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# Testing Biomonitoring Potential of Bryophytes. Note II: The Interrelations Between the Heavy Metal Content Identified in the Soil of Different Areas of Harghita County

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**Abstract**

Biomonitoring with bryophytes is a widely used method for assessing environmental quality, particularly air quality. Bryophytes, including mosses and lichens, are highly sensitive to environmental changes, making them effective biological indicators of air pollution. These plants absorb chemicals directly from the air, providing insights into pollutant levels in the surrounding environment. In this study, we aimed to test the interrelations between the heavy metal content identified in the soil of different areas of Harghita County. The study of the interrelations between heavy metals within analyzed sites performed using the cluster analysis highlights in all monitored areas the presence of two clusters, one of which corresponds in all cases to the concentration(s) of the microelement(s) that present the highest values, thus highlighting the high concentrations mainly of manganese and in some cases also of zinc, which are found mainly in the soil located in the vicinity of mining activities and/or those where the metallurgical industry is developed, but also railway traffic.

**Keywords:** cluster analysis, correlations, heavy metals, multivariate analysis.

## 1. Introduction

Monitoring heavy metal pollution in the environment is a very complex process, especially when it comes to heavy metal pollutants. Heavy metals are naturally found in the Earth's crust, from where they are released into the atmosphere and water bodies [7].

Some of the metals are essential for the metabolic functioning of organisms, but if they are deficient or present in excess, they can lead to physiological problems and have harmful consequences. Other metals, such as Pb, Cd, Al and Hg, are harmful regardless of their concentrations [5]. Heavy metals are particularly important from the perspective of the fact that they manifest themselves as pollutants. Once present in the environment, they are difficult to eliminate and tend to accumulate in the tissues of plants and other organisms through food chains. The increasing contamination requires monitoring of heavy metal concentrations in the environment and the influence of their effects on ecosystems [6]. Due to their widespread distribution and ability to accumulate large amounts of heavy metals, bryophytes have been used as an important biomonitoring system for heavy metal pollution since 1968 [11].

Also, the phenomenon that some bryophytes tend to grow on substrates containing certain heavy metals has led to their use as bioindicators that could indicate the presence of a particular metal in the environment.

In addition, the relative simplicity of these plants makes them an important model for investigating morphological and genomic changes in plants due to heavy metal toxicity [2].

Finally, the key phylogenetic position of bryophytes in plant evolution links their terrestrial and aquatic lifestyles [9], as well as the fact that they are the most conservative group of terrestrial plants, highlighting their importance for studies on the evolution of plant resistance mechanisms to these types and typologies of environmental pollution.

Bryophytes provide information on 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 of monitoring pollution with suspended metals in the air, where they are very important, but also in monitoring heavy metal pollution in aquatic environments [4].

However, these studies do not provide the absolute concentrations of elements that accumulate in the environment over a certain period. To obtain this information, it is advisable to establish and maintain a linear correlation between concentrations in bryophyte tissue and the concentrations of metals to which they are exposed, taking into account all factors that could disrupt this relationship [1].

Extracellular accumulation of heavy metals is mediated by ion exchange and complex formation between metals and organic functional groups in the cell walls of bryophytes [10]. Several methods have been developed to monitor global air pollution using bryophytes and applied in a wide range of in situ studies. Ground mosses have been the main target of experimental studies investigating the influence of atmospheric deposition on species and populations.

These experiments have been mainly conducted within the framework of research on global change in arctic and tundra ecosystems and have focused on nitrogen and phosphorus deposition [3].

Most experimental studies have been limited to a period of two to four years, and the results have been very controversial, depending on the species and ecosystems observed [8]. Long-term experiments or biomonitoring programs using bryophytes as reactive indicators for more than ten years have been almost non-existent until now. The study aims to test the interrelations between the heavy metals content identified in the soil of different areas of Harghita County.

## 2. Material and Method

In the spring of 2004, a study concerning the interaction of the heavy metals biomonitored with moss was performed in Harghita County. Soil samples were collected from seven locations: the Tironovul Luci protected area, the Romanian Railways near Sâncrăieni commune, Jigodin Baths, Central Park in Miercurea Ciuc, the Miercurea Ciuc Brewery, Șuta Forest, and the Șumuleu Ciuc area. The biomonitoring process utilized ground moss species as bioindicators.

Heavy metal concentrations were analyzed using X-ray spectroscopy with an XRF device.

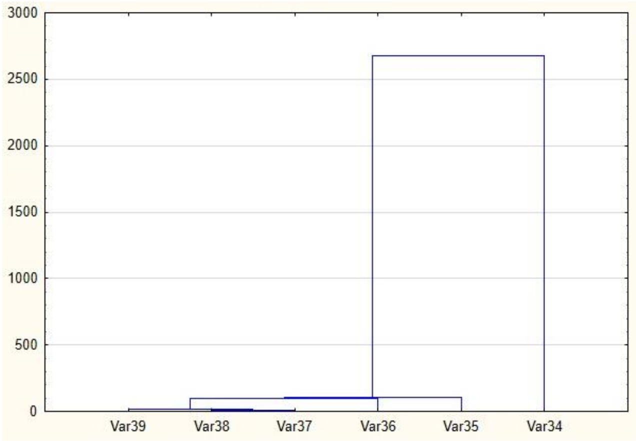
The data were statistically processed using Statistica for Windows. v.8.0. Simple correlations were calculated using Descriptive statistics option, and multivariate analysis for performing the cluster analysis.

## 3. Results and Discusions

The presence of two main clusters is observed. One consists of a single branch and corresponds to manganese, which is found in the highest concentration. The other consists of 5 subclusters, corresponding to the concentrations of the other microelements, much lower in value (Fig. 1). In the area of the nature reserve, strong and very strong positive correlations are observed between zinc on the one hand, chromium (R = 0.699) and gallium (R = +0.981), on the other hand, but also between chromium and gallium (R = +0.616). Also, a strong, but negative correlation is recorded between Mn and As (R = -0.789). This shows that an increase in the concentration of zinc also entails an increase in the concentrations of chromium and gallium, and an increase in the concentration of manganese entails a decrease in the concentration of arsenic (Table 1). In the central park area, heavy metal concentrations are also grouped into two main clusters. One of these corresponds to manganese, which is found in the highest concentration. The other subcluster is divided into two other subclusters. One of these corresponds to zinc and chromium, which are found in identical concentrations. The other subcluster presents two branches in which the other heavy metals are grouped in pairs according to their concentrations (Fig. 2).

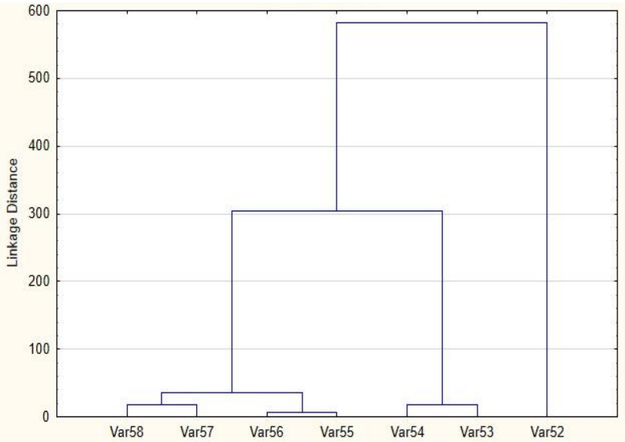
In the natural park, strong and very strong positive correlations are observed between manganese on the one hand, zinc (R = +0.844), chromium (R = +0.948) gallium (R = +0.822) and nickel (R = +0.894), on the other hand, between zinc on the one hand and chromium (R = + 0.815), gallium (R = + 0.742) and nickel (R = +0.793) on the other hand, between chromium and gallium (R = +0.707), but also between nickel on the one hand and chromium (R = +0.800) and gallium, and between chromium and gallium (R = +0.616).

A single strong negative correlation is highlighted, between silver and zinc equal to R = -0.707 (Table 2).




Var 34 – Mn, Var 35 – Zn, Var 36 – Cr, Var 37 – Ga, Var 38 – Ag, Var 39 – As

**Figure 1. The cluster analysis of the micro elemental content of moss collected from the Tinovul Luci natural reservation area**




Var 52 – Mn, Var 53 – Zn, Var 54 – Cr, Var 55 – Ga, Var 56 – Ag,Var 57 – Ni, Var 58 – Pb

**Figure 2. The cluster analysis of the micro elemental content of moss collected from the Central Park of Miercurea Ciuc**

Table 1. The simple correlations between the micro elemental content of moss collected from the Tinovul Luci natural reservation area

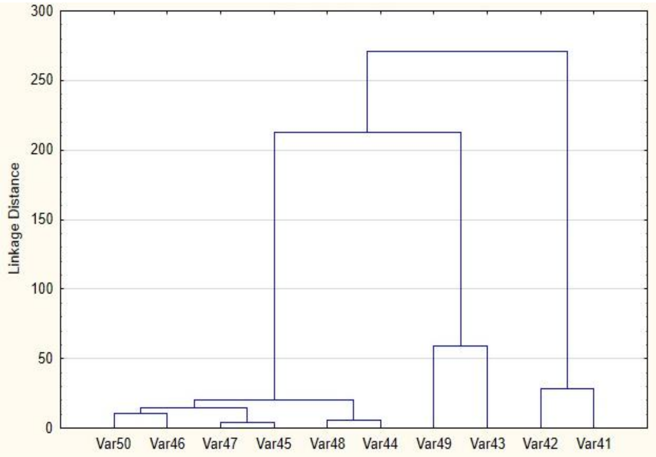
| Variable | Mn | Zn | Cr | Ga | Ag | As |
|---|---|---|---|---|---|---|
| Mn | 1.000 | 0.141 | 0.454 | -0.055 | 0.086 | -0.791 |
| Zn | 0.141 | 1.000 | 0.699 | 0.981 | 0.373 | 0.318 |
| Cr | 0.454 | 0.699 | 1.000 | 0.616 | -0.300 | 0.186 |
| Ga | -0.055 | 0.981 | 0.616 | 1.000 | 0.359 | 0.477 |
| Ag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

Table 2. The simple correlations between the micro elemental content of moss collected from the Central Park of Miercurea Ciuc

| 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 |

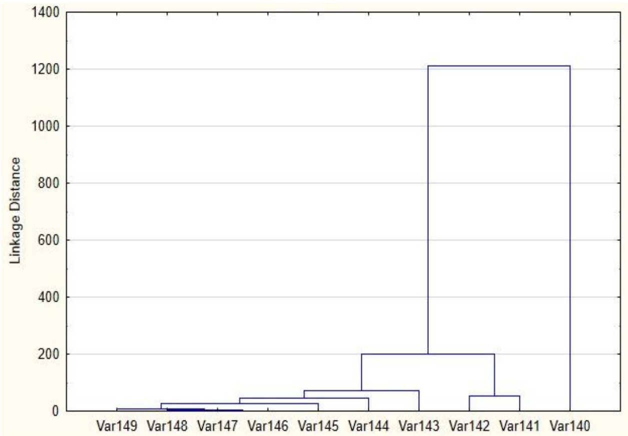
The concentrations of heavy metals detected in the bryophytes collected from the railway area can be seen to be grouped into two main clusters. One of these corresponds to manganese and zinc which are present in the highest concentrations. The other subcluster is divided into two other subclusters. One of these corresponds to copper and chromium which are also found in high concentrations. The other subcluster presents three branches in which the other heavy metals are grouped in pairs according to their concentrations (Fig. 3). Only strong and very strong positive correlations are observed between certain heavy metals identified around the central train station of Miercurea Ciuc municipality. Zinc is very strongly correlated and 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 is strongly correlated 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), but only strongly with lead according to the correlation coefficient value, R = +0.650 (Table 3). The concentrations of heavy metals detected in bryophytes collected from the Jigodin road traffic area can be observed to be grouped into two main clusters. One of these, consisting of a single branch, corresponds to manganese and is present in the highest concentration.

The other subcluster is divided into two others main subclusters. One of these corresponds to zinc and chromium, which are also found in high concentrations (Fig. 4, Table 4). The other subcluster presents seven branches in which the other heavy metals are grouped in pairs according to their concentrations (Fig. 4).




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 3. The cluster analysis of the micro elemental content of moss collected from the railway area of Miercurea Ciuc**




Var 140 – Mn, Var 141 – Zn, Var 142 – Cr, Var 143 – Cu, Var 144 – Ni, Var 145 – Sn, Var 146 – Ag, Var 147 – Cd, Var 148 – Pb, Var 149 – Ga

**Figure 4. The cluster analysis of the micro elemental content of moss collected from Jigodin area**

Table 3. The simple correlations between the micro elemental content of moss collected from the railway area of Miercurea Ciuc

| 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 |

Table 4. The simple correlations between the micro elemental content of moss collected from the Jigodin area

| Variabile | Mn | Zn | Cr | Cu | Ni | Sn | Ag | Cd | Pb | Ga |
|---|---|---|---|---|---|---|---|---|---|---|
| Mn | 1.000 | 0.333 | 0.804 | 0.792 | 0.962 | 0.929 | 0.836 | 0.773 | 0.915 | -0.381 |
| Zn | 0.333 | 1.000 | 0.156 | 0.519 | 0.266 | 0.290 | 0.199 | 0.295 | 0.276 | -0.418 |
| Cr | 0.804 | 0.156 | 1.000 | 0.858 | 0.855 | 0.946 | 0.995 | 0.986 | 0.968 | 0.238 |
| Cu | 0.792 | 0.519 | 0.858 | 1.000 | 0.880 | 0.927 | 0.898 | 0.898 | 0.857 | 0.000 |
| Ni | 0.962 | 0.266 | 0.855 | 0.880 | 1.000 | 0.973 | 0.896 | 0.822 | 0.911 | -0.232 |
| Sn | 0.929 | 0.290 | 0.946 | 0.927 | 0.973 | 1.000 | 0.971 | 0.931 | 0.970 | -0.050 |
| Ag | 0.836 | 0.199 | 0.995 | 0.898 | 0.896 | 0.971 | 1.000 | 0.983 | 0.971 | 0.177 |
| Cd | 0.773 | 0.295 | 0.986 | 0.898 | 0.822 | 0.931 | 0.983 | 1.000 | 0.954 | 0.244 |
| Pb | 0.915 | 0.276 | 0.968 | 0.857 | 0.911 | 0.970 | 0.971 | 0.954 | 1.000 | 0.000 |
| Ga | -0.381 | -0.418 | 0.238 | 0.000 | -0.232 | -0.050 | 0.177 | 0.244 | 0.000 | 1.000 |

And in the Jigodin road traffic area, like the railway traffic area of the Miercurea Ciuc municipality, only strong and very strong positive correlations are observed between the vast majority of the identified microelements.

Manganese is very strongly correlated with four microelements, namely nickel (R =+0.962), tin (R =+0.929), silver (R =+0.836) and lead (R = +0.915). Chromium is very strongly correlated with six microelements, namely nickel (R =+0.855), copper (R =+0.858), tin (R =+0.946), silver (R =+0.995), cadmium (R =+0.986) and lead (R = +0.968).

Copper is very strongly correlated with five microelements, namely nickel (R =+0.880), tin (R =+0.927), silver (R =+0.898), cadmium (R =+0.898) and lead (R = +0.857). Nickel is very strongly correlated with four microelements, namely tin (R =+0.973), silver (R =+0.896), cadmium (R =+0.822) and lead (R = +0.911). Tin is very strongly correlated with three microelements, namely silver (R =+0.971), and lead (R = +0.970).

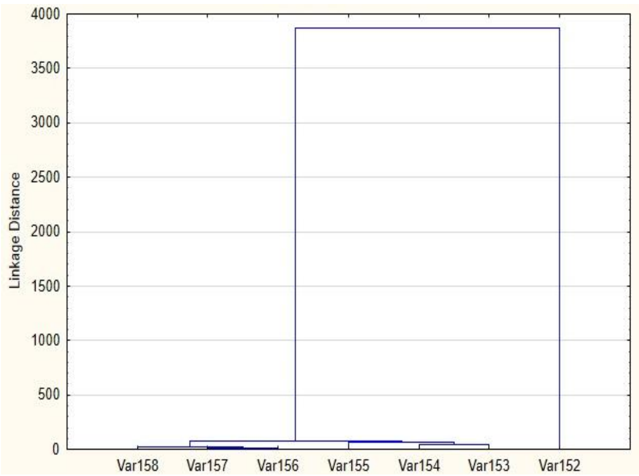
Silver is strongly correlated with lead (R = +0.971) and cadmium (R =+0.983). Cadmium is very strongly correlated with lead, the correlation coefficient being equal to R = +0.954 (Table 4).

The concentrations of heavy metals detected in bryophytes collected from the Suta forest area can be observed to be grouped into two main clusters. One of these, consisting of a single branch, corresponds to manganese and is present in the highest concentration. The other subcluster is divided into two other main subclusters. One of these comprises three branches and corresponds to zinc and chromium and copper which are found in higher concentrations close in value.

The other subcluster presents two branches, a single one corresponding to As which is found in the lowest concentration, and the other with two branches of tin and gallium found in similar concentrations higher than arsenic (Fig. 5). And in the Suta Forest area, strong and very strong positive correlations are observed between four of the seven identified microelements.

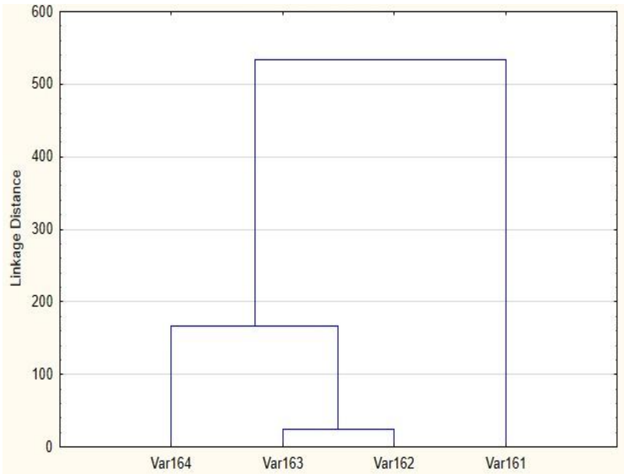
Manganese is very strongly correlated with three microelements, namely zinc (R =+0.982), chromium (R =+0.963) and copper (R = +0.864). Zinc is also very strongly correlated with three microelements, namely chromium (R =+0.825), copper (R =+0.980) and tin (R = +0.831). Copper is very strongly correlated only with tin, the correlation coefficient being equal to R = +0.918 (Table 5).

The concentrations of heavy metals detected in bryophytes collected from the Şumuleu Ciuc area can be observed to be grouped into two main clusters (Fig. 6, Table 6).




Var 152 – Mn, Var 153 – Zn, Var 154 – Cr, Var 155 – Cu, Var 156– Sn, Var 157 – Ga, Var 158 – As

**Figure 5. The cluster analysis of the micro elemental content of moss collected from the Suta Forest area**




Var 161 – Mn, Var 162 – Zn, Var 163 – Cr, Var 164 – Ni

**Figure 6. The cluster analysis of the micro elemental content of moss collected from the Şumuleu Ciuc area**

Table 5. The simple correlations between the micro elemental content of moss collected from the Suta Forest area

| Variabile | Mn | Zn | Cr | Cu | Sn | Ga | As |
|---|---|---|---|---|---|---|---|
| Mn | 1.000 | 0.892 | 0.963 | 0.864 | 0.649 | 0.412 | 0.259 |
| Zn | 0.892 | 1.000 | 0.825 | 0.980 | 0.831 | 0.681 | 0.426 |
| Cr | 0.963 | 0.825 | 1.000 | 0.760 | 0.464 | 0.483 | 0.089 |
| Cu | 0.864 | 0.980 | 0.760 | 1.000 | 0.918 | 0.631 | 0.405 |
| Sn | 0.649 | 0.831 | 0.464 | 0.918 | 1.000 | 0.469 | 0.469 |
| Ga | 0.412 | 0.681 | 0.483 | 0.631 | 0.469 | 1.000 | -0.038 |
| As | 0.259 | 0.426 | 0.089 | 0.405 | 0.469 | -0.038 | 1.000 |

Table 6. The simple correlations between the micro elemental content of moss collected from the Şumuleu Ciuc area

| Variabile | Mn | Zn | Cr | Ni |
|---|---|---|---|---|
| Mn | 1.000 | 0.915 | 0.582 | 0.936 |
| Zn | 0.915 | 1.000 | 0.754 | 0.963 |
| Cr | 0.582 | 0.754 | 1.000 | 0.828 |
| Ni | 0.936 | 0.963 | 0.828 | 1.000 |

One of these, consisting of a single branch, corresponds to manganese and is present in the highest concentration.

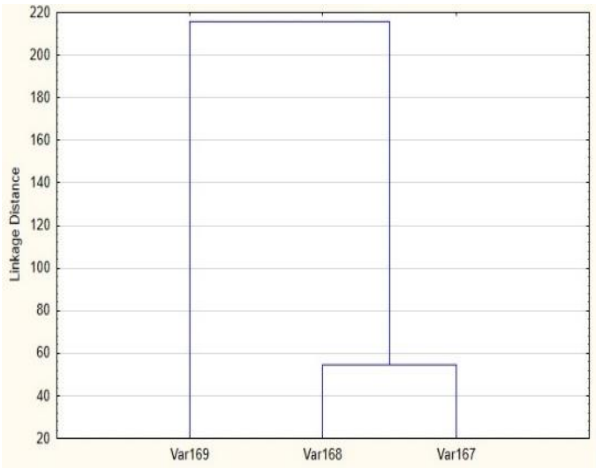
The other subcluster is divided into two others main subclusters. One of these comprises two branches corresponding to zinc and chromium found in similar concentrations, and the other branch consists of a single arm and corresponds to nickel, which is found in the lowest concentration (Fig. 6).

In the Şumuleu Ciuc area, strong and very strong positive correlations are observed between nickel and the other three identified microelements, but also between zinc and manganese. Manganese is very strongly correlated with zinc (R =+0.915).

Very strong correlations are observed between nickel and manganese (R =+0.936), but also between nickel and zinc (R = +0.63). A strong correlation is observed between nickel and chromium, the correlation coefficient being equal to R = +0.828 (Table 6).

The concentrations of heavy metals detected in biofuel collected from the brewery area of Miercurea Ciuc municipality can be seen to be grouped into two main clusters (Fig. 7, Table 7).

One of these, consisting of a single branch, corresponds to manganese and is present in the highest concentration. The other subcluster is divided into two other subclusters corresponding to zinc and chromium (Fig. 7). In the brewery area of Miercurea Ciuc municipality, only two strong positive correlations are observed. Nickel is strongly correlated with zinc (R = +0.809) and chromium, the correlation coefficient being equal to R = +0.895 (Table 7).




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

**Figure 7. The cluster analysis of the micro elemental content of moss collected from the brewery of Miercurea Ciuc**

Table 7. The simple correlations between the micro elemental content of moss collected from the brewery of Miercurea Ciuc

| 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 |

## 4. Conclusions

The cluster analysis highlights in all monitored areas the presence of two clusters, one of which corresponds in all cases to the concentration(s) of the microelement(s) that present the highest values, thus highlighting the high concentrations mainly of manganese and in some cases also of zinc, which are found mainly in the soil located in the vicinity of mining activities and/or those where the metallurgical industry is developed, but also railway traffic.

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