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INVESTIGATIONS ON ECOLOGICAL EFFECTS OF HEAVY METAL POLLUTION IN HUNGARY BY MOSS-DWELLING WATER BEARS (TARDIGRADA), AS BIOINDICATORS

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Abstract: The authors demonstrate a possible relationship between the concentrations of metals (Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) measured in mosses and the composition of Tardigrade species detected in the same samples. Cushions of *Hypnum cupressiforme* were collected at 18 sites distributed in the whole of Hungary to estimate the background air pollution, then analyzed by ICP-AES to determine the concentrations of heavy metals. Data reflect the expected correlation; elevated heavy metal contents decrease the number of water bear species and of specimens, and abundance of Tardigrada depends strongly on air pollution. Higher concentrations of cadmium and chromium seem to have particularly damaging and toxic influence on community structure. As the biologically relevant effects of pollution can only be evaluated by carrying out measurements on the organisms themselves, our method applied species of Bryophyte and of Tardigrade, as bioindicators were appeared to be an adequate method to show the effect of air pollution on abundance of water bears.

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INTRODUCTION

Effects of environmental pollutants can be well estimated by using bioindicators. These living organisms reflect the state of the environment and are suitable for indicating pollution because of their occurrence, absence or presence, frequency, distribution, abundance, vitality, reactions and responses change under certain environmental conditions [2, 4, 9, 11, 13, 14, 18, 19, 22].

During the past few decades, numerous studies have proved that mosses can be profitably used as bioindicators of atmospheric fallout because of their accumulation capability [2, 4, 5, 6, 8, 9, 11, 12, 13, 14, 18, 19, 21, 22]. Biomonitoring with cryptogams (mosses together with lichens) are a suitable, easy and cheap method to detect the possible emission sources of heavy metals, organic or radioactive pollutions. As bryophytes have no root system, their cuticle is lacking or reduced, their leaves are only a one cell-thick-layer, thus they get the nutrients and elements as well as heavy metals mainly from wet and dry depositions. The contact and effect of soil is negligible for most mosses, and contamination by metals from the substrate is insignificant. Mosses are valuable bio-accumulators, because they have high absorbing and ion

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exchange capacities; thus the concentrations of heavy metals in mosses closely correlate to atmospheric deposition. As many species of them are widespread, heavy metal concentrations from a distant area can be compared [9, 13, 18, 19].

Because of these useful characteristics of bryophytes, Nordic Council initiated the mean of moss analysis based on the same method to monitor the atmospheric background deposition of heavy metals in many European countries [18, 19]. A comprehensive moss survey of Europe started from 1990 to estimate the long term, long range and transboundary effects of atmospheric pollution [19]. Until now many studies have been carried out in different countries [2, 4, 5, 6, 8, 12, 18, 19, 21].

As mosses can serve permanently or temporarily habitats for many microbial and animal organisms [7, 10, 15, 16, 20, 23, 24, 25, 26] (e.g. bacteria, protozoa, rotifers, nematodes, tardigrades, collembola, etc.), so investigations of bryofauna dwelling on/in mosses mean a more complex type of monitoring. These studies can give information on populations and can detect the changes in the species structure of communities.

Bryofauna - especially species, whose their whole life cycle pass on mosses, and which take their nutrients from mosses - are able to indicate the biological effects of pollutants deposited on/in mosses before damaging of plant. Such are moss dwelling water bears; most of them eat mosses, except from predators feeding on small organisms of mosses. While mosses have been long used for bioindication purposes due to these advantageous specific features above mentioned, means of water bears in monitoring studies are rarely investigated. Effects of dust and of some environmental contaminants, as sulphur dioxide, DDT, heavy metals on Tardigrada have been already described [1, 10, 15, 16, 20, 23, 24, 25, 26]. Their sensitivity to polluted environment were also established as early as at the beginning of century [7].

Hungary had also joint the Nordic Council international program, and collection and analyses of mosses have been performed in 1995, 1997 and 1998 (data will be published soon). The aim of this paper was to describe the effects of atmospheric heavy metal deposition on the number of species and specimens of moss dwelling water bears (*Tardigrada*) in mosses collected in Hungary within the frame of the above project. *Hypnum cupressiforme* mosses were collected at 18 differently polluted sites distributed in whole Hungary to estimate the backgroung heavy metal pollution.

Figure 1. Area of study. Sampling sites numbered.

Table 1. Number of Tardigrade species and specimens found in 3g (dw) of moss samples collected in different localities of Hungary.

Sampling site (serial number)	Relative limit values of heavy metals		Number of Tardigrade	
	N	% (N = 8)	species	specimens
Bócsa – Bugac (3)	0	0	5	235
Vászoly (18)			8	197
Nemesvid (11)			4	136
Vág (17)			4	114
Sárkeresztúr (15)			5	103
Nagykőrös (10)			6	67
mean:			5.3	142
Gyula (6)	1	12.5	5	84
Győrszentiván (5)			4	57
Paks (13)			4	24
Baracs (2)			4	18
Mosonszentmiklós (9)			5	11
mean: 4.4			39	
Komló (8)	2	25	4	24
Kisszékely (7)			2	17
mean: 3.0			21	
Alsónémedi (1)	3	37.5	3	6
Nyíradony (12)	5	62.5	4 8	
Salgótarján (14)			l	l
mean:			2.5	5
Dunaújváros (4)	7	87.5	1	1
Százhalombatta (16)			1	l
mean:			1	1

Total number of specimens: 1,104.

MATERIALS AND METHODS

Our methods -regarding moss technique- are based on a guideline of an international mapping project initiated by Nordic Council. *(Atmospheric Heavy Metal Deposition in Europe)* [18, 19]. Samples of a widespread moss, *Hypnum cupressiforme* were preferred to collect at 18 differently polluted sites (Fig. 1) distributed over Hungary in the dry autumn of 1997 (11 sites) and 1998 (7 sites).

To estimate background air pollution, the samples were taken mainly from forests, where the sampling sites were not exposed directly to precipitation. The sampling sites were at least 300 m from main roads and built-up areas, and at least 100 m from any roads and buildings. 5–10 subsamples were collected and mixed from each sample point (50 × 50 m).

Table 2. Concentrations of heavy metals (µg.g^{-1} dw) measured in moss samples collected in different localities of Hungary.

Serial number	Sampling site	Sampling date	Element content (µg.g^{-1})							
			Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
1	Alsónémedi	1998.08.20	0.93	3.37	11.9	1644	4.88	22.9	5.22	59.6
2	Baracs	1997.10.03	0.94	2.35	10.3	1299	3.78	11.2	2.26	57.1
3	Bócsa-Bugac	1997.10.03	0.55	1.21	14.0	811	3.30	11.4	2.18	32.3
4	Dunaújváros	1998.08.20	1.83	7.33	13.4	6761	8.57	27.9	7.40	94.0
5	Győrszentiván	1997.10.24	0.61	1.39	12.8	851	4.93	5.4	1.88	60.6
6	Gyula	1997.11.23	0.63	2.98	14.3	1937	4.21	15.7	3.18	82.9
7	Kisszékely	1997.10.19	2.75	3.23	7.6	1744	3.48	9.6	2.97	41.6
8	Komló	1998.08.15	0.58	2.75	10.0	1977	5.51	26.4	3.99	54.1
9	Mosonszent-miklós	1997.10.24	0.40	1.79	11.0	1015	3.22	7.5	2.00	81.2
10	Nagykőrös	1998.08.15	0.32	0.61	10.6	577	3.02	11.6	1.48	45.5
11	Nemesvid	1997.10.25	0.25	0.00	3.8	240	0.97	5.3	0.70	27.3
12	Nyíradony	1997.10.23	0.98	4.01	16.3	2664	4.83	37.5	5.26	70.6
13	Paks	1998.08.15	0.44	1.73	24.3	1413	2.81	15.7	3.57	41.5
14	Salgótarján	1998.07.26	1.09	3.02	8.0	3483	6.15	13.4	4.04	72.2
15	Sárkeresztúr	1997.10.19	0.45	0.07	12.5	608	2.06	19.7	3.25	43.9
16	Százhalombatta	1998.08.20	1.87	6.00	13.9	4339	19.86	37.9	46.69	73.2
17	Vág	1997.11.09	0.69	2.07	12.1	1342	4.64	24.5	4.24	38.1
18	Vászoly	1997.12.16	0.87	1.37	7.1	471	1.79	12.9	2.28	22.6

In fold: above “relative limit value”.

Each sample was analyzed in 3 repetitions. The green parts of mosses were cleaned carefully without washing and then dried at 70ºC. Samples of 0.2 g were digested with 2 cm^3 of cc. HNO_3 and 2 cm^3 of cc. H_2O_2 at 130ºC under pressure in Teflon bombs for 45 min. Then it was filtered through Whatman No. 42 filter paper and brought to a volume of 10 ml with bi-distilled water. Cd, Cr, Cu, Fe, Ni, Pb, V and Zn concentrations were determined by ICP - AES (atomic emission spectrometry with inductively coupled plasma).

Tardigrade extraction was made from the moss samples, as described below:

1. 3 g of air-dry moss sample was soaked in tap water in a Petri dish for 24 h.
2. The moss samples were washed thoroughly 3 times in tap water on a sieve (2 mm mesh).
3. The washing water was collected and poured through on sieve (1 mm mesh) on to a glass filter (40 µm).
4. The remainder of the washing water collected in the filter glass was washed through the centrifuge tubes with tap water (10 ml). Centrifugation 10 min at 2,000 rev/min (g = 450).
5. The supernatants were poured out. Calcium-nitrate solution was added to the sediment (specific gravity 1.35 g.cm^{-3}) and centrifuged for 1 min at 3,000 rev/min (g = 1,050). The supernatants were poured on to a filter glass (40 µm). This procedure was repeated 10 times in fresh calcium-nitrate solution.
6. The remainder of the supernatants collected in the filter glass were thoroughly rinsed in tap water, washed through on to a 5 cm diameter Petri dish. Examination for Tardigrade specimens and eggs was carried out with a stereoscopic microscope at a magnification of × 25–50.
7. The Tardigrade specimens and eggs were preserved in polyvinyl-lactophenol and the species determined (taxonomic analysis) by microscope at magnification of × 400–500 [3, 17].

RESULTS

Concentrations of metals (Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) measured in the different moss samples varied greatly, depending on air quality. Heavy metal ranges were measured in dry weight as follows: Cd 0.25–2.75 µg.g^{-1}; Cr 0–7.33 µg.g^{-1}; Cu 3.8–24.3 µg.g^{-1}; Fe 240–6761 µg.g^{-1}; Ni: 0.97–19.86 µg.g^{-1}; Pb: 5.3–37.9 µg.g^{-1}; V: 0.70–46.69 µg.g^{-1}; Zn: 22.6–94.0 µg.g^{-1} (Tab. 2).

Maximum concentrations were found at the sampling sites surrounding industrial zones. High values of Cd, Cr, Fe, Ni and Zn were measured in Százhalombatta due to its oil-refinery and oil-fuelled power station, and in Dunaújváros because of its steel industry. As our purpose was to measure the background air contamination of the country, the effect of traffic compared with industry was negligible. Thus, Pb levels were found to be relatively low in mosses. Minimum heavy metal levels were

Table 3. Tardigrade species detected in 3 g (dw) of moss samples collected in different localities in Hungary (in order of abundance).

Sampling site	Relative limit values of heavy metals		Tardigrade species (see opposite)																				Number of Tardigrade	
	N	% (n = 8)	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	R	S	T	U	species	specimens
Bócsa–Bugac	0	0	6	222	-	1	-	4	-	-	-	2	-	-	-	-	-	-	-	-	-	-	5	235
Vászoly			2	122	-	9	42	4	-	8	-	-	-	-	-	-	-	9	-	1	-	-	8	197
Nemesvid			9	20	106	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	4	136
Vág			-	34	1	-	-	-	-	-	-	-	-	-	76	-	-	-	3	-	-	-	4	114
Sárkeresztúr			32	1	10	-	59	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	5	103
Nagykőrös			24	-	-	18	-	5	-	-	7	-	-	-	-	-	12	-	-	-	-	1	6	67
Gyula	1	12.5	3	-	23	7	50	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	5	84
Győrszentiván			-	-	5	50	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	4	57
Paks			1	-	-	4	18	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	4	24
Baracs			5	1	-	1	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	18
Mosonszentmiklós			5	-	1	-	-	3	-	-	1	-	1	-	-	-	-	-	-	-	-	-	5	11
Komló	2	25	-	8	-	-	-	-	3	-	-	-	-	1	-	12	-	-	-	-	-	-	4	24
Kisszékely			12	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	17
Alsónémedi	3	37.5	2	-	-	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	3	6
Nyíradony	5	62.5	2	2	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	4	8
Salgótarján			1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Dunaújváros	7	87.5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Százhalombatta			-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Number of occurrence			14	8	8	8	6	5	3	2	2	2	2	2	1	1	1	1	1	1	1	1		
Total number of specimens			105	41	150	93	185	17	5	9	8	3	2	2	76	12	12	9	3	1	1	1		1,104

detected in such unpolluted regions, as Vág, Nemesvid and Vászoly. Further, samples collected from protected natural areas, such as Bugac and Nagykőrös (Hungarian Great Plain) had low heavy metal contents.

A total of 20 Tardigrade species were determined in moss samples. The number of species and specimens varied greatly; in a few instances one individual only of a single species was present only, but in other cases 6–8 species could be found in their hundreds (Tab. 1, 3, 4).

To compare different heavy metal concentrations with the occurrence of Tardigrada-considering European metal averages in mosses [19], we assessed a scale with "relative limit values", above which metal levels might be damaging to the water bears and could decrease their abundance: Cd - 1 $\mu g.g^{-1}$ dw, Cr - 3 $\mu g.g^{-1}$ dw, Cu - 20 $\mu g.g^{-1}$ dw, Fe - 2000 $\mu g.g^{-1}$ dw, Ni - 5 $\mu g.g^{-1}$ dw, Pb - 25 $\mu g.g^{-1}$ dw, V - 5 $\mu g.g^{-1}$ dw, Zn - 55 $\mu g.g^{-1}$ dw.

Concentrations above our "relative limit values" were measured in less than a third of moss samples, except for zinc with a 50% ratio. To evaluate data, moss samples relative to numbers of species and specimens were classified on a scale ranging from 0–8, based on heavy metal contents. In the first category (Tab. 1), all 8 heavy metals were measured below "relative limit values"; the average of number of Tardigrade species was 5, and of specimens - 142. In the next group, only one metal level was found above the limit, 39 specimens of 4 species could be detected. In cases of 2 heavy metals above "relative values", 21 specimens of 3 species on average were determined, while the value with three metals was 6 specimens of 3 species. On average, 5 specimens of 2 species were found in the case of 5 heavy metals, and only 1 individual of 1 species was present in the moss sample, where 7 of 8 heavy metal concentrations were measured above our limit.

DISCUSSION

Heavy metal concentrations varied greatly in our moss samples, depending on environmental impact. High metal levels were definitely connected with emissions of industrial centers. It is well known [2, 4, 5, 6, 8, 9, 11, 12,

Table 4. Tardigrade species detected in moss samples collected in different localities in Hungary (in order of abundance).

	Tardigrade species	Collected by
A	Macrobiotus richtersi	Murray, 1911
B	Diphascon (Diphascon) pingue	Marcus, 1936
C	Hypsibius convergens	Urbanowicz, 1925
D	Isohypsibius prosostomus	Thulin, 1928
E	Macrobiotus hufelandi	Schultze, 1833
F	Isohypsibius bakonyiensis	Iharos, 1964
G	Isohypsibius schaudinni	Richters, 1909
H	Diphascon (Diphascon) bullatum	Murray, 1905
I	Hypsibius dujardini	Doyere, 1840
J	Isohypsibius silvicola	Iharos, 1966
K	Macrobiotus harmsworthi	Murray, 1907
L	Ramazzottius oberhaeuseri	Doyere, 1840
M	Astatumen bartosi	Weglarska, 1959
N	Astatumen ramazzotti	Iharos, 1966
O	Diphascon (Adropion) prorsirostre	Thulin, 1928
P	Diphascon (Diphascon) iharosi	Vargha, 1995
R	Hypsibius pallidus	Thulin, 1911
S	Macrobiotus pallari	Maucci, 1954
T	Milnesium tardigradum	Doyere, 1840
U	Minibiotus furcatus	Ehrenberg, 1859

13, 14, 18, 19, 21, 22], and our results also confirm, that mosses, as bioindicators and bioaccumulators, are suitable organisms for bioindication of atmospheric heavy metal pollution. The main intention of the present study was to show that moss dwelling water bears were also a useful for biomonitoring purposes. They were able to detect and indicate environmental contaminants by the changing of their occurrence, frequency and abundance [24, 25].

The investigation proved that the higher the metal concentrations were measured, the less species and specimens of Tardigrada could be detected in moss samples.

Cadmium and chromium have particularly harmful effects and toxicity for organisms. Cadmium is mainly spread to the environment by the use of phosphate fertilisers and through emissions from metal industry. The cadmium content of mosses is less than 0.3 $\mu g.g^{-1}$ dw in unpolluted areas of Europe [19]. The main source of chromium is also iron and steel mills. The baseline level for chromium in unpolluted regions is lower than 1 $\mu g.g^{-1}$ dw. [19]. These elements could decrease significantly the number of species and of specimens in our samples, mainly, if they were found together with other heavy metals in higher concentrations. Bioindicators generally seem to be the most sensitive to cadmium loading, which by itself can reduce the abundance. The number of Tardigrade species and of specimens may be informative as to potential damage of Cd.

Copper is an essential micronutrient to organisms, but at higher concentrations it is toxic. Low or only mildly elevated levels were found in the samples. Maximum value was not high (24 $\mu g.g^{-1}$ dw) and was measured near Paks, surrounding a nuclear power station. Copper does not seem to reduce the number of water bear species, but it may decrease the number of specimens.

Zinc can have a similar effect, but if this element occurred together with other metals in high quantities, it can be significantly damaging to water bears.

Iron is also an essential element to all organisms. Studies prove that the content of iron in mosses from unpolluted areas are generally lower than 500 $\mu g.g^{-1}$ dw [19]. Our maximum values were found around industrial regions strongly polluted by this element. Concentrations of nickel and of vanadium in mosses vary between 2–4 $\mu g.g^{-1}$ dw in most of Europe [19]. The main emission source of these metals is oil and coal burning and the steel industry. Extremely high nickel and vanadium levels were found in Százhalombatta due to its refinery and oil-fuelled power station.

Effects of heavy metals can be separately estimated only with difficulty in this study. They occured together and none of them reached extreme quantities in the samples in order that the damage and influence of abundance caused by each metal could be established.

If the metal concentrations measured were below “relative limit values”, the average of specimens was about 100, and 5–6 Tardigrade species could be detected in 3 g of mosses (in dry weight) by the method applied.

If only one metal reached a higher concentration in the sample, it could mildly decrease abundance of water bears.

The more the heavy metals are together in elevated quantities in the moss, the less number of indicator species and of specimens found in the sample (Tab. 1, 3, 4).

CONCLUSION

Our method was suitable for showing the ecological effects of heavy metals on abundance of moss dwelling water bears. An increase of heavy metal contents in mosses reduced the number of Tardigrade species and of specimens in the moss samples.

Results indicate that there was no significant effect damaging to the community structure, if there were metal concentrations measuring below “relative limit values”; the average of specimens was about 100, and 5–6 Tardigrade species could be detected in 3 g of moss samples (dw).

In the case of 1–2 heavy metals in elevated concentrations, 3–4 water bear species with a decreasing number of specimens were found. Abundance was greatly reduced due to harmful effect of more heavy metals detected in higher concentrations in the samples.

This method was proved to be a valuable technique for biomonitoring purposes and it was a useful mean for estimating the effects of distance from emission source.

Our results can serve as a database and as limit values for further monitoring investigations for following long-term and long range changes.

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