Bull Environ Contam Toxicol (2009) 83:174–179
DOI 10.1007/s00128-008-9601-8

Cross-Border Response of Moss, *Hypnum cupressiforme* Hedw., to Atmospheric Deposition in Southern Bulgaria and Northeastern Greece

Lilyana Yurukova · Evdoxia Tsakiri · Akin Çayir

Received: 29 May 2008 / Accepted: 17 October 2008 / Published online: 5 December 2008

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 Southern Bulgaria and Northeastern Greece. It is a part of the European moss survey 2005/2006 including first Greek results. Data obtained from 66 sites, covering 20,000 km^2, showed different deposition patterns particularly for As, Cd, Cr and Pb. The determined concentrations (ICP–AES), revealed no serious pollution by airborne heavy metals and toxic elements contaminants. Lead was the only element with proved statistical significant difference, between Bulgarian and Greek part of the study area.

Keywords Atmospheric deposition · *Hypnum cupressiforme* · Heavy metals

Moss technique used for atmospheric assessment was first described by Rühling and 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 (Grodzinska and Szarek-Lukaszewska 2001; Rühling and Steinnes 1998; Tyler 1990). Bulgaria was included in the project Atmospheric Heavy Metal Deposition in Europe using Mosses in 1994 (Rühling and Steinnes 1998; Yurukova 2007). Recently more than 28 countries were involved in the UNECE ICP Vegetation (United Nations Economic Commission for Europe International Co-operative Programme on Effects of Air Pollution on Natural Vegetation and Crops) - Heavy Metals in European Mosses (Buse et al. 2003; Harmens et al. 2007). 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). No official data are available for Greek total emissions.

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 (Southern Bulgaria and Northeastern Greece). Additionally, to compare the data with summarized results of European mosses, and to test temporal changes from previous periods as regards the Bulgarian territory. The first cross-border mapping of elements accumulation in mosses, as well as first data for Greece, attempts to locate heavy metal emission sources and the extent of transboundary pollution.

L. Yurukova (✉)
Institute of Botany, Bulgarian Academy of Sciences,
Acad. G. Bonchev Str., Bl. 23, Sofia 1113, Bulgaria
e-mail: yur7lild@bio.bas.bg

E. Tsakiri
Laboratory of Systematic Botany and Phytogeography,
Department of Botany, School of Biology, Aristotle University
of Thessaloniki, Thessaloniki 541 24, Greece

A. Çayir
Health Services Vocational College, Onsekiz Mart University,
17100 Chanakkale, Turkey

Materials and Methods

The study area is situated at Southern Bulgaria and Northeastern Greece (Fig. 1). Details for the geographical and economical characteristics of the area are presented in Table 1. The moss sampling followed the requirements of the European moss surveys (Buse et al. 2003; Rühling and Steinnes 1998). The sampling net included 66 sites (39 in Bulgaria and 27 in Greece) where the recommended pleurocarpous moss species in needed quantities could be found: *Hylocomium splendens* (Hedw.) Schimp., *Hypnum cupressiforme* Hedw., *Pleurozium schreberi* (Willd. ex Brid.) Mitt., *Rhytidium rugosum* (Hedw.) Kindb., *Pseudoscleropodium purum* (Hedw.) M.Fleisch., *Abietinella abietina* (Hedw.) M.Fleisch. (nomenclature according to Hill et al. 2006) (Fig. 1). Sampling was done in the autumn (October 2005), just at the end of the vascular plants growing season.

After the identification of the species, 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°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 (18 MΩ cm) to 50 mL. All solutions were stored in plastic flasks. Triplicates of each sample were prepared independently. 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. 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. Quality control was checked by standard moss reference materials M2 and M3. The measured concentrations were in good agreement with the recommended by Steinnes et al. (1997) values (Table 2). All concentrations are presented as mg kg^{-1} dry weight. For the statistical analysis levels of the investigated elements in mosses were compared between the two parts of the study area applying one-way ANOVA using SPSS 10. The single measurement for each part (Bulgarian and Greek) was included as the dependent variable. Principal component analyses with Rotated Component Matrix was used for data observed in the study area. ANOVA was applied to each element from the 3 moss surveys in Bulgarian territory for statistical assessment of temporal trends.

Results and Discussion

In this study are presented the results only of *Hypnum cupressiforme*, due to the fact that it is the main species proposed for moss surveys in Southeastern Europe (Yurukova 2007), and is the only moss species widely quantitative distributed in the study area. The analysed moss species was growing on soil (30 sites), on rock (26 sites) and on dead wood (10 sites).

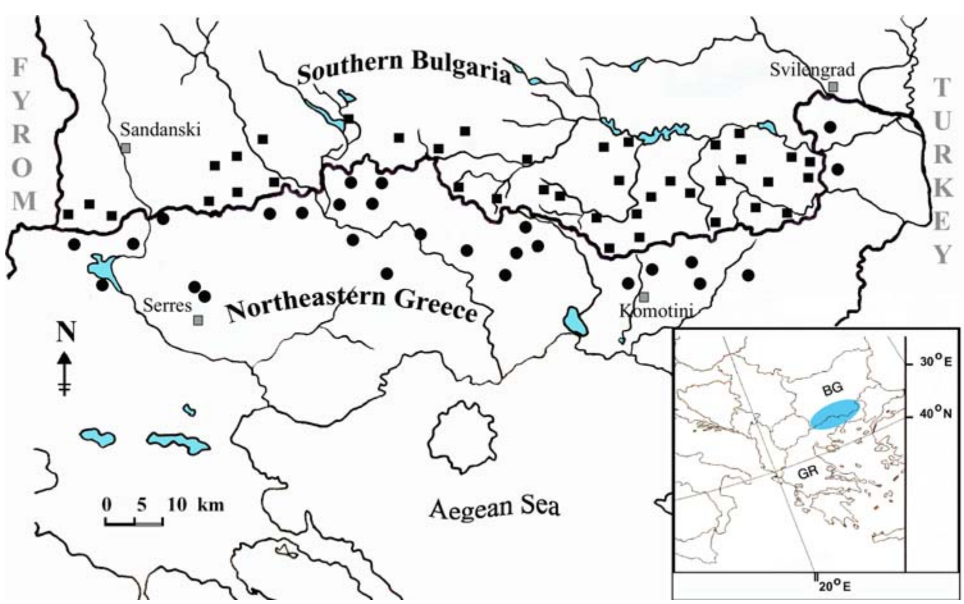

Fig. 1 Moss sampling net in 66 sites along the study area (Southern Bulgaria and Northeastern Greece)

Table 1 Geographical and economical data of the study area (Southern Bulgaria and Northeastern Greece)

Index	Bulgarian part	Greek part
Geographical coordinates	41°14′ N–42°00′ N 22°44′ E–26°10′ E	41°10′ N–41°40′ N 23°00′ E–26°15′ E
N–S the longest direction	30 km	25 km
W–E the longest direction	140 km	140 km
Area	8,400 km^2	11,596 km^2
Settlements	769	266
Agriculture area	2,220 km^2	3,200 km^2
Mountains (forest area)	7:5,179 km^2	7:6,491 km^2
Altitude – ranges; mean	74 – 2,212 m; 744 m	15 – 2,212 m; 119 m
Climate type	Temperately-continental, Continental-Mediterranean	Continental-Mediterranean
Annual precipitation	500–1,000 mm	500–1,000 mm
Number of rivers	7	3
Population	580,905	666,929
Population density	0–60 inhabitants per km^2	0–43 inhabitants per km^2
Urban population	271,078 (46.66%)	364,460 (54.65%)
Gross Domestic Product (GDP)		
1 – Agriculture (% of GDP)	14.5%	19.0%
2 – Industry and trade (% of GDP)	27.7%	22.5%
3 – Services (% of GDP)	57.8%	58.5%
Heavy industry	Lead-zinc smelter	None
Number of factories of heavy industry	12	3
Other industry (food, sugar, textile)	231 factories	194 factories
Power plants; fuel	None	1; natural gas and oil
Mine works	6 mines and 4 old U mines	11 old mines
Incineration plants	1	None
Cars per 1000 inhabitants	264	258
Dams (flood control)	4	5
Environmental protection of the region	15 NATURA 2000 sites. According to the international protection lows: 9 international bird areas. According to the Bulgarian environmental legislation: 3 reserves, 4 managed reserves, 3 protected areas	6 NATURA 2000 sites. According to the international protection lows: 3 biogenetic reserves, 2 international bird areas. According to the Greek environmental legislation: 3 natural monuments and landmarks, 3 game refuges

Table 2 Analytical results of Bulgarian certified laboratory of two moss reference materials, M2 and M3, prepared in Finland for the European moss surveys (average ± SD, mg kg^{-1} dry weight) and comparison with recommended values published by Steinnes et al. (1997)

Reference material	Al	As	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
M2	163 ± 9	0.82 ± 0.08	0.39 ± 0.01	0.78 ± 0.15	56.3 ± 1.0	224 ± 17	13.5 ± 0.5	5.72 ± 0.25	1.22 ± 0.05	33.0 ± 2.2
M3	141 ± 13	0.01 ± 0.004	0.08 ± 0.01	0.61 ± 0.05	3.57 ± 0.11	122 ± 20	0.98 ± 0.15	2.55 ± 0.15	0.73 ± 0.15	20.9 ± 2.3
[a]M2	175	0.92	0.44	0.91	68.1	245	14.8	5.86	1.21	35.2
[b]M3	160	0.098	0.11	0.82	3.64	137	0.92	2.94	1.07	25.9

[a] M2, [b] M3: After Steinnes et al. (1997)

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 3. Antimon (Sb) values were below the detection limit (<0.04 mg L^{-1}) in all moss samples. Comparing the last published median values in European mosses (Buse et al. 2003) and the median concentrations of this study, was found that the Fe and Ni values in

Table 3 Concentrations of heavy metals and toxic elements in mosses sampled in Southern Bulgaria and Northeastern Greece (mg kg^{-1} dry weight)

Index	Al	As	Cd	Cr	Cu	Fe	Pb	Ni	V	Zn
Number of sites	66	66	66	66	66	66	66	66	66	66
Average	2380	0.71	0.45	5.86	7.87	2354	42.6	8.88	5.97	37.6
SD	1886	0.93	0.78	8.40	6.95	1995	150	21.0	6.69	63.6
Min	553	0.10	0.10	0.84	2.45	454	2.39	1.52	1.36	10.1
Max	10394	4.75	4.75	39.7	37.6	9493	878	114	36.8	390
Median	1879	0.38	0.25	3.10	5.77	1653	8.46	3.34	3.75	20.5
Max in Europe[a]		118	11.0	265	3140	52200	887	302	77	2940
Median in Europe[a]		0.45	0.27	2.80	7.20	888	8.00	2.40	3.70	38.0

[a] Modified moss data of 28 European countries after Buse et al. (2003)

Hypnum cupressiforme were higher up to 2 times, than median values for Europe. The Pb median concentration in the study area was almost equal to the European median value. The obtained maximum values of all concerned elements, except Pb, were lower than the European maximum values (Buse et al. 2003): Cu (80 times), As (25 times), Zn, Cr and Fe (5–8 times), Cd, Ni and V (2–3 times). The European maximum concentrations of Pb in mosses were observed in Bulgaria during the last two European moss surveys, 1995/1996 and 2000/2001 respectively (Buse et al. 2003; Rühling and Steinnes 1998; Yurukova 2007).

The higher concentrations of all the examined heavy metals and toxic elements were found exclusively in the moss tissues of *Hypnum cupressiforme* collected in Bulgarian territory. The highest concentration values of Cd (up to 4.75 mg kg^{-1}), Cr (up to 39.70 mg kg^{-1}), Fe (up to 9,493 mg kg^{-1}), Pb (up to 878 mg kg^{-1}), Ni (up to 114 mg kg^{-1}), As (up to 4.75 mg kg^{-1}) appeared in the region of polymetal mines and influence of Pb-Zn complex (around Kardzhali town, Bulgaria). In the Greek part of the study area, the higher concentrations were those of As, Cr, Fe, Ni and V, which resulted from old mines, soil contamination by windblown dust and motorway construction. Further future investigations are needed to monitor the higher As concentration (up to 3.31 mg kg^{-1}) close to the 'Kerkini Lake', a protected area of the European network of the 'Natura 2000' Project (Dafis et al. 1996).

In this study, the concentrations of heavy metals and toxic elements showed two different groups of element distribution patterns. In the first group are Pb, Cd, Cu and Zn, mainly originated from anthropogenic sources, whereas the second group consisted of Fe, V, Al, Cr, Ni and As. Distribution maps of Al, As, Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in moss samples of *Hypnum cupressiforme* from the Southeastern part of Europe are presented in Fig. 2. Two factors affected heavy metals concentration in moss samples of the study area: soil dust as dry deposition and respectively wet atmospheric pollution. The Principle Component Analyses proved two components (F1 and F2) of heavy metals and toxic elements.

Applied ANOVA confirmed lack of statistically significant difference at the 99% confidence level between the means of analyzed Al, As, Cd, Cr, Cu, Ni, V and Zn in *Hypnum cupressiforme* sampled in Bulgarian and Greek part of the study area (*p*-values of the F-test were from 0.035 for Cd up to 0.853 for Cr, or greater than to 0.01). Statistically significant difference at the 99% confidence level was proved only for Pb (*p*-value was below 0.01) in Bulgarian and Greek part of study area.

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 still heavy local emissions, contaminated soils around ferrous industry, and site-specific characteristics as serpentine spots. However, all analyzed elements in *Hypnum cupressiforme* showed a small decline in time, the bigger decline between 2000 and 2005.

The observed results of the accumulation in moss tissues of the study area, during this 3-years period (2003–2005), should reflect the decrease of the important airborne pollutants Pb and Cd. Ilyin et al. (2005, 2007) pointed out the Pb and Cd decrease of the anthropogenic sources emissions during 2003–2005, to around 50% for Pb, and 40% for Cd in both countries. For the Bulgarian part of the study area the significant decrease of accumulation in *Hypnum cupressiforme* was not expected due to heavily polluted soils and open ore mines in some sites. For the Greek territory the data obtained were first attempt for partly moss survey in the country.

In conclusion, during the period 2003–2005, the territory of almost 20,000 km^2 in the part Southeastern Europe

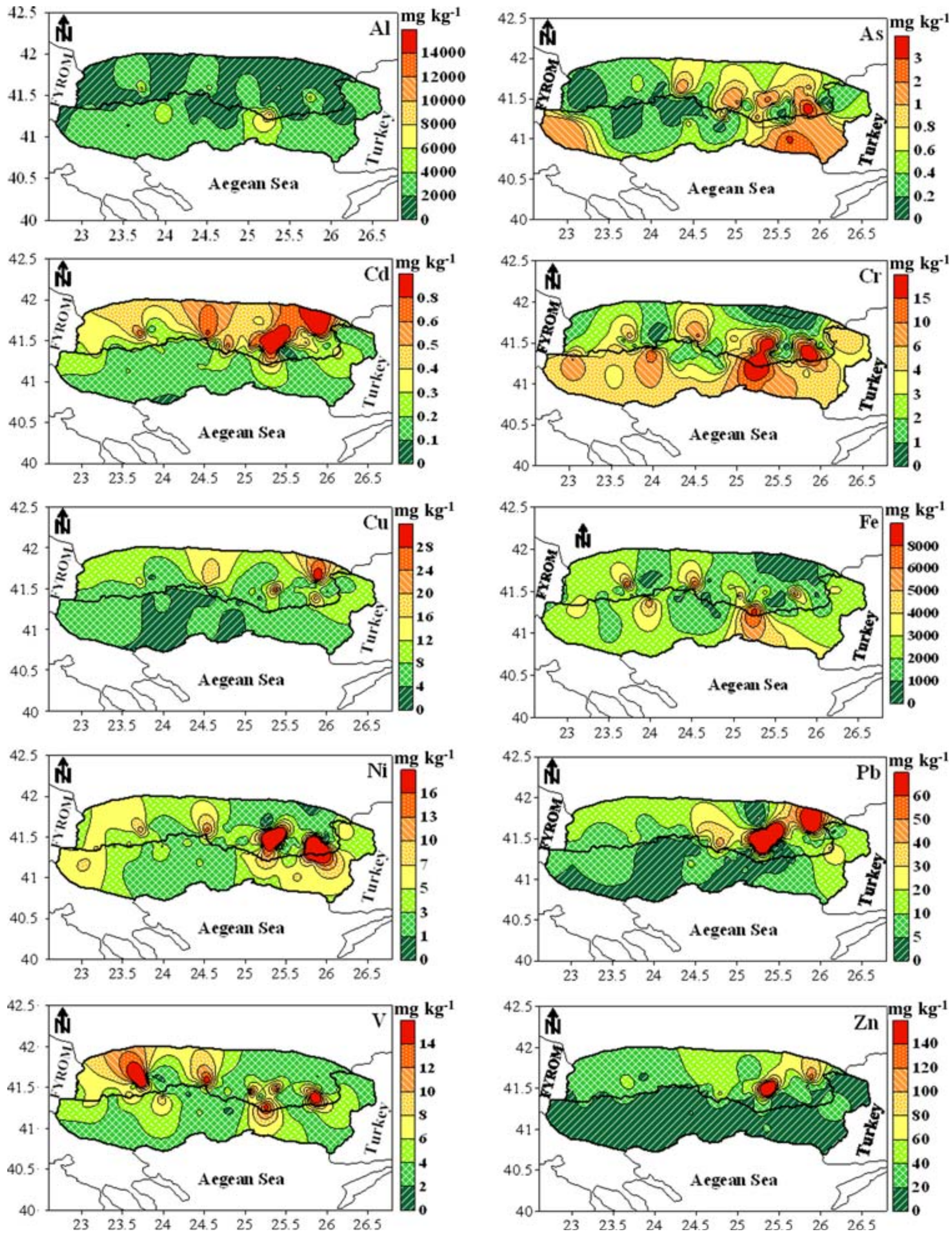

Fig. 2 Spatial distribution of heavy metal and toxic elements (mg kg^{-1}) in moss (*Hypnum cupressiforme*) in Southern Bulgaria and Northeastern Greece

(Southern Bulgaria and Northeastern Greece), was not seriously polluted by atmospheric heavy metal and toxic element contaminants, despite the fact that the area has more than 1 million inhabitants, nonferrous complex, 15 heavy industry factories, 425 other factories, 1 natural gas power plant, 21 polymetal works and old mines, 1 incineration plant and above 200 cars per 1,000 inhabitants. Data obtained of the moss *Hypnum cupressiforme*, used for the assessment of atmospheric dry and wet deposition in the study area, proved insignificant cross-border pollution.

Acknowledgements The authors are grateful to Dr. L. Thöni (FUB, Switzerland) for the partly financial support of the moss sampling in Bulgaria, and to Assoc. Prof. Dr. V. Karagiannakidou (Aristotle University, Greece) for her help on various subjects concerning the first moss sampling in Greece. We thank Mr. G. Derventzis for the transportation along the Greek sampling sites.

References

Buse A, Norris D, Harmens H, Büker P, Ashenden T, Mills G (eds) (2003) Heavy Metals in European Mosses: 2000/2001 Survey. CEH Bangor, UK, p 45

Clymo RS (1963) Ion exchange in *Sphagnum* and its relation to bog ecology. Ann Bot NS 27:309–324

Dafis S, Papastergiadou E, Georghiou K, Babalonas D, Georgiadis T, Papageorgiou M, Lazaridou T, Tsiaoussi V (1996) Directive 92/43/EEC The Greek "Habitat" Project NATURA 2000: an overview. Life Contract B4 -3200/94/756, Commission of the

European Communities DG XI, The Goulandris Natural History Museum, Greek Biotope/Wetland Centre, p 917
Grodzińska K, Szarek-Łukaszewska G (2001) Response of mosses to the heavy metal deposition in Poland – an overview. Environ Pollut 114:443–451. doi:10.1016/S0269-7491(00)00227-X
Harmens H, Norris DA, Koerber GR, Buse A, Steinnes E, Rühling Å (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. doi:10.1016/j.atmosenv.2007.03.062
Harmens H, Norris DA, Koerber GR, Buse A, Steinnes E, Rühling Å (2008) Temporal trends (1990–2000) in the concentration of cadmium, lead and mercury in mosses across Europe. Environ Pollut 151:368–376
Hill MO, Bell N, Bruggeman-Nannenga MA, Brugués M, Cano MJ, Enroth J, Flatberg KI, Frahm J-P, Gallego MT, Garilleti R, Guerra J, Hedenäs L, Holyoak DT, Hyvönen J, Ignatov MS, Lara F, Mazimpaka V, Muñoz J, Söderström L (2006) An annotated checklist of the mosses of Europe and Macaronesia. Bryological Monograph. J Bryol 3:198–267. doi:10.1179/174328206X119998
Ilyin I, Travnikov O, Aas W (2005) Heavy metals: transboundary pollution of the environment. EMEP/MSC-E Status Report 2/2005, p 58 (http://www.msceast.org)
Ilyin I, Rozovskaya O, Travnikov O, Aas W (2007) Heavy metals: transboundary pollution of the environment. EMEP/MSC-E Status Report 2/2007, p 85 (http://www.msceast.org)
Rühling A, Steinnes E (eds) (1998) Atmospheric heavy metal deposition in Europe 1995–1996. Nord 15:1–66
Rühling A, Tyler G (1968) An ecological approach to the lead problem. Bot Notiser 121:321–342
Steinnes E, Rühling Å, Lippo H, Mäkinen A (1997) Reference materials for large- scale metal deposition survey. Accred Qual Assur 2:243–249. doi:10.1007/s007690050141
Tyler G (1990) Bryophytes and heavy metals: a literature review. Bot J Linn Soc 104:231–253. doi:10.1111/j.1095-8339.1990.tb02220.x
Yurukova L (2007) Bulgarian experience during the last 3 EU moss surveys. Proceedings of the 7th Subregional Meeting on Effect-Oriented Activities in the Countries of Eastern and South-Eastern Europe, September 28–October 1, 2006. Baie Mare, Romania, Risoprint, pp. 157–164