ECOLOGIA BALKANICA
2011, Vol. 3, Issue 1 July 2011 pp. 89-94

Bryophytes in Protected Territories of Plovdiv City (Bulgaria): Preliminary Species List and First Data of Air Pollution Monitoring

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Abstract. The study comprises preliminary results of survey on bryophyte diversity in protected areas in the city of Plovdiv and biomonitoring urban air pollution with moss *Hypnum cupressiforme*. The finding of 2 species with conservational value (*Isothecium myosuroides* Brid., *Grimmia crinita* Brid.) suggests that surveys of bryophyte biodiversity across protected landscapes should be continued. The determined concentrations of 15 elements in *Hypnum cupressiforme* revealed no serious pollution by airborne heavy metals and toxic elements contaminants. The applied biomonitor and PCA distinctly separated anthropogenic influenced city sites and reference one.

Keywords: Bryophytes, Biodiversity, Monitoring of air pollution, *Hypnum cupressiforme*

Introduction

First account of Bulgarian bryophyte flora had been accomplished by PETROV (1975). Approximately 754 species are registered up to now (NATCHEVA & GANEVA, 2009). The present Red List of Bulgarian Bryophytes includes 251 species, of which 228 are Threatened (28 Critically Endangered, 42 Endangered, and 158 Vulnerable) (NATCHEVA *et al.*, 2006). Researches on species composition in urban conditions in Bulgaria are limited to bryoflora of Sofia (GANEVA, 2004), due to science interests focusing on anthropogenic undisturbed regions. Bryophyte diversity conservation worldwide and particularly in Bulgaria is of great importance.

Bryophyte species composition could enlighten micro-habitat conditions (GLIME, 2007). Morphological and anatomical characteristics (i.e. lack of epidermis and cuticle or their reduction, single layered leafs, absence of roots, etc.) result in considerable tolerance against contamination. Mapping of atmospheric pollution on the basis of mosses sensitivity appeared to allow precise determination of air quality (ANISHCHENKO, 2009).

Moss monitoring of atmospheric pollution was applied first by RÜHLING & TYLER (1968). Carpet-forming moss species have a number of advantages as biomonitors: wide geographical distribution; mineral supply obtained mainly by wet and dry precipitation; ability to accumulate elements in concentrations higher than the medium; fast uptake due to the lack of epidermis and cuticle, and the large surface-to-weight ratio,

Union of Scientists in Bulgaria – Plovdiv
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alive tissues of 3-4 years old and evergreen; easy and cheap technique (GRODZIŃSKA & SZAREK-ŁUKASZEWSKA, 2001; RÜHLING & STEINNES, 1998; TYLER, 1990). Bulgaria was included in the project Atmospheric Heavy Metal Deposition in Europe using Mosses in 1995 (YURUKOVA, 2000). During the second moss sampling, the project was transferred in UN/ECE ICP Vegetation (United Nations Economic Commission for Europe International Co-operative Programme on Effects of Air Pollution on Natural Vegetation and Crops) - European Heavy Metals in Mosses (BUSE *et al*., 2003; YURUKOVA, 2006). The third one took place in 2005/2006 (YURUKOVA, 2007, 2010). Recently more than 28 countries were involved in the UNECE ICP Vegetation - Heavy Metal Accumulation in Mosses in Europe (HARMENS *et al*., 2007, 2008). According to the EMEP data (Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutant in Europe) Bulgaria is one of the main sources of heavy metals in the Southeastern part of Europe. The contribution to the total 2005 emissions (including anthropogenic, natural and historical) of Bulgarian manufacturing industries and constructions for Pb was 77% and 91% for Cd (ILYIN *et al*., 2005; 2007).

The city of Plovdiv comprises area of 10198 ha, located at Upper Thracian Lowland and is the only city in Bulgaria with protected areas (PAs) at urbanized environment. There are 4 PAs with total area of 84556 ha – Nature monuments "Youth Hill" (DzhendemTepe) – 285.5 m above sea level, "Hill of the Liberators" (Bunardzhik) – 265 m a. s. l., "Danov Hill" (Sahat Tepe) – 227 m a. s. l. and Protected site "A roosting site of Pygmy Cormorant - Plovdiv". The main factor influencing the vegetation on the hills, is the anthropogenic. In the past the hills had been directly and indirectly subjected to human impact which resulted in the loss of primary vegetation due to the large-scale invasion of ruderal and weed species.

The city's passenger transport system consists mainly of private automobiles, taxis and buses. As of 2008, there were approximately 163400 private motor vehicles in the city, about 1500 taxis and 400 buses. Virtually buses are diesel powered and most taxis are gasoline powered. In recent years, automobile use has increased rapidly. One of the major negative effects of Plovdiv's current transportation system is high level of air pollution throughout much of the year. Plovdiv city has been classified as a region in which levels of total dust, coarse particles (also known as particulate matter PM_{10}) and Cd are above established norms according REGULATION 7 (1999). Much of Plovdiv's pollution problem stems from the city's climate and topography. A thermal inversion acts as a cap over the city during the fall and winter, inhibiting the dispersion and diffusion of pollutants. Pollutant dispersion is further obstructed by the city's location in a river valley.

After the analysis of 221 different European cities in 2008, the city of Plovdiv ranked first in the classification of the 30 worst cities in terms of air quality (ITALIAN NATIONAL INSTITUTE OF STATISTICS, 2010). With 208 days in 2008, it also ranked first for the number of days on which the PM_{10} limit value of 50 $\mu g/m^3$ was exceeded.

The aim of this study was to present preliminary results on the bryophyte species composition and the atmospheric pollution represented by bioaccumulation in the moss *Hypnum cupressiforme*.

Material and Methods

Bryophytes were recorded and sampled during 2010 from various substrates (rocks, stones, dead wood, and soil) in the area of three Nature monuments. The nomenclature accepted in HILL *et al.* (2006) is followed. Categories of threat are according NATCHEVA *et al.* (2006). The region of Kazandji Dere, Central Rhodopes was chosen as reference site for control sample of the biomonitor *Hypnum cupressiforme*.

Samples of *H. cupressiforme* were air-dried, cleaned carefully and age separated. About 1 g moss material was treated with 5 ml 65% nitric acid (Merck) overnight. The wet-ashed procedure was followed by Microwave Digestion System CEM MDS 81D, duration 5 minute at 600 W. After 3 ml 30% hydrogen peroxide addition, vessels were cooled for 1 hour at room temperature. Vessels were treated by the Microwave Digestion System for 10 min again at 600 W for full digestion of the organic matter. The filtrate was diluted with double distilled water up to 50 ml.

The elements Pb, Cd, Co, Ni, As, Cr, Se, B, Sr, Be, V, Mo, Bi, U and Hg were determined by inductively coupled plasma mass spectrometry (ICP-MS) via Agilent 7700 ICP-MS, DF 1000. 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_3 (p.a.) Monostandard of Hg 100 ppt was also used in the calibration. Signals of suitable isotopes for the tested elements have been measured twice in both modes - without and with helium gas collision cell.

Data analyses using package Canoco ver. 4.5 were conducted (TER BRAAK & SMILAUER, 2002). Linear method Principal Component Analysis (PCA) was carried out on data on analyzed 15 elements in moss tissues at 3 sampling sites. Scaling was focused on sample distances. The data were transformed (x′ = log (x+1)), automatically centered and standardized.

Results and Discussion

The species list of studied urban protected areas in Plovdiv includes 7 mosses, 2 of which with conservational value (Table 1). These preliminary results compared to the total of 19 species registered in parks, gardens and meadows in Sofia (GANEVA, 2004), suggest high bryophyte diversity in the city of Plovdiv could be expected. Despite numerous pressures in urban environment, bryophyte flora in cities appeared to be rich. For example in the metropolitan city of Cologne (W. Germany) were accounted 143 taxa (SABOVLJEVIĆ & SABOVLJEVIĆ, 2009).

Table 1. List of registered species in the protected territories of the city of Plovdiv. Categories (according to IUCN, 2010): CR - *Critically Endangered*; LR - *Lower risk*/Not *threatened*; VU – Vulnerable.

Species	Locality	**Category of threat**
***Bryum dichotomum* Hedw.**	**Danov Hill**	LR
***Bryum moravicum* Podp.**	**Dzhambaz Hill***	LR
***Dicranella schreberiana* (Hedw.) Schimp.**	**Dzhambaz Hill**	LR
***Hypnum cupressiforme* Hedw.**	**Youth Hill, BunardzhikHill**	LR
***Isothecium myosuroides* Brid.**	**Danov Hill**	CR
***Grimmia crinita* Brid.**	**Youth Hill**	VU
***Tortula modica* R.H.Zander**	Dzhambaz Hill	LR

* part of the "Three Hills" (also known as "Trimontsium") comprising Teksim Hill, Dzhambaz Hill and Nebet Hill)

Among the above species *Isothecium myosuroides* showed poor or restricted growth during the maximum phase of SO_2 pollution (ADAMS & PRESTON, 1992). Species growth assessed in the monitored site could be related to the summer season without use of fossil fuels for heating.

Measured content of 15 elements in selected biomonitor are presented at Table 2. The results for five elements: Pb, Cd, Cr, V and Ni showed increased level of pollution at the NM "Youth Hill". The prevailing winds in the city of Plovdiv move in west-to-east direction and thus industrial contamination could be excluded. Moreover after the assessment of emissions dispersion from main industrial sources, the contribution of these sources to the air pollution was found to be negligible in comparison with the air pollution caused by the other sources (ATANASSOV *et al.*, 2006). The immediate proximity of railways could be assumed as a factor exerting greatest influence on the polluted site.

Table 2. Analyzed concentrations of 15 elements in moss *Hypnum cupressiforme*.

Index	Reference site		NM "Bunardzhik Hill"		NM "Youth Hill"	
	mg kg^{-1}	RSD %	mg kg^{-1}	RSD %	mg kg^{-1}	RSD %
Pb	4.03	0.5	9.12	0.7	21.6	0.6
Cd	0.35	0.9	1.26	1.1	2.5	1.4
Co	2.2	0.6	0.8	0.6	0.93	1.1
Ni	1.7	0.9	2.8	1.2	4.4	1.5
As	<0.5		0.57	12	0.52	12
Cr	1.9	2.6	1.7	2	3	1.5
Se	<0.6		<0.6		<0.6	
B	<10		<10		<10	
Sr	11	1.5	57	0.9	54	0.6
Be	<0.1		0.17	14	<0.1	
V	1.6	0.4	4	0.5	7.7	0.5
Mo	<0.4		<0.4		<0.4	
Bi	0.8		0.6		0.7	
U	0.05	3.8	0.19	0.7	0.64	1.2
Hg	0.08	13.5	0.17	10.5	0.13	11.6

Relationship between analyzed elements in *Hypnum cupressiforme* at selected sites was assessed by PCA (Fig. 1). The first axis with eigenvalue 0.722 explained 72.2% of data variation. First and second axes together explained 100% of the variation. The ordination plot represents a gradient between sites located at the city of Plovdiv and normally subject to anthropogenic pressure (left part of the plot) to locality at the reference region of the Central Rhodopes Mountain characterized by higher values only of Bi and Co. NM Bunardzhik Hill with elevated values of Be, Hg, As and Sr lied in the top-left quadrant of the diagram above more impacted NM Youth Hill represented by higher loads of 6 elements (Cr, U, Pb, Ni, V, Cd).

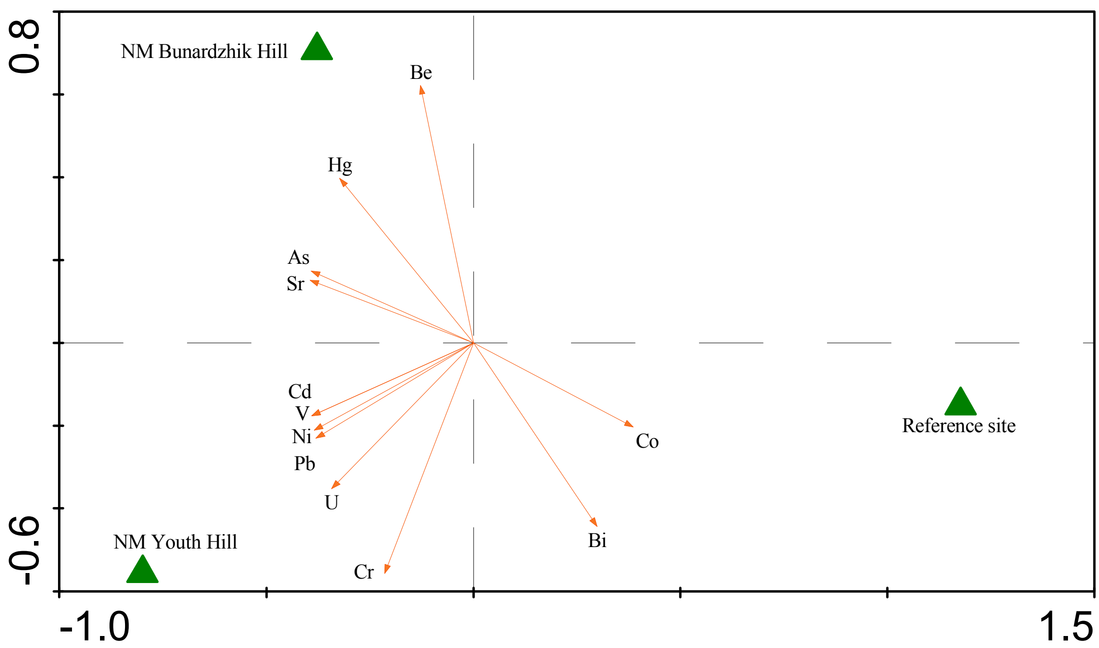

Fig. 1. PCA ordination diagram with data for reference and 2 city sites.

Maximum concentration of cadmium measured at NM "Youth Hill" was 2 times lower and close to the Bulgarian and European maximum of 5.23 mg kg^{-1} (YURUKOVA & GECHEVA, 2009). Arsenic had similar levels at the 2 protected territories which were about 20 times lower compared to the maximum concentration of 12.6 mg kg^{-1} for Bulgaria and approximately 40 times lower than European maximal value of 21.6

mg kg^{-1}. Lead showed average load at the selected sites, holding position between minimal and maximal values for the country. Levels of nickel and chromium in both city sites were close to the minimum concentrations assessed at Bulgaria and Europe. Mercury was approximately 2.5 times lower as compared to the maximum values analyzed in FYR of Macedonia.

Conclusion

According to our preliminary results, the list of moss species in protected territories of Plovdiv city could be characterized by high diversity in future detail studies.

Comparatively low content of assessed 15 elements in biomonitor *Hypnum cupressiforme* confirmed tendency towards diminishing air pollution in the city. Results of the PCA clearly opposed selected two city sites under anthropogenic pressure to reference region.

The bryomonitoring of total air pollutants certainly plays a role in moss protection and conservation.

Acknowledgements

We would like to thank to Dr. Veselin Kmetov, Head of the Analytical Chemistry and Computer Chemistry Department, University of Plovdiv and the team of NSFB project GAMA DO 02-70 11/12/2008 for chemical analyses of bryophyte samples.

We are also thankful to Student's Council of Plovdiv University who supported financially a B.Sc. Thesis, part of which are the results discussed.

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Received: 31.01.2011
Accepted: 03.03.2011