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ENVIRONMENTAL FACTORS AFFECTING TRACE METAL ACCUMULATION IN TWO MOSS SPECIES

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Abstract: Transplants of two morphologically contrasted moss species (*Hylocomium splendens* and *Pleurozium schreberi*) were exposed at urban site affected by heavy industry. Irrigated moss bag method was implemented and moss samples were placed in two heights above ground (1 m, 2 m). The samples were subsequently analysed for As, Al, Cd, Cr, Cu, Hg, Fe, Ni, Pb, V and Zn content using atomic absorption spectrophotometry. Overall, only few significant correlations between metals accumulation were found when assessed separately, mainly mimicking their prevalence in the environment (e.g. strong correlation between Al and Fe). Simultaneously collected – during two exposition periods – meteorological data were then analysed for possible effects on bioaccumulation patterns by the means of multivariate analysis (PCA, RDA). Models revealing significant dependencies, both on the factors of design (species and treatment) and on the environmental factors, were obtained. Species was by far the most important factor – *Hylocomium splendens* was found to be a better accumulator, placement of the transplants in 2 m was proved to enhance the metal accumulation in comparison to the placement in 1 m above ground. Two of the environmental factors – irradiance and temperature – had an observable positive effect on the metal bioaccumulation in case of Cd, Cu, Fe and V, and negative effect on the bioaccumulation of Hg.

Key words: trace metal, atmospheric pollution, monitoring, mosses, Ostrava

1. INTRODUCTION

Mosses are widely used for biomonitoring of atmospheric pollutants, especially trace metals and metalloids (Falla et al., 2000; Chakrabortty & Paratkar, 2006). In 28 European countries including the Czech Republic, trace metal deposition in mosses has been evaluated through internationally coordinated programmes such as “The international bio-monitoring programme UN ECE ICP-vegetation” (Sucharová & Suchara, 1998; Schröder et al., 2008; Harmens et al., 2008). Major part of monitoring studies concerning mosses is focused solely on passive biomonitoring. This approach provides us with useful information on bioaccumulation in native moss species but it is not exploitable in intensively polluted localities. In such localities native mosses are not present or they are but they have already adapted to pollution and therefore are inert to its effects (Fernández et al., 2000; Fernández & Carballeira, 2000). Also, different climatic conditions of biomonitoring sites may affect the bioaccumulation of elements of interest thus aggravating the potential comparison.

In addition, analysis of possible environment-induced bias of accumulation patterns obtained from active biomonitoring leads us to better understanding of factors affecting the process in native species. This is usually not practicable when applying passive biomonitoring approach, for the material collected is subjected to unknown environmental conditions prior to exposition. The variety of active biomonitoring methods, on the other hand, allows the researcher to ensure the initial conditions of the moss material that is used. Applicability of terrestrial moss in passive biomonitoring have been recently thoroughly discussed by Reimann et al., (2001) and Aboal et al., (2010).

Recently, several active moss biomonitoring techniques have been developed in order to assess trace element deposition. In general, a well-known method is the moss bag monitoring method (Adamo et al., 2003; Fernández et al., 2004; Sun et al., 2009), nevertheless, it is not widely used. This method is being constantly improved (Vázquez et al., 1999; Aboal et al., 2008), but the main disadvantage – in comparison with passive biomonitoring – is that inevitable drying out of moss material (especially in less humid areas) is present. Moreover, some recent studies suggest that devitalisation of samples prior to the exposition reduces the initial variability of accumulation capability of the material as well as it makes the results much more revealing (Giordano et al., 2009; Tretiach et al., 2007). This may be favourable in active biomonitoring survey, nevertheless, it is disadvantageous when assessing the factors affecting bioaccumulation in standard passive biomonitoring. This inherent contrast between the need for the highest accuracy when carrying out of the particular biomonitoring study and the need for the fittest simulation of native conditions (live moss, humid conditions) makes the moss bag monitoring method not exploitable for the assessment of factors altering the native moss bioaccumulation patterns.

Al-Radady et al., (1993) promoted the irrigated moss bag monitoring method that allows us to avoid the disadvantage mentioned above and is exploitable both indoors and outdoors. This technique, where moss material is placed on continuously wet capillary matting and then exposed, was further improved by Fernández & Carballeira (2000). Despite its apparent advantages, it has only been used few times and it has not been incorporated into any continuous biomonitoring programme yet. However, it has been used by Aničić et al., (2009) recently.

Another major disadvantage of using moss for biomonitoring of atmospheric trace elements lies in underestimation of weather conditions affecting the bioaccumulation. It is known that these conditions influence accumulation rates – as pointed out by Pesch & Schröder (2006) – though particular effect is yet unknown. Microclimatic conditions of native mosses are difficult to follow, and when using transplants, heed is usually not paid to weather variability at all. The effort to analyse at least some of the factors – but not those of weather – was made by Fernández & Carballeira (2000) by taking canopy, substrate and season into account. Understanding the factors that may affect bioaccumulation is crucial for normalization of the biomonitoring techniques which have been disparate and rather confusing so far. The study hereby presented is an attempt to enhance this understanding.

2. MATERIAL AND METHODS

Two species of terrestrial mosses used as transplants, *Hylocomium splendens* and *Pleurozium schreberi*, were collected from presumably unaffected site in Protected Landscape Area of Beskydy Mts. (northeast of Czech Republic). Both species are abundant in the Czech Republic and in the site of interest in particular. Moreover, they are commonly used in passive biomonitoring and thus were subjected to various comparisons in terms of bioaccumulation (Halleraker et al., 1998). Samples were thoroughly washed with distilled water and their 4-cm apical segments were excised based on the recommendations by Sidhu & Brown (1996) and several others (Paulissen et al., 2004; Toet et al., 2006; Nguyen-Viet et al., 2007). The segments were subsequently placed on capillary matting as in Fernández & Carballeira (2000) and wrapped in 0.8 aperture nylon net to prevent them from being blown away by the winds. The total exposed area was 18.5 × 13 cm.

Capillary mattings were placed on the lid of plastic box filled with 2 litres of demineralized water (to avoid other uptake of elements under study than from the atmosphere) with both ends drawn through slits in the lid. The water was refilled when necessary.

The boxes with both moss species were mounted on the construction of measuring equipment Czech Hydrometeorological Institute (CHMI) uses to monitor weather and pollution conditions. The boxes were placed at two heights, 1 and 2 meters above ground, in order to assess the effect of height on accumulation rates. Boxes with samples were prevented from rain by metal construction, which was part of the equipment, so there would be no precipitation-driven bias of the outcomes.

The site of the experiment was located in a heavily polluted area of so called Black Triangle II. in the northeast of the Czech Republic (Markert et al., 1996) in the city of Ostrava which is known for its heavy industry (mainly coal mining and steelworks). The pollution coming from traffic is still increasing (Houthuijs et al., 2001) and the city in general has the highest air pollution levels in the Czech Republic (Hunova, 2001).

Transplants were exposed twice for one month period; during the first month, fourteen-day sampling period was used, in the subsequent exposition, seven-day sampling period was applied. The samples obtained were put in polyethylene bags and forthwith moved to a laboratory where they were washed for 30 s in distilled water to remove particles adhering to the surface. Longer rinsing is known to cause leakage of already accumulated elements (Wells & Brown, 1990; Fernández et al., 2010).

Then the samples were put into covered Petri dishes and dried at 50°C to constant weight – this temperature was chosen to prevent the loss of volatile elements that can be induced by higher temperatures (MacNaeidhe, 1995) – and stored in exsiccator until the time of the analysis. Total number of 28 samples was collected, 10 in the first exposition, 18 in the second exposition, including blinds of both species.

The samples were analysed in the accredited laboratory of Nanotechnology Centre of VŠB-TUO. The concentrations of Al, As, Cd, Cr, Cu, Fe, Ni, Pb, V and Zn were determined after total decomposition of samples in acid mixture using atomic emission spectroscopy with inductively coupled plasma (AES – ICP); SPECTRO Ciros Vision was used. Instrument was operated at 1.25 kW. The liquid samples were nebulised by cross nebuliser, than the aerosol of the sample pass through Scott spray chamber to axial plasma torch. Measured intensities of spectral lines of elements intensities were evaluated by Smart Analyzer software. The Merck calibration standards (concentration of elements 1g/L) were used for preparation of multielement and multipoint calibration standards. The concentration of Hg was determined using Advanced Mercury Analyser AMA 254. The instrument is based on thermal decomposition of the sample and collection of the evolved Hg vapour on a gold amalgamator. The estimated measured uncertainties were Al – 4.2 %, As – 10 %, Cd – 6.5%, Cr – 12.5 %, Cu and Fe – 2.0 %, Ni – 6.3 %, Pb – 8.5 %, V – 4.8 %, Zn – 6.2 % and Hg – 12 %.

Simultaneously, meteorological data were collected at the site of the experiment by CHMI, temperature (7:00, 14:00, 21:00 and average), air humidity (7:00, 14:00, 21:00 and average) and irradiance (kJ m^{-2}) for every day of exposition.

The effects of treatment and weather conditions on element accumulation in moss material were evaluated by multivariate gradient analyses (principal component analysis and redundancy analysis) in CANOCO for Windows 4.5 (Braak & Smilauer, 1998).

3. RESULTS AND DISCUSSION

Overall concentrations showed considerably different patterns of bioaccumulation among treatments. *Hylocomium splendens* showed higher and sturdier accumulation rates. Any correlation found in accumulation of a particular element between one treatment and the other was noted in Table 1. It is apparent that in case of majority of the measured elements, there is a lack of correlation between two treatments even within one species. However, there is a clear positive correlation between accumulation rates of Fe and Zn in both heights of *H. splendens* and between Cr accumulation rates in both heights of *P. schreberi*. A notable negative correlation was found also between accumulation rates of Cu in both of the species when placed at 1 m height.

Table 1. Pearson correlation coefficient of element accumulation among treatment

	hs1hs2	ps1ps2	hs1ps1	hs2ps2
Fe	N	N	N	0.92
Cr	N	0.76	N	N
Cu	N	N	-0.83	N
Zn	0.86	N	N	-0.76

hs1, hs2 – *H. splendens*, 1 m and 2 m above ground height respectively, ps1, ps2 – *P. schreberi*, 1 m and 2 m above ground height respectively. Critical values (six degrees of freedom) are 0.73, 0.81 and 0.92 for $\alpha < 0.1$, 0.05, 0.01 respectively

Table 2 shows an example of correlations between particular elements concentrations, in this case those of *H. splendens* exposed in 1 m above ground height; among most of the treatments, strong correlation of Al and Fe was apparent (which is striking in its solitude even when the data are pooled) but the interpretation of this fact is rather difficult. According to Hashimoto et al., (1992), anthropogenic source of both elements in atmospheric fly ash are mainly iron works, smelters and mining, all of them can play some role in the site of interest. Fossil fuel combustion (including transportation) (Twardovska et al., 2003) as well as agriculture driven erosion from the nearby fields (WHO, 2003) that are largely of cambisol type may be important nonetheless. Ongoing acidification of Czech soils may contribute to availability of Al and Fe in eroded particles (Hruška & Krám, 1994; Hruška et al., 2002).

Multivariate analysis of data was carried out. In prefatory analysis (CA), linear response of accumulation was observed; therefore principal component analysis (PCA) and redundancy analysis (RDA) were implemented. Principal component analysis (Fig. 1) displayed clear distinction of accumulation values in two species-determined groups, regardless of the treatment.

Table 2. Significant Pearson correlation coefficients of element accumulations, *H. splendens*, 1 m above ground height

	Hg	**As**	**Cd**	**Pb**	**Ni**	**Al**	**Cr**	**Cu**	**Fe**	**V**	**Zn**
Hg	-										
As	N	-									
Cd	N	N	-								
Pb	N	N	N	-							
Ni	N	N	N	N	-						
Al	-0.87	N	N	N	N	-					
Cr	-0.74	0.87	N	N	N	0.92	-				
Cu	N	-0.78	N	N	N	N	N	-			
Fe	-0.94	0.79	N	N	N	0.93	0.90	N	-		
V	N	N	N	N	N	N	N	N	N	-	
Zn	N	0.78	N	N	N	N	0.81	N	N	N	-

Critical values (six degrees of freedom) are 0.73, 0.81 and 0.92 for α < 0.1, 0.05, 0.01 respectively; N – no significant correlation

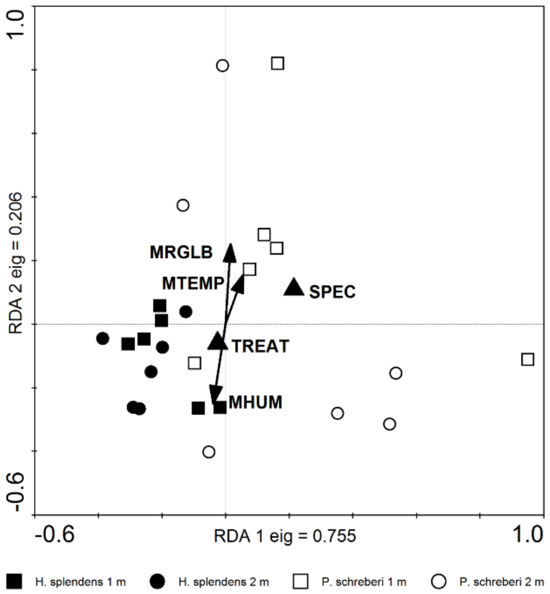

Figure 1. PCA biplot with variables of species and treatment as nominal variables.

Samples of *H. splendens* are aggregated predominantly in quadrant III, in contrast to *P. schreberi* samples being rather scattered in all the quadrants. Intrinsic variability of *P. schreberi* is thus much higher than the one of *H. splendens*. *P. schreberi* samples regression coefficients are far larger as well; its samples thus tend to have greater – positive – response when independent variable grows in value. When environmental factors are projected to the diagram – with treatment and species as nominal variables – it is apparent that apart from species-related division, there is an irradiance-humidity related second axis, alongside which the *Pleurozium* samples have much more extreme coordinates as well.

First two axes of this model account for 96.1 % of data variance (first axis itself 75.5 %), which is present in CANOCO tabular summary of PCA analysis (Table 3). Amongst all the factors, species was by far the most correlated with the first axis (r = 0.687). The rest of the variables correlated mainly with the second axis, as indeed indicated by the diagram.

Table 3. PCA summary

	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalues:	0.75	0.20	0.02	0.01
Species-environment correlations:	0.697	0.527	0.611	0.462
Cumulative percentage				
of species data:	75.5	96.1	98.9	100
of species-environment relation:	84	97.1	99.5	100
Sum of all eigenvalues:	1			
Sum of all canonical	0.437			

Concluding from the redundancy analysis outcomes, variance explained by variables of interest is quite high – 43.7 % with Monte Carlo permutation test (4999 permutations under full model) revealing the significance of the first and all canonical axes at α = 0.0236 and α = 0.0364, respectively. The first axis itself accounted for 91.7 % of the variance explained by environmental variables.

Species is by far the most determining factor in the model affecting mainly accumulation of As, Al, Ni and Fe whose scores align strongly with the first axis that has the strongest correlation with this factor.

In the biplot (Fig. 2), the factors are set in pattern clearly dividing vector of humidity from vectors of temperature and irradiance. Factor of species is perpendicular to the line they create and treatment slightly aligns to the vector of humidity. Scores of the remaining element accumulation were affected by more factors at the same time. Particularly remarkable are alignments of Zn and Pb and those of Cu and Cr. Relative intactness of scores of V accumulation should be pointed out as well.

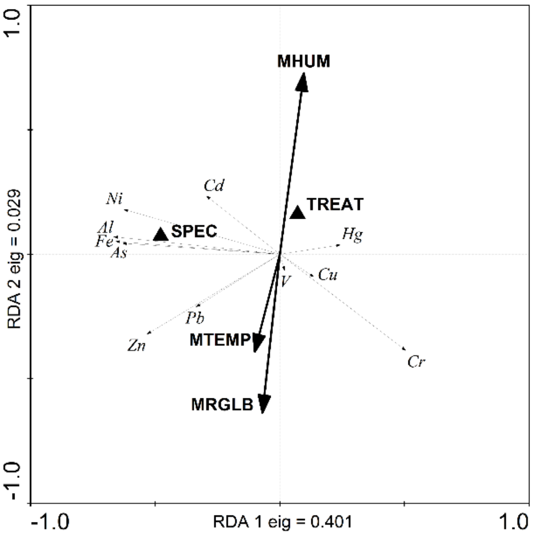

Figure 2. RDA biplot with variables of species and treatment as nominal variables.

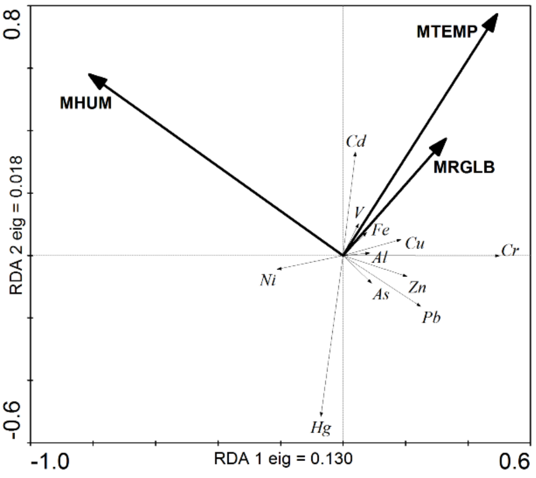

Figure 3. RDA biplot with variables of species and treatment as covariates.

Overall, the model describes the main gradients of the factors accounting for the accumulation patterns but, as previously indicated, the most significant variables in the model are those arising from the design (species, treatment). It is, however, not an easy task to simply leave them aside, since they apparently do have some important impact on the accumulation, though their influence bonds with the effect of environmental variables.

Since the effect of environmental factors was of the highest concern, the variables of species and treatment were taken as covariates in consequent analysis, so that their effect could be partialled out. In this case, logarithmic transformation of accumulation data was applied in order to reduce extreme values and therefore lessen disproportionality of accumulation between common and less common elements (Dragović & Mihailović, 2009).

According to the diagram (Fig. 3), the first RDA axis explains 13 % variability, all canonical axes then account for 15.8 % of the variability in bioaccumulation (Table 4). Accumulation patterns of particular elements were affected by the variables varying in degrees of synergy. The exceptions are V being in a tight relation with temperature, Fe with irradiance and Hg in negative correlation with all the selected variables.

Table 4. RDA summary.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues:	0.13	0.018	0.011	0.227
Species-environment correlations:	0.664	0.629	0.413	0
Cumulative percentage variance				
of species data:	20	22.7	24.4	59.3
of species-environment	82.3	93.3	100	0
Sum of all eigenvalues:	0.65			
Sum of all canonical eigenvalues:	0.158			

Monte Carlo permutation test summary is presented in Table 5, the significance of the first RDA axis was at α = 0.0236 and of all canonical axes at α = 0.0364, the model can be thus deemed to be representative.

Table 5. Monte Carlo permutation test summary

Test of significance of first canonical	0.13
F-ratio:	4.509
P-value:	0.0236
Test of significance of all canonical	0.158
F-ratio:	1.931
P-value:	0.0364
(4999 mutations under full model)	

4. CONCLUSION

The most important factors altering the bioaccumulation patterns of the moss material were found to be those of species and treatment. These factors are usually the ones followed in passive biomonitoring studies for they tend to be focused on one species and above ground height is always zero. Nevertheless, it is indicated that collection of more species (usually applied when one is not present in the site of interest) must be carried out carefully and with respect to possible bias of such kind.

Meteorological factors under study, irradiance, temperature and humidity, were found to have effect on the bioaccumulation as well. It is therefore implied that it is not convenient to ignore meteorological factors when pursuing a biomonitoring survey, as even the three environmental factors under examination can alter the accumulation rates in a significant way. However, the results obtained show that it is not optimal to assess these factors separately since every element is affected by their specific combination. Consequently, the knowledge of possible environment-caused bias needs to be obtained and partialled out of the trace element accumulation models in the future. Monitoring of the three factors studied (temperature, humidity, irradiance) may claim more effort from the researcher, however, it is technically accessible and provides us with the information needed not only for particular biomonitoring studies but moreover, for further improvement of biomonitoring techniques in general.

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