ЮБИЛЕЙНА НАУЧНА КОНФЕРЕНЦИЯ ПО ЕКОЛОГИЯ (СБОРНИК С ДОКЛАДИ)
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TRANSBOUNDARY AIRBORNE DEPOSITION ESTIMATED BY THE MOSS *Hypnum cupressiforme* Hedw. IN SOUTHEASTERN BULGARIA – EUROPEAN TURKEY

Lilyana D. Yurukova[1], Mahmut Coşkun[2], Gana M. Gecheva[3], A. Çayir[2], Münevver Coşkun[2]

[1]Institute of Botany, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria
E-mail: yur7lild@bio.bas.bg
[2]Chanakkale Onsekiz Mart University, Chanakkale 17100, Turkey
[3]University of Plovdiv, Faculty of Biology, Plovdiv 4000, Bulgaria
E-mail: ggecheva@uni-plovdiv.bg

Abstract. This study aimed at first cross-border mapping of 10 heavy metals and toxic elements accumulation in moss *Hypnum cupressiforme* reflecting wet and dry atmospheric deposition in Southeastern Bulgaria and European Turkey. It is a part of the European moss survey 2005/2006. Data obtained from 114 sites, covering 35,736 km^2, showed different deposition patterns particularly for As, Cu, Cr and Ni. The determined concentrations (ICP–AES), revealed no heavily pollution by airborne heavy metals and toxic elements. The elements Pb, Cr, Cu, Zn and Al were found with proved statistical significant difference, between Bulgarian and Turkish part of the study area. The significant transboundary pollution was not proved.

Key words: Atmospheric deposition, *Hypnum cupressiforme*, heavy metals, toxic elements.

INTRODUCTION

Moss monitoring technique used for atmospheric assessment was described by RÜHLING & TYLER (1968). For monitoring heavy metal airborne pollution, moss species are especially suitable due to the high cation-exchange capacity (CLYMO, 1963). Carpet-forming moss species have a number of advantages as biomonitors: vast 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, 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). Second moss sampling was done

in the whole country in September-October 2000 and March 2001 during the project transfer as 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, 2005). The third one, as well as the first cross-border studies in Serbian, FYROM`s, and Greek territory took place in 2005/2006 (YURUKOVA, 2007). Parallel to these studies, there has started the joint project on atmospheric assessment of dry and wet deposition of 11 elements using mosses in the European part of Turkey and the southeastern part of Bulgaria. Recently more than 28 countries were involved in the UNECE ICP Vegetation - Heavy Metal Accumulation in Mosses in Europe (HARMENS *et al*., 2007, 2008). Bulgaria is one of the main sources of heavy metals in the Southeastern part of Europe; the official EMEP data (Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutant in Europe) for the total 2005 emissions (including anthropogenic, natural and historical) of Pb and Cd in Bulgaria were 115 and 12 t y^{-1} respectively (ILYIN *et al*., 2005; 2007). The contribution to the total Bulgarian emissions in 2005 was as follows: manufacturing industries and constructions - 77% for Pb and 91% for Cd. No such available data for Turkey, especially for the European part.

The aim of this study was to present atmospheric pollution during 3-years period (2003 - 2005), assessed by using mosses, in a part of Southeastern Europe (Southeastern Bulgaria and European Turkey). Additionally, to compare the data with summarized results of European mosses, and to test temporal changes from previous moss surveys. The first cross-border mapping of elements accumulation in mosses attempts to locate heavy metal emission sources and the extent of transboundary pollution.

MATERIALS AND METHODS

The study area is situated at Southeastern Bulgaria and European Turkey and covers 35,736 km^2 (Fig. 1). The area is mainly plain and hilly, the range of altitudes

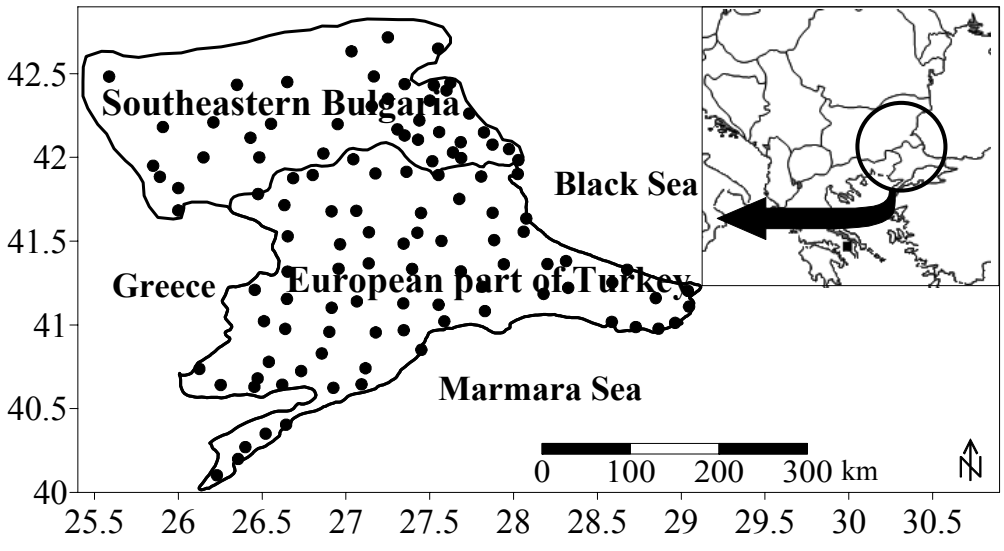

Fig. 1. *Location of the moss sampling sites in Southeastern Bulgaria and European Turkey.*

from sea level up to 1,031 m. Out of the entire territory 1,143 km^2 are covered with forests. The climate is temperate-continental and temperate-mediterranean, and the annual mean precipitation is around 590 mm. The moss sampling followed the requirements of the European moss surveys (BUSE *et al*., 2003; RÜHLING & STEINNES, 1998). The sampling net included 114 sites (40 in its Bulgarian part and 74 sites in its Turkish part) where the recommended pleurocarpous moss species in needed quantities could be found. The current paper comments on results of *Hypnum cupressiforme* Hedw. (nomenclature according to HILL *et al*., 2006) which is found to be the main moss species for Southeastern Europe (YURUKOVA, 2007) and is the only moss species widely quantitative distributed in the whole study area. The moss sampling was done in early spring (April 2006) before vascular plants started growth.

After the identification of the species, Bulgarian samples were air-dried, cleaned very carefully and age separated (3-years part). Moss samples were not washed, but homogenized by hands, using nylon gloves. They were stored deep frozen until further analytical treatment. Before analysis the samples were dried at 40^{o}C and then wet-ashed. About 1 g moss material was treated with 15 ml nitric acid (9.67 M) overnight. The wet-ashed procedure was continued with heating on a water bath, following by addition of 2 ml portions of hydrogen peroxide. This treatment was repeated till full digestion. The filtrate was diluted with double distilled water (0.06 μS cm^{-1}) up to 50 ml. All solutions were stored in plastic flasks. Duplicates of each sample were prepared independently.

The moss samples collected in the European part of Turkey were cleaned from residuals, soil and other litter in the laboratory. Green and yellow green parts of mosses were selected and then dried in oven at 40 ^{0}C till dry weight. The samples were homogenised using plastic mill and digested in microwave digestion unit (CEM Mars X-press). Sample of 0.5 g moss was digested with 10 ml 14.5 M HNO_3. After the digestion procedure, the filtered solution in a volumetric flask was completed up to 25 ml with deionised water (18 MΩ cm). Two parallel digestions were performed for each sampling site.

Elements Al, As, Cd, Cr, Cu, Fe, Ni, Pb, Sb, V and Zn were determined by atomic emission spectrometry with inductively coupled plasma (ICP–AES) using VARIAN VISTA-PRO instrument in the Bulgarian ICP lab and VARIAN LIBERTY SERIES II in the Turkish one. The detection limits were 0.004 mg L^{-1} for Cd, Cr, Cu, Ni and Zn, 0.02 mg L^{-1} for As, 0.03 mg L^{-1} for Pb and V, 0.04 mg L^{-1} for Al, Fe and Sb. The analytical precision was verified by replicating (deviation between the duplicates was below 5% in all cases) and by use of blanks and stock standard solutions (1000 μg L^{-1} Merck) for the preparation of working aqueous solutions. All concentrations are presented as mg kg^{-1} dry weight.

Quality control was checked by standard moss reference materials M2 and M3 analyzed in Bulgarian and Turkish laboratories. The measured concentrations were in good agreement with the recommended by STEINNES *et al*. (1997) values. Additionally 4 moss samples were collected and prepared for analysis in Bulgaria and respectively in Turkey, which were parallely analyzed in the both labs.

RESULTS AND DISCUSSION

Applied ANOVA analysis, using SPSS 10.0 software, for the parallel measurements of 2 moss reference materials M2 and M3, and 8 moss samples (4 Bulgarian and 4 Turkish) confirmed lack of statistically significant difference at the 99% confidence level between the means of all analyzed elements measured in Bulgarian and Turkish labs (P-values of the F-test are from 0.181 to 0.957 or greater than to 0.01).

The main results of 10 heavy metals (Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) and toxic elements (Al, As) of the first cross-border survey in the study area of Southeastern Europe are presented in Table 1. Antimony (Sb) values were below the detection limit (< 0.04 mg L^{-1}) in all moss samples.

Table 1. *The concentration of heavy metals and toxic elements in the mosses of Southeastern Bulgaria and European Turkey (mg kg^{-1} dry weight).*

Index	Al	As	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Number of Sites	114	89	111	114	114	114	114	114	114	114
Average	2567	2.82	0.31	4.87	7.56	1882	4.70	8.01	5.09	28.8
SD	2024	3.35	0.19	4.00	6.79	1213	3.51	6.81	3.04	15.8
Min	766	0.16	0.09	1.33	2.28	377	0.79	1.78	1.10	13.8
Max	11900	16.8	1.11	21.4	50.3	7013	20.5	48.7	18.2	126
Median	1956	1.35	0.28	3.63	5.45	1584	3.53	5.70	4.31	25.3
Number of Sites in EU*	4149	5001	5689	5581	5699	5566	5904	5621	5522	5930
Max in EU*	31180	21.6	5.23	90.7	672	23490	1016	249	68.5	694
Median in EU*	804	0.18	0.21	2.04	6.32	409	1.7	4.1	2.13	31.4

*Modified moss data of 28 European countries after HARMENS *et al*., 2008.

When the last median values of European mosses found during the Moss Survey 2005/2006 (HARMENS *et al*., 2008) and these results are compared, As, Fe, Al, Cr and Ni values are higher than median values of European data, 7, 4, 2, 2, 2 times respectively. However, the obtained maximum values of all concerned elements (Ni – 50-fold, Cu – 13-fold, Cd, Pb and Zn – 5-fold, Cr and V – 4-fold, Al and Fe – 3-fold) are lower than European maximum values. Taking into account that the prevailing part of European countries has developed economy the citied higher European maximum values probably have resulted from heavy industry facilities, i.e. the so called "hot spots".

Box plots for each element of all Bulgarian and Turkish results (BG+TR) and for each country, drown using Statistica 6 software, are presented in Figure 2. The boxes of the heavy metals Pb, Cu and toxic element Al were quite different for both countries. The one-way ANOVA proved statistically significant difference at the 99% confidence level for Pb, Cr, Cu, Zn and Al (P-values of the F-test are from 0.000 to 0.005 or lower than 0.01). However, no significant different between both countries was found for Ni, Fe and V at the 99% confidence level.

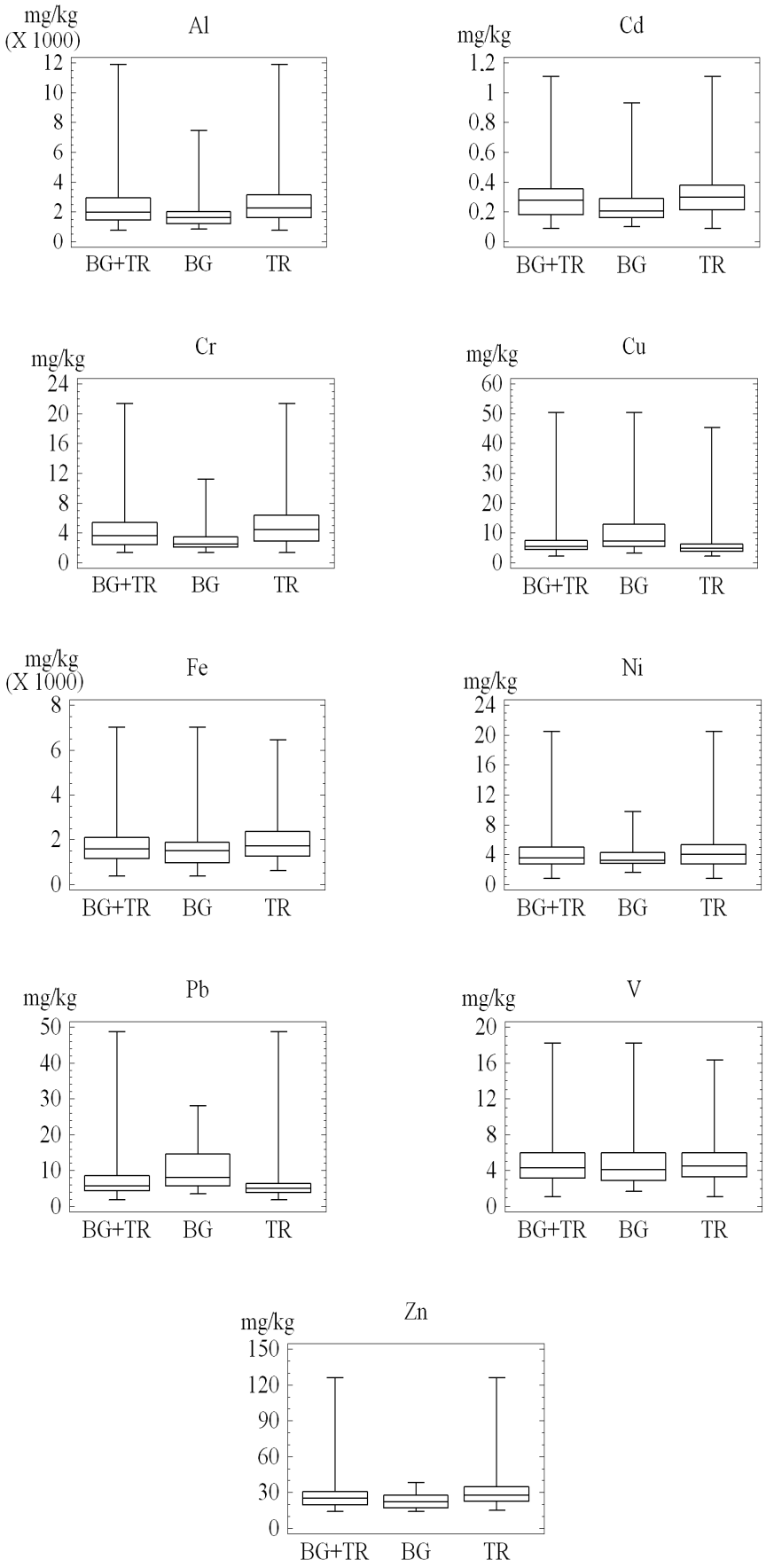

Fig. 2. *Box plots of heavy metals and toxic elements in mosses of Southeastern Europe.*

Distribution maps of heavy metals and toxic elements assessed by mosses in the studied part of Southeastern Europe are not presented in this publication. High concentrations of Pb and Cu at the vicinity of Istanbul and Burgas cities indicate heavy atmospheric pollution. Zinc contamination was obvious around Istanbul. Cadmium and vanadium distributions are very similar; both could originate from anthropogenic sources and/or soil dust. Their concentrations near to Istanbul and Burgas, large cities in the Southeastern Europe, have high values. Apart from Cu, Pb and Zn, the rest elements studied show similar distributions and have approximately low concentrations at the observed large cities regions for the researched period. An exception is copper polluted region south of Burgas due to ores and mines. Maximum values of As, Cd, Cr, Ni, Fe and Ni are obtained at the European Turkey. Atmospheric pollution with As could be pointed at the line Tekirdag - Edirne to the Bulgarian territory. Two large glass factories and intensively applied fertilizers in the surrounded agriculture area appear to be plausible reason for possible but not statistically proved transboundary transfer of As.

No statistical significant temporal trends of airborne heavy metals and toxic elements were found in mosses, during the last 3 moss surveys, reflecting the dry and wet atmospheric deposition (1993-1995, 1998-2000, 2003-2005) for the Bulgarian territory of the study area. The significant decreasing trend of the element content of As, Cu, V, Zn, Cd and Pb, in most of the European countries involved in the last moss surveys (HARMENS et al. 2007; 2008), was not proved in the Bulgarian part of study area due to local emissions, especially around big cities, contaminated soils around industry, and site-specific characteristics as geochemical anomalies. However, all analyzed elements in *Hypnum cupressiforme* showed a small decline in time, the bigger decline between 2000 and 2005. The comparison of previous Turkish (COŞKUN *et al.,* 2005) and present results for each element showed that the distribution pattern and median concentrations of Cu, Zn, Ni, and Pb and As were similar, but there were remarkable decrease of maximum value of Pb and As. Previous maximum value of Pb and As were 293 and 42 mg/kg respectively, whereas the corresponding values from the present study are 48 and 16.8 mg/kg - probably due to leaded gasoline banning since 2000 in Turkey. Decrease of As could be connected with unqualified coal prohibition during 5 last years.

In conclusion, during the period 2003-2005, the territory of almost 40,000 km^2 in the part of Southeastern Europe (Southeastern Bulgaria and European Turkey), was not seriously polluted by atmospheric heavy metal and toxic element contaminants, despite the fact that the area has more than 12 million inhabitants, oil refinery, nonferrous complex, 13 heavy industry factories, 1,474 other factories, 9 coal and 1 natural gas power plants, 11 polymetal mine works, 6 incineration plants and 160 cars per 1,000 inhabitants. Data obtained with mosses, mainly *Hypnum cupressiforme*, used for the assessment of atmospheric dry and wet deposition in the study area, proved insignificant cross-border pollution.

REFERENCES

BUSE A., D. NORRIS, H. HARMENS, P. BÜKER, T. ASHENDEN, G. MILLS (Eds). 2003. Heavy Metals in European Mosses: 2000/2001 Survey. UK, CEH, Bangor, 45 p.

CLYMO R.S. 1963. Ion exchange in *Sphagnum* and its relation to bog ecology. – Ann Bot NS, 27: 309-324.

COŞKUN M., M.V. FRONTASYEVA, E. STTEINNES, A.Y. ÇOTUK, S.S. PAVLOV, M. COŞKUN, A.S. SAZONOV, A. ÇAYIR, M. BELIVERMIS. 2005. Atmospheric deposition of heavy metals in Thrace studied by analysis of moss (*Hypnum cupressiforme*). - Bull. Environ. Contam. Toxicol., 74, 1, 201-209.

GRODZIŃSKA K., G. SZAREK-ŁUKASZEWSKA. 2001. Response of mosses to the heavy metal deposition in Poland – an overview. – Environ. Pollut., 114: 443-451.

HARMENS H., D.A. NORRIS, G.R. KOERBER, A. BUSE, E. STEINNES, Å. RÜHLING. 2007. Temporal trends in the concentration of arsenic, chromium, copper, iron, nickel, vanadium and zinc in mosses across Europe between 1990 and 2000. - Atmos. Environ., 41: 6673–6687.

HARMENS H., D.A. NORRIS, G.R. KOERBER, A. BUSE, E. STEINNES, Å. RÜHLING. 2008a. Temporal trends (1990-2000) in the concentration of cadmium, lead and mercury in mosses across Europe. – Environ. Pollut., 151: 368-376.

HARMENS H., D. NORRIS, H.G. ZECHMEISTER, Y. ALEKSIAYENAK, L. TEMMERMAN, L. YURUKOVA, Z. SPIRIC, etc. 2008b. Spatial and Temporal Trends in Heavy Metal Accumulation in Mosses in Europe (1990-2005). United Kingdom, CEH, Bangor, 51 p.

HILL M.O., N. BELL, M.A. BRUGGEMAN-NANNENGA, M. BRUGUÉS, M.J. CANO, J. ENROTH, K.I. FLATBERG, J-P. FRAHM, M.T. GALLEGO, R. GARILLETI, J. GUERRA, L. HEDENÄS, D.T. HOLYOAK, J. HYVÖNEN, M.S. IGNATOV, F. LARA, V. MAZIMPAKA, J. MUÑOZ, L. SÖDERSTRÖM. 2006. An annotated checklist of the mosses of Europe and Macaronesia. - Bryological Monograph. J. Bryol., 28, 3: 198-267.

ILYIN I., O. TRAVNIKOV, W. AAS. 2005. Heavy metals: transboundary pollution of the environment. EMEP/MSC-E Status Report 2/2005, 58 p. (http://www.msceast.org)

ILYIN I., O. ROZOVSKAYA, O. TRAVNIKOV, W. AAS. 2007. Heavy metals: transboundary pollution of the environment. EMEP/MSC-E Status Report 2/2007, 85 p. (http://www.msceast.org)

RÜHLING Å., E. STEINNES. (Eds). 1998. Atmospheric heavy metal deposition in Europe 1995-1996. - Nord 15: 1-66.

RÜHLING Å., G. TYLER. 1968. An ecological approach to the lead problem. – Bot. Notiser, 121:,321-342.

STEINNES E., Å. RÜHLING, H. LIPPO, A. MÄKINEN. 1997. Reference materials for large- scale metal deposition survey. – Accred. Qual. Assur. 2: 243-249.

TYLER G. 1990. Bryophytes and heavy metals: a literature review. Bot. J. Linn. Soc., 104: 231-253.

YURUKOVA L. 2000. First Bulgarian Data in European Atmospheric Heavy Metals Bryomonitoring. ISBN 954-9746-03-8.

YURUKOVA L. 2005. The second Bulgarian Data of the European Bryomonitoring of Heavy Metals. ISBN 954-9746-08-9.

YURUKOVA L. 2007. Bulgarian experience during the last 3 EU moss surveys. – In: Proceedings of the 7th Subregional Meeting on Effect-Oriented Activities in the Countries of Eastern and South-Eastern Europe, September 28-October 1, 2006. Romania, Risoprint, Baie Mare, pp. 157-164.

АТМОСФЕРНО ОТЛАГАНЕ НА ТЕЖКИ МЕТАЛИ И ТОКСИЧНИ ЕЛЕМЕНТИ В ЧАСТ ОТ ЮГОИЗТОЧНА ЕВРОПА, ОЦЕНЕНО С МЪХА *Hypnum cupressiforme*

Лиляна Д. Юрукова[1], Махмут Джошкун[2], Гана М. Гечева[3], А. Чаир[2], Мюнивер Джошкун[2]

[1] Институт по ботаника, Българска Академия на Науките, ул.„ Акад. Г. Бончев“, бл. 23, София 1113, България, E-mail: yur7lild@bio.bas.bg
[2] Университет Онсекиз Март, Чанаккале 17100, Турция
[3] ПУ „П. Хилендарски“, Биологичен Факултет, Пловдив 4000, България

(Резюме)

Настоящото проучване е с цел първо трансгранично картиране на акумулацията на 10 тежки метали и токсични елементи в мъха *Hypnum cupressiforme*, отразяващо мокрото и сухото атмосферно отлагане в Югоизточна България и Европейската част на Турция. Изследването е част от Европейския проект за проучвания с мъхове 2005/2006. Данните, получени за 114 пункта, покриващи 35736 km^2 показват различни модели на разпределение на отлагането, особено за As, Cu, Cr и Ni. Анализираните концентрации (ICP–AES) не показват сериозно замърсяване на атмосферата с тежки метали и токсични елементи. Елементите Pb, Cr, Cu, Zn и Al са с доказани статистически разлики между българската и турската територия, които са обект изследване. Не е доказано статистически значимо трансгранично замърсяване.