Assessment of the biomonitoring potential of bryophytes

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Abstract. The consequences of pollution are devastating not only for living organisms and not necessarily through direct action but, in particular, through the long-term effects on the components of the environment: water, air, soil. After the awareness of the existence of the danger of the imbalance of the ecosystems that support life on the whole planet, it was sought to find some ways to supervise them. Biomonitoring is the continuous observation of an area with the help of biomonitors represented by indicators of atmospheric pollution. In the present study, bryophytes are used to monitor air pollution in the biomonitored area. Bryophytes, popularly known as "mosses", are plants, generally small and mostly perennial. Compared to other plants, bryophytes grow on all possible substrates and in all vegetation formations, including aquatic environments. The methods used in the evaluation of bioindicators include the analysis of the chemical composition of the selected species, measurements of physiological and biochemical parameters, as well as observations of morphological or behavioral changes. The results of these analysis provide important information about the degree and type of air pollution as well as its evolution over time.
Keywords: atmospheric pollution, biomonitoring, bryophytes, bioindicators.

Introduction. Understanding how environmental change influences ecosystems and biodiversity has become a central topic in environmental research, particularly in regions affected by increasing anthropogenic pressure. This knowledge is essential for informing pollution management strategies and supporting environmental policy. In this context, biomonitoring offers a practical approach for assessing environmental quality through the use of living organisms as indicators.

These organisms—ranging from plants and animals to microorganisms—respond to variations in environmental conditions such as pollution load, temperature shifts, or chemical exposure. Their sensitivity allows them to reflect changes across different environmental compartments, including air, water, and soil, as well as the overall intensity of human impact.

Within this framework, bryophytes represent a particularly useful group of bioindicators. Their simple morphology, combined with the absence of true roots and a well-developed cuticle, enables direct uptake of water and dissolved substances over their entire surface, primarily from atmospheric deposition. As a result, they are highly responsive to airborne contaminants. In addition, bryophytes are capable of accumulating pollutants, including heavy metals, within their tissues, making them suitable for monitoring long-term environmental contamination.

In air quality assessments, bryophytes are widely applied as non-invasive indicators of atmospheric pollution. Variations in their distribution, abundance, and chemical composition can provide relevant information on spatial differences in pollution levels and environmental quality.

The present study focuses on a bibliographic review of bryophyte-based biomonitoring approaches, the identification of effective bryophyte groups for monitoring purposes, and the statistical analysis of available data regarding heavy metal accumulation in the study area.

Material and Method. Anthropogenic sources of pollution, such as vehicle emissions, coal combustion, non-ferrous metal smelting, iron and steel production, waste incineration, and the production of chemicals and fertilizers, emit large quantities of pollutants into the atmosphere worldwide, with a negative impact on biogeochemical cycles in ecosystems and on human health.

Environmental pollution, as a general concept, represents the introduction into the environment of physical, chemical, or biological agents that have harmful effects on human health, ecosystems, and natural resources. Environmental pollution surveillance networks are organized monitoring systems that collect data and information on air quality, water, soil, and other aspects of the surrounding environment. These networks are essential for understanding and assessing the level of pollution and its impact on human health, ecosystems, and the environment in general (Markert 2007). Markert B. stated in 2007 that human activities, through the development of industry, agriculture, transportation, etc., have released into the environment significant quantities of substances that do not exist in nature (xenobiotics, many radionuclides), which are potentially harmful both to environmental health and to human health. Heavy metals are metallic elements with relatively high densities (greater than 5 g/cm^3) and which exhibit toxicity at low concentrations for living organisms. They are often characterized by high atomic mass and specific chemical behaviors, such as the formation of complex ionic and covalent bonds.

Biomonitoring refers to the use of living organisms to assess the state of the environment and the impact of various factors on it. This process involves monitoring species of plants, animals, and microorganisms in order to detect the presence and effects of chemical pollutants, climate change, and other ecological stressors.

In biomonitoring, biomonitors are used as indicators, representing organisms or communities of organisms used to assess environmental quality and to monitor pollution. They can indicate the presence and level of contaminants, as well as their effects on ecosystems and human health. As early as 2007, Larsen and collaborators (Larsen et al., 2007) reported the use of lichens as indicators and monitors of atmospheric pollution with heavy metals in the scientific literature since the beginning of the Industrial Revolution (Figure 1).

Figure 1. Air bioindicators
(source: https://revista.newprojects.org).

Among biological organisms, plants have proven to be very useful in the monitoring and detection of heavy metals due to their absorption capacity (Cooper et al., 2020). Plants are widely used as bioindicators because of their visible and distinct responses, in which bryophytes play a very important and specific role due to their extreme sensitivity to pollutants in the immediate substrate.

Such species serve as good bioindicators and act as an environmental "warning system." In addition, bryophytes have the ability to absorb and retain pollutants at concentrations much higher than those absorbed and retained by higher plants growing in the same habitat (Jiang et al., 2020).

The use of bryophytes in biomonitoring is an effective and accessible practice, as these organisms are sensitive to changes in their environment and can provide valuable information on environmental quality and the impact of human activities. Bryophytes also have the potential to grow on substrates containing specific metals and can therefore be used as bioindicators of these elements. In addition, their simple structure makes them suitable candidates for studying morphological and genetic changes that occur due to toxicity or metal-induced stress (Stanković et al., 2018). The ability of bryophytes to retain toxic elements has led to their use as indicators of air pollution (Rühling & Tyler, 2004). Bryophytes consist of both sensitive and tolerant species and respond to pollution in different ways.

***Biomonitoring of heavy metal pollution using bryophytes**.* Monitoring heavy metal pollution in the environment is a very complex process, especially when it involves persistent pollutants. Heavy metals occur naturally in the Earth's crust, from where they are released into the atmosphere and into water bodies (Nagajyoti et al., 2010). Some of these metals are essential for the metabolic functioning of organisms, but if they are deficient or present in excess, they can lead to physiological disorders and have harmful consequences. Other metals, such as Pb, Cd, Al, and Hg, are harmful regardless of their concentration (Krzesłowska, 2011). Heavy metals are particularly important from the perspective that they act as pollutants. Once released into the environment, they are difficult to remove and tend to accumulate in plant tissues and other organisms through trophic chains. Increasing contamination requires continuous monitoring of heavy metal concentrations in the environment and of their effects on ecosystems (Markert & Weckert, 1989).

Bryophytes provide information regarding the interactions between different heavy metals and their effects on living systems, which cannot be obtained through instrumental measurements. Due to all these characteristics, bryophytes have been used for decades not only in studies monitoring airborne metal pollution, where they are very important, but also in monitoring heavy metal pollution in aquatic environments (Kelly et al., 1987). However, these studies do not provide the absolute concentrations of elements accumulating in the environment over a given period. To obtain this information, it is necessary to establish and maintain a linear correlation between concentrations in bryophyte tissues and the metal concentrations to which they are exposed, taking into account all factors that could disturb this relationship (Boquete et al., 2014). In this context, heavy metal toxicity may also alter the accumulation characteristics of metals by bryophytes, which could affect their reliability as biomonitoring systems or could serve as a physiological marker of heavy metal pollution.

Establishing biomonitoring points. The establishment of target areas for passive biomonitoring of heavy metals at the level of Harghita County was carried out by developing a map of the county, as shown in Figure 2.

The biomonitoring points were established in such a way as to include four sampling points per county. In this way, the monitoring of the presence and accumulation of heavy metals is ensured, as well as the estimation of their temporal and spatial dynamics across the entire territory of Harghita County. For the selection of locations, areas with different pollution sources were taken into account, as follows:

- Luci peat bog – A protected area of national interest corresponding to IUCN category IV, located in eastern Transylvania, on the territory of Harghita County.
- Romanian railway (CFR) – The railway is located in Sâncrăieni commune, near the city of Miercurea Ciuc.
- Central Park – The park is located in the heart of the city; it is one of the most popular meeting points and leisure destinations in the area.
- Miercurea Ciuc Brewery – The brewery in Miercurea Ciuc is located in a strategic area of the city, being a distinctive element of the local industrial landscape.

From the selected points, samples of natural sorbents in the form of mosses bryophyte class were collected.

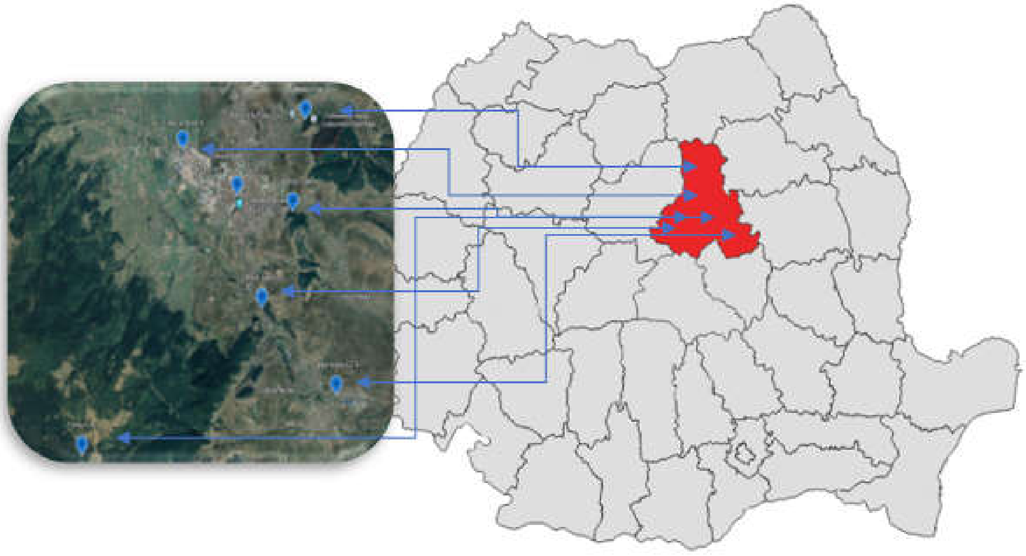


Figure 2. Area with selected biomonitoring points (original).

Collection, preparation, and processing of plant material. The species selected for active biomonitoring was the bryophyte *Polytrichum commune* (haircap moss).

This species was selected for its biomonitoring qualities, such as its ability to tolerate, retain, and accumulate a wide range of atmospheric pollutants, including heavy metals, and its wide distribution across all areas. From each sampling point, 100 grams of material were collected in three replicates. The plant material corresponding to each sample was sealed in Ziploc bags, which were then labeled. For each collected sample, a field sheet was completed, specifying: the name of the person who performed the collection, the sample number, the identification data of the sampling point, the species name, the date of collection.

The collected material was divided into several sample sets for dry matter analyses and XRF analyses (X-ray fluorescence spectrometry). Each type of sample was labeled with the corresponding destination label, according to an analysis sheet. All samples were sealed in labeled Ziploc bags and stored until analysis in the dark and at low temperatures.

For analysis, the plant material was dried at 105°C for 5 hours, ground into a fine powder, and then analyzed.

In order to identify the heavy metals that may represent the greatest environmental threats, approximations were made for the calculation of the Pollution Index (PI), for heavy metals quantified in bryophytes depending on the sampling area.

According to the formula:

$$PI = Cn / GB$$

where:

Cn – concentration of the heavy metal in the sample, ppm
GB – geochemical background values, ppm

In the present case, the following were considered:
Cn – concentration of the heavy metal in the bryophyte sample, ppm
GB – geochemical background values for soil, ppm

For the geochemical background values, tolerable levels available in the specialized literature were taken into account, as reported by Kloke (1979), and for elements not available in that source, the composition of the upper layers of the crust was considered. (Table 1).

Table 1

Tolerable levels of heavy metals in soil, according to the specialized literature

Specification	*Kloke, 1979*	*Rudnick & Gao, 2003*
Mn	-	438,59
Zn	300	-
Cr	-	92
Ga	-	17,5
Ag	-	53
As	20	-
Ni	100	-
Pb	100	-
Cu	100	-
Cd	3	-

To verify the previously presented hypothesis, the calculation of the Bioaccumulation Index (BI) was also considered, using as a reference the concentration of heavy metals present in the natural reserve, regarded as the least exposed area.

According to the formula (Kowalska et al., 2018):

$$BI = Cm / Crn$$

where:

Cm – concentration of the heavy metal measured in different areas, ppm
Crn – concentration of the heavy metal measured in the natural reserve, ppm

The statistical interpretation was performed using the XLSTAT software.

Results and Discussion. In the Luci peat bog nature reserve area, six microelements were identified in the collected bryophyte samples. The concentrations of the analyzed heavy metals range between 1,300 ppm for Mn and 5 ppm for As (Table 2).

Table 2

Heavy metal content of bryophyte samples collected from the Luci peat bog nature reserve (ppm) (original)

Specification	*n*	*X*	*s*	*CV%*
Mn	5	1300.00	73.14	5.63
Zn	5	106.00	3.39	3.20
Cr	5	60.00	1.58	2.64
Ga	5	15.00	3.08	20.55
Ag	5	11.00	3.16	28.75
As	5	5.00	1.05	2100

In the central park area of Miercurea Ciuc municipality, six microelements were identified in the collected bryophyte samples. The concentrations of the analyzed heavy metals range between 430 ppm for Mn and 27 ppm for Pb (Table 3).

In the railway area of Miercurea Ciuc municipality, ten microelements were identified in the collected bryophyte samples. The concentrations of the analyzed heavy metals range between 261 ppm for Zn (a value very close to that identified for Mn, equal to 260 ppm) and 4 ppm for As. In this area, a higher number of heavy metals is observed compared to those detected in the Luci peat bog nature reserve and the central park area of Miercurea Ciuc (Table 4).

Table 3

Heavy metal content of bryophyte samples collected from the central park area of Miercurea Ciuc (original)

Specification	*n*	*X*	*s*	*CV%*
Mn	5	430.00	17.68	4.11
Zn	5	170.00	14.16	8.33
Cr	5	170.00	7.91	4.65
Ga	5	11.00	2.24	20.33
Ag	5	11.00	2.12	19.28
Ni	5	34.00	3.16	9.30
Pb	5	27.00	2.55	9.44

Table 4

Heavy metal content of bryophyte samples collected from the railway area of Miercurea Ciuc (original)

Specification	*n*	*X*	*s*	*CV%*
Mn	5	260.00	10.61	4.08
Zn	5	261.00	8.69	3.33
Cr	5	140.00	15.81	11.29
Ga	5	5.00	1.58	31.62
Ag	5	14.00	2.55	18.21
As	5	4.00	1.87	46.77
Ni	5	22.00	1.58	7.19
Pb	5	16.00	2.55	15.93
Cu	5	118.00	4.69	3.97
Cd	5	23.00	4.74	20.62

In the brewery area of Miercurea-Ciuc municipality, only three microelements were identified in the collected bryophyte samples. The concentrations of the analyzed heavy metals range between 167.20 ppm for manganese and 46.80 ppm for chromium (Table 5).

Table 5

Heavy metal content of bryophyte samples collected from the brewery area of Miercurea Ciuc (original)

Specification	*n*	*X*	*s*	*CV%*
Mn	5	167.20	5.17	3.09
Zn	5	143.20	6.14	4.29
Cr	5	46.80	6.83	14.60

As previously mentioned, in order to identify the heavy metals that may represent the greatest environmental threats, approximations were made for the calculation of the Pollution Index (PI), taking into account tolerable levels available in the specialized literature for geochemical background values, and for those not available, the composition of the upper layers of the Earth's crust as reported in the scientific literature.

According to the obtained Pollution Index (PI) values, it is noted that in the nature reserve area, only Mn shows a supra-unitary value (PI = 2.96). In the railway area, supra-unitary values are observed for Cr (PI = 1.52), As (PI = 1.10), Cu (PI = 1.18), and Cd (PI = 7.67). In the central park, only one supra-unitary value was obtained for Cr (PI = 1.85). Regarding the brewery area of Miercurea Ciuc municipality, although only three microelements were identified, chromium also showed a supra-unitary value of PI = 2.37.

For the Bioaccumulation Index, supra-unitary values are observed for microelements depending on the area, as follows:

➢ in the railway area of Miercurea Ciuc municipality for zinc (BIZn = 2.46), chromium (BICr = 2.33), and silver (BIAg = 1.27);

➢ in the central park area of Miercurea Ciuc municipality for zinc (BIZn = 1.60), chromium (BICr = 2.83), and silver (BIAg = 1.00);

➢ in the brewery area of Miercurea Ciuc municipality for zinc (BIZn = 1.35).

***Interrelations between the heavy metal content of bryophytes collected from various areas of Harghita County*.** Two main clusters can be observed. One consists of a single branch and corresponds to manganese, which shows the highest concentration. The other is formed by five sub-clusters, corresponding to the concentrations of the remaining microelements, which are significantly lower in value (Figure 3).

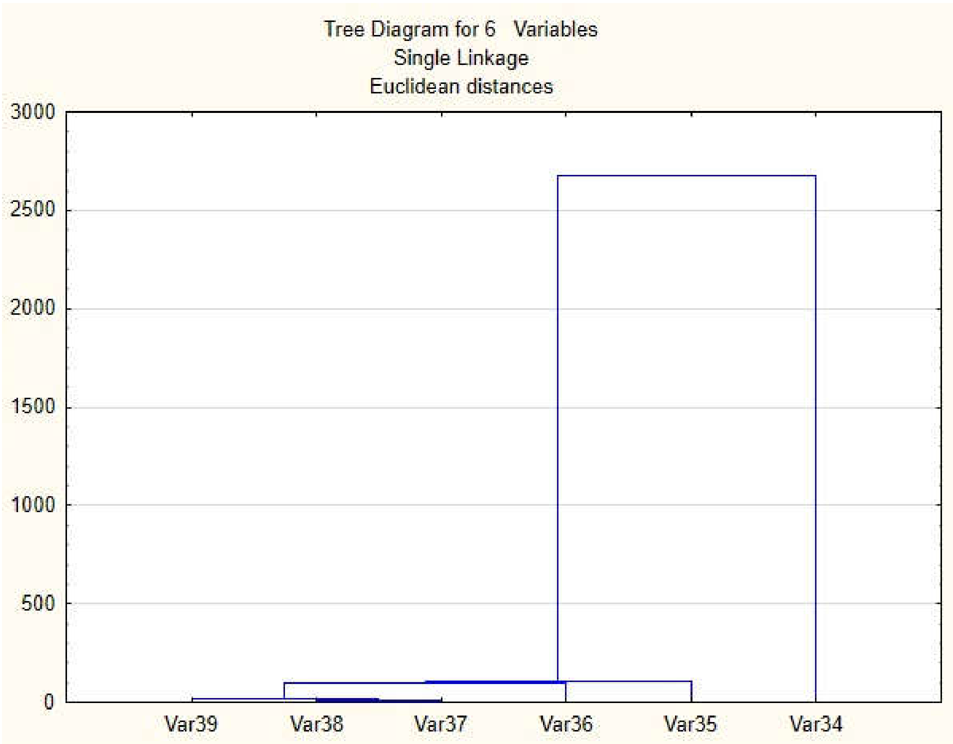


Figure 3. Cluster analysis applied to the microelement content of bryophyte samples collected from the Tinovul Luci Nature Reserve area (original).

In the natural reserve area, strong and very strong positive correlations are observed between zinc on the one hand and chromium (R = 0.699) and gallium (R = +0.981) on the other, as well as between chromium and gallium (R = +0.616). In addition, a strong negative correlation is recorded between Mn and As (R = -0.789). This indicates that an increase in zinc concentration is accompanied by increases in chromium and gallium concentrations, while an increase in manganese concentration is associated with a decrease in arsenic concentration (Table 6).

Table 6

Simple correlations between the microelement content of bryophytes collected from the Tinovul Luci Nature Reserve area (original)

Variabile	*Mn*	*Zn*	*Cr*	*Ga*	*Ag*	*As*
Mn	1.000	0.141	0.454	-0.055	0.086	-0.791
Zn	0.141	1.000	0.699	0.981	0.373	0.318
Cr	0.454	0.699	1.000	0.616	-0.300	0.186
Ga	-0.055	0.981	0.616	1.000	0.359	0.477
Ag	0.086	0.373	-0.300	0.359	1.000	-0.310
As	-0.791	0.318	0.186	0.477	-0.310	1.000

In the Central Park area, heavy metal concentrations are also grouped into two main clusters. One of these corresponds to manganese, which is found at the highest concentration. The other sub-cluster is further divided into two additional sub-clusters. One of them corresponds to zinc and chromium, which are present at identical concentrations. The other sub-cluster shows two branches, where the remaining heavy metals are grouped in pairs according to their concentrations (Figure 4).

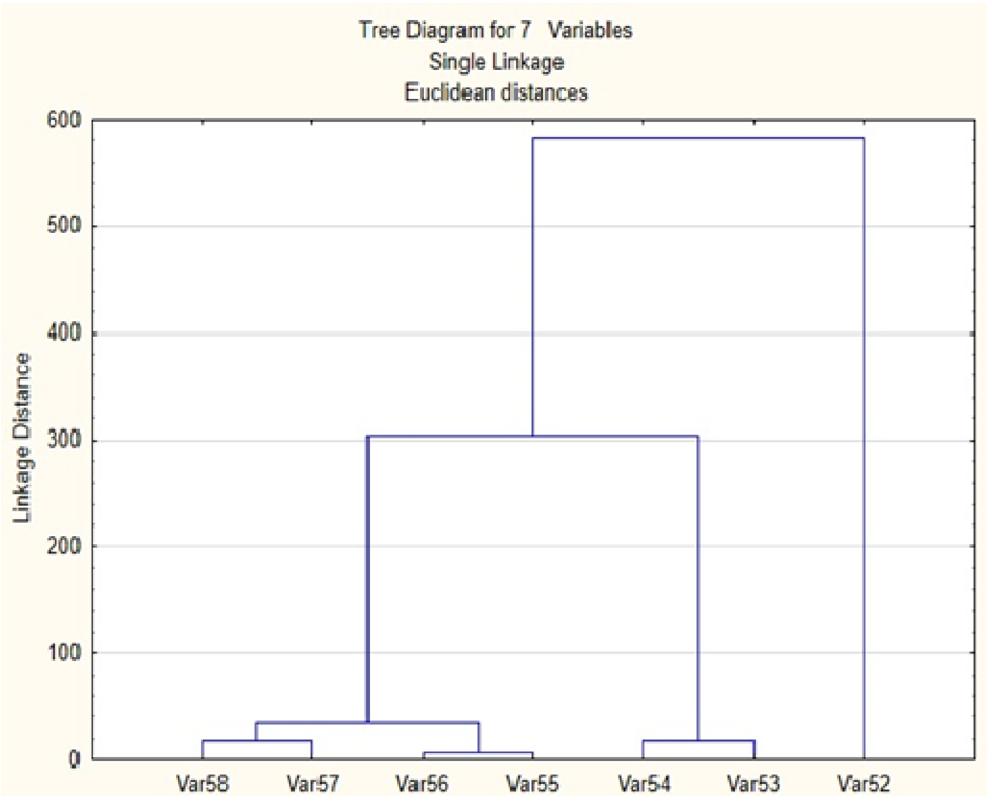


Figure 4. Cluster analysis applied to the microelement content of bryophyte samples collected from the Central Park area of Miercurea Ciuc municipality (original).

In the natural park area, strong and very strong positive correlations are observed between manganese on the one hand and zinc (R = +0.844), chromium (R = +0.948), gallium (R = +0.822) and nickel (R = +0.894) on the other. Likewise, strong positive correlations are also identified between zinc and chromium (R = +0.815), gallium (R = +0.742) and nickel (R = +0.793), as well as between chromium and gallium (R = +0.707). In addition, nickel shows strong positive correlations with chromium (R = +0.800) and gallium, as well as further associations between chromium and gallium (R =

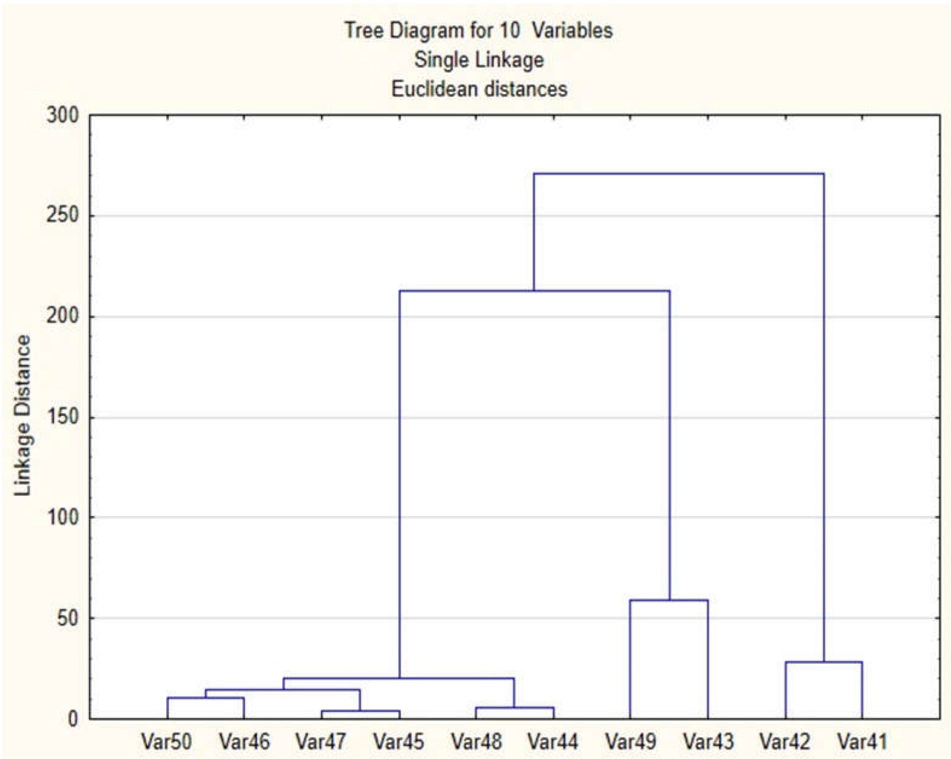
+0.616). A single strong negative correlation is observed between silver and zinc (R = -0.707) (Table 7).

Table 7

Simple correlations between the microelement content of bryophytes collected from the Central Park area of Miercurea Ciuc municipality (original)

Variabile	*Mn*	*Zn*	*Cr*	*Ga*	*Ag*	*Ni*	*Pb*
Mn	1.000	0.844	0.984	0.822	-0.267	0.894	-0.055
Zn	0.844	1.000	0.815	0.742	-0.707	0.793	-0.132
Cr	0.984	0.815	1.000	0.707	-0.298	0.800	0.124
Ga	0.822	0.742	0.707	1.000	-0.105	0.990	-0.614
Ag	-0.267	-0.707	-0.298	-0.105	1.000	-0.149	-0.185
Ni	0.894	0.793	0.800	0.990	-0.149	1.000	-0.496
Pb	-0.055	-0.132	0.124	-0.614	-0.185	-0.496	1.000

The concentrations of heavy metals detected in bryophytes collected from the railway area can be grouped into two main clusters. One of these corresponds to manganese and zinc, which are present at the highest concentrations. The other sub-cluster is further divided into two additional sub-clusters. One of them corresponds to copper and chromium, which are also found at relatively high concentrations. The second sub-cluster shows three branches, in which the remaining heavy metals are grouped in pairs according to their concentrations (Figure 5).



Var 41 – Mn, Var 42 – Zn, Var 43 – Cr, Var 44 – Cu, Var 45 – Ni, Var 46 – Sn, Var 47 – Ag, Var 48 – Cd, Var 49 – Pb, Var 50 – Ga

Figure 5. Cluster analysis applied to the microelement content of bryophyte samples collected from the railway area of Miercurea Ciuc municipality (original).

Only strong and very strong positive correlations are observed between certain heavy metals identified in the Central Railway Station area of Miercurea Ciuc municipality. Zinc shows a very strong correlation only with manganese (R = +0.964) and nickel (R = +0.761). Chromium is very strongly correlated with gallium (R = +0.930) and arsenic (R

= +0.930). Silver shows a strong correlation with nickel (R = +0.676). Lead is strongly correlated with chromium (R = +0.794), gallium (R = +0.739), silver (R = +0.758) and arsenic (R = +0.739). Copper is very strongly correlated with chromium (R = +0.900) and arsenic (R = +0.992), and shows a strong correlation with lead, according to the correlation coefficient value (R = +0.650) (Table 8).

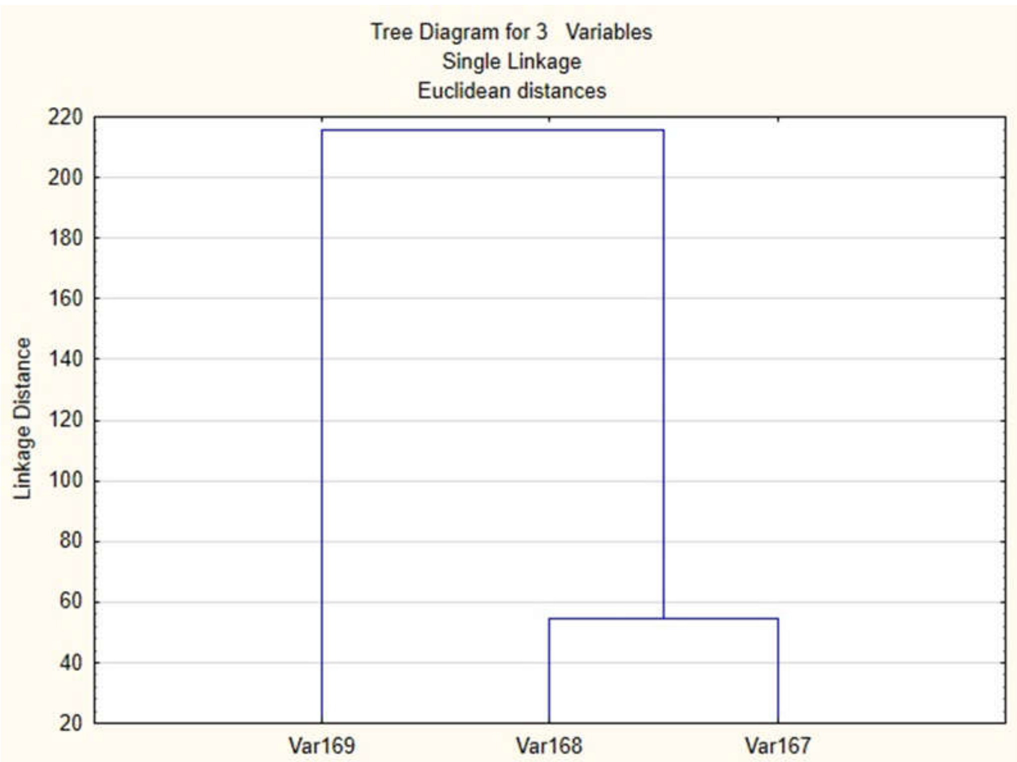
The concentrations of heavy metals detected in bryophytes collected from the brewery area of Miercurea Ciuc municipality can be grouped into two main clusters. One of these, consisting of a single branch, corresponds to manganese, which is present at the highest concentration. The other sub-cluster is divided into two additional sub-clusters corresponding to zinc and chromium (Figure 6).

In the brewery area of Miercurea Ciuc municipality, only two strong positive correlations are observed. Nickel shows a strong correlation with zinc (R = +0.809) and with chromium, the correlation coefficient being R = +0.895 (Table 9).

Table 8

Simple correlations between the microelement content of bryophytes collected from the railway area of Miercurea Ciuc municipality (original)

Variabile	Mn	Zn	Cr	Ga	Ag	As	Ni	Pb	Cu	Cd
Mn	1.000	0.964	0.473	0.147	0.164	0.147	0.569	0.151	0.127	0.268
Zn	0.964	1.000	0.300	-0.062	0.300	-0.062	0.761	0.108	-0.100	-0.033
Cr	0.473	0.300	1.000	0.930	0.300	0.930	-0.085	0.794	0.900	0.569
Ga	0.147	-0.062	0.930	1.000	0.124	0.025	-0.419	0.739	0.250	0.413
Ag	0.164	0.300	0.300	0.124	1.000	0.124	0.676	0.758	0.001	0.123
As	0.147	-0.062	0.930	0.025	0.124	1.000	-0.419	0.739	0.992	0.292
Ni	0.569	0.761	-0.085	-0.419	0.676	-0.419	1.000	0.153	-0.507	-0.107
Pb	0.151	0.108	0.794	0.739	0.758	0.739	0.153	1.000	0.650	0.250
Cu	0.127	-0.100	0.900	0.250	0.001	0.292	-0.507	0.650	1.000	0.155
Cd	0.268	-0.033	0.569	0.413	0.123	0.292	-0.107	0.250	0.155	1.000



Var 167 – Mn, Var 168 – Zn, Var 169 – Cr

Figure 6. Cluster analysis applied to the microelement content of bryophyte samples collected from the brewery area of Miercurea Ciuc municipality (original).

Table 9
Simple correlations between the microelement content of bryophytes collected from the brewery area of Miercurea Ciuc municipality (original)

Variabile	*Zn*	*Cr*	*Ni*
Zn	1.000	0.566	0.809
Cr	0.566	1.000	0.895
Ni	0.809	0.895	1.000

Conclusions. Following the research conducted within the project, a series of conclusions were obtained, presented below.

➢ The highest number of heavy metals present in concentrations exceeding the detection limit were identified in areas with railway and road traffic, corresponding to the railway area of Miercurea Ciuc municipality and the Jigodin area, with 10 elements, while the lowest number was recorded in the brewery area of Miercurea Ciuc municipality, with 4 and respectively 3 elements. The highest concentrations were reported for manganese (1300 ppm in the LUCI peat bog nature reserve area). The lowest concentrations corresponded to arsenic (5 ppm in the LUCI peat bog area, 4 ppm in the railway area of Miercurea Ciuc) and gallium (5 ppm in the railway area of Miercurea Ciuc).

➢ According to the approximated Singular Pollution Index values, possible manganese pollution (typically originating from combustion processes and improper management of electrical and electronic waste) is observed in the LUCI peat bog nature reserve area, the central park of Miercurea Ciuc, and the brewery area of Miercurea Ciuc. The presence of chromium (with a potential origin in combustion processes and industrial activities) may represent a threat in railway and road traffic areas of Miercurea Ciuc railway zone and the brewery area of Miercurea Ciuc. The presence of arsenic and copper may constitute a problem in the railway area of Miercurea Ciuc, while cadmium is mainly associated with railway and road traffic areas of Miercurea Ciuc.

➢ Bioaccumulation index values suggest elevated levels for zinc and chromium, as well as for silver and manganese (with possible origins in industrial wastewater and mining activities). It can be observed that, according to both calculated indices, chromium pollution may represent a real environmental threat in all monitored areas. Additionally, cadmium shows a high pollution potential, particularly in traffic areas, both railway and road.

➢ Cluster analysis highlights, across all monitored areas, the presence of two clusters, one of which consistently corresponds to the concentration(s) of the microelement(s) with the highest values, mainly indicating elevated concentrations of manganese and, in some cases, zinc. These elements are mainly found in soils located near mining activities and/or metallurgical industries, as well as railway traffic zones.

Authors Contributions. Endre Molnar wrote, read and revised the manuscript.

Conflicts of Interest. The author declares that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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