

## LICHEN-BAGS AS A BIOMONITORING TECHNIQUE IN AN URBAN AREA

PETROVA, S.P.<sup>1\*</sup> – YURUKOVA, L.D.<sup>2</sup> – VELCHEVA, I.G.<sup>1</sup>

<sup>1</sup> *University of Plovdiv “Paisii Hilendarski”, Faculty of Biology, Department of Ecology and Environmental Conservation, Plovdiv 4000, Tzar Assen Str. 24, Bulgaria  
(phone: +359-32-261519)*

<sup>2</sup> *Bulgarian Academy of Sciences, Institute of Biodiversity and Ecosystem Research, Sofia 1113, Acad. G. Bonchev Str., Bl. 23, Bulgaria*

*\*Corresponding author:  
e-mail: slaveya\_petrova@uni-plovdiv.bg*

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**Abstract.** The suggested work presents the first application of the lichen-bags technique in the city of Plovdiv (Bulgaria). Plovdiv was pointed as one of the most polluted cities in Europe for 2008 by National Institute of Statistics of Italy (ISTAT, 2011). The study was carried out to estimate the air quality in the areas with high traffic activity, industrial and suburban zones by means of lichens. For this purpose, the concentrations of 21 chemical elements (Al, As, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, U, V and Zn) were investigated using *Pseudevernia furfuracea* (L.) Zopf. samples in bags, which were exposed at 4 different sites in the city of Plovdiv. The elements Na, Mn, Cu, Zn and Sr increased significantly their concentrations in the lichen-bags, whereas K, S and Cd decreased theirs in comparison with the unexposed lichenized fungi. Highest values of RAF (relative accumulation factor) were found for Na (3.49), Zn (2.92) and Cu (3.33).

**Keywords:** *atmospheric pollution, urbanization, active biomonitoring, lichen-bags, Pseudevernia furfuracea*

### Introduction

Since the beginning of the twentieth century, air pollution has worsened as a consequence of urbanization and industrialization, together with the rapid growth of the motorized transportation and population. Of the main components of air pollution, an important group to be considered is that of heavy metals, mostly associated with airborne particulate matter. This is particularly true in city centers, where vehicle engines, as well as the heating systems inside buildings, represent the most important sources of air pollution (Sawidis et al., 1995; Monaci et al., 2000).

Lichens are widely used in biomonitoring studies of air pollution, either as bioindicators of air quality or as bioaccumulators of atmospheric deposition (Conti and Geccheti, 2001; Sczepaniak and Bizuik, 2003; Cansaran-Duman et al., 2012). These organisms can be easily sampled, are low in cost, and allow wide areas to be monitored. Moreover, lichens do not have root systems or waxy cuticles, and thus they are strongly dependent on wet and dry deposition for their mineral nutrients (Bermudez et al., 2009). Unlike many other plants, they lack variability in morphology throughout the growing season, thus they are used for biomonitoring purposes in many ways (Giordano et al., 2005).

Some lichen species were successfully applied from many years for assessing the atmospheric deposition of heavy metals and toxic elements in background and polluted areas. Lichens naturally occur in many terrestrial ecosystems, but the anthropogenic

factors can be responsible for their lack in urban areas. In such cases, the passive biomonitoring is not useful and the active biomonitoring comes to replace it. One of the techniques is exposing bags containing lichen in the polluted area to measure concentrations of contaminants affecting the samples (Adamo et al., 2003; Giordano et al., 2005; Yildiz et al., 2008).

This technique has a lot of advantages: well-defined exposure time, known original concentration of elements in the biomonitor, flexibility both in site selection and in number of stations that can be chosen. The major limitations of the method are in the unknown collection efficiency for different contaminants in different biomonitors and, therefore, the impossibility to standardize the exposure time for the exact species in bags and/or for urban, industrial or other source of pollution.

The suggested work shows the first application of the lichen-bags technique in the city of Plovdiv (Bulgaria). Furthermore, Plovdiv was pointed as one of the most polluted cities in Europe for 2008 by National Institute of Statistics of Italy (ISTAT, 2011). The study was carried out to estimate the air quality in the areas with high traffic activity, industrial and suburban zones by means of lichens. For this purpose, the concentrations of 21 chemical elements (Al, As, Ca, Cd, Cr, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, U, V and Zn) were investigated using *Pseudevernia furfuracea* (L.) Zopf. samples in bags, which were exposed at 4 different sites in the city of Plovdiv.

## Materials and methods

### *Study area and sampling sites*

Plovdiv (42°9' N, 24°45' E) is the second-largest city after the capital Sofia with a population of over 338 000 inhabitants (NSI, 2011). It is situated in Southern Bulgaria on the two banks of the Maritsa River. Air quality in Plovdiv is quite worsened due to the motor vehicle emissions and residential heating, than the industrial sector, which has a minor impact (Atanassov et al., 2006; RIOSV, 2010).

For the main purpose of our study, four sites (Northern, Eastern, Western and Central) were selected on the basis of the type of anthropogenic impact (*Fig. 1*).

The Northern site (N 42°10'32.3", E 24°45'53.4") is located in the city outskirts and was chosen because it is close to an industrial nonresidential area (Northern industrial zone in the city of Plovdiv) where there are a fossil fuel power plant, cosmetic and glass factories, as well as other small-point sources of contaminants.

The Eastern site (N 42°08'24.3", E 24°47'06.2") is located in the "Trakiya" suburb, which is characterized by a high traffic and moderate household pressure.

The Western site (N 42°07'54.4", E 24°42'18.0") was chosen in the "Smirnenski" suburb, which is subjected to a moderate anthropogenic impact.

The Central site (N 42°08'22.8", E 24°44'24.1") is located in the main center of the city of Plovdiv, along one of the major streets (Ruski Boulevard) with a very intensive daily traffic.

### *Experimental set up*

*Pseudevernia furfuracea* (L.) Zopf. was applied as a suitable biomonitor of air pollution. Lichen material was collected from the Vitosha Natural Park, Bulgaria (42°37' N, 23°19' E, altitude 1710 m) in April 2010. Samples were cleaned from bark

particles and air-dried in the laboratory. The unwashed material (3 g) was placed in a nylon mesh (10 × 10 cm) and 12 lichen-bags from each site were prepared. A novel hanging system (Fig. 2) at the same height from the ground (approximately 6-7 m) was used in the four studied sites in the city of Plovdiv (north, east, central and west part). Lichen-bags were exposed to the ambient air from May to October 2010.

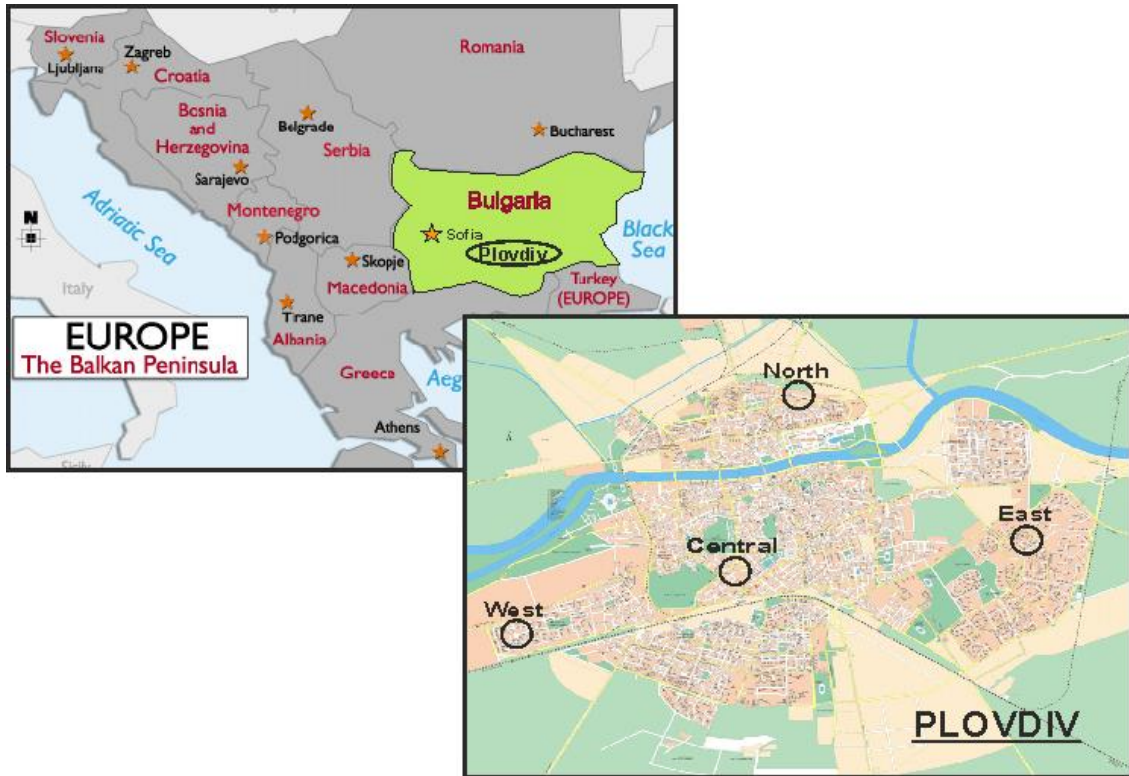


Figure 1. Plovdiv map, which shows the studied sites' location

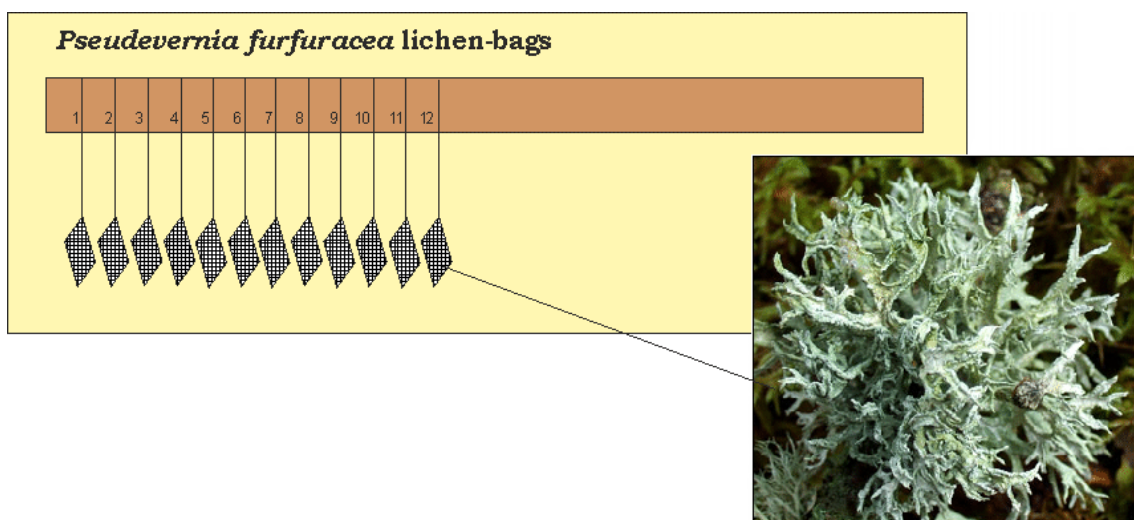


Figure 2. The novel hanging system with lichen-bags

At the beginning of the experiment and every week during the study, respectively each lichen-bag was carefully moistened with drops of distilled water. Lichens, which were exposed for one, three and five months, respectively were collected at the end of each period and stored in clean, labeled polyethylene bags until chemical analysis.

### **Analytical procedures**

Approximately 1 g ground plant material was treated with 5 ml 65% HNO<sub>3</sub> (Merck) for 24 h at room temperature. The wet-ashed procedure was carried out with a Microwave Digestion System CEM MDS 81D. Samples were treated for 5 min at maximum power (600W) in closed vessels. After cooling, the vessels were opened and 2 ml HNO<sub>3</sub> and 3 ml 30% H<sub>2</sub>O<sub>2</sub> were added and left to react for 1 hour. The vessels of the Microwave Digestion System were closed and treated for 10 min at 600W once again for full digestion of the organic matter. The filtrate was diluted with double distilled water up to 50 ml.

All samples, blanks and standards were spiked with internal standards – Ge 50 ppb and Rh 5 ppb final concentration in the solutions. Calibration standards Multy VI (Merck) were freshly prepared from 1 to 1000 ppb in 0.05 volume % HNO<sub>3</sub> (p.a.). Monostandard of Hg 100 ppt was also used in the calibration. Signals of suitable isotopes for the tested elements were measured twice in both modes – without and with helium gas collision cell.

Concentrations of 22 macro- and microelements were analyzed by FAAS (Zn, Fe, K, Mg, Mn, Na, Cu, Ca) using Atomic Absorption Spectrometer PERKIN-ELMER 4000 and ICP-MS method (Al, S, P, Cr, V, Co, Ni, As, Sr, Mo, Cd, Hg, Pb, U) with instrument Agilent 7700 (2009).

### **Statistical analysis**

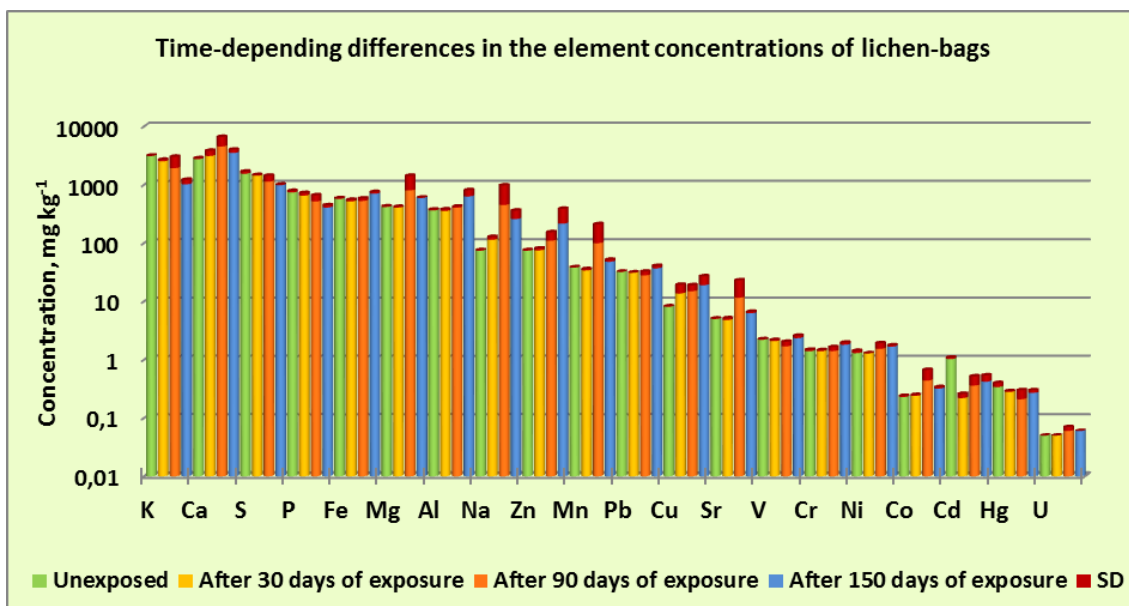
Data analyses using STATISTICA 7.0. for Windows (StatSoft Inc., 2004) were conducted. One-way ANOVA was applied in order to determine the significant differences ( $p < 0.05$ ) occurring between the element concentrations in the lichen bags from the four studied sites and the unexposed sample.

### **Results and discussion**

Element concentrations in the transplant material of the lichen biomonitor before and after exposure could be compared to track the anthropogenic load in time (*Fig. 3*). Macroelements K (up to 4 times), P and S decreased their concentrations by extending the exposure period ( $p < 0.05$ ) in comparison with the unexposed *Pseudevernia furfuracea*. Loss of K in moss-bags after exposure has been observed in Sofia by Culicov and Yurukova (2006) and in many other studies (Yurukova and Ganeva, 1997; Adamo et al., 2003; Culicov et al., 2005), but no additional data in respect to loss of macroelements in lichen-bags were found in the literature.

Elements As, Cd, Hg and U had insignificantly higher concentrations in the unexposed *Pseudevernia furfuracea* in comparison with the exposed samples. A possible reason for this result could be the higher content of these elements in the background area for the material in Vitosha Mountain as similar results were reported for the moss *Sphagnum girgensohnii*, collected in the same region (Yurukova et al., 2013). Furthermore, an interesting result for this group of elements (As, Cd, Hg, U) was

the dynamics during the three exposure periods: an insignificant decrease of the concentrations between the control (unexposed sample) and exposed lichen after 30 days period (for Hg – till 90 day), then an increase to 150 day of exposure. These elements are connected with burning processes of the fuels, which during the summer were not so heavy as in the winter. Thus a possible explanation for this result could be the emissions emanated from the non-ferrous metallurgy plant, located nearby the city of Plovdiv (to the South direction), as well as the predominant wind from south to north during the period of exposure. This suggestion is supported by the highest concentrations of As, Cd, Hg and U, found in the lichen-bags from the Southern site.



**Figure 3.** Element concentrations in *Pseudevernia furfuracea* bags after 30, 90 and 150 days of exposure in the city of Plovdiv

Overall, the reasons for these dynamics in the elements concentrations could be explained by the hypotheses of Sanita di Toppi and Gabrielli (1999). According to them, the Cd ions are transported into the cell vacuole of phytochelatins and they are accumulated until the ionic binding to the cell wall is less effective. Metal ions are able to bind to the negative charges on the pectin compounds (e.g., galacturonic acid) from the wall by the dynamic conversion process (passive ion exchange). Moreover, the authors state that the dead plant tissue showed a higher cation exchange capacity compared with the live cells, probably due to the destruction of the membrane, allowing the cations to bind more protein regions than in the normal state. On the other hand, some abiotic factors, such as rainfall, can dissolve and wash out the associated cell-wall elements more easily than in the vacuole.

Elements Al, Cu, Ni and Zn significantly increased their content in lichen-bags with extending the exposure period ( $p < 0.05$ ). These data correspond with the finding of many authors that used *Pseudevernia furfuracea* (Basile et al., 2008) and other lichens (Sujetoviene and Sliumpaite, 2013) in urban areas. Element concentrations, which we measured in the lichen-bags from Plovdiv in the present study were higher compared to the results of Basile et al. (2008) from Italy (Cd 1.5 times, Cu 4 times, Mn 1.5 times, Pb, 2 times and Zn 9 times). was quite higher when compared to the data from Italy.

Relative accumulation factors (RAF) were calculated for all elements as follows: the lichen-bags mean content of each element after 150 days of exposure ( $C_{\text{exposed}}$ ) in all studied sites was divided by the content of each element before exposure ( $C_{\text{unexposed}}$ ).

$$\text{RAF} = C_{\text{exposed}}/C_{\text{unexposed}}$$

The values of RAF are given in *Table 1*. Highest values were found for Na (3.49), Zn (2.92) and Cu (3.33). Culicov and Yurukova (2006) reported similar RAF for Na in lichen-bags from Sofia.

**Table 1.** Relative accumulation factors (RAF) of the analyzed elements in *Pseudevernia furfuracea* bags after 150 days of exposure in Plovdiv

Element	Mean content in lichen-bags after 150 days of exposure, mg kg <sup>-1</sup>	Content in unexposed sample <i>Pseudevernia furfuracea</i> , mg kg <sup>-1</sup>	RAF
Al	633	364	1.74
As	0.55	2.0	-0.275
Ca	3535.5	2761	1.28
Cd	0.425	1.03	-0.41
Cr	1.8	1.4	1.29
Co	0.32	0.23	1.40
Cu	18.9	8.1	2.33
Fe	686.75	573	1.20
Hg	0.27	0.34	-0.79
K	1020	3114	-0.33
Mg	595.25	417	1.43
Mn	47.75	38	1.26
Na	258	74	3.49
Ni	1.675	1.3	1.29
P	407.5	740	-0.55
Pb	37	32	1.16
S	984	1547	-0.64
Sr	6.3	5	1.26
U	0.06	0.05	1.2
V	2.35	2.2	1.07
Zn	215.75	74	2.92

Bioaccumulation capability of lichens was determined by their significant cation exchange capacity, which is due to the negatively charged cell wall bases (mostly acidic carboxyl groups), which may also be connected via ionic bonds with the dissolved cations. Elements can be detained and particles can be suspended in the intercellular spaces or on uneven surfaces (Freitas, 1994). Many factors determine the volume and speed of the processes of uptake and release of various elements in lichens, such as chemical characteristics of the element, chemical composition and size of the particles associated with the element, and impact of toxic substances contained in the organism.

In the present study 40 positive and 21 negative correlations were proved for the analyzed elements ( $p < 0.05$ ). Strongest positive one was found for P, S and K; followed

by the correlation between Na, Ca, Mg, Mn, Ni, Sr Cd and U; as well as Al, V, Pb, Cr and Fe. Negative relationships existed between the macroelements and the group of heavy metals.

Site-depending differences are presented in Fig. 4. Mean concentrations of K, Ca, S, P, Fe and Mg proved insignificant differences in the lichen-bags exposed in the studied sites.

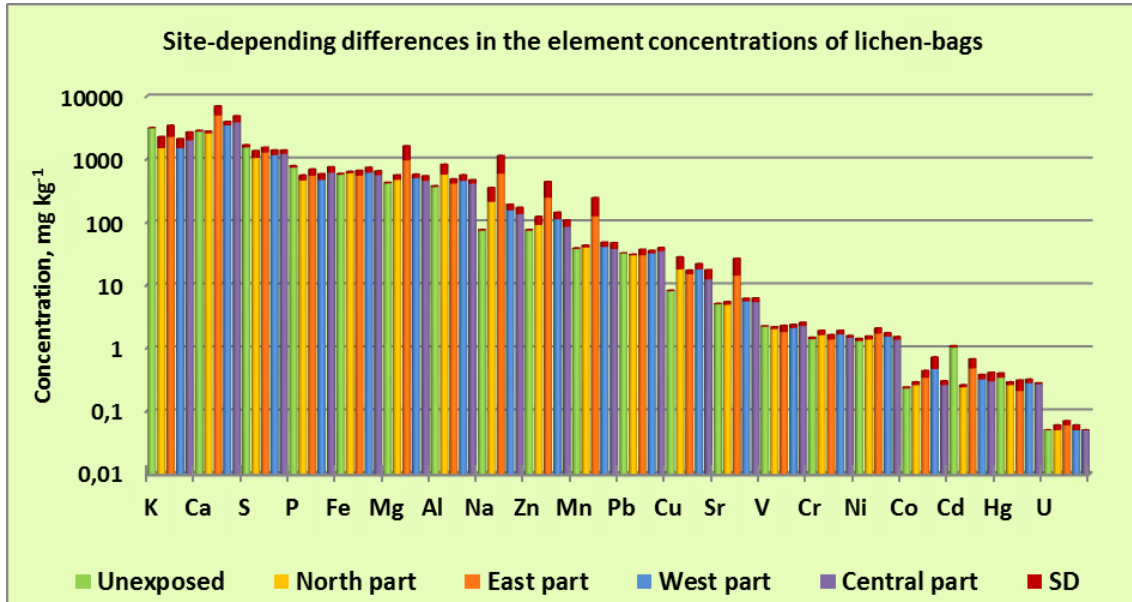


Figure 4. Element concentrations in *Pseudevernia furfuracea* bags after 150 days of exposure in four selected sites in the city of Plovdiv

A tendency to enlarge the number of maximal values of the elements (P, Pb, V, Cr, Hg, Fe) by approaching the central part of Plovdiv was found. The Eastern zone was characterized with highest concentrations of K, Ca, S, Mg, Na, Zn, Mn, Sr, Ni, Cd and U. However, none of the elements had highest concentrations in the Western site. In addition, the applied cluster analysis showed a close relationship between the Northern and Western sites, connected then with the Central zone but quite separated from the Eastern site (Fig. 5).

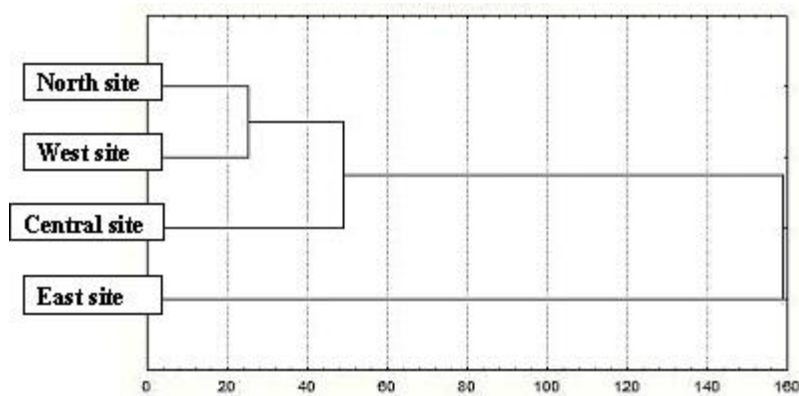


Figure 5. Cluster analysis of the studied sites in the city of Plovdiv based on element content in *Pseudevernia furfuracea* bags after 150 days of exposure

## Conclusions

In general, in the present study it was attempted to describe and assess the atmospheric pollution levels in the city of Plovdiv using lichen-bags technique for three periods. Na, Mn, Cu, Zn and Sr increased significantly their concentrations in the lichen-bags, whereas K, S and Cd decreased theirs in comparison with the unexposed lichenized fungi. Highest concentrations of 11 elements were measured in the collectors, which were exposed in the Eastern site of the urban area, probably due to non-ferrous complex nearby.

Pearson`s analysis showed a strong positive correlation between the macronutrients P, S and K, followed by the correlation between Na, Ca, Mg, Mn, Ni, Sr, Cd and U, and lastly between Al, V, Pb, Cr and Fe, which mainly indicates the vehicle emissions impact.

We can conclude that this study confirms that the species *Pseudevernia furfuracea* is an efficient element accumulator in lichen-bags exposed in urban areas. Moreover, the results from this preliminary study can be successfully used in future comparative analyses for the atmospheric pollution and risk assessment in similar urban areas.

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