Use of Lichen and Moss in Assessment of Forest Contamination with Heavy Metals in Praded and Glacensis Euroregions (Poland and Czech Republic)

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Abstract The concentrations of selected metals—Cr, Ni, Cu, Zn, Cd, and Pb—were determined in the samples of *Hypogymnia physodes* lichen and *Pleurozium schreberi* moss collected in Polish and Czech Euroregions Praded and Glacensis. More specifically, the samples were collected in Bory Stobrawskie, Bory Niemodlińskie, and Kotlina Kłodzka (Poland) and in Jeseniki (Czech Republic). The concentration of metals in the samples was measured using the atomic absorption spectrometry (flame AAS technique and electrothermal atomization AAS technique). The results were used to calculate the comparison factor (CF) that quantifies the difference in concentration of a given bioavailable analyte × accumulated in lichens and mosses: $CF = 2 (c_{x,lichen} - c_{x,moss}) (c_{x,lichen} + c_{x,moss})^{-1}$. The values of CF greater than 0.62 indicate the most probable location of heavy metals deposited in the considered area. In this work, the method was used to show a significant contribution of urban emissions to the deposition of heavy metals in the area of Bory Stobrawskie and in the vicinity of Kłodzko City.

Keywords Heavy metals · Biomonitoring · Lichen · Moss · Comparison factor

1 Introduction

The living organisms that react to changes in the chemical composition of the environment are more and more often employed in the assessment of the environmental pollution (Wolterbeek 2003; Markert et al. 2003; Smodiš et al. 2004; Markert 2007). Good examples are the use of lichens in the Integrated Monitoring of Natural Environment System and the use of biological material in the quality evaluation of natural waters (Directive 2000/60/EC).

Lichens and mosses dominate among organisms used for evaluation of pollution by atmospheric aerosols (Fraenzle and Markert 2007; Markert et al. 2008). Most often, the biomonitoring is done by analyzing the chemical composition of lichens and mosses collected from their natural environment (e.g., Loppi and Bonini 2000; Szarek-Łukaszewska et al. 2002). The accumulation of micro- and macroelements in biomonitors exposed to polluted atmosphere is measured with instrumental analytic methods (Frontasyeva et al. 1995; Markert 1996).

In urban and industrial areas, the exposition techniques often used are those that utilize living

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organisms transferred from the relatively pristine areas (e.g., Culicov and Yurukova 2006; Kosior et al. 2010). The time-controlled exposition of these organisms provides more information on the deposition of pollutants in such areas.

The results of biomonitoring are usually interpreted with the comparative analysis (Daillant et al. 2003; Bergamaschi et al. 2004) and other statistical methods: factor analysis (Jasan et al. 2004), regression analysis (Riga-Karandinos and Karandinos 1998), principal component analysis—PCA (Sorbo et al. 2008), cluster analysis (Fuga et al. 2008), and time series—TS (Reis et al. 2003). A good example is the application of data clustering and PCA to the interpretation of results on the concentration distribution of elements accumulated in mosses exposed in the urban area of the Gdańsk City in Poland (Szczepaniak et al. 2007). The work evaluated the usability of dry and living moss *Sphagnum palustre* in the biomonitoring studies.

In order to assess the sources of analytes accumulated in mosses or lichens, the results are analyzed using the enrichment factor (EF), which compares the relative concentration of an analyte accumulated in lichens or mosses to that in soil. The reference elements— scandium and aluminum—rarely appear in atmospheric aerosols from anthropogenic sources (Bargagli et al. 1995; Bergamaschi et al. 2005). The sum of concentrations of the rare-earth elements was also suggested as a suitable reference quantity (Chiarenzelli et al. 2001). By definition, the enrichment factor close to unity (EF=1) indicates that a given element originates from soil (Chiarenzelli et al. 2001). However, some authors argue that the values of EF<10 correspond to the measurement background; hence, the values do not show whether the source of analytes accumulated in lichens and mosses was the soil or some other remote source of emission (Chiarenzelli et al. 2001; Freitas et al. 2007). Disagreement between the real source of the analytes and the remote sources indicated by the high values of EF can result from the uncertainty of the measuring method, from inhomogeneity of the chemical composition of the samples used, and from the very definition of EF. Enrichment factors are determined from the concentrations of analytes in the soil, lichens, and mosses; but mosses and lichens accumulate only bioaccessible forms of the pollutants. Moreover, bio-accumulation is affected by the chemical characteristics of compounds which contain the examined analytes (mainly the ability of compounds to form ionic forms),

as well as by the affinity of the ionic forms of compounds to the sorption structures of mosses and lichens.

The idea of using lichens and mosses as bioindicators and biomonitors is still a matter of discussion between scientists. The authors argue on the sorption preferences of lichens and mosses, both living under natural conditions and then transferred for exposition from the ecologically pristine regions to the polluted areas (Kashulina et al. 1998). The research showed that under natural conditions, mosses preferred to accumulate Al, Ca, Cr, and Ni (Gałuszka 2005) or Al, Cr, Fe, Mn, Ni, and Ti (Bargagli et al. 2002); while lichens preferred Cu, Hg, Na, Ti, and Zn (Gałuszka 2005) or Cd, Cu, Hg, Pb, V, and Zn (Bargagli et al. 2002). Metals such as Cd, Fe, and Pb were accumulated comparably (Gałuszka 2005). On highly contaminated soils, mosses usually accumulated more analytes than lichens (Reimann, et al. 1999). The opposite tendency was observed in regions with volcanic exhalations that enriched the atmospheric aerosol (Loppi et al. 1999).

This work presents a statistical assessment of pollution in the considered regions and introduces a new evaluation method which is based on the comparison of analyte contents in epiphytic lichens and epigeal mosses living close to each other. The epigeal mosses accumulate comparable or larger amounts of elements from the soil, including the previously deposited anthropogenic elements. On the contrary, the epiphytic lichens accumulate comparable or larger amounts of analytes that had not been locally translocated to the atmospheric aerosol from the soil (Kłos et al. 2010). In epigeal mosses, the sorption of microelements transported from remote sources is limited by the easily available macroelements from the soil, which are less available for the epiphytic lichens. The influence of macroelements on sorption of microelements was discussed elsewhere (Hyvärinen and Crittenden 1996; Hauck et al. 2002; Kłos et al. 2005, 2007). The presented research was part of the Operational Programme for Transboundary Cooperation between Czech Republic and Poland, entitled "Biomonitoring research in Polish–Czech transboundary region."

2 Materials and Methods

The research was carried out in the forested areas of the Praded and Glacensis Euroregions—Bory Stobrawskie

(BS), Bory Niemodlińskie (BN), and Kotlina Kłodzka (KK) in Poland and Jeseniki (J) in the Czech Republic. The selected areas had similar tree cover (pine and spruce forests), but differed in the geological structure of soil. In Bory Niemodlińskie and Bory Stobrawskie, the sand soil was covered with humus layer at least 3 cm thick. The geologically inhomogeneous soil in Kotlina Kłodzka and Jeseniki was typical for the Sudety Mountain range. Samples of the epiphytic lichen *Hypogymnia physodes* and the epigeal moss *Pleurozium schreberi* were collected from April to September 2010. Figure 1 shows the localization of sampling sites.

2.1 Collection and Preparation of Samples

The samples of lichen and moss were collected at the sites shown in Fig. 1, from six points at each site. Lichen was collected from horizontally growing branches of spruce and larch trees, at 1.5–2 m above the ground level. Lichen samples from six points at a site were mixed together. The moss samples were mixed together accordingly. The total mass of samples collected at each site was 20 g of lichen and 20 g of moss. The samples were cleaned from mechanical impurities and dried at temperatures not higher than 303 K. Dried samples were homogenized in agate mortars and subjected to AAS measurements.

2.2 Analysis

The prepared samples were analyzed independently in the accredited laboratory of the Health Institute in Hradec Králové (Zdravotního ústavu v Hradci Králové—ZÚHK) and at the Chair of Biotechnology

and Molecular Biology, University of Opole (UO). Metals were determined with the flame AAS technique (F-AAS). Concentrations of Cr smaller than the detection limit of F-AAS were determined with the electrothermal atomization AAS technique (ETA-AAS). Table 1 shows the details of analytical procedures, reagents, and equipment used.

2.3 Quality Assessment/Quality Control

For the proper quality assurance and control in the analysis of metals in lichen and moss samples, the method quantification limit values were determined both in the ZÚHK (μg g^{-1}: 0.13 (Cd), 0.5 (Cr, Cu, Ni, Zn, Pb), and 0.025 (Cr, ETA-AAS)) and in the UO (μg g^{-1}: 0.5 (Cu, Zn, Cd), 0.7 (Ni), and 1.3 (Pb)) laboratories.

The reference material BCR-482 lichen from the Institute for Reference Materials and Measurements, Belgium was also analyzed. The results of analysis are compared to the certified values in Table 2.

Maximal deviations between the mean values of metal concentrations determined in the collected samples in ZÚHK and in UO were (respectively for lichen and moss): Ni (9.7% and 7.5%), Cu (8.7% and 3.9%), Zn (5.8% and 9.3%), Cd (9.6% and 10.3%), and Pb (7.1% and 10.5%). Chromium was determined only in the ZÚHK laboratory.

2.4 Statistical Analysis and Data Interpretation

All statistical computations were done using the R language (R Development Core Team 2009). R is a free software environment for statistical computation

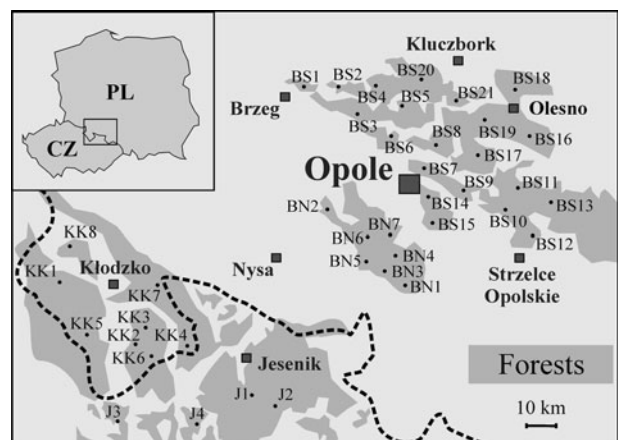**Fig. 1** Map with localization of sampling sites

Table 1 Details of analytical procedures, reagents, and equipment

Description	ZÚHK	UO
Mass of mineralized samples	1.0 g	1.0 g
Type of digestion	Mineralization by dry MLS-1200 MEGA	Microwave digestion MARS-X
Acid used for digestion	HNO_3 (65%) and H_2O_2	Aqua regia
Type of AAS	SOLAAR M5, AVANTA SUMA, from UNICAM	SOLAAR 969 from UNICAM
Standards for calibration	ANALYTIKA Ltd. (CZ)	ANALYTIKA Ltd. (CZ)
Reagents	MERCK	Scharlau

and graphics. The capabilities of R can be extended through packages for specialized statistical techniques, graphical devices, programming interfaces, and import/export function to many external data formats.

The results of research were analyzed using the comparison factor (CF) defined as a ratio of a difference between the analyte concentrations in lichen ($c_{i,lichen}$) and in moss ($c_{i,moss}$), to the average of these concentrations: $CF = 2 \cdot (c_{i,lichen} - c_{i,moss}) \cdot (c_{i,lichen} + c_{i,moss})^{-1}$.

Kłos et al. (2010) showed that $CF > 0.62$ determined the deposition zones of heavy metals which had been introduced to the atmosphere by primary emission and not by the secondary enrichment of atmospheric aerosol with local contaminants from the soil. Obviously, this interpretation applies to the bioavailable forms of considered analytes. The limiting value of CF was determined by the analysis of a data set containing the concentrations of heavy metals accumulated in samples of epiphytic lichens and epigeal mosses from our own measurements and from 257 published measurements in various regions of Europe. In the range $-0.62 < CF < 0.62$, which

contained 67% of all results, a statistically significant relation $c_{i,moss} = c_{i,lichen}$ was valid (Kłos et al. 2010).

3 Results and Discussion

Table 3 shows the mean concentrations of analytes accumulated in lichen and moss samples collected in BS, BN, KK, and J. The table includes the uncertainties of the analytical method, $\pm SD_{AM}$ (%), determined from the results for samples collected at sites BS1, BN1, KK1, and J1 (Fig. 1). For this purpose, five samples of lichen and five samples of moss were collected at each site. Each sample was independently cleaned, homogenized, mineralized, and analyzed. The determined values of $\pm SD_{AM}$ are comparable with the corresponding values obtained from a study carried out in 2006 in BS (Kłos 2009).

The distribution of concentrations of each metal accumulated in lichen and moss samples collected in the forested areas BS, BN, KK, and J was described by specifying the maximum and the minimum values,

Table 2 Comparison of measured and certified data for BCR 482 lichen ($\mu g\ g^{-1}$)

Element	BCR-482 lichen	AAS (UO; %)				AAS (ZÚHK) ND[b] (%)
	Certified value ($\mu g\ g^{-1}$)	±Uncertainty	Mean	±SD	D[a]	
Cr	4.12	0.15	n.d.	n.d.	n.d.	20
Ni	2.47	0.07	2.16	0.32	−13	20
Cu	7.03	0.19	6.54	0.18	−7.0	10
Zn	100.6	2.2	93.9	2.5	−6.7	10
Cd	0.56	0.02	0.50	0.04	−6.3	20
Pb	40.9	1.4	37.6	0.9	−7.6	20

[a] Deviation, the instrumentally measured/certified value expressed (in %) between the results obtained by AAS and the certified value

[b] Uncertainty of the analytical method (in %) determined for various organic reference materials, as specified in the laboratory accreditation

Table 3 Heavy metal content in lichens (L) and mosses (M; µg g^{-1})

Sample no.	Cr		Ni		Cu		Zn		Cd		Pb	
	L	M	L	M	L	M	L	M	L	M	L	M
BS1	2.00	0.600	3.00	1.91	6.25	5.03	61.5	23.0	0.41	0.174	13.5	2.42
BS2	0.88	0.412	1.14	14.0	3.49	3.23	58.7	26.4	0.42	0.183	9.55	2.23
BS3	0.65	0.484	1.08	0.645	3.25	4.22	65.5	36.9	0.44	0.285	9.35	2.50
BS4	0.80	0.390	1.92	0.580	5.72	7.09	63.6	48.0	0.55	0.377	9.81	5.34
BS5	0.38	0.445	1.18	0.715	4.55	9.11	64.0	41.3	0.98	0.402	6.14	2.67
BS6	1.30	0.500	0.78	0.722	5.23	5.02	75.0	40.3	0.64	0.882	13.7	3.58
BS7	1.42	1.20	0.78	0.572	4.19	5.14	71.3	50.3	0.65	0.780	13.0	5.41
BS8	1.10	0.513	1.52	0.279	3.45	2.98	104	31.8	0.63	0.433	13.1	3.74
BS9	1.66	0.910	2.23	1.72	4.64	7.36	134	43.5	1.03	0.485	14.1	4.02
BS10	1.18	0.875	0.94	0.965	4.33	5.12	97.0	54.8	1.36	0.822	17.2	6.20
BS11	0.50	0.380	0.85	0.538	4.48	5.15	68.9	115	1.04	0.485	11.8	3.21
BS12	1.03	1.25	2.21	0.980	4.37	4.55	152	34.4	2.23	0.428	25.2	8.88
BS13	1.65	1.03	1.08	1.06	3.28	3.78	114	41.9	1.46	0.843	22.0	8.07
BS14	1.30	1.23	0.94	0.959	5.59	8.42	104	58.5	1.16	0.683	28.6	7.51
BS15	1.58	0.525	1.09	1.71	6.05	10.4	133	74.9	1.50	0.798	34.7	11.9
BS16	1.53	1.00	1.47	1.27	4.59	10.1	129	63.7	1.25	0.823	20.3	6.01
BS17	1.28	1.30	1.20	1.00	4.56	6.66	120	113	0.85	0.248	17.4	7.17
BS18	1.80	1.00	1.59	1.13	8.61	7.21	110	54.2	0.87	0.373	17.3	1.38
BS19	1.50	0.850	1.29	0.625	4.36	5.64	125	49.3	0.97	0.415	14.8	2.28
BS20	1.30	1.55	0.95	1.79	4.07	4.01	63.8	26.6	0.39	0.0975	9.85	1.83
BS21	2.28	0.950	1.23	0.627	4.52	6.67	65.7	18.4	0.71	0.295	16.0	4.09
BN1	1.25	1.25	2.01	1.43	5.10	7.55	60.0	49.5	3.00	3.00	20.1	17.2
BN2	1.20	2.60	1.53	2.98	6.05	14.2	76.5	81.5	1.90	1.25	15.3	35.7
BN3	1.25	1.50	1.52	1.95	4.40	9.35	84.5	89.5	1.35	5.50	15.2	23.5
BN4	1.00	0.95	1.43	1.50	6.85	7.35	44.5	87.0	1.95	1.50	14.3	18.0
BN5	1.65	3.70	2.31	1.39	12.6	9.90	85.5	82.0	1.25	1.80	23.1	16.7
BN6	2.45	4.30	2.34	2.49	7.30	13.7	75.0	94.0	1.75	1.65	23.4	29.9
BN7	1.90	2.35	2.36	1.60	7.35	7.70	64.5	106	3.55	2.15	23.6	19.2
KK1	1.58	0.800	1.30	0.880	4.34	5.76	141	36.2	1.26	0.160	13.6	5.81
KK2	2.50	4.69	1.83	4.24	5.80	5.95	80.7	39.7	0.72	0.665	12.5	2.55
KK3	2.89	3.81	2.13	2.05	6.85	7.45	109	47.8	1.07	0.563	10.8	2.89
KK4	2.27	3.33	1.35	1.81	3.70	7.05	47.5	48.5	1.64	0.795	11.6	7.61
KK5	1.87	3.23	1.33	1.30	5.15	3.65	55.9	32.7	0.81	0.595	6.27	4.31
KK6	2.64	3.43	2.03	1.63	7.05	4.65	79.4	33.0	1.52	0.258	14.4	3.68
KK7	2.26	9.47	3.91	4.20	16.9	11.9	100	50.7	1.68	0.920	18.0	4.84
KK8	1.76	4.45	1.54	1.42	7.28	13.0	84.8	34.1	0.66	1.03	10.9	4.33
J1	3.50	5.25	2.89	2.53	6.35	12.2	125	70.0	1.35	1.45	28.9	25.3
J2	3.45	3.30	2.38	1.56	7.40	10.1	102	55.5	1.05	1.10	23.8	15.6
J3	3.10	3.00	0.740	1.19	6.35	12.6	99.0	170.5	1.20	1.40	7.40	11.9
J4	3.10	3.45	1.26	1.69	7.30	5.50	71.0	51.0	1.40	0.950	12.6	16.9
± SD$_{AM}$ (%)	20	20	18	16	10	12	7.7	10	9.9	12	8.4	13

the upper and the lower quartiles, the median value, and the outlying points (McGill et al. 1978). Graphical representation of this description is shown in Figs. 2 and 3.
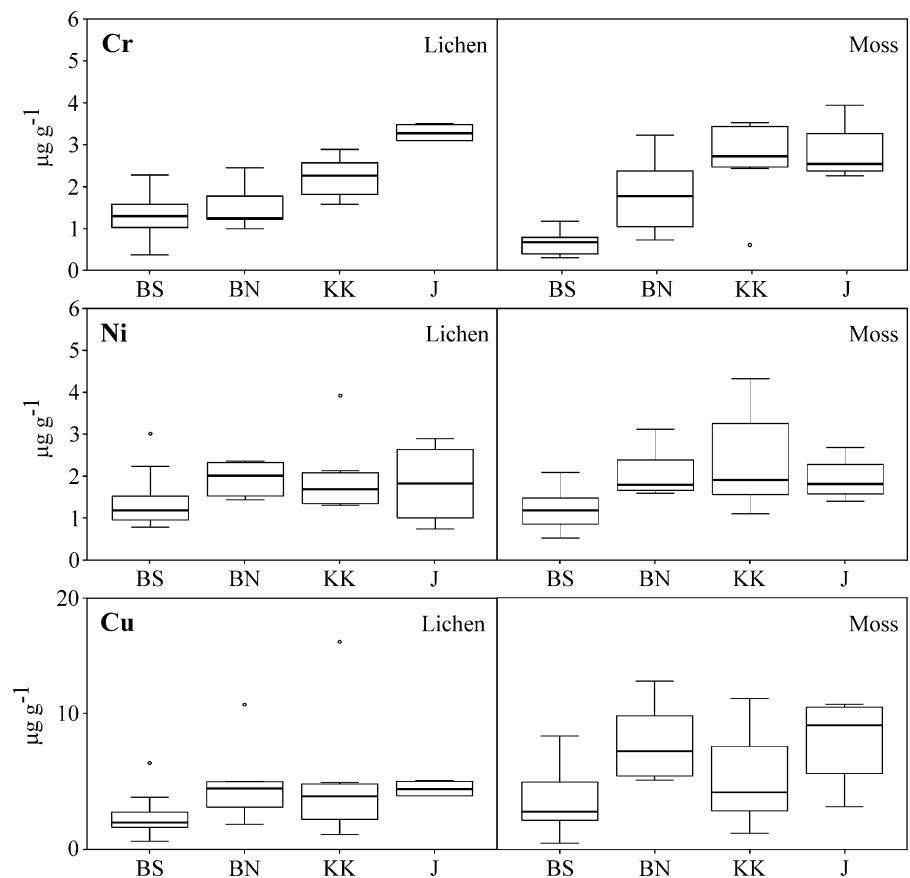
The statistical description presented in Figs. 2 and
3 indicates differences in concentration of analytes in
the samples of lichen and moss collected in BS, BN,
KK, and J. The concentration of Cu, Zn, Cd, and Pb
was higher in mosses from BN and J. Lichen from
these areas contained higher concentration of Cu, Cd,
and Pb. Plots in Figs. 2 and 3 indicate significant
inhomogeneity of concentrations of some analytes in
lichen and in moss samples from some areas, e.g., the
inhomogeneity of Zn and Pb concentrations in lichen
samples from BS.

When assessing the pollution of a specified area by
determination of analytes in lichen and moss samples,
it is important to compare the obtained results to the
results from similar monitoring in the other regions of
Europe. The available data for lichen are incomplete,
since the systematic research has been done only in
Portugal. On the other hand, many countries carry out
systematic determination of heavy metals in mosses
every 5 years, starting from 1990. In 2005, a more or
less intensive research was done in 28 European

countries (Harmens et al. 2010). The obtained results,
presented as minimum/mean/maximum values in
$\mu g\ g^{-1}$, were: Cr (0.28/1.81/12.7), Ni (0.24/1.74/
50.6), Cu (0.69/6.25/38.8), Zn (5.2/33.0/77.4) Cd
(0.031/0.18/0.57), and Pb (0.67/4.19/23.6). For the
Opole region (Fig. 1), which was included in the
research, the mean values in $\mu g\ g^{-1}$ were: Cr (2.74),
Ni (2.56), and Zn (64.3; Cu, Cd, and Pb were not
determined). These values compare well to the results
contained in Table 3 and presented in Figs. 2 and 3.
The contents of Cd determined in the present work
were much higher than the maximum values observed
in the aforementioned 2005 project. This is in good
agreement with results from the joint project of the
Vysehrad Group countries (Hungary, Czech Republic,
Poland, and Slovakia) which showed high accumula-
tion of Cd in mosses (0.55–7.12 mg g^{-1}) in the Upper
Silesia, an industrial region in Poland located close to
the area monitored in the present work (Suchara et al.
2007). High accumulation of Cd was also observed in
the Czech Republic, and in the Holy Cross Mountains

Fig. 3 Statistical parameters of distributions of Zn, Cd, and Pb concentrations in lichen and moss samples collected in BS, BN, KK, and J
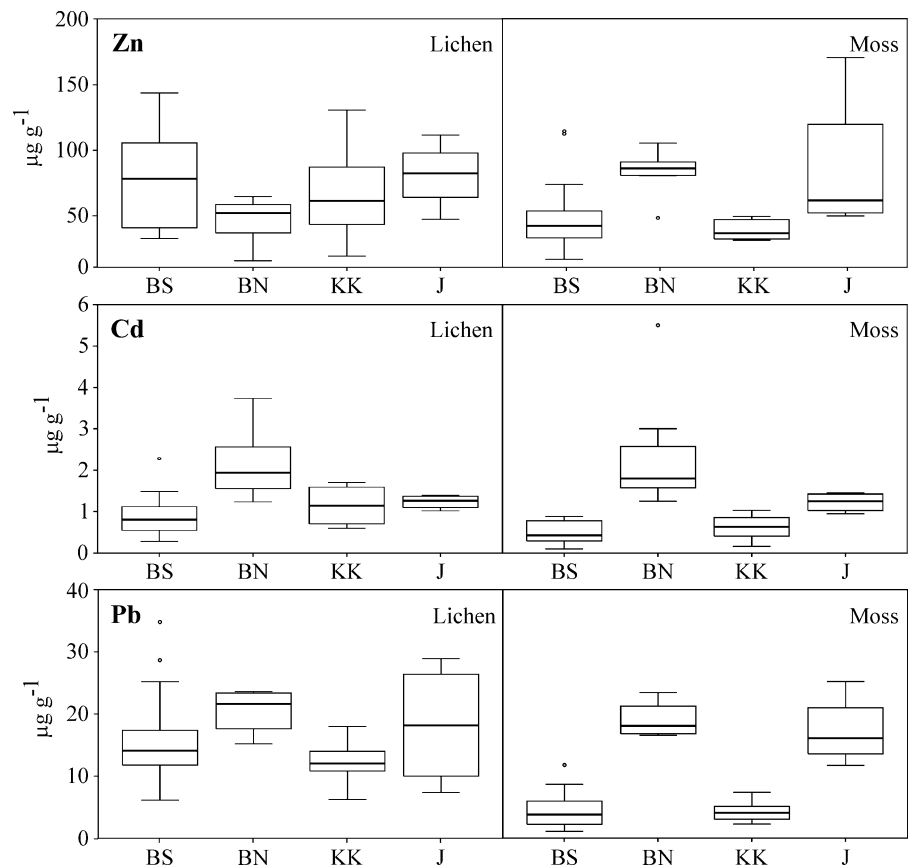
in South-Central Poland, where 0.8–1.1 mg g^{-1} of Cd was determined in moss samples (Gałuszka 2005).

The comparison of concentrations of heavy metals accumulated in lichen or in moss allows the general assessment of pollution in a given region, while the kind and the quantitative proportion of these metals indicate their sources (Lucaciu et al. 2004). The evaluation of current deposition of pollutants originating from the remote sources of emission requires additional analysis, such as the TS analysis mentioned in Section 1. The inflowing character of pollutants can also be judged from the highly uncertain enrichment factor.

The deposition of bioavailable forms of heavy metals injected to the atmosphere with primary emissions was evaluated using the determined values of comparison factor, CF. The maps in Fig. 4 show the areas for which CF>0.62.

Plots in Fig. 4 indicate significant deposition of all considered metals except Cu in the area north-east to the city of Opole (the BS region), and around the city

of Kłodzko (the KK region). This distribution resulted from the local meteorological conditions.

North and south winds, which dominate in the vicinity of the Opole City, transport urban and industrial pollutants from the city and from the local-district towns Brzeg and Strzelce Opolskie directly to the BS area. In Kłodzko, the south winds slightly prevail, but windless weather occurs often—e.g., for about 26.1% of time in 1971–2000 (Ecophysiographic Development 2005). Therefore, the deposition of metals in the KK area results rather from the local emissions.

The main sources of Zn emission in the BS area are cement works located directly in the Opole City, in the Górażdże village located about 20 km south-east from Opole, and in Strzelce Opolskie located 30 km south-east from Opole. The recorded average emission of Zn was 0.293 kg per 1 Gg of manufactured cement (Olendrzyński et al. 2003). The cement works incinerate their wastes on-site, which can increase the emission of considered

analytes. Other serious emission sources include urban emissions resulting mostly from combustion of fossil fuels, vehicle emissions, and emissions from numerous minor industrial facilities located in the area. For instance, in the Brzeg town, there is a tannery—a potential emitter of Cr.

The concentration of industrial facilities in the area of Kotlina Kłodzka is low, so the main sources of Zn, Cd, and Pb emission are household facilities and local electric power stations fired with fossil fuels.

The determined CF values do not indicate any current deposition of metals in the BN and J areas. However, these areas exhibited high concentrations of most considered analytes accumulated in moss (Figs. 2 and 3), which may indicate earlier deposition and contamination of soil. Before the 1990s systemic transformation in Poland, the BN area contained a military training ground as well as small industrial facilities with now inestimable emission of pollutants, such as a porcelain factory in Tułowice.

The distribution of sites with CF>0.62 does not indicate the considered research region is affected by remote emissions of heavy metals from large industrial centers—Górnośląski Okręg Przemysłowy in Poland, located 150 km to the east and south-east, and Mostecká Basin in the Czech Republic, located 200 km to the west.

4 Conclusions

The presented results of biomonitoring studies utilizing lichens and mosses provided significant information on the level of environmental pollution, directions of pollutant transport, and bioavailability of pollutants.

The comparison of results obtained for the Praded and Glacensis Euroregions with the corresponding results from other European countries showed that the concentrations of considered analytes accumulated in mosses were of similar magnitude, with the exception of cadmium. The highest concentrations of cadmium in mosses, which were determined in Bory Niemodlińskie, exceeded by several times the maximal concentrations determined in other regions of Europe.

This work presents a new method of analyzing the biomonitoring results, which utilizes the CF defined as a ratio of a difference between the analyte concentrations in lichen and in moss, to the average of these concentrations. It was hypothetically assumed that the values CF>0.62 indicate that the deposited bioavailable forms of heavy metals were introduced to the atmosphere with primary emissions, and not by the secondary enrichment of the atmospheric aerosol with local emissions from soil. Several quoted studies on sorption mechanisms and on the comparison of concentrations of analytes accumulated in lichens and mosses support this hypothesis. However, further support is required,
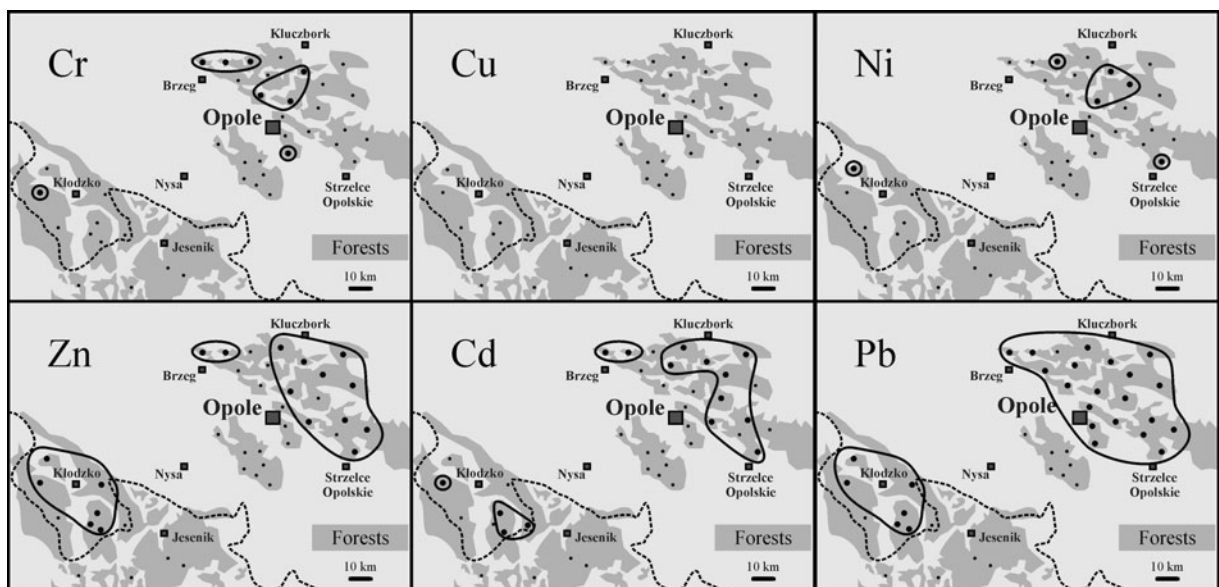

Fig. 4 Areas of the research region for which the comparison factor for specified metals exceeded 0.62 (CF >0.62)

stemming from studies in other geographical regions. In this work, the method was used to show a significant contribution of urban emissions to the deposition of heavy metals in the area of Bory Stobrawskie and in the vicinity of Kłodzko City.

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