.; Urošević, M.A. The Effect of Heavy Industry on Air Pollution Studied by Active Moss Biomonitoring in Donetsk Region (Ukraine). *Arch. Environ. Contam. Toxicol.* **2021**, *80*, 546–557. [[CrossRef](#)]
49. Demková, L.; Árvay, J.; Bobul'ská, L.; Obona, J. The Risk Elements Biomonitoring in the Ambient Air of an Underground Parking Lot. *Polish J. Nat. Sci.* **2018**, *33*, 545–559.
50. Carrieri, V.; Varela, Z.; Aboal, J.R.; De Nicola, F.; Fernández, J.A. Suitability of Aquatic Mosses for Biomonitoring Micro/Meso Plastics in Freshwater Ecosystems. *Environ. Sci. Eur.* **2022**, *34*, 1–10. [[CrossRef](#)]
51. Debén, S.; Fernández, J.A.; Giráldez, P.; Vázquez Arias, A.; Aboal, J.R. Methodological Advances to Biomonitor Water Quality with Transplanted Aquatic Mosses. *Sci. Total Environ.* **2020**, *706*, 136082. [[CrossRef](#)]
52. Debén, S.; Fernández, J.A.; Carballeira, A.; Kosior, G.; Aboal, J.R. Improving Active Biomonitoring in Aquatic Environments: The Optimal Number and Position of Moss Bags. *Ecol. Indic.* **2018**, *93*, 753–758. [[CrossRef](#)]
53. Debén, S.; Fernández, J.A.; Carballeira, A.; Aboal, J.R. Using Devitalized Moss for Active Biomonitoring of Water Pollution. *Environ. Pollut.* **2016**, *210*, 315–322. [[CrossRef](#)]
54. Krakovská, A.S.; Svozilík, V.; Zinicovscaia, I.; Vergel, K.; Jančík, P. Analysis of Spatial Data from Moss Biomonitoring in Czech-Polish Border. *Atmosphere* **2020**, *11*, 1237. [[CrossRef](#)]
55. Chaligava, O.; Nikolaev, I.; Khetagurov, K.; Lavrinenko, Y.; Bazaev, A.; Frontasyeva, M.; Vergel, K.; Grozdov, D. First Results on Moss Biomonitoring of Trace Elements in the Central Part of Georgia, Caucasus. *Atmosphere* **2021**, *12*, 317. [[CrossRef](#)]
56. Domańska, W. *Ochrona Środowiska 2019*; Główny Urząd Statystyczny, Departament Badań Przestrzennych i Środowiska: Warsaw, Poland, 2019; pp. 186–188.
57. García-Seoane, R.; Fernández, J.A.; Chilà, A.; Aboal, J.R. Improving the Uptake of Pollutants in Moss Bags: The Wind Effect. *Ecol. Indic.* **2019**, *107*, 105577. [[CrossRef](#)]
58. Davies, B.E.; White, H.M. Environmental Pollution by Wind Blown Lead Mine Waste: A Case Study in Wales, U.K. *Sci. Total Environ.* **1981**, *20*, 57–74. [[CrossRef](#)]
59. Rajfur, M.; Klos, A.; Waclawek, M. Algae Utilization in Assessment of the Large Turawa Lake (Poland) Pollution with Heavy Metals. *J. Environ. Sci. Health—Part A Toxic/Hazard. Subst. Environ. Eng.* **2011**, *46*, 1401–1408. [[CrossRef](#)] [[PubMed](#)]
60. Nemčić-Jurec, J.; Jazbec, A. Point Source Pollution and Variability of Nitrate Concentrations in Water from Shallow Aquifers. *Appl. Water Sci.* **2017**, *7*, 1337–1348. [[CrossRef](#)]
61. Liang, N.; Yang, L.; Dai, J.; Pang, X. Heavy Metal Pollution in Surface Water of Linglong Gold Mining Area, China. *Procedia Environ. Sci.* **2011**, *10*, 914–917. [[CrossRef](#)]
62. Świsłowski, P.; Nowak, A.; Waclawek, S.; Ziembik, Z.; Rajfur, M. Is Active Moss Biomonitoring Comparable to Air Filter Standard Sampling? *Int. J. Environ. Res. Public Health* **2022**, *19*, 4706. [[CrossRef](#)]