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Bioindicative comparison of the fern *Athyrium distentifolium* for trace pollution in the Sudety and Tatra mountains of Poland

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Abstract Concentrations of the elements Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were measured in the fronds of the fern *Athyrium distentifolium* from the Sudety and Tatra mountains (Poland). The *A. distentifolium* sites in the Sudety mountains which were influenced by long-range metal transport from the former Black Triangle were distinguished by the principal component and classification analysis (PCCA). These sites were situated on the west side slopes of one of the ranges in the Sudety mountains (within a 150-km radius of the heart of the former Black Triangle) at an altitude of 700 m asl, and exposed to prevailing winds. This most affected area had significantly higher foliar concentrations of Cu, Cr and Ni which are typical for long-range transported airborne elements occurring in coal fly ash emitted by lignite combustion industry.

Keywords Monitoring · Metal · *Athyrium distentifolium* · Transboundary pollution

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Introduction

The determination of concentrations of metals in the environment and variants of biota responses to various doses of anthropogenic pressure factors requires constant monitoring of the increasing number of pollutants and it is an important part of the understanding of biogeochemical processes and gauging ecosystem health (Schilling and Lehman 2002; Solenská et al. 2006; Zechmeister and Hohenwallner 2006). The elemental composition of plant tissues in nutrients as well as non-essential elements is of interest for ecology and environmental protection, and it is used in the assessment of air and soil pollution (Fernández et al. 2000; Markert et al. 2003; Conti 2005; Kłos et al. 2010). Ferns have often been associated with contaminated soils, particularly with mining operations. Some fern species, such as

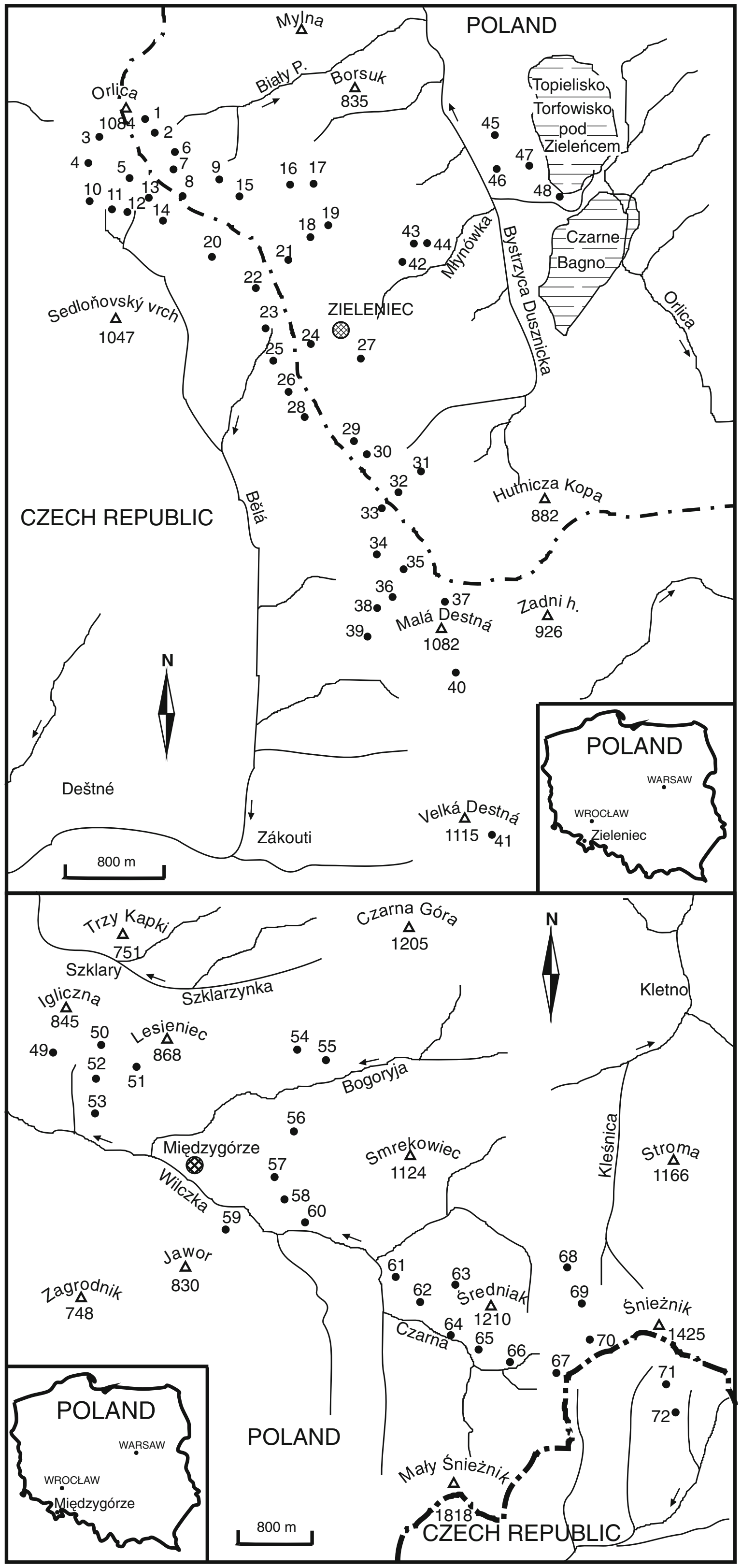

Fig. 1 Location of the *A. distentifolium* sampling sites (*solid circle*) in the Sudety mountains

Athyrium filix-femina, are important bioindicators of metalliferous soils (Shan et al. 2003; Cornara et al. 2007; Kachenko et al. 2007). Vacek et al. (1999) found that the share *Athyrium distentifolium* as a dominating forest understory species in the Karkonosze mountains increased over a period of air pollution stress. Among ferns, there are also species which hyperaccumulate metals (Shan et al. 2003). This ability makes them ideal environmental indicators of contamination, especially that ferns can tolerate a wide range of environmental extremes (Chang et al. 2009).

The former 'Black Triangle', the border area between Poland, Germany and the Czech Republic, is one of the

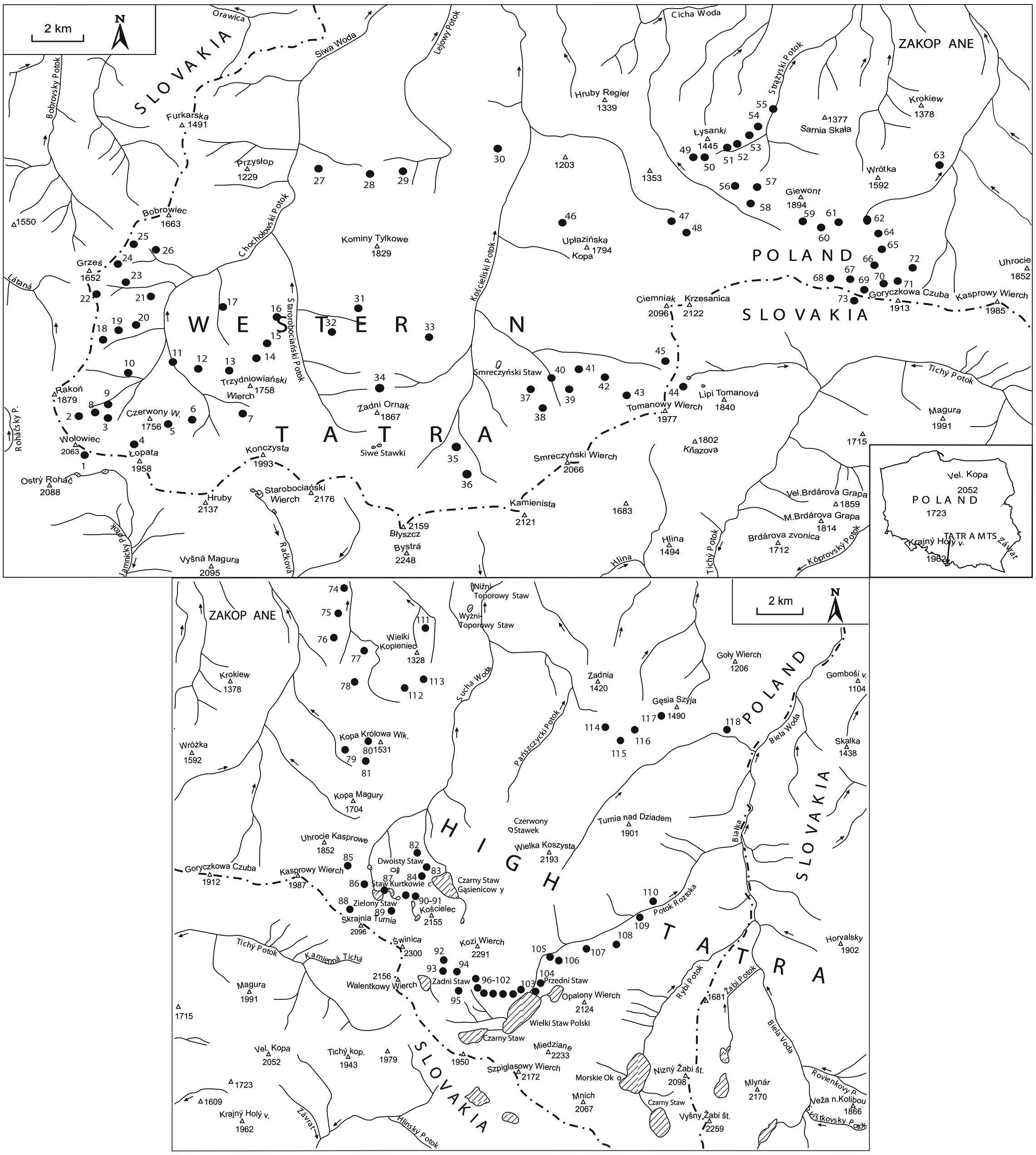

Fig. 2 Location of the *A. distentifolium* sampling sites (*solid circle*) in the Tatra mountains

most heavily industrialised and polluted areas in Europe. The 'Black Triangle' was for decades a source of metals and acidifying species in choking coal dust emitted by electric power and heating plants, ferrous and non-ferrous smelters, cement and glass production plants and mining facilities which could be found within a 150-km radius of the heart of the Black Triangle. These industrial activities caused the most damage, especially at altitudes above 700 m asl (Markert et al. 1996a; Świetlicki and Krejci 1996; Fanta 1997). Despite overall improvements in pollution control during the 1990s, the former Black Triangle region is still a source of air pollution (Bridgman et al. 2002). In terms of industry structure, the Czech and Polish parts of this region are still highly affected by mining industry in the lignite basins. Nowadays, chemical industry and electric power plants are the largest sources of pollution affecting the state of the environment in the region (Abraham et al. 2004; Andruszkiewicz 2008).

The Sudety mountains are situated within the area influenced by the former Black Triangle exhausts, exposed to prevailing, humid maritime air masses from the West, which enables efficient transport of air pollution. The Tatra mountains and the adjacent lowlands along the Polish–Slovak border which form part of the Biosphere Reserve, created in 1993 in the context of the UNESCO MAB program, are one of the most protected areas in the country (Bytnerowicz et. al. 2003).

Therefore, it was of interest to compare the accumulation abilities of long-range transported elements, especially metals, by the fern *A. distentifolium* between the Sudety mountains and a nature reserve of the Tatra mountains. This species is very common in both areas. We tested the hypothesis that *A. distentifolium* is a bioindicator of long-range transboundary pollution.

Materials and methods

Sampling design

In the Polish Sudety mountains (Fig. 1) and Tatra mountains (Fig. 2), a total of respectively 72 and 118 sampling sites with *A. distentifolium* were selected. There were no any local pollution sources where samples were collected. At each site, five frond samples were collected within a 25×25 m square. Each sample consisted of a mixture of three sub-samples. As required by the rules set by the Environmental Monitoring and Data Group (Markert et al. 1996b) and the European Heavy Metal Survey (Harmens et al. 2004), the collected ferns had not been exposed directly to canopy throughfall. The total number of fern samples was $N=190\times5=950$.

Plant analysis

Before analysis, the plant material was washed for a few seconds in distilled water and dried at 50°C to constant weight. Fern samples were homogenised in a laboratory mill. Samples (300 mg dry weight, in triplicate) were digested with nitric acid (ultra pure, 65%) and perchloric acid (ultra pure, 70%) in a CEN Corporation MARS5 microwave. After dilution to 50 mL, the plant and soil

Table 1 Analysis of certified reference material

Bush branches and leaves DC73348 LGC

Element	Certified (μg g^{-1})	Found (μg g^{-1})	Recovery (%)	CV (%)
Cd	0.140±0.06	0.134±0.003	95.71	2.2
Cr	2.30±0.30	2.28±0.06	99.13	2.6
Cu	5.20±0.50	5.05±0.11	97.12	2.2
Fe	1020±67.00	1041±21.00	102.06	2.0
Mn	58.00±6.00	58.97±1.43	101.67	2.4
Ni	1.70±0.40	1.68±0.06	98.82	3.6
Pb	7.10±1.10	6.81±0.21	95.91	3.1
Zn	20.60±2.20	20.79±0.30	100.92	1.4

CV coefficient of variance

Table 2 Range, mean and SD of concentration (milligrams per kilogram on a dry weight basis) of elements in *A. distentifolium* of the Sudety and Tatra mountains

	Sudety range	Mean	SD	Tatra range	Mean	SD	*t* test	Probability level
Cd	0.04–2.2	0.36	0.3	0.10–0.38	0.2	0.1	4.35	<0.001
Cr	0.09–5.97	0.73	1.0	0.03–0.93	0.4	0.2	4.12	<0.001
Cu	4.55–29.6	9.0	4.8	2.1–7.1	4.3	1.3	10.15	<0.001
Fe	57–461	85	64	41–103	68	16	2.75	<0.050
Mn	58–1,030	243	227	8–225	103	65	6.26	<0.001
Ni	0.79–18	3.6	3.0	0.01–2.3	0.8	0.5	9.73	<0.001
Pb	0.73–8.3	2.4	1.5	0.6–3.0	1.9	0.6	2.94	<0.050
Zn	13–118	28	20	6–28	14	4.1	7.22	<0.001

$t_{0.05(205)}$ tabular=1.97

digests were analysed for Fe, Mn and Zn using Flame and Cd, Cr, Cu, Ni and Pb were analysed using Furnace Atomic Absorption Spectrophotometry AVANTA PM GBC instrument. All elements were determined against standards (Atomic Absorption Standard Solution from Sigma Chemical Co.) and blanks containing the same matrix as the samples and were subjected to the same procedure. All results for plants were calculated on a dry weight basis.

The accuracy of the methods applied for the determination of elements in plants was checked by analysis of Certified Reference Materials. We used DC73348 LGC standards of bush branches and leaves as certified reference materials. The coefficient of variance was calculated for the determined concentrations of the elements in the reference material. The results are shown in Table 1.

Statistical analysis

Differences between sampling sites in terms of concentrations of the elements in ferns were evaluated by ANOVA on log-transformed data to obtain a normal distribution of features according to Zar (1999). The normality of the analysed features was checked by means of Shapiro-Wilk's *W* test, and the homogeneity of variances was checked by means of Bartlett's test (Zar 1999; Sokal and Rohlf 1994). Concentrations of the elements in ferns from the Sudety and the Tatra mountains were examined with the *t* test applied to log-transformed data.

The matrix of Cr, Cu Ni and Zn concentrations in plant samples from 72 sampling sites in the Sudety mountains as well as the matrix of concentrations of the same four elements in plant samples from all the 190 sites from the Sudety and the Tatra mountains were subjected to ordination to reveal possible gradients of element levels, using principal component analysis (PCA) and classification analysis to reduce the amount of data and stabilise subsequent statistical analyses (Vaughan and Ormerod 2005; Martin and Falko 2009). PCCA has previously been applied in environmental sciences (Deng et al. 2007; Otto et al. 2008). PCCA is based on PCA and offers a practical and clear classification of a set of data for a number of objects (Legendre and Legendre 1998). Plot of PCCA ordination of the plant samples and projections of element concentrations in ferns on the factor plane provides information about similarities between samples and shows correlations between the original variables and the first two factors and practically and clearly classifies a set of data for a number of

Table 3 Concentrations (milligrams per kilogram) of elements in ferns from literature

	Cd	Cr	Cu	Fe	Mn	Ni	Zn
1	0.3–0.8	0.4–1.7	6.4–13	58–115	60–1,062	2.2–11	11–58
2	0.4–5.1	2.2–2.8	6.2–18	130–198		26–34	15–45

1 Kozanecka et al. (2002) in *D. filix-mas* and *P. aquilinum* from pollution-free regions in Poland, *2* Cornara et al. (2007) *A. filix-femina* from serpentine and metalliferous soils

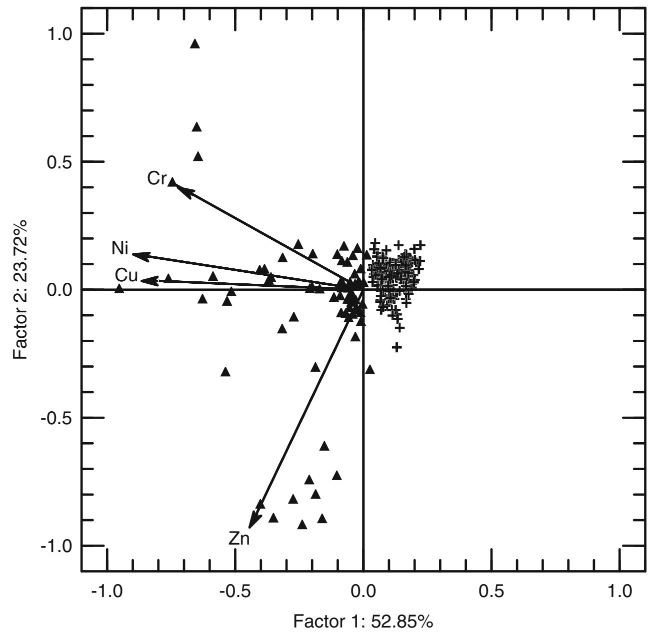

Fig. 3 Ordination of the 72 sampling sites from the Sudety (*solid triangle*) and 118 sites from the Tatra mountains (*plus sign*) by PCCA based on concentrations of Cu, Cr Ni and Zn in *A. distentifolium* and projection of the metal concentrations in ferns on the factor plane

objects (Legendre and Legendre 1998). In the PCCA ordination, Cr, Cu Ni and Zn were included because the concentration of these elements in *A. distentifolium* made it possible to clearly distinguish ferns between the Sudety and the Tatra mountains.

The calculations were done with the Statistica version 8.0 (StatSoft, Inc 2011).

Results and discussion

The ranges of concentrations of metals in ferns from the Sudety and the Tatra mountains are displayed in Table 2. The ferns tested differed significantly in terms of the concentrations of the elements assessed (ANOVA, $p=0.05$).

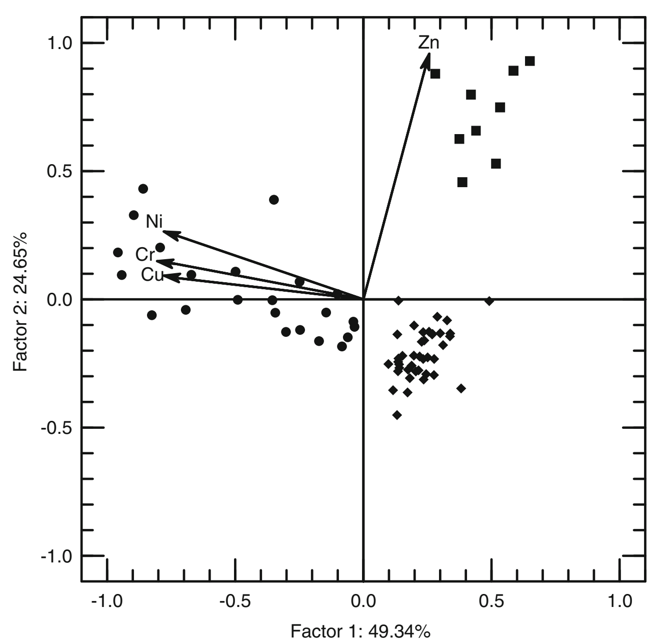

Fig. 4 Ordination of the 72 sampling sites from Sudety mountains by PCCA based on concentrations of Cu, Cr, Ni and Zn in *A. distentifolium* and projection of the metal concentrations in ferns on the factor plane

A. distentifolium from the Sudety mountains contained significantly higher concentrations of all the elements (Table 2) than the same species from the Tatra mountains.

According to Kozanecka et al. (2002), *Dryopteris filix-mas* and *Pteridium aquilinum* from pollution-free regions in Poland contained lower upper limit concentrations of Cd, Cr, Cu, Fe, Ni and Zn and comparable concentrations of Mn than *A. distentifolium* from the Sudety mountains and higher concentrations of these elements than *A. distentifolium* from the Tatra mountains (Table 3). *A. filix-femina* from serpentine and metalliferrous sites (Cornara et al. 2007) contained higher concentrations of Cd and Ni and lower concentration of Cr, Cu, Fe and Zn than *A. distentifolium* from the Sudety mountains (Table 3).

The results of the PCCA ordination of sampling sites from both the Sudety and the Tatra mountains are shown in Fig. 3. The first principal component discriminates between ferns growing in the Sudety mountains (negative scores) and those growing in the Tatra mountains (positive scores). Ferns from the Tatra mountains form a very homogeneous group in respect to metal concentrations distinguishing them from the Sudety mountains ferns. The projection of the variables on the factor plane indicates that factor 1 is negatively related to Cr, Cu and Ni while factor 2 is negatively related to Zn. Ferns growing in the Sudety as well as those growing in the Tatra were differentiated by the value of factor 1, which relates negatively to Cr, Cu and Ni.

The results of PCCA ordination of sampling sites from the Sudety mountains are shown in Fig. 4. Three groups of ferns are clearly distinguished. The first group (solid circle) of elemental concentration collections projects more closely with Cu, Cr and Ni. The second group (solid square) of elemental concentration collections projects more closely with Zn. The third group (solid diamond) relates to positive scores of factor 1. When both Figs. 3 and 4 are compared, it is noted that collections projecting more closely with Cu, Cr and Ni are formed by ferns from the same sites (3, 4, 5, 10–14, 20, 22, 23, 25–26, 28, 33–37, 38–41) on both ordering types. These ferns are from sites from the most affected zone, situated on the west side slopes of the mountain range (Fig. 1) at an altitude above 700 m asl, exposed to prevailing winds and within a 150-km radius of the heart of the former Black Triangle (Markert et al. 1996a; Fanta 1997). According to Mateu et al. (1996), Świetlicki and Krejci (1996), Riga-Karandinos and Karandinos (1998), Sawidis et al. (2001) and Sarris et al. (2009), among others, Cr, Cu, Ni and Zn are all significantly enriched in coal fly ash emitted by lignite combustion industry and transported in large amounts over long distances in the air. The area of the Czech Republic influenced by the former Black Triangle emission was characterised by the highest deposition loads of Al, Cd, Co, Cr, Cu, Fe, Ni, Pb, V and Zn (Sucharová and Suchara 1998). Ferns of the sites tested projecting more closely with Zn (9, 15, 21, 24, 31, 57–60) are also the same for PCCA presenting both the Sudety and the Tatra mountains (Fig. 3) as well as for PCCA classifying the Sudety mountains (Fig. 4). These are ferns collected in sites which might be additionally influenced by gasoline exhausts as Zn from motor oil additives, tyres, brake liners, metal corrosion and road surface material is one of the main polluting metals in the roadway environment (Denier van der Gon et al. 2007). Since leaded petrol has been phased out, Zn has been proposed to be a more reliable tracer of motor vehicle emissions than Pb (Oliva and Rautio 2004). The third group is composed of ferns from sites classified as unaffected (Fig. 4) in which concentrations of Cr, Cu, Ni and Zn are lower than in other sites.

Conclusion

The *A. distentifolium* sites which were influenced by long-range metal transport were distinguished by the principal component and classification analysis (PCCA). These sites were situated on the west side slopes of one of the Sudety mountain ranges (within a 150-km radius of the heart of the former Black Triangle) at an altitude of 700 m asl and exposed to prevailing winds. This most affected area was distinguished by significantly higher foliar concentrations of Cu, Cr and Ni.

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