Environ Monit Assess (2009) 157:529–537
DOI 10.1007/s10661-008-0553-6

Cross-border response of mosses to heavy metal atmospheric deposition in Southeastern Bulgaria and European Turkey

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Received: 3 April 2008 / Accepted: 11 September 2008 / Published online: 10 October 2008

Abstract First cross-border atmospheric pollution of 11 heavy metals and toxic elements assessed by *Hypnum cupressiforme* was reported for a part of Southeastern Europe (Southeastern Bulgaria and European Turkey). Moss monitoring technique followed the main requirements of European Moss Survey. Moss samples were collected in April 2006 both in Bulgaria and Turkey. Concentration of Al, As, Cd, Cr, Cu, Fe, Ni, Pb, Sb, V, and Zn were determined by ICP-AES. Interlaboratory parallel calibration (exchanged four moss samples from each country), standard reference moss materials (M2 and M3) results ensured the study. ANOVA showed no differences between measured results in both laboratories at the 99% confidence level. Principle Component Analyze proved two factors: F1 group of Al, As, Cd, Cr, Fe, Ni, and V and F2 of Cu, Pb, and Zn as main atmospheric pollutants. Results obtained showed approximately Cu and Pb high concentrations around Istanbul and Burgas and Zn pollution in Istanbul district. Arsenic cross-border atmospheric pollution in the study area of Southeastern Europe was found.

Keywords Biomonitoring · *Hypnum cupressiforme* · Atmospheric deposition · Cross-border pollution

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Introduction

For more than three now decades the possibility of observing the element status of our environment using living organisms instead of direct measurements of the emission into the ecosystems has been intensively discussed (Martin and Coughtrey 1982). Moss monitoring technique used for atmospheric assessment was described by Rühling and Tyler (1968). Moss species are especially suitable for monitoring of the atmospheric heavy metal pollution due to the high cation-exchange capacity (Steinnes 1980; Grodzińska et al. 1999; Gerdol et al. 2000; Lucaciu et al. 2004). The main mechanisms of metal uptake are: ion exchange, intracellular uptake and particulate entrapment (Clymo 1963; Tyler 1990). Mosses are utilized quite extensively in assessing atmospheric wet and dry deposition, and they have a number of advantages in their use as bioindicator organisms: (a) vast geographical distribution of many species and occurrence in natural, industrial, urban, and rural areas; (b) ability to accumulate elements in higher concentrations than the other higher plants; (c) lack of epidermis and cuticle (or reduction), large surface-to-weight ratio, one cell layer of the leaves, no organs for uptake in most carpet-forming species ensure only supply by wet and dry atmospheric deposition; (d) biomonitoring throughout the whole year—mosses are evergreen; (e) element content in some pleurocarpous mosses reflects an average exposure during period of 3–4 years period when living moss tissues are built up (Rühling 1994; Yurukova and Damianova 1995; Grodzińska and Szarek-Łukaszewska 2001).

Bulgaria was included in the project Atmospheric Heavy Metal Deposition in Europe by Mosses in 1994 (Rühling and Steinnes 1998; 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 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. Bulgarian analytical and certified ICP labs were included in the Intercalibration of moss and humus reference samples in 1995. The obtained results of anonymous six samples from ICP-AES method done in authorized/certified analytical laboratory were very close to the recommended values published by Steinnes et al. (1997) on the base of the results obtained in all participating Labs (29) from 20 European countries. Atmospheric air pollution in European Turkey using moss monitoring technique was studied for the first time in 2001 (Coşkun et al. 2005). Second sampling in European Turkey was conducted in April 2006 in the frame of European Moss Survey 2005/2006 (ICP Vegetation). Within the region of Southeastern Europe similar moss survey was done in FYROM in 2002 covering 73 sites with the following moss species *Hypnum cupressiforme*, *Homalothecium lutescens* (given as *Camptothecium lutescens*) and *Homalothecium sericeum* by nuclear activation analysis (Dubna, Russia) (Barandovski et al. 2007).

The aim of this study is to present atmospheric pollution during a period of 3 years (2003, 2004, 2005), as assessed by mosses, in the part of Southeastern Europe (Southern Bulgaria and European Turkey). In our discussion hereafter we will seek to position the region in question within wider locations by referring to findings from other regions in Europe. Additionally, we will include a comparative perspective by employing data from previous periods which will be indicative of temporal changes.

Material and methods

The study area is situated as a part of Southeastern Bulgaria and the European part of Turkey and covers 35736 km^2. The area is mainly plain and hilly, the range of altitudes from sea level up to 1031 m. Out of the entire territory 1143 km^2 are covered with forests. The climate is temperate-continental and temperate-Mediterranean, and the annual mean precipitation is around 590 mm. The geographical and economic characteristics of the above-mentioned area are presented in detail in Table 1. Phytogeographically, the Bulgarian part of the study area belongs to Euro-Westsiberian region, whereas the Turkish part is mainly in the Euro-Siberian and Mediterranean regions with some Irano-Turanian elements (Papp and Sabovljević 2003).

The selection of the moss sampling sites in the studied area is concordant with the following requirements: (a) the requirement in the European moss surveys for 1.5 moss sample per 1000 km^2;

Table 1 Geographical and economical characteristics of the studied part of Southeastern Europe (Southeastern Bulgaria and European Turkey)

	Southeastern Bulgaria	European Turkey
Geographical coordinates	41°41′ N–42°43′ N 25°35′ E–28°01′ E	40°01′ N–42°08′ N 26°00′ E–29°01′ E
N–S the longest direction	108 km	163 km
W–E the longest direction	196 km	260 km
Area	11972 km^2	23764 km^2
Settlements	360	900
Agriculture area	6225 km^2	12600 km^2
Mountains (forest area)	7 (part of Eastern Balkan Mts., Bakadzhik Mt., Sakar Mt., Strandzha Mt., Manastirski hills, Derventski hills, Huhla Mt.); 513 km^2	3 (Istranca Mountains, Ganos and Koru Mountains); 630 km^2
Altitude—ranges; mean	0–856 m, 428 m	0–1031 m
Climate type	Temperate-continental, Temperate-Mediterranean	Temperate-continental, Continental-Mediterranean
Annual precipitation	530–860 mm; 590 mm	550–1500 mm; 600 mm (Dönmez 1990)
Number of rivers, big lakes	6; 4	2; 3
Hydroelectric power plants	2	0
Population	784541	11603342
Population density	10–100 inhabitants per km^2	10–32,000 inhabitants per km^2
Urban population	686,060	10,839,792
1st sector (agriculture) % of GDP	27.1%	11.8%
2nd sector (industry & trade) % of GDP	30.4%	53.2%
3rd sector (services) % of GDP	42.5%	35%
Heavy industry	Oil refinery, nonferrous complex	Glass, paint and cement factories
Number of factories of heavy industry	5	8
Number of factories of other industry	482 (textile, milk, meat, fish, vegetable oil, tobacco, wine, beer, ceramic, plastic, carpets, clothes)	992
Power plants; fuel	9 coal	1 natural gas
Mine works	9 coal, Cu, Fe, polymetal ores; 1 old U mines (shut down 20 years ago)	1 lignite in Saray-Tekirdag
Incineration plants	2	4
Cars per 1,000 inhabitants	140	184
Dams (flood control)	5 (plus 70 micro-dams)	8
Environmental protection of the region	42 NATURA 2000 protected sites: 12 sites according to the Birds Directive (Council Directive 79/409/EEC) with total area 151937 ha; 30 sites according to the Habitats Directive (Council Directive 92/43/EEC) covering 292855 ha	National Park; Çanakkale—Gelibolu Peninsula Historical Park

Table 1 (continued)

	Southeastern Bulgaria	European Turkey
	According to the international Protection laws among them 4 Ramsar Convention sites According to the Bulgarian environmental legislation (Biological Diversity Law, 2002; Protected Areas Law, 1998) 5 reserves 4 managed reserves 1 nature park	Nature Park; Istanbul—Türkmenbasi Natural Park Natural protection areas: Kirklareli—Kasatura Estuary Nature Reserve, Kırklareli—Saka Lake Nature Reserve, Edirne Gala Lake Nature Reserve

(b) the possibility to locate the recommended moss pleurocarpous species in needed quantities due to the fact that a large part of Bulgarian and Turkish Trace are covered with agricultural fields; (c) the necessity to include more sampling sites in a few areas with local sources of heavy metal emissions.

The moss sampling net included 114 sites (40 in its Bulgarian part and 74 sites in its Turkish part) (Fig. 1). The current paper comments on results of *Hypnum cupressiforme* Hedw. (nomenclature according to Corley et al. 1981) 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 distribution of the substrates under the mosses was 76 on soil, 24 on dead wood, and 14 on rocks.

Sampling followed basic recommendations pointed out for the moss surveys in Europe by Rühling (1994) and Rühling and Steinnes (1998), i.e. each sample consists five subsamples in a site,

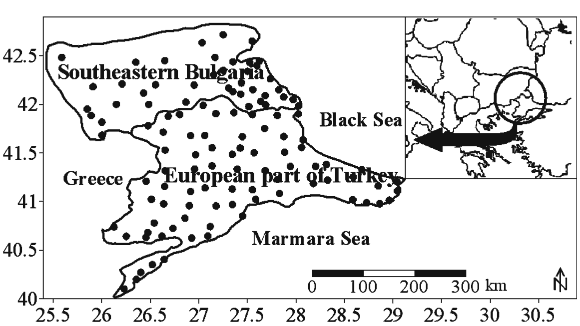

Fig. 1 Location of the moss sampling sites in Southeastern Bulgaria and European Turkey

50 × 50 m; in forest gaps; 300 m from main motor roads and cities; 100 m from small roads or single houses; using nylon gloves, analytically clean plastic bags and without smoking during the sampling. During the current study epiphytes were avoided. The samples were taken under the upper forests frontier in the mountains and with the majority not higher than 400 m above the main valley. The moss sampling was done in early spring (April 2006) before vascular plants started growth.

The mosses collected in the Bulgarian part of the overall study region were stored in a refrigerator after determination of species; or dried, cleaned very carefully and age separated (3-years apart). The samples were not washed, but homogenized by hands. They were stored deep-frozen until further analytical treatment. The samples were dried at 40°C and then wet-ashed. About 1–2 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 hydrogen peroxide. This treatment was repeated till full digestion. The filtrate was diluted with double distilled water to 25 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°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

Table 2 The parallel average results of Bulgarian and Turkish labs for two reference moss materials (M2 and M3) and exchanged eight moss samples (mg/kg d.w.)

		*M2	*M3	M2	M3	BG1	BG2	BG3	BG4	TR1	TR2	TR3	TR4
Al	BG	175	160	163	141	1040	2681	962	832	744	806	1765	959
	TR			162	188	1060	3339	1056	905	766	1077	1985	1231
As	BG	0.92	0.09	0.80	< 0.5	< 0.5	0.85	0.50	0.64	< 0.5	< 0.5	< 0.5	< 0.5
	TR			1.05	< 0.5	< 0.5	1.26	0.52	0.76	< 0.5	1.00	2.24	< 0.5
Cd	BG	0.44	0.11	0.385	0.08	0.21	0.24	0.35	< 0.2	0.20	0.18	0.62	0.49
	TR			0.43	0.11	0.23	0.25	0.37	0.10	0.14	0.19	0.27	0.28
Cr	BG	0.91	0.82	0.78	0.61	2.52	5.95	1.91	1.78	1.45	1.43	2.75	2.50
	TR			0.88	0.61	2.73	6.56	2.98	2.07	1.36	2.42	3.12	2.16
Cu	BG	68.1	3.64	56.3	3.57	4.13	5.37	4.98	3.04	2.75	3.63	4.23	4.07
	TR			52.5	3.31	3.30	5.13	4.90	3.28	2.28	3.27	4.51	4.84
Fe	BG	245	137	224	122	781	1809	724	524	513	468	1237	897
	TR			229	167	779	1826	812	647	642	1248	1483	1001
Ni	BG	14.8	0.92	13.5	0.98	1.65	3.39	1.92	1.21	1.44	1.16	3.60	2.85
	TR			13.3	0.84	1.59	3.89	1.98	1.20	0.79	1.69	3.48	1.99
Pb	BG	5.86	2.94	5.72	2.55	5.67	6.42	7.73	3.47	3.53	2.96	8.27	14.4
	TR			6.26	2.43	6.10	5.92	8.56	4.34	3.46	3.16	8.60	11.9
V	BG	1.21	1.07	1.22	0.73	2.53	3.55	2.46	1.74	1.40	1.96	3.08	2.52
	TR			1.23	1.13	2.65	4.77	2.87	2.23	1.97	3.06	5.08	3.15
Zn	BG	35.2	25.9	33.0	20.8	20.2	18.1	16.6	14.3	13.9	10.7	22.1	25.4
	TR			33.7	25.4	20.2	20.3	23.7	16.2	15.2	18.1	25.6	24.9

*M2 and *M3 are reference materials for European moss surveys with recommended values published by Steinnes et al. (1997). Deviation between the parallel measurements was below 5% in all cases

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.

The elements Al, As, Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn have been 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 maximum relative standard deviations were as follows Cd and Cr (36.0%), Pb (33.1%), Sb (30%), Al (28.8%), As (23.5%), V (18.2%), Cu (15.0%), followed by Ni (8.6%), Zn (6.2%), Fe (2.4%). Analytical precision was checked with replicating, blanks, stock standard solutions, as well as moss reference materials prepared during the European moss surveys in 1995 and 2005. The results for each site are based on the means of two moss samples and for separate samples are taken the means of three analytical determinations. The results are presented in milligram per kilogram.

Table 3 The concentration of heavy metals and toxic elements in the mosses of Southeastern Bulgaria and European Turkey (mg/kg d.w.)

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[a]		6202	6865	6865	6865	6865	6865	6865	6865	6865
Max in EU[a]		118	11.0	265	3140	52200	302	887	77	2940
Median in EU[a]		0.45	0.27	2.80	7.20	888	2.40	8.00	3.70	38.0

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

In order to determine whether any difference in the moss data net could be due to different labs analyzing the moss materials or are real border effects, four moss samples were collected and prepared for analysis in Bulgaria and respectively in Turkey, which were parallely analyzed in the both labs. Reference materials, M2 and M3 prepared for European moss survey, with certified element values, published by Steinnes et al. (1997), were used for this purpose.

Results and discussion

The results obtained for two reference moss materials, M2 and M3, as well as data of four Bulgarian and four Turkish mosses are presented in Table 2. The applied ANOVA analysis for the parallel measurements of two moss reference materials and eight BG and TR mosses 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 ten heavy metals and toxic elements of the first cross-border moss survey in Southeastern Bulgaria and European Turkey are presented in Table 3. When the last median values of the European and the present study are compared, As, Cr and Fe values are two times higher than median values of EU. However, the obtained maximum values of all concerned elements are lower than European maximum values. Southeastern Europe has mineral soils with low humus content since large area of Europe, especially the Northern part has rich in humus soil (Akalan 1988). This feature could be the main reason for the difference concerning median values. Taking into account that the prevailing part of European countries has developed economy, the citied higher EU maximum values probably have resulted from heavy industry facilities, i.e. the so called "hot points".

In this study, two different groups of heavy metals and distribution patterns were observed. The first group including Pb, Cu and Zn have predominantly anthropogenic origin compared to the second group comprising As, Cd, Cr, Fe, Ni and V. The distribution of Al is slightly different from the second group. It is well known that Al in moss tissues comes mainly from solved soil particles as dry deposition and represents the resuspension of soil and soil originated contamination. The Principle Component Analyses proved two components (F1 and F2) of heavy metals: Al, As, Cd, Cr, Fe, Ni, V and Cu, Pb, Zn, respectively (Table 4).

Distribution maps of Al, As, Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn in moss samples from the studied part of Southeastern Europe are presented in Fig. 2. High concentrations of Pb and Cu at the vicinity of Istanbul and Burgas cities indicate atmospheric pollution. Zinc contamination is obvious around Istanbul. Cadmium and vanadium distributions are very similar; both could originate from anthropogenic sources or soil dust. Their concentrations near Istanbul and Burgas, large cities in Southeastern Europe, have high values. Apart from Cu, Pb and Zn, the rest of the studied elements show similar distributions and have relatively low concentrations in the observed large cities regions for the researched period. An exception to the above pattern is the copper polluted region south of Burgas due to ore deposits and mines. Maximum values of As, Cd, Cr, Ni, Fe and Ni are obtained in the European part of Turkey. Transboundary atmospheric pollution with As could be seen at the line Tekirdag-Edirne to the Bulgarian territory. Two large glass fac-

Table 4 Principal component analyses with rotated component matrix

	Component	
	F1	F2
Al	0.932	0.048
As	0.870	0.143
Cd	0.846	0.260
Cr	0.954	0.036
Cu	0.119	0.894
Fe	0.932	0.100
Ni	0.828	0.047
Pb	−0.022	0.948
V	0.858	0.252
Zn	0.264	0.805
% Total variance	59.58	21.82

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. Rotation converged in three iterations

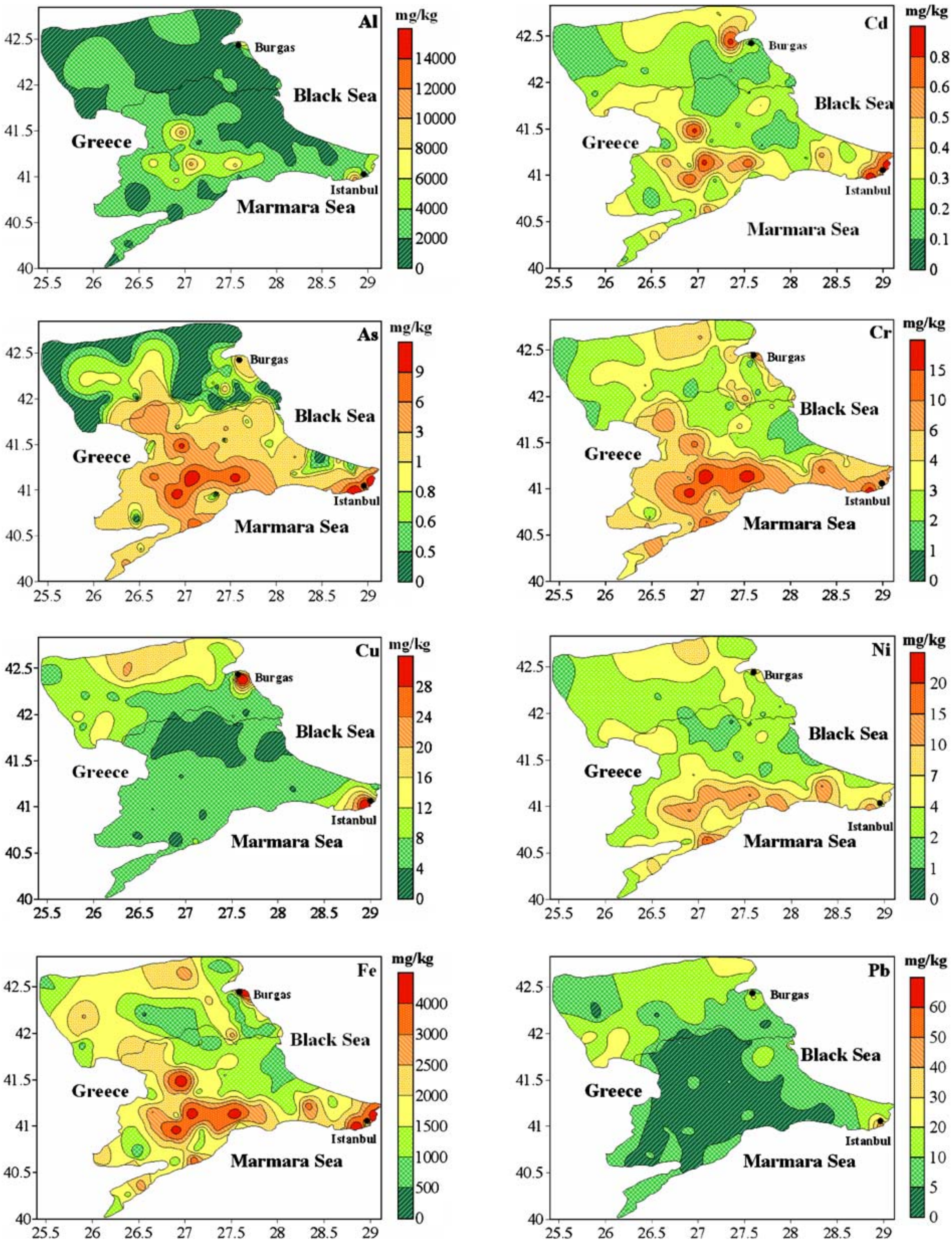

Fig. 2 Distributions of heavy metals assessed by mosses in the studied area of Southeastern Europe

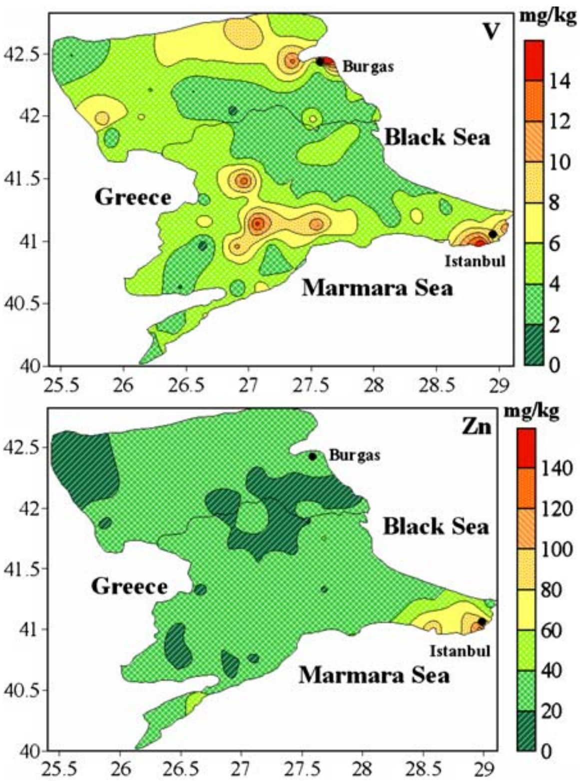

Fig. 2 (continued)

tories as well as intensively applied fertilizers in the surrounded agricultural area appear to be the plausible reason for the observed As cross-border transfer.

Coşkun et al. (2006) approached the distribution of the heavy metals in the soils in the Turkish part of the studied area. The content of the elements Cu, Cr, Ni, Pb and Zn correlates positively to their soil amount. There is only one moss monitoring data obtained previously (Coşkun et al. 2005) in European Turkey showing atmospheric heavy metal pollution. The comparison of previous and present results for each element showed that the distribution pattern and median concentrations of Cu, Zn, Ni, and Pb and As were similar. Although the past and present distribution patterns of Pb and As were similar, there was a 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 also related to unqualified coal prohibition during last 5 years. No statistical proved temporal changes of heavy metals in mosses were found during the last three moss surveys for the Bulgarian territory of the studied area.

In conclusion, having more than 12 million inhabitants, oil refinery, nonferrous complex, 13 heavy industry factories, 1474 other factories, nine coal and one natural gas power plants, 11 polymetal mine works, six incineration plants and 160 cars per 1000 inhabitants, the territory of 35736 km^2 in the studied part of Southeastern Europe is not seriously polluted by atmospheric heavy metal contaminants according to the established results in this study.

Acknowledgement The authors are grateful to the Turkish Scientific and Technological Council (TUBITAK) for the financial support of this study as a joint project (no:105Y144) with the Bulgarian Academy of Sciences.

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