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Original Research

Comparison of Chemical Composition of Two Co-Occurring Chasmophytes, *Asplenium ruta-muraria* L. (Pteridophyta) and *Cymbalaria muralis* Gaer., Mey. & Scher. (Spermatophyta) from Habitats Differing in Air Pollution

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Abstract

Concentrations of the elements N, P, K, Ca, Mg, Na, S, Fe, Mn, Zn, Cu, Cd, Pb, Co and Cr were measured in two co-occurring wall-fissure plants, fern *A. ruta-muraria* and seed plant *C. muralis*, collected at 14 sites in urban, urban-rural and rural areas in Lower Silesia (SW Poland). Airborne particulate matter < 10 µm (PM_{10}) was used as indicators of metal contamination at the site where they were growing. Principal component analysis showed that in contrast to *C. muralis, A. ruta-muraria* from urban sites contained higher levels of Ca than those from urban-rural and rural sites. However, *A. ruta-muraria* from urban sites contained lower levels of K than those from urban-rural and rural sites. The Zn and Cd concentrations both in *A. ruta-muraria* and *C. muralis* were higher in urban sites, in the direct vicinity of the river, than those at other sites in the study area. PM_{10} influenced some features of foliar elemental composition of the two species in different ways. In *A. ruta-muraria*, Ca, Zn and Cd increased in proportion to PM_{10}, while levels of K decreased and in *C. muralis*, Cd increased in proportion to PM_{10}, while levels of Ca and Cr decreased.

Keywords: wall-fissure plants, macronutrients, heavy metals, airborne particulate matter, PM_{10}

Introduction

The chemical composition of plants reflects, in general, the elemental composition of the growth media. The growth media, nutrient solution or soil are the main sources of elements in plants [1, 2]. Few data are available on chemical composition of vascular plants able to grow in fissures or cracks of man-made walls [3, 4]. The man-made walls represent extreme environments, with edaphic factors such as the low availability of water and nutrients, and exposure to wind and sun [5]. The wall-fissure vegetation consist of species specialized to grow in fissures or cracks of the rocks (chasmophytes), as well as species that grow on the ground [6, 7]. Plants usually take more than 10 years

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to colonize a wall [8]. When plants grow in fissures or cracks, the gap between these cracks and fissures may be increased further on account of the increase in the volume of the roots and root tip pressure. However, the roots tend to grow by taking advantage of zones that present lower resistance to growth. In the case of man-made walls, usually it is the layer of mortar that joins stones or bricks that provides the weak zones [9]. Climate and atmospheric pollution contribute to the crumbling of mortar and increase in size of pre-existing cracks and fissures in the wall [8]. The wall-fissure plants contribute to chemical weathering of the substrate. This is affected by the release of exudates that react chemically that the substrate or may result in the erosion of the surface on account of uptake of calcium or other ions present in the substrate [9]. For wall-fissure plants, exposure and moisture are the most crucial environmental factors on vertical walls. Water supply in fissures or cracks is limited mainly because of small soil volume [6, 10]. The nutrient supply represents an additional serious problem next to the water supply [7]. Dust along with dead organic matter may induce the formation of soil in fissures or cracks of walls [7-9]. Dust is an important source for many trace metals [2]. Metallic pollutants as well as gaseous deposition can profoundly alter the chemistry of soil and it can affect some features of tissue elemental composition of wall-fissure plants. The heavy metals may cause inhibition of macronutrient absorption and imbalances in the chemical equilibrium in plant tissues. The imbalanced reactions between elements may cause chemical stress in plants [2].

In Europe, fern *Asplenium ruta-muraria* and seed plant *Cymbalaria muralis* are reported as two of the few typical chasmophytic species of the walls that survive in urban areas [7, 10-12]. At some localities, the two species grow closely together on the same vertical wall, and they are sometimes the dominant species in wall plant communities [12, 13]. All the plants growing on vertical wall fissures face similar environmental conditions [14]. Thus, they have to cope with limited nutrient supply and other resources as well as with aerial pollution. In urban areas and rural areas, *A. ruta-muraria* has been found to accumulate high concentrations of iron in fronds [3, 4]. Nothing is known about the uptake of nutrients or heavy metals by *C. muralis*. Co-occurring species usually have different means of nutrient resource use, and they are able to employ different strategies for survival when exposed to heavy metals [15, 16]. Study of their tissue chemical characteristics, such as macronutrient and heavy metal concentrations provides useful information about differences in a species ability to use nutrient resources and species response to environmental pollution. In this context, the aims were:

(1) to determine the macronutrient and heavy metal concentrations in two co-occurring chasmophytes, *A. ruta-muraria* and *C. muralis*, from sites that ranged from polluted urban areas to relatively uncontaminated rural areas,
(2) to verify whether a correlation exists between element concentration in chasmophytes and pollution state of the site where they are growing, and which tissue elements depend on particulate pollution such as PM_{10} (particulate matter) and
(3) to identify the major differences in features of tissue elemental composition between these two species.

Materials and Methods

Study Species and Sampling Sites

A. ruta-muraria (wall rue) is a small (5-15 cm), delicate wintergreen calciphilous fern. It is one of the most widespread and common ferns in Europe. The natural habitat of wall rue is fissures of calcareous rocks, but it has been able to expand its range by colonizing mortar in man-made old walls [5]. Wall rue is concentrated to north-facing or otherwise shaded sides of walls and also the growing places on rocks of this fern are mostly somewhat protected against direct insolation [17].

C. muralis (ivy-leaved toadflax) is a small size, creeping flowering plant with thread-like stems (sometimes up to 60 cm) and tiny leaves. It is not frost tender. It is a plant native to Mediterranean Europe but widely naturalized elsewhere. The natural habitat of ivy-leaved toadflax is shady rocks and woods, somewhat calcicole. It is naturalized on man-made walls, or more rarely, on stony ground, e.g. in old quarries [14]. It is the most characteristic plant of old towns and castles in Europe [15]. It is cultivated for ornament [14].

Fourteen sites were chosen, differing for type of environment, building material of the walls on which chasmophytes were grown and atmospheric pollution grades (Table 1). All sites are situated in southwest Poland. These sites are located at about 100-680 m above sea level. Six sites were classified as 'urban' sites (u1-6), four of them (u1-4) were situated in the town centre, on the walls along the river, in close proximity to roads, in a zone where metal contamination can be carried by heat and power generating plant activity, while the other two (u5-6) were situated in the suburbs, in close proximity to roads. Five sites classified as 'urban–rural' and these sites (ur1-5) were situated in little towns, in average population density, in close proximity to roads. Three sites were classified as 'rural' and these sites (r1-3) were situated in a mountain zone between 415 and 680 m a.s.l. at low population density, two of them (r2 and r3) were in close proximity to roads. Both plant species at all the sites are growing at the top of the wall except one rural site (r2). In r2 site, chasmophytes are growing at the bottom of the wall. Plant samples were collected from small fissures on mortar of vertical old walls. The fissures, although sometimes quite deep, contained very little soil, eliminating the possibility of soil element analysis. Plant samples were collected in August 2004. At all the locations, the two plant species grew together on the same wall at a distance from about 2-10 m and in most of the sites were the sole vascular plants.

Airborne Particulate Matter

Airborne particulate matter (having diameter of less than 10 μm, measured in $\mu g\ m^{-3}$ air per day, i.e. PM_{10}) was used as an indicator of metal contamination at the site.

Table 1. Characteristics of sampling sites.

Site (coordinates) Type of area Abbreviation No.	Type of wall building material	Exposure	Environmental conditions	Mean PM_{10} ($\mu g\ m^{-3}$)
Wrocław (51°06'N 17°01'E)				
urban				
u1	brick	N	Plants grow on riverside wall 20 m from road; area with tree cover	37.8
u2	brick	NE	Plants grow on riverside wall 10 m from road; area with tree cover	37.8
u3	granite	N	Plants grow on riverside wall 2 m from road; area without tree cover	37.8
u4	brick	NE	Plants grow on riverside wall 2 m from road; area without tree cover	37.8
u5	brick	NW	Plants grow on high boundary wall 2 m from road; area with tree cover	30.7
Oleśnica (51°21'N 17°23'E)				
urban				
u6	brick	NE	Plants grow on town wall 3 m from road; area with tree cover	35.9
Niemcza (50°42'N 16°50'E)				
urban–rural				
ur1	granite	NE	Plants grow on high boundary wall 5 m from road; area with tree cover	22.7
ur2	schist	N	Plants grow on high boundary wall 2m from road; area with tree cover	22.7
Strzelin (50°46'N 17°03'E)				
urban-rural				
ur3	schist	NW	Plants grow on town wall 2 m from road; area with tree cover	25.8
Ząbkowice (50°35'N 16°49E)				
urban-rural				
ur4	brick	NW	Plants grow on castle wall 4 m from unpaved road; area with tree cover	28.6
Sobótka (50°54'N 16°44'E)				
urban-rural				
ur5	schist	NW	Plants grow on high boundary wall 2 m from road; area without tree cover	27.0
Książ (50°50'N 16°18'E)				
rural				
r1	granite	N	Plants grow on supporting wall 150 m from unpaved road; area with tree cover	28.6
Janowice (50°52'N 15°55'E)				
rural				
r2	schist	NW	Plants grow on low boundary wall 1 m from road; area without tree cover	25.1
Srebrna Góra (50°34'N 16°39'E)				
rural				
r3	schist	NW	Plants grow on supporting wall 2m from road; area with tree cover	27.1

The levels of PM_{10} are generally higher in urban areas relative to rural locations. Airborne particulate matter is known to contain metals and is an overall good indicator of the extent of soil metal contamination at a site [18, 19]. A measure of the annual mean 24-h PM_{10} at each sampling was determined from the Regional Inspectorate for Environment Protection (RIEP, Wrocław, Poland) database by averaging measures of PM_{10} deposition over the 5 years prior to sample collection [19] (Table 1). Five sites (u1-4 and ur4) were located within 1 km of a RIEP monitor and the only data from that monitor was used to estimate the PM_{10} at those sites. Nine (u5-6, ur1-3, ur5 and r1-3) sites were located more than 1 km (up to approx. 30 km) from the nearest monitor and the PM_{10} at those sites were calculated by averaging the values from the two or three closest surrounding monitors [16].

Table 2. Macroelement concentrations (mg kg^{-1} d.w.) in the fronds of *A. ruta-muraria* and in the leaves of *C. muralis* sampled from the 14 site studies. Site numbers correspond to those in Table 1.

Site	N	P	K	Ca	Mg	Na	S
A. ruta-muraria							
u1	26880	1846	8431	2420	3294	400	1375
u2	23240	1235	8499	2582	1405	456	2091
u3	25660	1769	7159	3359	3345	420	1269
u4	25830	1437	8497	2166	2120	231	1780
u5	34770	1500	8422	2849	1450	238	1695
u6	26600	1021	9525	2871	2859	223	1752
ur1	38430	1845	10322	1997	2480	119	1870
ur2	21000	2072	10285	2148	1752	64	1122
ur3	30730	1906	11700	1657	2109	100	1065
ur4	38430	1778	10495	2410	1639	109	2297
ur5	15260	1045	12487	1635	1693	1074	2596
r1	25750	1891	15774	2066	3119	280	2192
r2	22201	1679	16231	2376	2193	182	2044
r3	26700	1274	15580	2354	1514	655	1673
C. muralis							
u1	23800	2190	12637	2702	3236	4995	3017
u2	28420	3361	18764	2936	1128	3017	4067
u3	27658	2895	15326	3568	2654	2894	3965
u4	21840	2174	18248	2381	3322	4733	1849
u5	18200	1554	10108	2032	1651	1389	2426
u6	24710	1600	11635	3350	1805	1085	2423
ur1	24500	1044	15555	7100	2311	1952	2982
ur2	28700	2367	14503	6906	4137	1304	5468
ur3	22960	2141	13944	8708	2835	4821	2398
ur4	36400	1983	19811	7381	2646	3456	2239
ur5	23425	1698	13689	7231	1792	4865	2139
r1	23460	1891	15491	6925	1249	960	2724
r2	26530	1927	14966	9614	2292	2323	3154
r3	25460	1965	14326	6235	3965	1426	2435
mean (SD)[1/]	26534 (5683)	1593 (340)	10957 (3009)	2349 (470)	2212 (696)	325 (272)	1737 (399)
mean (SD)[2/]	25433 (4200)	2056 (569)	14928 (2672)	5504 (2562)	2501 (938)	2801 (1538)	2949 (969)

[1/] *A. ruta-muraria*
[2/] *C. muralis*

Plant Analysis

Frond samples of *A. ruta-muraria* and leaf samples of *C. muralis* (cleaned of foreign matter, briefly washed) were dried in paper bags at 60ºC for 48 h and immediately ground in a mill (FexIKA M 20). The metals were extracted using microwave digestion with HNO_3 (conc.) and HCl (conc.) in Teflon vessels. Total Mg, Fe, Mn, Cu and Zn levels were measured by flame atomic absorption spectrophotometery (FAAS; GBC Avanta PM), Pb, Cd, Co and Cr levels were measured by electrothermal atomic absorption spectrophotometery (ETAAS; GBC Avanta PM), K, Ca and Na levels

Table 3. Metal concentrations (mg kg^{-1} d.w.) in the fronds of *A. ruta-muraria* and in the leaves *C. muralis* sampled from the 14 site studies. Site numbers correspond to those in Table 1.

Site	Fe	Mn	Zn	Cu	Cd	Pb	Co	Cr
A. ruta-muraria								
u1	1100	47.1	84.1	12.5	0.24	5.7	0.25	4.8
u2	611	25.8	99.5	11.9	0.30	3.3	0.22	2.6
u3	1022	42.5	85.3	11.2	0.20	5.0	0.22	4.8
u4	514	39.1	73.2	10.2	0.24	2.7	0.15	3.2
u5	586	23.4	47.1	17.1	0.12	0.5	0.13	1.6
u6	526	19.2	56.2	7.6	0.15	1.0	0.11	3.5
ur1	922	28.8	56.8	8.9	0.09	1.9	0.15	2.2
ur2	477	31.9	39.8	7.4	0.08	3.0	0.18	3.4
ur3	319	24.4	52.0	9.2	0.09	2.0	0.18	6.1
ur4	467	32.4	33.6	19.6	0.08	5.0	0.40	6.0
ur5	415	36.4	30.5	8.4	0.09	1.8	0.20	6.4
r1	555	26.1	41.9	9.2	0.12	0.7	0.16	2.1
r2	1908	75.4	58.4	16.3	0.05	1.8	0.25	12.3
r3	707	27.7	47.9	7.7	0.12	1.9	0.14	3.8
C. muralis								
u1	253	67.5	69.0	8.7	0.23	4.2	0.12	2.1
u2	1188	124	58.2	19.8	0.19	3.8	0.25	4.0
u3	989	112	65.3	16.3	0.08	3.6	0.29	4.1
u4	1454	147	79.9	20.7	0.08	7.2	0.34	9.5
u5	335	40.5	50.0	11.7	0.03	0.8	0.14	3.2
u6	313	214	26.8	8.5	0.03	3.7	0.19	2.5
ur1	387	128	49.1	12.2	0.05	2.2	0.18	11.7
ur2	933	59.3	49.4	9.5	0.03	2.1	0.13	11.0
ur3	759	73.1	39.7	9.8	0.03	1.5	0.24	7.4
ur4	678	79.3	37.6	22.3	0.04	1.0	0.26	4.8
ur5	342	76.3	45.7	11.3	0.05	2.3	0.20	10.3
r1	487	58.9	26.4	28.9	0.03	2.3	0.12	2.7
r2	1947	78.8	51.5	18.9	0.05	5.5	0.72	15.3
r3	387	59.8	37.5	12.3	0.04	1.1	0.23	4.8
mean (SD)[1/]	724 (412)	34.3 (14.2)	57.6 (20.7)	11.2 (3.9)	0.14 (0.07)	2.6 (1.6)	0.18 (0.05)	4.5 (2.7)
mean (SD)[2/]	747 (506)	94.2 (46.4)	49.0 (15.4)	15.1 (6.2)	0.07 (0.06)	2.9 (1.8)	0.25 (0.15)	6.7 (4.2)

[1/]*A. ruta-muraria*
[2/]*C. muralis*

were measured by flame atomic emission spectrophotometery (FAES; PFP 7 Jenway). All metals were measured against standards (BDH Chemicals Ltd, pro analyze quality) and blanks prepared in concentrated nitric and hydrochloric acid. The reference material consisted of poplar leaves (GBW 07604). In order to determine P concentration with the molybdate blue method, the powdered plant tissue was burned in a muffle furnace at 450ºC, and ash was dissolved in 20% HCl. A plant's total N content was measured using Kjeldahl's method in a Kjeldhal Autoanalyzer (Vapodest 40 Distillation Unit, Gerhardt) with a previous acid digestion (with H_2SO_4 + sodium thiosulfate + salicylic acid + reactive catalyst) conducted with a Kjeldatherm Digestion block KB-8 Digester (Gerhardt). Total S content was measured by a LECO-144 SC Analyzer. All the analyses were done in duplicate. All results for the plants were calculated on a dry-weight basis.

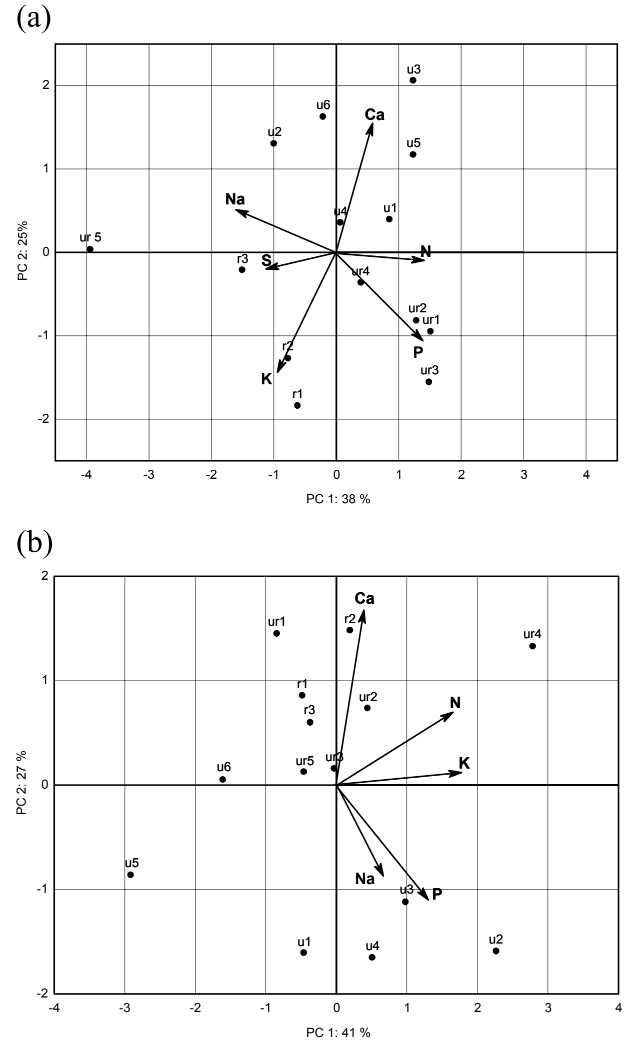

Fig. 1. Ordination of the 14 sites (dots) along two axes extracted by a PCA analysis based on six and five variables related to macroelement concentrations, (a) in the fronds of *A. ruta-muraria* and (b) in the leaves of *C. muralis*, measured at each site.

Statistical Analysis

To investigate the correlation between sites and plant tissue, macronutrient content as well as between sites and plant tissue heavy metal concentration, two separate principal components analyses (PCA) were performed for each species. The preliminary PCAs showed that Mg and Cu (in *A. ruta-muraria*) and Mg, S and Mn (in *C. muralis*) made variance drop below 1%. Based on the results of both analyses, these variables were eliminated and analyses were repeated on subsets of all the plant variables. The plant variables having loading ≥ 0.6 in a factor were considered related to that factor. Results of the PCA analysis were visualized by means of diagrams obtained by overlapping scores and loading plots for PC1 versus PC2 [20]. Relationships between foliar element concentrations across sites as well as foliar element concentrations and PM_{10} across sites were examined using regression analysis, i.e. Pearson correlation analysis [21]. For comparison of leaf element contents between plant species, t-test was performed. Data were log-transformed to satisfy normality and homogeneity of variance assumptions. All calculations were performed using the CSS Statistica 8 program [22].

Results

For *A. ruta-muraria*, the first two PCA axes accounted for 63% of the total variation in the plant variables related to plant tissue macroelement concentrations. The first factor, accounting for 38% of variation, was mainly related to plant tissue N and P concentrations in the positive side, and Na in the negative side (Fig. 1a). The second factor, accounting for 25% of variation, was mainly related to Ca concentration in the positive side and K in the negative side (Fig. 1a). Plant tissue concentrations of P and N were related to each other and were strongly negatively related to tissue Na concentration. Plant tissue K concentration was positively related to tissue S concentration and negatively related to tissue Ca concentration. Results from Fig. 1a agree with the correlation table between plant tissue macroelement concentrations across sites (Table 4).

In the space defined by the two factors extracted by PCA, urban or urban-rural and/or rural sites do not tend to form distinct groups (Fig. 1a). The lack of clustering among these sites is indicative of the variation in the concentrations of each macroelement across these sites. However, some trends are worth noting. Most of the urban sites showed the highest foliar Ca content and the lowest foliar K content. On the other hand, rural sites showed the highest foliar K content and low foliar Ca content and they were more similar to some urban-rural sites (ur2 and ur4). Plant tissue macroelements, like P and N, were more abundant in the most urban-rural sites (ur1-4). These sites were strongly characterized by their lowest foliar Na concentration. Only one urban-rural site (ur5) showed the highest foliar Na content and the lowest foliar N content. Regression analysis revealed significant correlations between only plant tissue K and Ca concentrations and the PM_{10} at a site (negative and positive, respectively) (Table 5).

For *C. muralis*, the first two PCA axes accounted for 68% of total variation in the plant variables related to plant tissue macroelement concentrations. The first factor, accounting for 41% of variation, was mainly related to plant tissue N, P and K concentrations in the positive side (Fig. 1b). The second factor, accounting for 27% of variation, was mainly related to plant tissue Ca concentrations in the positive side (Fig. 1b). Plant tissue N and K concentrations were strongly related to each other and were also positively related to plant tissue Ca and P concentrations. Results from Fig. 1b agree with the correlation table between plant tissue macroelement concentrations across sites (Table 4).

The first two principal components do not indicate a clear tendency for sites of a particular sampling location type (urban vs. urban-rural vs. rural) to cluster together (Fig. 1b). However, some trends are worth noting. Urban-rural and rural sites showed the highest foliar Ca concentration, while urban sites showed the lowest foliar Ca concentration. Foliar N, P and K concentrations at the rural, urban-rural (except ur1 and ur4) and urban sites (except u2 and u5) were similar to each other and they showed intermediate value for these variables. At urban sites, u2 showed the highest foliar P concentration while u5 had the lowest foliar N and K concentrations. At urban-rural sites, ur4 showed the highest foliar N and K concentrations, while ur1 showed the lowest foliar P concentration (Fig. 2b). Regression analysis revealed a negative, significant correlation between only foliar Ca concentrations and PM_{10} at a site (Table 5).

For *A. ruta-muraria*, the first two PCA axes accounted for 80% of the total variation in the plant variables related to plant tissue heavy metal concentrations. The first factor, accounting for 48% of variation, was mainly related to plant tissue Mn, Fe, Co and Cr concentrations in the negative side (Fig. 2a). Plant tissue Pb concentration also presented negative loading (-0.57) in the first factor. The second factor, accounting for 32% of variation, was mainly related to plant tissue Zn and Cd concentrations on the negative side (Fig. 2a). Foliar Mn, Fe, Co and Cr concentrations were strongly related to each other. Foliar Cd concentration was negatively related to foliar Cr concentration, but not to foliar Mn, Fe and Co concentrations. Foliar Co and Pb concentrations and foliar Zn and Cd concentrations were strongly related to each other. Results from Fig. 2a agree with the correlation table between foliar heavy metal concentrations across sites (Table 4). Regression analysis also revealed negative, significant correlations between foliar Cd and K concentrations (Table 4).

The first two principal components show little differentiation in overall foliar metal composition with respect to sampling location type (urban vs. urban-rural vs. rural; Fig. 2a). It is noteworthy that the foliar Cd and Zn concentrations were the highest in four of six urban sites (u1-4). One of them (u1) also showed the highest foliar Pb concentration. These sites have unique features (Table 1) and they tend to form a distinct group. On the other side, foliar Zn and Cd concentrations at the urban-rural, rural and two remaining urban sites (u5 and u6) were similar to each other and some of them showed lowest values for these variables. These sites tend to form a second distinct group and they are subjected to similar environmental conditions (Table 1). Foliar Cd concentration was the lowest and concentrations of Cr, Mn and Fe were the highest at one of the rural sites (r2). This site (r2) is isolated from all other sites (Fig. 2a). Regression analysis revealed positive, significant correlations between foliar Zn and Cd concentrations and PM_{10} at one site only (Table 5).

For *C. muralis*, the first two PCA axes accounted for 70% of the total variation in the plant variables related to plant tissue heavy metal concentrations. The first factor, accounting for 45% of variation, was mainly related to plant tissue Fe, Pb and Co concentration in the negative side (Fig. 2b). Plant tissue Cr concentration also presented a negative loading (-0.59) in the first factor. The second factor, accounting for 28% of variation, was mainly related to plant tissue Cd concentration on the negative side (Fig. 2b). Plant tissue Zn concentration revealed similar loadings (≈-0.63) on the negative side of both factors.

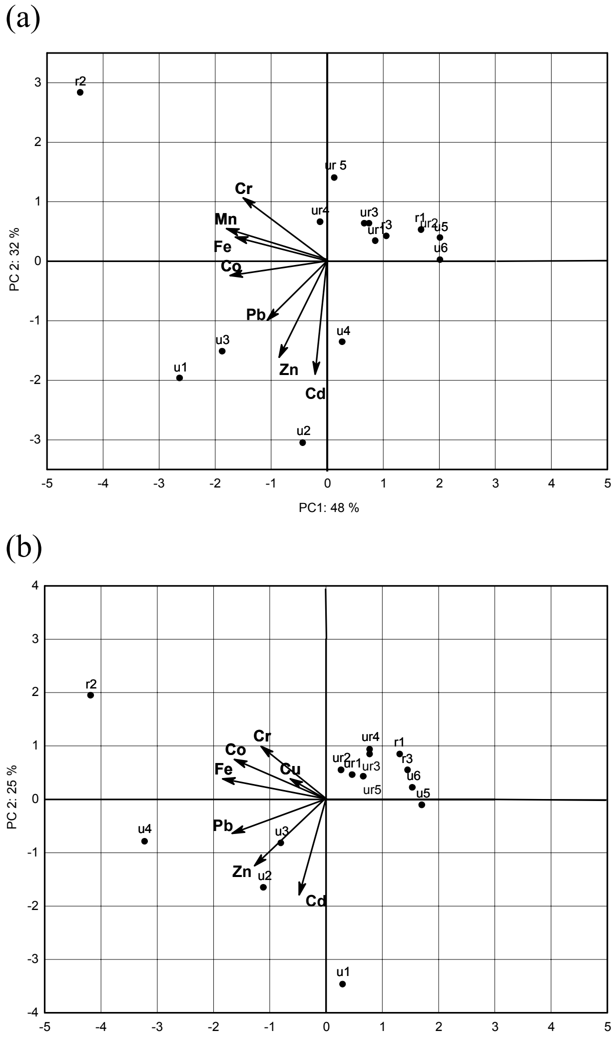

Fig. 2. Ordination of the 14 sites (dots) along two axes extracted by PCA analysis based on seven variables related to heavy metal concentrations, (a) in the fronds of *A. ruta-muraria* and (b) in the leaves of *C. muralis*, measured at each site.

Table 4. Correlation matrix of simple linear coefficient values (r) for each pair of all elements analyzed (a) in the fronds of *A. ruta-muraria* (n=14) and (b) in the leaves of *C. muralis* (n=14); r value in italics indicates an element significantly (P < 0.05) correlated with a given element.

	N	P	K	Ca	Mg	Na	S	Fe	Mn	Zn	Cu	Cd	Pb	Co
(a)														
P	0.315													
K	-0.240	0.019												
Ca	0.142	-0.138	-0.469											
Mg	0.101	0.312	-0.119	0.270										
Na	***-0.560***	***-0.631***	0.156	-0.123	-0.143									
S	-0.128	-0.392	0.347	-0.097	-0.232	0.227								
Fe	-0.024	0.181	0.254	0.283	0.317	-0.093	0.076							
Mn	-0.347	0.202	0.259	0.047	0.195	0.019	0.073	***0.859***						
Zn	0.053	-0.062	-0.522	0.480	0.324	-0.026	-0.199	0.332	0.192					
Cu	0.206	0.159	-0.112	0.324	-0.210	-0.267	0.369	0.334	0.369	0.018				
Cd	-0.006	-0.266	***-0.603***	0.398	0.196	0.177	-0.068	-0.086	-0.128	***0.854***	-0.071			
Pb	-0.122	0.324	-0.472	0.262	0.235	-0.012	-0.217	0.156	0.295	0.452	0.285	0.421		
Co	-0.466	0.268	-0.014	-0.033	0.127	0.211	-0.036	0.514	***0.710***	0.364	0.330	0.164	***0.645***	
Cr	-0.408	0.053	0.430	-0.127	0.004	0.056	0.085	***0.635***	***0.816***	-0.090	0.342	-0.403	0.154	***0.622***
(b)														
P	0.352													
K	***0.664***	0.432												
Ca	0.318	-0.289	0.157											
Mg	0.170	0.050	0.033	0.115										
Na	-0.014	0.232	0.248	-0.041	0.145									
S	0.334	0.539	0.070	-0.018	0.209	-0.310								
Fe	0.225	0.453	0.492	0.186	0.081	0.128	0.264							
Mn	0.045	0.001	0.141	-0.345	-0.203	-0.026	-0.122	0.099						
Zn	-0.141	0.416	0.221	-0.487	0.286	***0.540***	0.174	0.437	0.055					
Cu	0.290	0.234	***0.676***	0.110	-0.367	-0.009	-0.116	0.411	-0.068	-0.035				
Cd	0.044	***0.552***	0.153	-0.511	-0.042	0.443	0.240	0.061	0.059	***0.611***	0.043			
Pb	-0.132	0.271	0.248	-0.291	0.034	0.318	-0.004	***0.652***	0.499	***0.642***	0.214	0.407		
Co	0.181	0.103	0.269	0.369	0.001	0.097	0.057	***0.833***	0.095	0.208	0.277	-0.075	***0.536***	
Cr	0.035	-0.241	0.170	***0.607***	0.233	0.113	0.146	***0.559***	-0.054	0.189	-0.054	-0.301	0.279	***0.590***

Foliar Cd and Zn concentrations were strongly related to each other and were highest at four urban sites (u1-4). One of them (u4) also showed the highest foliar Pb concentration. These sites tend to form a distinct group. On the other side, rural, urban-rural and remaining urban sites (u5 and u6) tend to cluster together and some of them showed the lowest foliar Cd and Zn concentrations. Foliar Fe, Co and Cr concentrations were strongly related to each other and the highest at one of the rural sites (r2). This site (r2) is isolated from all other sites (Fig. 2b). Results from Fig. 2b agree with the correlation table between foliar heavy metal concentrations across sites (Table 4). Regression analysis also revealed positive, significant correlations between foliar Cr and Ca concentrations, foliar Cd and P concentrations, foliar Cu and K concentrations and foliar Zn and Na concentrations (Table 4). Concentrations of foliar Cd and Cr were also significantly correlated with PM_{10} at a site (positive and negative, respectively) (Table 5).

Discussion

Higher terrestrial plants reveal a variable and sometimes specific ability to absorb elements from growth media [2, 23]. *A. ruta-muraria* and *C. muralis* are calciphilous plants. Calciphilous plants possess adaptive mechanisms (avoidance and tolerance) for coping with often high calcium and bicarbonate concentrations in the soil solution. On the other hand, calcicole species possess mechanisms for coping with constraints on mineral nutrition, such as low P, K, Fe, Zn and Mn availability in the soil solution [1]. In the present study, only concentrations of Ca, P, K and Mn in both studied species (in all the sites) were lower than average values presented by Marschner [1] for various plant species with the exception of Mn content in *C. muralis*. Thus, the chemical composition of the chasmophytes presented in this study, reflects the avoidance strategies of higher concentrations of Ca and the limiting concentrations of P, K and Mn in the environment. In this context, it is worth noting that the relatively low amounts of Ca and sometimes other nutritional elements also in the plants, in general, may be caused by high levels of heavy metals present in the environment [24, 2, 16]. Concentrations of Ca, P, K, and Mn as well as S in the examined *C. muralis* were significantly (t-test) higher than the concentrations of these elements found in the examined *A. ruta-muraria* ($P < 0.05$). Therefore, in *A. ruta-muraria* the uptake of P, K, Ca, Mn and S was more restricted compared with *C. muralis*. These differences may be related to remarkable morphological differences among those vascular plants (fern vs. seed plant). Moreover, concentrations of Na in *C. muralis* were also significantly higher than those found in *A. ruta-muralis* ($P < 0.05$). Concentrations of this element in *C. muralis* (in all the sites) were also higher than the average values reported by literature for plants [25]. Sodium, especially, is known to readily replace potassium functions in plants [1]. However, the high concentrations of Na observed in the leaves *C. muralis* were lower than those considered as excessive for plants [1], but rather seem to be the property of *C. muralis*.

Table 5. Correlation analysis of element concentrations in the fronds of *A. ruta-muraria* and in the leaves *C. muralis* and annual mean 24-h particulate matter (PM_{10}) for 14 sites. Values presented are Pearson correlation coefficients (r); r value in italics indicates element significantly ($P < 0.05$) correlated with PM_{10}.

A. ruta- muraria	PM_{10}	*C. muralis*	PM_{10}
N	-0.091	N	-0.088
P	-0.359	P	0.522
K	***-0.618***	K	0.090
Ca	***0.600***	Ca	***-0.840***
Mg	0.349	Mg	-0.191
Na	0.153	Na	0.289
S	-0.026	S	-0.096
Fe	-0.026	Fe	0.054
Mn	-0.038	Mn	0.465
Zn	***0.743***	Zn	0.492
Cu	0.097	Cu	0.116
Cd	***0.887***	Cd	***0.635***
Pb	0.400	Pb	0.519
Co	0.012	Co	-0.082
Cr	-0.237	Cr	***-0.609***

Wide ranges of foliar metal concentrations both in *A. ruta-muraria* and *C. muralis* were observed for all elements across the study sites. In both investigated species, only concentrations of Fe and Cr (in all sites), Zn (in most of the sites), Cd (only some urban sites), Pb (in *A. ruta-muraria* at some urban and urban-rural sites, whilst in *C. muralis* at some urban and rural sites) and Cu (only in *C. muralis* in three sites) were higher than the average values presented by Kabata-Pendias and Pendias [26] and Kabata-Pendias [2] for plants from unpolluted areas. The elevated levels of aforementioned elements in both studied plants point to possible pollution of those sites with Fe, Cr, Zn, Pb, Cd and Cu; that is with those elements that occur in high concentrations in the atmospheric dust of urban and industrial areas of Poland [31-33]. However, the maximum concentrations of Cd and Pb detected in both plant species in this study were lower than those considered either excessive or toxic [2, 26]. On the other hand, in both studied plants concentrations of Zn (in four of urban sites on a riverside walls), Fe (in two of urban sites and one of rural sites) and Cu (for this element only in *C. muralis* in three sites) were approximate to those documented as either excessive or toxic [2, 26]. The concentrations of Cr detected in *A. ruta-muraria* (at some urban-rural and rural sites) and in *C. muralis* (at some urban, urban-rural and rural sites) were above the value reported in literature as excessive or toxic [2, 26]. However, we observed no evidence of aberration in morphology of the plants. Our results suggest that *A. ruta-muraria* as well as *C. muralis* has specific capacity to accumulate considerable amounts of Cr and that they use Cr for some beneficial purposes. On the other hand, they may have the ability to isolate the unwanted elements in some organelles which is, to a certain degree, supported by the fact that the these plants also showed high concentrations of Pb and Cd. Comparably high concentrations of Fe, Zn and Pb have been reported for *A. ruta-muraria* growing on the man-made walls and also calcareous rocks in some relatively uncontaminated mountain areas as well as in polluted urban areas [3, 4], and for *A. cuneifolium* ssp. *cuneifolium* growing on the serpentine soils [27], as well as for some other *Asplenium* species and other ferns growing in the ground [27-29]. Similar high concentrations of Cr to those found by us in *A. ruta-muraria* was found by Ozaki et al. [28] in fronds of *A. trichomanes* and Coronara et al. [27] in fronds of *A. cuneifolium* ssp. *cuneifolium*. In the present study, there was no statistically significant difference between *A. ruta-muraria* and *C. muralis* in concentrations of the metals examined (Fe, Zn, Cu, Pb, Co, Cr) except for Cd. Concentrations of Cd in fronds of *A. ruta- muraria* were significantly higher than those found in the leaves of *C. muralis* ($P < 0.05$).

Phytoxic effects of heavy metals, in general, are due to their interactions with other elements [16]. These interactions may refer to the ability of the one element to inhibit the absorption of other elements in plants [2, 26]. Both the studied species were characterized by significant positive correlations between such variables as Fe and Cr contents in plant tissue; Cr and Co; Zn and Cd; and Pb and Co. A significant positive relationship between concentrations of Cr and Co has been documented by Ozaki [28] for ferns. Moreover, *A. ruta muraria* was characterized by significant positive correlations between Fe-Mn; Cr-Mn; Co-Mn and negative correlation between K-Cd and *C. muralis* was characterized by significant positive correlations between Fe-Pb; Fe-Co; Zn-Pb; Ca-Cr; P-Cd; K-Cu and Na-Zn. Some of these correlations are different from those presented by Kabata-Pendias and Pendias [26] and Kabata-Pendias [2], who reported that relations between Fe-Cr; Fe-Mn; Cr-Mn; Co-Mn; Fe-Co; Ca-Cr and P-Cd are usually negative, and the relation between Zn-Cd may be either negative or positive. However, these authors also state that some synergistic effects have also been observed for antagonistic pairs of elements, depending on the specific reaction of the plant genotype or species. Such correlations between elements may change, depending on the quantitative proportions not only of heavy metals but also of macroelements in the environment [2, 26].

In the present study, concentrations of airborne particulate matter were used as an indicator of metal contamination at a site where the two species are growing. Heavy metals, mainly the particulate form of atmospheric pollutants, are emitted into the atmosphere from anthropogenic sources such as road components, traffic, power plants, industries and residential heating [2, 30-33]. As would be expected, the concentrations of PM_{10} at the rural and urban-rural sites are generally lower than at urban locations. None of the PM_{10} values at the sites sampled exceeded the maximum allowable concentration (40 µg m^{-3}) for air quality established by the RIEP. Airborne particulate matter influenced some features of tissue elemental composition of the two species in different ways. In *A. ruta-muraria*, concentrations of Ca, Zn and Cd showed positive correlations with PM_{10}, while concentrations of K were negatively correlated with PM_{10}. In *C. muralis*, concentrations of Cd showed positive correlation with PM_{10}, while concentrations of Ca and Cr were negatively correlated with PM_{10}. According to Markert and Wtorova [24], Uhling and Junttila [16], the presence of high levels of heavy metals in the environment seems to be directly associated with exclusion of Ca and sometimes also K, which may be indicated by a lower concentration of Ca in *C. muralis* and lower concentration of K in *A. ruta-muraria* from urban areas. In contrast to leaves of *C. muralis*, fronds of *A. ruta-muraria* from urban sites contained more Ca as compared with the studied fern species from urban-rural and rural sites. This indicates that Ca in this fern fulfils important metabolic functions. Atmospheric aerosol particles in urban areas contain not only anthropogenic metals but also crust metals, among others Ca [32, 34]. Many studies indicated that the concentrations of certain heavy metals, such as Zn and Cd, increase in urban areas mainly due to traffic pollution [30, 33-36]. Tyres, motor oils, and diesel fuel are considered major sources of Zn and Cd from road traffic [32-34]. *A. ruta-muraria* and *C. muralis* growing in four urban sites, in the direct vicinity of the river, beside roads contained more Zn and Cd as compared with plants from the remaining urban, urban-rural and rural sites. It seems, therefore, that an increase in humidity level may enhance the effect of aerial pollution on chemical composition of plant tissue.

Conclusions

1. Two co-occurring chasmophytes, fern *A. ruta-muraria* and seed plant *C. muralis*, differed in uptake of macronutrients and heavy metals. Seed plant contained significantly more P, K, Ca, Mn, S and Na and fern significantly more Cd. There was no statistically significant difference between fern and seed plant in concentrations of N, Mg, Fe, Zn, Cu, Pb, Co and Cr.
2. Concentrations of Fe, Cr, Cd, Zn and Pb in both species were greater than background values without any morphological aberrations in the plants.
3. In contrast to *C. muralis*, *A. ruta-muraria* from urban sites contained higher levels of Ca than those from urban-rural and rural sites. However, *A. ruta-muraria* from urban sites contained lower levels of K than those from urban-rural and rural sites. Zn and Cd concentrations both in *A. ruta-muraria* and *C. muralis* were higher in urban sites, in the direct vicinity of the river, than those at other sites in the study area. In *A. ruta-muraria*, Ca, Zn and Cd increased in proportion to PM_{10}, while levels of K decreased and in *C. muralis*, Cd increased in proportion to PM_{10}, while levels of Ca and Cr decreased.

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