RESEARCH ARTICLE

Atmospheric pollution assessment with mosses in Western Rhodopes, Bulgaria

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ABSTRACT
The moss analysis technique was applied to monitor 10 heavy metals and toxic elements deposition. Our study was the first attempt to assess spatial patterns in a border mountain region (area 8732 km^2) with a low population density and high proportion of protected territories. The obtained results did not correlate to the results from areas with low air pollution and could be linked to the impact of old and open mines.

Key words: *Hypnum,* biomonitoring, heavy metals, Bulgaria

Introduction

Mosses were successfully applied for more than 30 years for assessing atmospheric pollution based on their ability to obtain nutrients from precipitation and dry deposition. Among the advantages of the carpet-forming mosses as biomonitors are: mineral supply mainly by wet and dry atmospheric deposition; fast uptake due to lack of epidermis and cuticle; high surface-to-volume ratio and thus high cation exchange capacity.

Trends in heavy metal accumulation in mosses have been monitored every five years in Europe (Yurukova, 2010).

We focused our attention on the region selected for the study because it comprised a large number of protected areas, unpopulated areas, and old and open mines. The above combination between pristine and affected sites on a small scale was selected as a pilot "background-impact" region for application of the moss biomonitoring technique. Thus the aims of this study were: (i) to assess concentrations of the main 10 heavy metals in the most distributed moss species *Hypnum cupressiforme* Hedw. in a border mountain region; (ii) to evaluate the bioaccumulation levels regarding last Bulgarian and European moss data.

Materials and Methods

Study area and sampling

The guidelines in the "Heavy metals, nitrogen and POPs in European mosses: 2015 survey", published by the UNECE ICP Vegetation were followed (Frontasyeva et al., 2014). Moss samples were collected during October – November 2014 in Western Rhodopes, Bulgaria. Mean altitude is 1098 m a.s.l.; the climate is mountain and Continental-Mediterranean in the lower parts. Population density is 28.5 persons per km^2. Protected territories cover about 11% of the territory of Western Rhodopes. Fifteen sites were chosen in the region with the aim to collect 1 moss sample per 600 km^2. Each sample consisted of up to 5 sub-samples after the standardized European methodology (Frontasyeva et al., 2014).

Hypnum cupressiforme Hedw. was collected at 14 sites from the Rhodopes network (8732 km^2), and *Homalothecium lutescens* (Hedw.) H.Rob. at one site. Current nomenclature was followed (Hill et al., 2006).

Analytical methods

The standard treatment procedure was followed: samples were air-dried, cleaned, and segregated on the basis of age. Moss samples were dried at 40°C and then 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. The elements Al, As, Cd, Cr, Cu, Fe, Ni, Pb, V and Zn were determined by ICP-OES (iCAP 6300 Duo, Thermo Scientific) and ICP-MS (Agilent 7700). A reported value for each sample is the average of three independently dissolved portions, each of which is measured with 5 repetitions. The content of As and Se in some of the tested moss samples was below the limits of quantitation (LOQ), which were: As 0.5 mg kg^{-1} and Se 0.6 mg kg^{-1}.

Statistical analysis

Principal component analysis (PCA) was used to study relationships between the different elements in each site (Ter Braak & Šmilauer, 2002). The element values were divided by their standard deviation and PCA was species-centered. The spatial trends were assessed and evaluated. The maps were produced using ArcMAP, part of ArcGIS, an integrated geographical information system (GIS).

Results and Discussion

The first data for the border mountain region – Western Rhodopes showed that measured maximum concentration of V was higher than assessed maximum levels in Bulgaria and Europe during the last European survey: 5 and 2 times, respectively (Table 1). Moreover, minimum values in the studied mining region were higher than the corresponding European moss tissues.

The major anthropogenic point sources of vanadium emissions are metallurgical work, followed by burning of crude or residual oil and coal. Vanadium concentrations were found to decrease in Bulgaria over the 3 measurement periods (1995-2005; Yurukova, 2010). Ten years later the assessed high V levels in the whole territory of the Western Rhodopes (Figure 1) probably reflected the influence of the old mines in the region, as well as coal combustion. The median for V in the studied region was 5 times higher with the V level in Norway (1.4 mg kg^{-1}), representing an area with low levels of

Table 1. *Concentrations of heavy metals and toxic elements in mosses sampled in Western Rhodopes, Bulgaria and Europe (* - after Yurukova et al. (2014); ** - modified moss data from 28 European countries after Harmens et al. (2008)).*

Element, mg kg^{-1}	Al	As	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Western Rhodopes 2014										
Number of sites	15	15	15	15	15	15	15	15	15	15
Min	1711	<0.5	0.08	1.5	3.8	399	0.8	4.3	1.4	14.4
Max	6008	3.4	1.92	10.5	11.3	3596	12	60.1	101	143
Mean	3349	2	0.53	3.3	6.2	1187	2.5	20.71	17.3	48
Median	2457	3.4	0.40	2.3	5.2	856	1.4	9.4	6	36.5
Bulgaria 2010-2011*										
Number of sites	129	60	129	129	129	129	129	129	129	129
Min	402	0.15	0.043	0.72	2.00	307	0.84	1.69	0.96	8.22
Max	8886	10.8	7.75	38.1	270	8546	82.1	333	22.4	286
Mean	1493	1.08	0.39	3.46	12.2	1534	4.37	16.8	3.96	30.6
Median	1245	0.63	0.21	2.06	7.01	1101	2.61	8.00	3.07	22.2
Europe 2010-2011**										
Number of sites	3707	3624	4350	4223	4170	4119	4171	4174	4137	4398
Min	25.0	0.02	0.003	0.11	0.23	27.0	0.14	0.27	0.11	0.6
Max	34400	51.1	24.0	293	672	29500	857	333	58.3	1440
Mean	1049	0.38	0.31	3.21	8.58	848	3.47	6.27	2.33	35.2
Median	534	0.2	0.15	1.45	5.67	416	1.57	3.11	1.37	30.8

air pollution (Harmens et al., 2015), while measured maximum value in Western Rhodopes was 100 times higher.

Maximum concentrations of the rest 9 studied metals were lower in comparison with European maximum levels: Ni 70 times, Cu 60 times, Cr 30 times, As 15 times, Cd 12 times, Zn 10 times, Fe 9 times, and Al 6 times. Maximum lead concentration during the last European survey was measured in Bulgaria (333mg kg^{-1}), but moss tissues from Western Rhodopes had more than 5 times lower lead maximum level.

Minimum values of Al, Cr, Cu, Fe, Pb and Zn measured in Western Rhodopes were higher than minimums for Europe. Moreover, medians for all elements except Ni were considerably higher in comparison with Bulgarian and European moss data.

In general, the results for the studied elements in the Rhodopes – low populated region covering several protected territories, did not correlate to the results from areas with low air pollution.

Principal component analysis (PCA) was carried out on data for 10 elements measured in moss tissues from 15 sites in Western Rhodopes, Bulgaria (Figure 2). The element values were divided by their standard deviation and PCA was species-centered. The sum of all eigenvalues was 1.000. The first axis explained 92.7% of the variation in the data set and was positively correlated with Al, Fe, V, Cr, Ni, As and Cu, while negative correlation was established for Zn and Pb. The second axis correlated with cadmium. Sites in the upper-right part of the ordination plot were over serpentines, while those located in the left of the diagram represented old uranium and open mines. The most pristine areas were separated in the bottom-right quadrant.

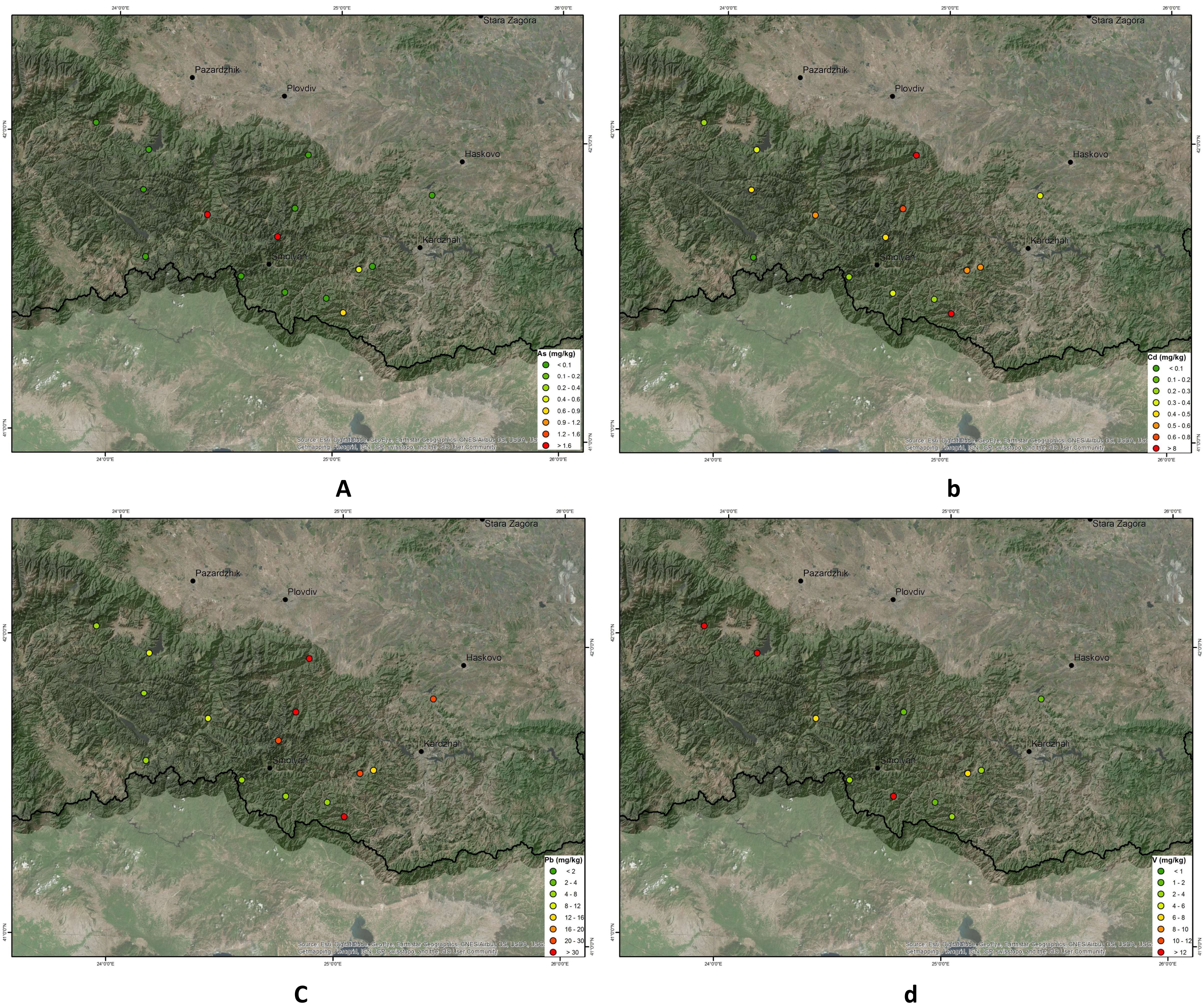

Figure 1. *Element concentration in mosses in Western Rhodopes, Bulgaria for a) arsenic (As), b) cadmium (Cd), c) lead (Pb) and d) vanadium (V).*

Conclusion

PCA classified the sites into 3 depositions types which correspond with each other. These types are characterized by: (1) old and open mines; (2) serpentines; (3) regions in greater altitudes, low population density and lower concentration in mosses.

Concentrations of the enriched elements from this study reflected the need for developing practices towards reducing the environmental impact of mining operations. Continuing the moss monitoring is crucial for future evaluations of the success of implemented environmental measures.

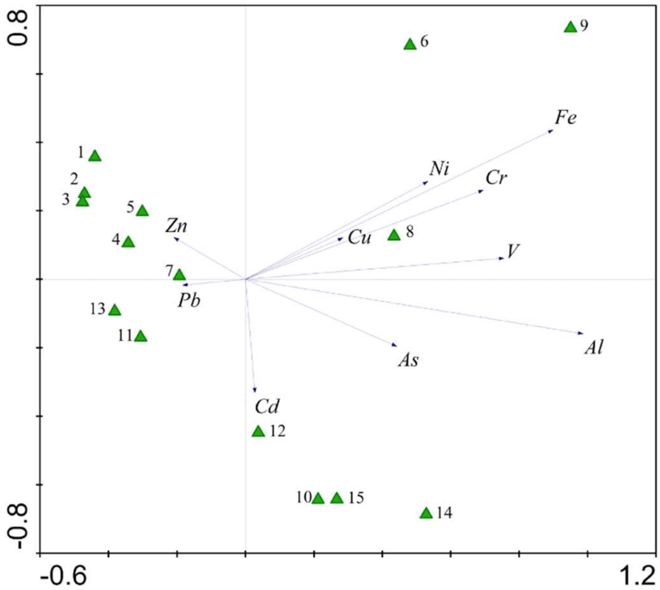

Figure 2. *PCA-ordination diagram of studied sites and 10 analyzed heavy metals and toxic elements.*

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