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ACTIVE AND PASSIVE MOSS MONITORING OF TRACE ELEMENTS IN URBAN AND MOUNTAIN AREAS, BULGARIA

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Abstract

The moss *Hypnum cupressiforme* Hedw. was used as biomonitor in three urban and five mountain sites with different pollution loads. The concentrations of 22 macro- and microelements were determined in moss-bags and were compared with those of the same elements in moss samples naturally growing in the Rhodope Mountains area. Most of the elements had highest concentrations in moss-bags exposed in Plovdiv. Strong accumulation of Cd and Pb was registered in all moss-bags with maximum in moss-bags from Sofia, reflecting that urban activities could induce more severe air pollution than mining. Cd reached saturation at the 30th day of exposure, while Pb continued to accumulate. Analysed levels of Hg and Pb during passive monitoring confirmed the Rhodope Mountains as a region with constant high toxic element levels.

Key words: biomonitoring, *Hypnum cupressiforme*, moss-bags, trace elements.

Introduction

Air pollution monitoring is a complex issue, and techniques for air quality monitoring have been the objects of intensive research in past decades (Adamo et al. 2003). Instrumental monitoring cannot be applied for longer periods and large areas due to cost barriers. Biomonitoring is a simple, cost-effective alternative for large-scale monitoring, revealing the spatial pattern and temporal trends of pollution (Goodman and Roberts 1971, Markert et al. 1997, Harmens et al. 2010, Nickel et al. 2014, Bargagli 2016).

Biomonitoring techniques consider organisms, based on their different sensitivity to air pollution or actively, through their capacity to accumulate pollutants in the tissues (Falla et al. 2000, Wolterbeek 2002). Two types of biomonitoring are differentiated in the use of mosses to evaluate atmospheric contamination: (i) passive biomonitoring, using moss that grows naturally in a particular area, and (ii) active biomonitoring, by transplanting moss from other locations. Whilst the use of native moss is more appropriate for extensive studies in large areas (i.e. regional or national studies), active biomonitoring is more useful for intensive studies in smaller areas (i.e. urban or industrial areas, where mosses absent due to predominantly paved and landscaped surfaces) (Aničić et al. 2009). Active monitoring by moss-bags has been applied on anthropogenic emissions of industry and traffic (Adamo et al. 2003, Zechmeister et al. 2006, Yurukova et al. 2013). The 'moss-bag technique' is the most common type of active biomonitoring with mosses reported in the literature. The technique, which was originally introduced by Goodman and Roberts (1971), involves exposure of moss samples held within mesh bags in order to monitor the presence of contaminants in the air in particular time span.

The aim of this study was to: (i) to identity the spatial distribution of (trace) elements in selected regions of Bulgaria with *Hypnum cupressiforme* Hedw.; (ii) comparing results from active and passive monitoring; (iii) testing the adequate sampling period for active monitoring.

Materials and Methods

Study sites

The combination of background and impacted sites, determined the interest in a comprehensive study and implementing the biomonitoring approach with mosses. The sites were chosen on the basis of results obtained in previous studies (Gecheva et al. 2016, Gribacheva et al. 2019). The active biomonitoring was carried out in two of the largest Bulgarian cities (Sofia and Plovdiv) – the exposure sites were located in the centres, near main boule-

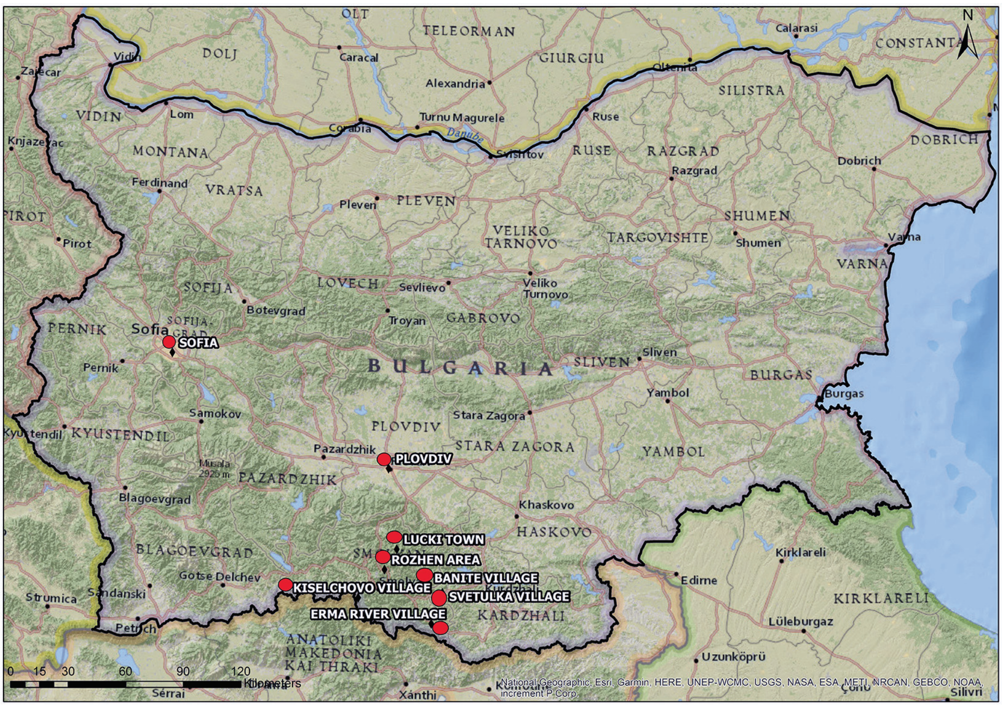


Fig. 1. Map of the studied sites.

vards and in a town affected by mining activities (Luki). Five sites on the territory of the Western Rhodopes were selected for passive biomonitoning, among them three background sites (Svetulka and Banite villages, and Rozhen area), an old uranium mine (Kiselchovo village), a tailing pond (Erma reka village) (Fig. 1, Table 1).

Table 1. Characteristics of the studied sites.

Sites	Geographical position		Altitude, m a.s.l.	Population, No. of inhabitants	Environmental protection of the region
	Longitude E	Latitude N			
Sofia	23°21’5.14”	42°39’15.07”	601	1,308,412	2 Natura 2000 protected areas
Plovdiv	24°44’42.34”	42° 8’41.43”	166	342,048	2 Natura 2000 protected areas 4 protected territories under the Bulgarian Protected Areas Ac.
Luki town	24º48’33.4”	41º45’58.4”	1000	2393	2 Natura 2000 protected areas
Svetulka village	25°06’05.7”	41°33’59.0”	705	861	-
Banite village	25° 0’22.10”	41°37’0.23”	729	3547	1 Natura 2000 protected area
Rozhen area	24º44’08.6’’	41º40’12.03”	1430	-	-
Kiselchovo village	24°34’36.1’’	41°32’04.6’’	1066	23	2 Natura 2000 protected areas
Erma reka village	25°02’09.7”	41°25’15.05”	580	895	1 Natura 2000 protected area

Note: the data for the population are as of 31.12.2020 (NSI 2021).

Moss sampling and preparation

Active monitoring

H. cupressiforme samples were collected in March 2019 from pristine background mountain area (41.6574 N, 24.7716 E, 1260 m a.s.l. in Western Rhodopes). In the laboratory, the moss was cleaned of soil particles and other foreign substances and air-dried. Moss-bags were prepared by weighing about 3 g of air-dried moss, packed loosely in nylon meshes (10×10 cm) and were exposed at three selected cities (Sofia, Plovdiv, Luki) from April to June 2019. Moss-bags were hung on holders at approximately 10 m above the street level (Vuković et al. 2013). The mosses exposed for fifteen, thirty and seventy-five days were collected by the end of each period, stored and transported to the laboratory in clean polyethylene bags and proceeded for further chemical analysis as described below.

Passive monitoring

H. cupressiforme was collected at 5 sites (Svetulka and Banite villages, Rozhen area, Kiselchovo and Erma reka villages) in May 2019.

The moss sampling followed the requirements of ICP-Vegetation manual – each sample consisted of up to 5 sub-samples according to the standardized European methodology. Sampling, transport, storage and pre-treatment followed the standardized protocol (Frontasyeva and Harmens 2014).

Chemical analytical methods

The samples collected by active and passive monitoring were carefully cleaned from mechanical particles and other organic material, dried at 40 ^{o}C and wet-ashed. About 1 g moss material was treated with nitric acid (65 %) overnight, followed by addition of 2 mL portions of hydrogen peroxide. Samples were sealed and irradiated in Milestone Ethos One microwave digestion system.

Two moss reference materials developed within the European Moss Surveys (EMS) (Frontasyeva and Harmens 2014) (M2 and M3, *Pleurozium schreberi* (Brid.) Mitt.) were applied in the current study. To ensure permanent quality control of the analytical measurements, a portion of the reference material was digested and analysed together with each series of samples. The contribution of the reagents used in the analytical procedure was evaluated with the corresponding blank samples for each batch.

A portion of moss reference material used within EMS (Frontasyeva and Harmens 2014) (M2 and M3, *P. schreberi* was digested together with every sample series and corresponding blank samples were prepared as well.

The elements P, K, Ca, S, Na, Mg, Mn, Fe, Al, Zn, Cu, Pb and Sr were determined by atomic emission spectrometry with inductively coupled plasma (ICP-AES) using iCAP 6300 Duo, S, Thermo Scientific. The elements Cr, Co, Cd, V, Ni, As, Hg, Se and Sb were determined by ICP-MS (Agilent 7700). Multielement standard solutions Merck (Darmstadt, Germany), traceable to NIST, were used as calibrators for both methods.

All concentrations were presented as mg·kg^{-1} dry weight.

Statistical analysis

We performed principal component analysis (PCA) to synthetize our findings and see whether there are any correlations between the studied elements. **PCA** included into CANOCO (Ter Braak and Smilauer 2002) was used to study relationships between different elements in each studied site. The element concentrations were divided by their standard deviation and PCA was species-centered.

The map was produced using ArcMAP, part of ArcGIS, an integrated GIS.

Results and Discussion

Active monitoring

Background concentrations in *H. cupressiforme* exposed had similar levels to *H. cupressiforme* from natural area in Italy (Giordano et al. 2009) except for Al (5 times higher values), Fe and Co (3 times higher values) and Mn (2 times higher values) in the Rhodope Mountains.

Macroelements K, Ca, Mg, Mn and P, as well as Hg did not vary between background sample and exposed moss bags. Al, Co and Fe had no significant accumulation during the 3 exposition periods in all studied cities. Cr and Ni increased twice at the end of the experiment in Plovdiv and Luki, as well as Mn and As in moss bags from Luki, and Cu in Plovdiv.

Cd and Pb were the metals with strong accumulation in moss-bags at all locations with maximum measured in Sofia (Table 2). Cd reached its maximum on the

Table 2. Descriptive statistics of element concentrations (mg·kg^{-1}).

Element	Active biomonitoring (n = 10)				Passive biomonitoring (n = 5)		
	background	min	max	median	min	max	median
Na	60.1	53.3	127.7	71.3	156.5	201.5	183.0
K	5144.3	5144.3	6154.4	5756.7	1892.5	2833.3	2258.2
Mg	1827.4	1817.6	2718.8	1939.2	1160.5	2024.5	1552.0
Ca	7772.6	7600.4	12 298.7	7910.7	7002.8	11 034.2	8652.5
Mn	154.1	134.7	242.8	191.2	59.4	180.7	80.3
Fe	2298.5	2091.9	4600.9	2506.7	1317.4	2941.8	1498.7
Al	3300.2	2982.3	5588.0	3468.2	1242.4	2768.8	1469.4
P	1789.6	1714.1	1947.3	1824.8	602.4	1139.1	917.2
Cr	4.3	4.0	11.1	4.7	12.3	29.7	20.8
Co	1.0	0.9	2.1	1.1	6.1	6.4	n.a.
Ni	2.8	2.6	7.2	3.1	11.1	25.7	15.2
Cu	6.7	6.4	15.4	6.8	3.3	8.7	4.3
Zn	24.8	24.8	101.5	28.5	20.1	97.8	49.4
As	0.7	0.6	1.1	0.7	1.3	26.8	4.8
Cd	0.3	0.3	4.2	1.8	1.4	3.4	2.3
Hg	0.045	0.045	0.045	0.045	3.0	22.3	9.2
Pb	5.5	5.5	29.8	8.8	31.8	93.9	48.6

30th day of exposition, while Pb after the first increase at the 15th day, recorded second increase at the end of the experiment (75th day). Maximum levels of Cd and Pb in two of the largest Bulgarian cities exceeded the thresholds for medium pollution: 0.8 and 12 mg·kg^{-1} (Ștefănuț et al. 2019). These exceedances reflected that the air pollution as a consequence of urban activities (mainly road transport) is more severe than in settlements affected by mining activities.

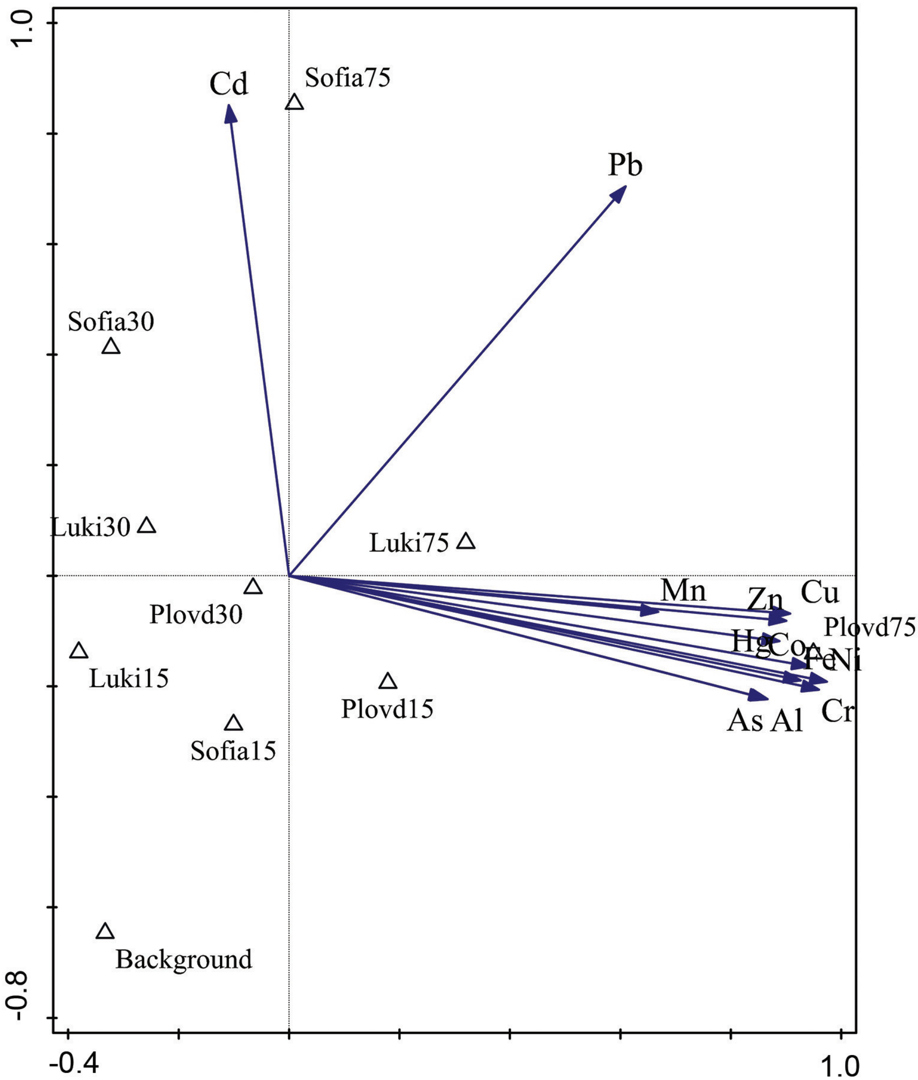
Passive monitoring

Medians of K, Mn, Al and P concentrations were more than 2 times lower in samples from passive monitoring in comparison with levels in moss-bags, while Na followed the opposite model (Table 2). Lower Na content could be a result of low retention capacity in moss. Both passive and active samples' medians of the above elements were lower than medians reported for 97 locations on Bulgarian territory (Marinova et al. 2010). This finding reflected specific regional values of these elements.

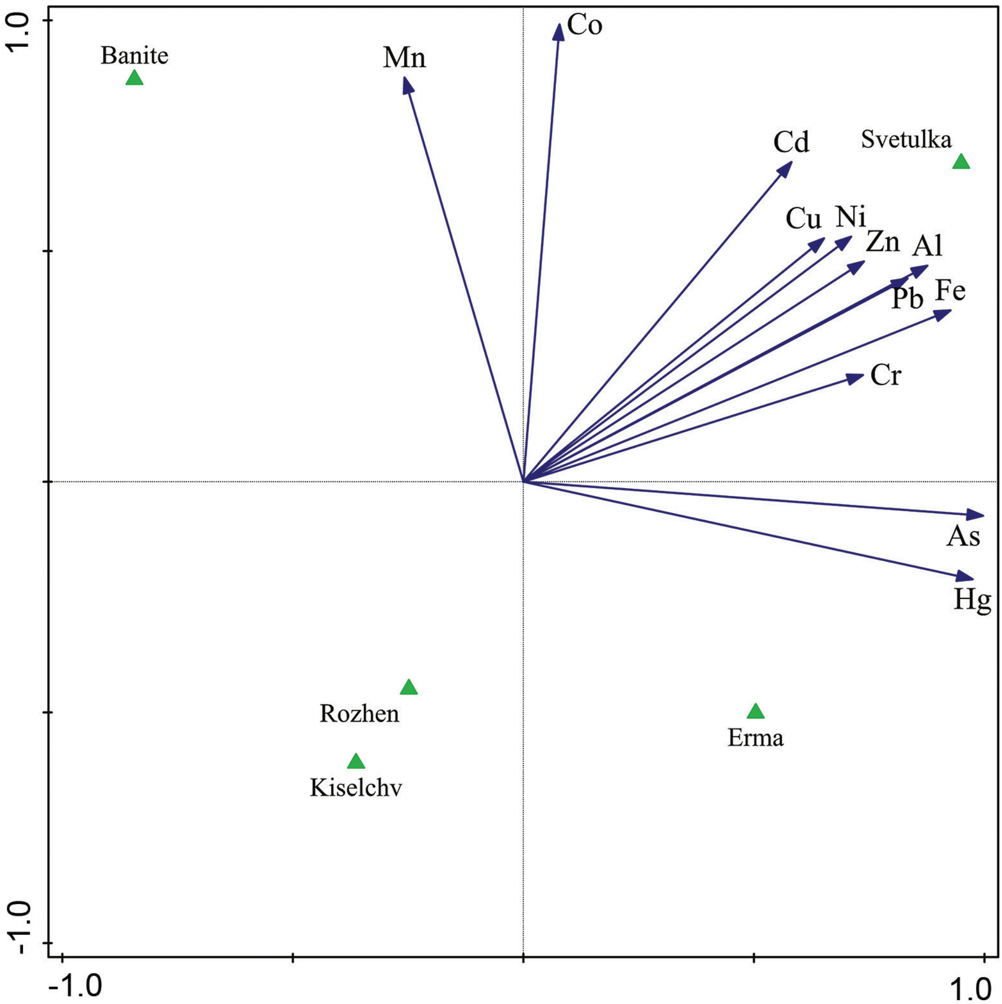
Mercury varied between 3 and 22.3 mg·kg^{-1}, Pb ranged between 31.8 and 93.9 mg·kg^{-1} in samples from Western Rhodopes. Maximum concentrations of Hg and Pb were 113 and 5 times (respectively) higher than their maximum levels in Germany during 2015 (Schröder and Nickel 2019). Maximum levels of As were higher than in other countries participating in EMS, except Serbia – 71.1 mg·kg^{-1} (Frontasyeva et al. 2020). The maximum concentrations of Zn were almost 6 times lower than registered in the Czech Republic (578 mg·kg^{-1}), and 10 times lower for Cr in comparison the Russian Federation – 301 mg·kg^{-1} (Frontasyeva et al. 2020). Although the Rhodope Mountains are essential in terms of water supply balance and possess high biodiversity and great potential for tourism (Sabev and Yordanov

2014), this study confirmed them as a region with constantly increased toxic element levels due to the mining activities.

PCA of analysed elements in moss-bags showed that the first axis (eigenvalue 0.589) correlated with all heavy metals and toxic elements except Cd (Fig. 2a). Cd accumulation in moss-bags from Sofia contained 4- and 3-times higher concentrations during the second and third exposition period. Pb had also maximum in moss-bags from Sofia at 75th day. Moss-bags from Plovdiv at the 75th day (right bottom) contained accumulated maximum of most of the trace elements. The observed maximum values at the end of the experimental period suggested that element saturation level had not been



a



b

Fig. 2. PCA-ordination diagrams of analysed elements and studied sites: a – active and b – passive monitoring.

reached.

First axis (eigenvalue 0.991) of the ordination diagram from passive monitoring correlated with most of the elements, except Mn and Co (Fig. 2b). Among the studied sites, moss from Svetulka village revealed highest levels of 11 elements (upper right of the plot), probably due to the serpentinites covering the area. Three locations (Banite, Rozhen and Kiselchovo) were with minimum accumulated levels (left part of the diagram).

Conclusions

Despite natural and anthropogenic specific characteristics of the selected 3 cities, the accumulation of 11 heavy metals and toxic elements increased at the end of the bag exposure period (except for Hg). Highest number of maximums was registered in moss-bags from Plovdiv (Fe, Al, Cr, Co, Ni, Zn, Cu) probably due to the high number of days with temperature inversion and low wind speed. Pb exceedances of the thresholds for medium pollution in Sofia and Plovdiv, as well as Cd exceedances in the 3 cities, reflected the strong negative transport effect. Based on the results, 4-week of exposure is recommended for rapid assessment of pollution in an urban environment, while 10-week is suggested for detailed monitoring.

We registered high levels of some heavy metals (Pb, Hg) in native mosses due to old and open mines, appeared local emissions, and site-specific characteristics.

This study confirms that both active and passive moss monitoring is suitable for detecting temporal and spatial trends in elements deposition.

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