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DETERMINATION OF HEAVY METAL DEPOSITION IN THE COUNTY OF OBRENOVAC (SERBIA) USING MOSSES AS BIOINDICATORS. I: ALUMINUM (AL), ARSENIC (AS), AND BORON (B)

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Abstract **-** In the present study, the deposition of three heavy metals (Al, As and B) in the county of Obrenovac (Serbia) is determined using four moss taxa (*Bryum argenteum*, *Bryum capillare*, *Brachythecium* sp., and *Hypnum cupressiforme*) as bioindicators. Distribution of average heavy metal content in all mosses in the county of Obrenovac is presented in maps, while long-term atmospheric deposition (in the mosses *Bryum argenteum* and *B. capillare*) and short-term atmospheric deposition (in the mosses *Brachythecium* sp. and *Hypnum cupressiforme*) are discussed and given in tables. Areas of the highest contaminations are highlighted.

Key words: Heavy metal deposition, mosses, bioindicators, Serbia

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INTRODUCTION

Surveillance of heavy metals in mosses was originally established in the Scandinavian countries in the 1980s. However, the idea of using mosses to measure atmospheric heavy metal deposition was developed alredy in the late 1960s (Rhüling and Tyler, 1968; Tyler, 1970). It is based on the fact that mosses, especially the carpet-forming species, obtain most of their nutrients directly from precipitation and dry deposition. In the present investigations, we decided to use two acrocarpous moss species (*Bryum argenteum* Hedw. and *Bryum capillare* Hedw.) that can give us an idea of long-term atmospheric deposition, inasmuch as they are attached to the substrate and also accumulate metals deposed during the last few decades in the surface layers of the substrate.

Two pleurocarpous taxa (*Brachythecium* sp. and *Hypnum cupressiforme* Hedw.) were used to scan short-term atmospheric deposition of heavy metals, considering that these taxa are not strongly attached to the substrate and accumulate mostly from precipitation.

Mosses are better than other higher plants in scanning heavy metal deposition because:

- they are perennial without deciduous periods;
- they have high cation exchange capacity that allows them to accumulate great amounts of heavy metals between the apoplast and symplast compartments without damaging vital functions of the cells;
- mosses do not possess thick and strong protective layers like cuticles.

Also, this time-integrated way of measuring patterns of heavy metal deposition from the atmosphere to terrestrial ecosystems, besides being spatially oriented, is easier and cheaper than conventional precipitation analyses, as it avoids the need for deploying large numbers of precipitation collectors. The higher trace element concentration in mosses compared to rain water makes analysis more straightforward and less prone to contamination (Berg *et al.* 1997).

Use of mosses to investigate heavy metal deposition shows transboundary heavy metal pollution and can indicate the paths by which atmospheric pollutants enter from other territories or reveal their sources within the investigated area.

Although 15 heavy metals were analyzed in all, only deposition and distribution of aluminum, arsenic and boron are treated in the present study.

MATERIAL AND METHODS

The acrocarpous mosses *Bryum argenteum* and *Bryum capillare* were used to research long-term atmospheric deposition, while the pleurocarpous *Brachythecium* sp. and *Hypnum cupressiforme* were used to scan short-term atmospheric deposition in the county of Obrenovac (Serbia). *Hypnum cupressiforme* is one of the standard species used in Europe to survey heavy metal deposition (B u s e *et al.* 2003.), while the other three are standard in Europe, but do not grow in this region. In estimating which other species are eligible for use in heavy metal deposition monitoring, we relied on the experience of Thöni (1996), Herpin *et al.* (1994), Siewers and Hairpin (1998) Zechmeister (1994), and Ross (1990).

As far as possible, moss sampling was conducted according to guidelines, set out in the experimental protocol for a survey carried out in 2000/2001 (UNECE, 2001). Details of the procedure are given in Rühling *et al.* (1998).

Each sampling site was located at least 300 m from main roads and populated areas and at least 100 m from any road or single house. In forests or plantations, samples were collected in small open spaces to preclude any effect of canopy drip. Sampling and sample handling were carried out using plastic gloves and bags. About three moss samples were collected from each site. Dead material and litter were removed from the samples. Green parts of mosses were used for the analyses.

The county of Obrenovac was chosen for this investigation because of its industry and location. Each sampling site was GPS located with a precision of ±10 m, and GPS dates (Germin) were digitalized on the maps using the following softwares: OziExplorer 3.95.3b, © D&L Software Pty Ltd, and WinDig 2.5 Shareware, © D.Lovy. All material was collected during November of 2002. Not more than one site was chosen in a 50x50 m square. Seventy-five localities were chosen out of 129 for comparisons and further analyses. The selection was based on the presence of all investigated species and yearly biomass. More than 500 samples were analyzed.

After collecting, samples were dried as soon as possible in a drying oven to constant dry weight (dw) at a constant temperature of 35^{0}C, then stored at -20^{0}C. Following homogenization in porcelain, the samples were treated with 5+1 parts of nitric acid and perchloric acid (HNO_3:$HClO_4$ = 5:1) and left for 24 hours. After that, a Kjeldatherm digesting unit was used for digestion at 150-200^{0}C for about one hour. Digested samples were filtered on qualitative filter paper to dispose of silicate remains. Sample volume was then normated to 50 ml. Aluminum (Al) was detected by AAS on a Pye Unican SP9 atomic absorbance spectrophotometer from Philips using the flame of acetylene/nitrogen-suboxide. Boron (B) was detected by ICP-ES, while arsenic (As) was determined by AAS using hydride techniques.

For explanation of the results and their presentation on maps, the folloving statistical statistics parameters were used: average values, standard deviation, minimum, maximum, and percent deviation. Map making and interpolation of exact data were made using Agis software (v1. 71 32 byte, © Agis Software, 2001).

RESULTS AND DISCUSSION

Aluminum in Obrenovac County probably comes from heavy road traffic, intensive coal burning, and incorrect waste disposal. The most contaminated areas according to deposition in mosses are the town of Obrenovac and the northernmost part of the county by the Sava River (>40 mg/g). A somewhat lower level of Al deposition (20-30 mg/g) occurs in the central part of the county, where Al is probably spread by traffic on the main roads.

Aluminum represents 8% of the Earth's crust. It comes from bauxite (M e r i a n , 1984), is very widespread in all forms of industry (machine construction, electro engineering, civil engineering, the food industry, agriculture), and is also present in aluminum foil, iron plates, and other widely used articles. Aluminum emission into the atmosphere was great during the last century. Only in the 70s it was 7.2 million tons per year (L a n t z y and M a c k e n z i e , 1979). It is known that the atmospheric concentration of aluminum is significantly elevated (>10mg/m3) in areas with a cement indu-

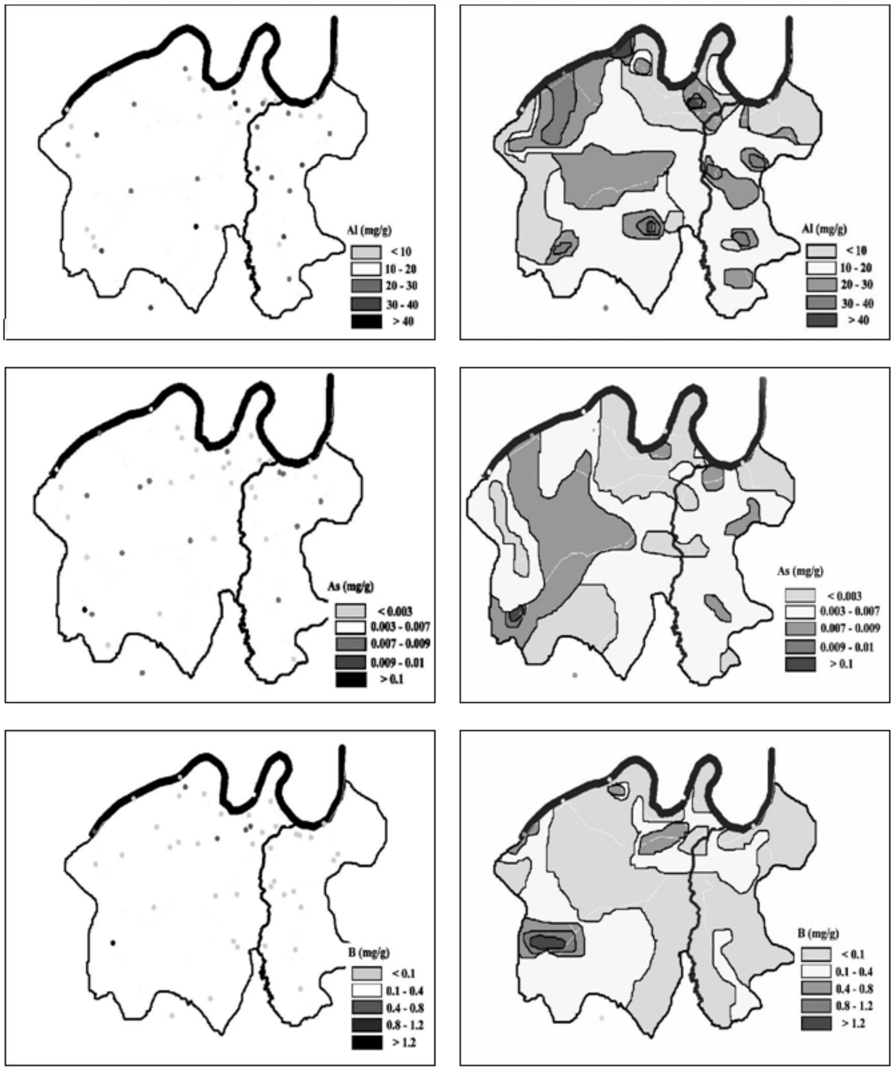

Fig. 1. Exact locations of moss sampling (left) and extrapolated maps (right) of selected heavy metal deposition in the county of Obrenovac (Serbia).

stry (M e r i a n , 1984).

In slightly acid and neutral soils, dissolving of aluminum is inconsiderable, while in acid soils (pH<5.0) the concentration of Al^{3+} is significantly elevated. High concentrations of Al^{3+} in the substrate exert toxic action on plants by damaging the roots and by lowering the uptake of essential phosphorous, calcium, and magnesium (B e r g m a n n , 1988). Aluminum is also toxic for animals and man, especially if its concentration is high in water and if air with a high concentration of it is inhaled over longer period. It can cause Alzheimer's disease (M e r i a n , 1984). In drinking water, the maximal tolerated value of aluminum concentration is 0.2mg/l (T h ö n i and S e i t l e r , 2004).

Arsenic and boron are probably spread in the county of Obrenovac by wind blowing from coal ash deposit sites. According to the mosses analyzed, the center of arsenic deposition is the southwestern part of the county around the village of Vučičevica. However, there is a rather large area in the western part of county with average arsenic deposition of 000.7-000.9 mg/g (dw of mosses). Some smaller areas with high arsenic deposition are also are present in the town of Obrenovac and around Barič. The highest presence of boron is in the southwestern part of Obrenovac County, where its concentration is more than 1.2 mg/g. Some zones with medium concentrations (0.4-0.8 mg/g) are found in the town of Obrenovac and places by the Sava (Mladost and Ušće).

Table 1. Deposition of Al, As, and B in the county of Obrenovac (Abbreviations: H - *Hypnum cupressiforme*, BRA - *Brachythecium* sp., Bc - *Bryum capillare*, Ba - *Bryum argenteum.*

No.	Locality	Longitude	Latitude	Al mg/g	As mg/g	B mg/g
1	Vinogradi - H	20.163702	44.391758	3.2455	0.0010	0.4032
2	Moštanica 1 - H	20.183672	44.384249	13.7534	0.0050	0.5001
3	Iskra 1 - Bc	20.155235	44.392722	15.7587	0.0052	0.2750
4	Iskra 2 - Ba	20.152826	44.393284	34.8813	0.0078	<0.0261
5	Iskra 1 - H	20.155235	44.392722	9.5772	0.0052	0.3451
6	Iskra 2 - Bc	20.152826	44.393284	13.6424	0.0067	<0.0111
7	Rvati 1 - Bc	20.118796	44.396930	34.6702	0.0152	<0.0223
9	Deponija B ulaz 1 - Bc	20.023331	44.383735	35.1069	0.0087	0.0162
10	Zabrežje 1 - Bc	20.121273	44.411245	21.8641	0.0056	0.1374
11	Ušće 2 - Bc	20.066441	44.419235	14.5886	0.0058	0.0707
12	Vinogradi - Bc	20.163702	44.391758	7.5440	0.0035	0.3632
13	Iskra 1 - Ba	20.155235	44.392722	20.1420	0.0064	<0.0080
14	Ušće 2 - H	20.066441	44.419235	44.8494	0.0063	0.0252
15	Ušće 1 - Bc	20.070343	44.414738	11.1272	0.0040	0.4919
16	Urozv - BRA	20.079770	44.389043	26.1097	0.0074	<0.0167
17	Zabrežje 2 - H	20.133796	44.408293	12.8400	0.0041	0.0613
18	Orašac 1 - H	20.021819	44.336717	13.4694	0.0044	0.1429
19	Hotel - Ba	20.127451	44.394049	24.8599	0.0054	0.0781
20	Moštanica 1 - H	20.183672	44.384249	6.4935	0.0017	0.1623
21	Grabovac 1 - H	20.046934	44.359997	7.4700	0.0026	0.2916
22	Šab.put nadv. - Bc	20.094085	44.391367	31.2722	0.0104	<0.0127
23	Vranić - H	20.152122	44.347529	13.6044	0.0041	0.1922
24	Jasenak 2 - BRA	20.156246	44.360071	24.2931	0.0078	0.0617
25	Dren 1 - BRA	20.023224	44.358238	26.9551	0.0055	<0.0171
26	Veliko Polje 1 - H	20.108648	44.365954	17.7903	0.0023	<0.0390
27	Grabovac 1 - Bc	20.046934	44.359997	10.7424	0.0032	0.0461
28	Belo Polje 1 - Bc	20.118064	44.382783	17.7177	0.0055	<0.0250
29	Brović 1 - Bc	20.072201	44.335108	18.5153	0.0072	0.1608
30	Ljubinić 2 - BRA	20.026762	44.334832	12.1507	0.0021	0.1458
31	Hotel - H	20.127451	44.394049	24.8571	0.0055	0.0802
32	Grabovac 1 - BRA	20.046934	44.359997	26.5043	0.0081	0.0456
33	Ljubinić 2 - Bc	20.026762	44.334832	30.2326	0.0075	0.1525
34	Veliko Polje 4 - H	20.109057	44.341908	17.8296	0.0055	<0.0070
35	Zabran 3 - H	20.137615	44.396905	7.0804	0.0023	0.1702
36	Zabran 1 - H	20.139396	44.398268	20.0140	0.0070	<0.0117
37	Orašac 3 - H	20.016612	44.343855	5.3107	0.0053	1.8056
38	Orašac 2 - H	20.020860	44.340639	18.0890	0.0044	0.0162
39	Zabran 2 - Ba	20.142377	44.401672	9.9973	0.0027	0.1583
40	Belo Polje 1 - Ba	20.118064	44.382783	17.8883	0.0052	0.2356
41	Orašac 2 - BRA	20.020860	44.340639	8.6845	0.0035	0.1769
42	Ljubinić 1 - BRA	20.037630	44.322132	14.8620	0.0015	0.1696
43	Grabovac nad. - Ba	20.092788	44.365167	15.3791	0.0022	0.0918
44	Joševa - H	20.060545	44.310742	23.0646	0.0046	0.0688
45	Brović 2 - BRA	20.088929	44.318537	18.0171	0.0037	0.0487
46	Jasenak 2 - Ba	20.156246	44.360071	12.8943	0.0031	0.0904
47	Garbovac nadv. - BRA	20.092788	44.365167	25.8155	0.0079	0.0381

Table 1. Continued

No.	Locality	Longitude	Latitude	Al mg/g	As mg/g	B mg/g
48	Baljevac 1 - Bc	20.152044	44.340743	30.7846	0.0071	0.0291
49	Joševa - Bc	20.060545	44.310742	37.1464	0.0059	0.0190
50	Joševa - BRA	20.060545	44.310742	35.1556	0.0073	0.0593
51	EPS - Bc	20.120401	44.388845	7.5956	0.0018	0.0550
52	Konatice II - BRA	20.148928	44.337410	18.2688	0.0033	0.0631
53	Zabran 1 - Ba	20.139396	44.398268	10.1677	0.0011	0.0797
54	Mislođin 1 - BRA	20.136579	44.383096	15.2645	0.0023	0.2971
55	Brović 1 - H	20.072201	44.335108	11.4323	0.0016	0.2473
56	Mislođin 4 - H	20.134067	44.369616	14.5418	0.0024	0.1195
57	Stubline 2 - H	20.091649	44.345095	76.9157	0.0062	0.1634
58	Konatice 1 - Bc	20.162150	44.316265	6.1409	0.0016	0.1317
59	Zabran 3 - Ba	20.137615	44.396905	6.5014	0.0021	0.0468
60	Jasenak - H	20.143804	44.365736	21.0274	0.0026	0.0697
61	Konatice 2 - Ba	20.155831	44.322960	24.9250	0.0041	0.0879
62	Veliko Polje 4 - Bc	20.109057	44.341908	18.2531	0.0047	<0.0167
63	Mislođin 1 - BRA	20.133857	44.387041	26.4333	0.0028	0.0616
64	Veliko Polje 3 - Bc	20.106117	44.344670	7.0910	0.0062	0.0522
65	Konatice II - Bc	20.148928	44.337410	43.0922	0.0046	0.0817
66	Mislođin 6 - Ba	20.164676	44.371027	0.2029	<0.0003	0.0330
67	Stubline 1 - H	20.086353	44.357185	12.3300	0.0036	0.1989
68	Šab.put nadv. - Ba	20.094085	44.391367	23.3058	0.0099	0.0286
69	Dren 1 - H	20.023224	44.358238	13.3679	0.0029	0.2303
70	Zabran 2 - Bc	20.142377	44.401672	13.8389	0.0035	0.3460
71	Baljevac 2 - H	20.129432	44.342383	19.1194	0.0044	0.0546
72	Mislođin 5 - Ba	20.150813	44.367274	13.8926	0.0034	<0.0096
73	Orašac 1 - BRA	20.021819	44.336717	24.4505	0.0073	0.1282
74	Konatice II - H	20.148928	44.337410	5.9067	0.0031	0.1136
75	Šab.put 1 - BRA	20.049094	44.396566	20.0018	0.0022	0.2788
76	TENT<B 3 - Bc	20.003761	44.379624	15.2160	0.0082	0.3272
77	Šab.put 1 - H	20.094085	44.391367	4.7800	0.0030	0.5008
78	Ratari 2 - BRA	20.058939	44.387315	17.1296	0.0055	0.0343
79	TENT<B 1 - H	19.593841	44.380930	2.7789	0.0015	<0.0496
80	TENT<B 2 - H	20.010398	44.374841	3.7931	0.0021	<0.0172
81	Ratari 1 - H	20.065292	44.389672	13.6849	0.0066	<0.0074
82	Ušće<Skela - Bc	20.031781	44.409564	19.0549	0.0067	<0.0476
83	Ratari 2 - Ba	20.058939	44.387315	15.6052	0.0070	0.0253
84	TENT<B 4 - Ba	20.005827	44.387162	15.1136	0.0065	0.0605
85	TENT<B 2 - BRA	20.010398	44.374841	18.8041	0.0041	<0.0157
86	TENT<B 1 - BRA	19.593841	44.380930	4.5290	0.0014	0.3442
87	TENT<B 4 - Bc	20.005827	44.387162	3.4083	0.0015	0.1607
88	TENT<B 3 - Bc	20.003761	44.379624	23.1495	0.0049	<0.0145
89	Orašac 1 - BRA	20.021819	44.336717	7.0589	0.0164	0.2594
90	Ušće<Skela - BRA	20.031781	44.409564	37.7415	0.0068	<0.0189
91	Šab.put 1 - Bc	20.049094	44.396566	29.0161	0.0040	0.0971
92	TENT<B ulaz - BRA	20.002958	44.394451	7.3122	0.0042	0.5631
93	Depoija 1 - BRA	20.087035	44.407417	28.8883	0.0108	<0.0154

Table 1. Continued

No.	Locality	Longitude	Latitude	Al mg/g	As mg/g	B mg/g
94	TENT<B 2 - Bc	20.010398	44.374841	1.5475	0.0004	0.2987
95	Konatice 1 - H	20.162150	44.316265	16.1006	0.0019	0.1115
96	Mislođin 6 - BRA	20.164676	44.371027	16.9469	0.0037	0.0722
97	Mislođin 3 - BRA	20.136294	44.371922	12.6024	0.0044	<0.0158
98	Mislođin 6 - Bc	20.164676	44.371027	31.3750	0.0067	0.2588
99	Jasenak 2 - Ba	20.156246	44.360071	23.8202	0.0041	0.0861
100	Mislođin 4 - Bc	20.134067	44.369616	32.0245	0.0051	<0.0116
101	Zabran 1 - BRA	20.139396	44.398268	6.6987	0.0028	0.1005
102	Rojkovac 1 - Bc	20.117592	44.401807	17.6951	0.0021	0.1247
103	Rojkovac 1 - BRA	20.117592	44.401807	20.3806	0.0061	<0.0307
104	Rvati 1 - BRA	20.118796	44.396930	48.2657	0.0025	0.4908
105	Rojkovac 1 - Ba	20.117592	44.401807	24.8176	0.0068	<0.0212
106	Moštanica 3 - BRA	20.175487	44.380355	39.3572	0.0046	0.1082
107	Razu - BRA	20.065879	44.410726	5.6224	0.0015	0.3203
108	Ušće 3 - BRA	20.084220	44.411524	22.0240	0.0029	0.0182
109	Duboko 3 - H	20.176888	44.391497	5.5357	0.0009	0.1744
110	Zabrežje 1 - BRA	20.121273	44.411245	8.7102	0.0030	0.1075
111	Zabran 3 - BRA	20.137615	44.396905	25.3142	0.0054	0.0726
112	Moštanica 2 - Bc	20.180515	44.381400	18.8143	0.0065	<0.0109
113	Razu - Ba	20.065879	44.410726	10.0591	0.0031	0.3138
114	Rvati 3 - BRA	20.115276	44.395254	6.1793	0.0029	0.7912
115	TENT<A 1 - BRA	20.096950	44.402553	18.6353	0.0052	<0.0200
116	Moštanica 3 - Bc	20.175487	44.380355	16.3106	0.0033	0.3996
117	Zabrežje 2 - BRA	20.133796	44.408293	15.1400	0.0038	<0.0189
118	Urozv - Bc	20.079770	44.389043	6.7581	0.0101	0.1791
119	Depoija 1 - H	20.087035	44.407417	4.5273	0.0021	0.2632
120	Zabrežje 2 - Ba	20.133796	44.408293	13.9746	0.0040	0.1424
121	Moštanica 1 - Bc	20.183672	44.384249	20.7035	0.0058	0.0639
122	Vinogradi - Ba 2	0.163702	44.391758	13.3177	0.0035	<0.0098
123	Urozv - H	20.079770	44.389043	10.7742	0.0026	0.0724
124	Razu - H	20.065879	44.410726	10.2135	0.0037	0.0738
125	Duboko 1 - Ba	20.173260	44.398253	5.9324	0.0027	<0.0159
126	Vinogradi - BRA	20.163702	44.391758	1.7874	0.0031	0.0777
127	Duboko - BRA	20.146281	44.397974	4.9484	0.0024	0.1834
128	TENT<A 1 - Bc	20.096950	44.402553	3.0319	0.0011	0.0693
129	Zabrežje 2 - Bc	20.133796	44.408293	19.2847	0.0011	0.0464
Median				17.3828	0.0045	0.1346

Arsenic is present in the Earth's crust with ca. 1.7 g per ton (Thöni and Seitler, 2004). It is often present in the form of arsenictrioxide (As_2O_3) as a side product in production of copper, lead, zinc, and nickel. Because arsenic induces cancer, it is used to only a limited extent in industry (production of screens and some other articles). However, it is still present in glass and substances for preservation of wood products. Use of arsenic in production of herbicides, insecticides, fungicides, and pigments is strictly excluded due to its easy binding in organisms. Arsenic is mainly present in the atmosphere as a side product of copper and nickel production and of brown coal burning. It can be present in brown coal in concentrations up to 1500 mg/kg, although the average is

18 mg/kg (Thöni and Seitler, 2004). Further sources of arsenic are the metal industry, agriculture industry, and volcanic activity. Global emission of arsenic fell from 1983 (19000 t per year) to 1995 (5000 t per year) (Pacyna and Pacyna, 2001). Normally, it is present in the atmosphere with 2800 t (Lantzy and Mackenzie, 1979).

There are no data on the effects of arsenic on plants (Thöni and Seitler, 2004). For animals and man, arsenic is known to be toxic, carcinogenic, teratogenic and mutagenic. The chronic presence of arsenic pollution can lead to serious hematological and neurological diseases (Merian, 1984). Borderline values of arsenic concentration are as follows: 1 mg/m^3 in the air, 0.1 mg/l in water, and 0.05 mg/l in drinking water (Thöni and Seitler, 2004).

Since all of the sampled species could not be found at each locality, average values of all specimens were extrapolated on the maps to get an idea of heavy metal deposition in the county of Obrenovac (Fig. 1). However, if one separates the values of deposition obtained from pleurocarpous mosses (*Brachythecium* sp. and *Hypnum cupressiforme*) and ones obtained from acrocarpous mosses (*Bryum argenteum* and *Bryum capillare*), it is readily discernible that the first two give us an idea of short-term and the last two of long-term deposition, respectively (Tab. 1).

These differences are easily explained in terms of the life forms of these mosses and their uptake of heavy metals. Pleurocarps are not closely attached to the substrate and so get the bulk of deposited heavy metals directly from the atmosphere (during their pauciennial life period), while acrocarps are strictly attached to the substrate and receive the bulk of deposited heavy metals from the substrate solution (metals deposited over a period that is longer than their pauciennial life span).

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ДЕПОЗИЦИЈА ТЕШКИХ МЕТАЛА НА ПОДРУЧЈУ ОПШТИНЕ ОБРЕНОВАЦ (СРБИЈА) ПРАЋЕНА ПРЕКО МАХОВИНА КАО БИОИНДИКАТОРА I: АЛУМИНИЈУМ (AL), АРСЕН (AS), И БОР (B)

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У овом раду представљена је депозиција три тешка метала (алуминијум, арсен и бор) на подручју општине Обреновац (Србија) праћена преко четири маховине (*Bryum argenteum, Bryum capillare, Brachythecium* sp. и *Hypnum cupressiforme).* Распоред просечног садржаја ових метала у маховинама представљен је на картама са тачним подацима и екстраполирано, док се дугорочна (вредности код маховина *Bryum argenteum* и *B. capillare*) и краткорочна (вредности код маховина *Brachythecium* sp. и *Hypnum cupressiforme*) депозиција тешких метала може видети из табела. Подручја територије општине Обреновац са највишим степеном контаминације су посебно означена.