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Comparative study of the suitability of three lichen species to trace-element air monitoring

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"Capsule": *Evernia prunastri was the most suitable lichen for trace-element air monitoring.*

Abstract

To investigate the suitability of three lichen species (*Cetraria islandica*, *Evernia prunastri*, and *Ramalina farinacea*) as transplants to trace-element air biomonitoring, they were exposed on substratum-free supports, from July 1996 until July 1997, in three European countries with different climates (Germany, Italy, Romania), at six sites with different types of air pollutants (two in each country). After 2, 4, 6, and 12 months of exposure, some portions of thallus were collected, prepared, and measured by instrumental neutron activation analysis (INAA) at the Institute of Physics and Nuclear Engineering in Bucharest and by energy dispersive X-ray fluorescence analysis (EDXRFA) at the University of Hohenheim in Stuttgart. Fifteen environmentally relevant elements: As, Br, Ca, Co, Cr, Cu, Fe, K, Mn, Ni, Pb, S, Sb, V, and Zn were determined. The analytical results were compared statistically. To study the distribution of the trace-elements between the lichens and the lichen throughfall water inside a virtual column, the throughfall water was collected under the lichen transplants during 6 and 12 months. The dried residues were analysed by INAA at Bucharest. The accumulating capacity for all investigated species is evident. For a comparative evaluation, the initial element contents, the "accumulation factors" relative to the bulk deposition, the interspecies "calibration factors", and the "retention efficiencies", defined as ratios of the lichen enrichment to the sum of this enrichment and the content in the lichen throughfall water, were considered. These criteria attest the best suitability for *Evernia prunastri*, followed by *Ramalina farinacea* and *Cetraria islandica*.

Keywords: Biomonitoring; Bioaccumulation; Lichen transplants; Trace-elements; Air pollution; Atmospheric deposition; Bulk deposition; Interspecies calibration; Lichen throughfall water

1. Introduction

Besides the mosses, the lichens belong to the "classical" bioaccumulators applied in trace-element air monitoring programmes (e.g. Pilegaard, 1979; Boileau et al., 1982; Walther et al., 1990; Herzig and Urech, 1991; Sloof and Wolterbeek, 1991a,b; Freitas, 1994; Freitas et al., 1999; Rodrigo et al., 1999). Especially in the last decade, the use of lichen transplants gained in importance owing to their advantages of a substratum-free exposure during a defined period and of knowledge of the initial element contents (Sloof, 1993, 1995; Caniglia et al., 1994; Freitas et al., 2000). Efforts are being made to quantify, by mathematical modelling, the relationship between environmental availability and elemental accumulation in lichens (Reis et al., 1999).

For practical purposes, it is very useful to test and to quantify the suitability of various lichen species to the monitoring of heavy metal air pollution, allowing in this way the best choice for the transplants. This was the objective of the joint project of our institutions. The main results concerning the accumulating capacity of lichens, averaged at different climate conditions and types of pollutants, are presented in this paper.

2. Materials and methods

Three lichen species: *Cetraria islandica, Evernia prunastri,* and *Ramalina farinacea* (Nimis, 1993) were investigated.

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Cetraria islandica (L.) Ach. is a very common terricolous acidophytic species. It has a very wide circumboreal-montane and bipolar distribution with optimum from lowland up to well above the tree line. It usually grows on very acid, low nutrient sand and sandy loam, turf, and stony soils, but also on level soils rich in lime, normally on well-lit habitats, as in silicate and calcareous turfs, dwarf shrub heaths, open Scots pine forest, high moors, and moor forests. However, it also survives on moderately well-lit sites and is of rather wide ecological amplitude.

Evernia prunastri (L.) Ach. is a circumboreal-temperate species, widespread only in the Northern hemisphere. This lichen is often abundant with a very wide ecological amplitude. It is one of the most common epiphytic lichens, usually found up to the tree line on neutral to acid-barked trunks of deciduous and conifer trees on sunny, often windswept, places.

Ramalina farinacea (L.) Ach. has a wide distribution in Europe and a wide ecological amplitude. It grows on bark, more rarely on lignum. It is rather frequent from the upper montane belt to the lowlands, being more common near the coast, in sites with high atmospheric humidity and in humid beech forest in the mountains. This species is quite sensitive to air pollution.

The samples of *Cetraria islandica* were collected on the ground in the Prealps area, near "Vette Feltrine" 2000 m a.s.l. (Belluno, North-East Italy), while those of *Evernia prunastri* and *Ramalina farinacea* on the bark of larch near "Croce d'Aune" 1200 m a.s.l., in the same geographical area.

E. prunastri was already used in a previous study of the heavy metal accumulation in transplants exposed for 12 months in the industrial zone of Padua at different distances from a steel works (Caniglia et al., 1994).

In this study one epigaeic and two epiphytic species were used, to find out if the different lichen ecology influences the accumulating capacity of the transplants.

After collection, the lichens were washed with deionised water and 10 samples from each species were analysed to obtain the initial element contents (before exposure).

Portions of lichens with a mass between 20 and 40 g were mounted on appropriate plastic supports ("little traps": 15×15×1.5 cm, with mesh of 1 cm^2) and fixed horizontally on bars about 1.5 m above the soil at six sites with different air pollution types, situated in three countries with different climates (Germany, Italy, and Romania). The exposure sites are briefly characterized in Table 1. The exposure equipment was provided with "crowns of thorns" of lacquered metal as a protection against birds (Fig. 1).

The simultaneous exposure of the three lichen species began in July 1996. After 2, 4, 6, and 12 months, some portions of thallus (about a quarter of the exposed material) were collected, dried for 2 days at 35 °C, ground, homogenised, pressed into pellets of about 100 mg, and then investigated by two analytical methods. **E**nergy **D**ispersive **X**-**R**ay **F**luorescence **A**nalysis (EDXRFA) was used at the University of Hohenheim in Stuttgart and **I**nstrumental **N**eutron **A**ctivation **A**nalysis (INAA) at the Institute of Physics and Nuclear Engineering in Bucharest-Măgurele. Fifteen elements: As, Br, Ca, Co, Cr, Cu, Fe, K, Mn, Ni, Pb, S, Sb, V, and Zn were determined (11 of them by both methods, Pb and S only by EDXRFA, Co and Sb only by INAA).

For EDXRFA, a device (Finnigan Inc.) with Rh tube excitation and Si(Li) detector was employed. The

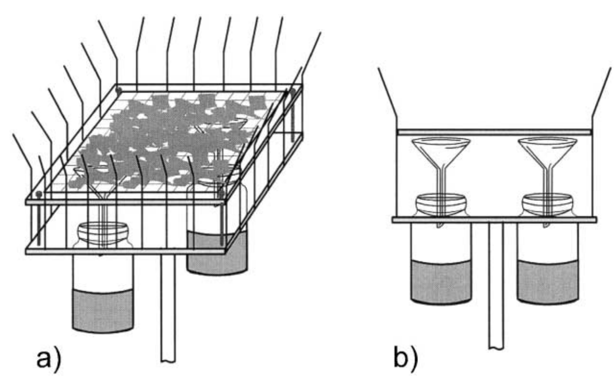

Fig. 1. Equipment to expose lichen transplants and to collect lichen throughfall water: (a) general view, (b) diagonal section.

Table 1
Short characterisation of the exposure sites

Country	Locality	Abbreviation	Climate characteristics	Measured precipitations in l/(m^2 a)	Air pollution type
Germany	Stuttgart-Hohenheim	D-1	Temperate with maritime and continental influence, humid	637	Urban + mixed industry
	Esslingen am Neckar	D-2			Car + rail traffic
Italy	Padua	I-1	Mediterranean, humid		Urban + mixed industry
	Venice-Porto Marghera	I-2		672	Oil refineries
Romania	Bucharest-Măgurele	RO-1	Continental, subhumid	560	Mixed industry + power plant
	Târgovişte	RO-2			Metal-working industry

Table 2
Initial element contents (before exposure) in mg/kg

Element	*Cetraria islandica*		*Evernia prunastri*		*Ramalina farinacea*		*Evernia prunastri* IAEA-336	*Parmelia sulcata*	
	Average	S.D.	Average	S.D.	Average	S.D.	Stone et al. (1995)	Sloof (1995)	Freitas (2000)
As	0.23	0.05	0.32	0.02	0.35	0.07	0.64		0.791
Br	3.0	0.9	8.2	1.2	3.7	0.6	12.9		
Ca	6100	4800	8200	1500	6100	1400	2600		7090
Co	0.23	0.12	0.18	0.04	0.18	0.07	0.29	1.37	
Cr	1.1	0.6	1.1	0.5	1.4	0.7	1.03		4.78
Cu	3.7	2.3	4.6	2	5.8	2.2	3.55		
Fe	250	160	280	24	260	41	425		
K	2180	440	2150	190	1670	115	1840		
Mn	11.2	3.7	53	6.7	28.8	4.8	64		
Ni	5.3	3.3	4.6	1.2	4.8	1.6			
Pb	10.5	2.9	13.9	7.2	12.4	3.8	5.0		
S	520	70	770	82	1000	144			
Sb	0.08	0.03	0.28	0.04	0.38	0.13	0.073		0.21
V	1.0	0.5	1.4	0.14	1.8	0.3	1.5		3.44
Zn	27.8	5.6	26.7	2.6	26.8	3.2	31.5	143	

Table 3
Element contents of *Cetraria islandica* in mg/kg

Site	Exposure time	As	Br	Ca	Co	Cr	Cu	Fe	K	Mn	Ni	Pb	S	Sb	V	Zn
All	"0" months	0.23	3.0	6100	0.23	1.1	3.7	250	2180	11.2	5.3	10.5	520	0.08	1.0	27.8
D-1	2 months	0.17	1.8	2880	0.21	0.7	5.0	213	1799	7.6	5.0	7.0	420	0.12	1.0	25.5
	4 months	0.27	2.4	2225	0.21	1.4	6.2	316	1794	8.7	5.7	12.0	669	0.31	1.4	33.9
	6 months	0.22	2.5	2185	0.19	1.2	4.6	255	1340	6.6	3.2	6.6	630	0.32	1.0	28.1
	12 months	0.30	1.9	2345	0.30	1.6	5.8	343	1515	9.6	5.1	10.0	760	0.39	1.4	36.1
D-2	2 months	0.19	1.7	5022	0.25	1.3	5.6	399	1954	11.7	4.2	7.0	590	0.30	0.8	40.0
	4 months	0.32	3.5	1853	0.31	2.9	9.6	731	1838	13.5	7.4	18.0	690	0.70	1.4	51.8
	6 months	0.29	2.8	3350	0.26	2.9	13.7	802	1294	12.2	5.7	18.0	830	0.85	1.4	64.0
	12 months	0.42	3.3	3325	0.50	5.3	21.0	1590	1535	17.5	6.8	20.0	810	1.30	2.3	81.7
I-1	2 months	0.17	2.1	5870	0.16	0.6	6.1	203	1569	9.6	6.8	3.0	540	0.16	0.9	39.5
	4 months	0.26	3.9	2363	0.20	1.7	6.5	332	1653	8.3	6.1	21.0	640	0.59	1.5	53.7
	6 months	0.37	5.0	4080	0.23	2.0	10.2	448	1540	11.5	5.4	30.2	860	0.81	2.0	63.0
	12 months	0.43	3.2	7010	0.31	2.7	13.3	651	1145	17.8	6.9	48.3	860	1.30	3.1	83.0
I-2	2 months	0.21	3.6	20256	0.19	1.0	4.3	243	809	9.9	7.4	18.0	630	0.12	2.4	32.4
	4 months	0.32	5.2	2400	0.18	1.4	4.0	309	756	7.0	6.8	17.0	720	0.21	3.7	28.0
	6 months	0.37	3.7	2630	0.31	2.3	7.9	489	1030	9.2	7.3	13.0	830	0.30	5.8	36.7
	12 months	0.58	9.0	13440	0.52	4.4	11.6	712	1195	18.0	12.9	23.0	1260	0.50	11.4	60.9
RO-1	2 months	0.28	3.1	6516	0.35	1.0	3.2	280	1989	13.5	6.9	16.3	529	0.12	1.9	34.4
	4 months	0.44	3.6	5063	0.36	2.3	5.5	643	2067	15.3	7.3	32.0	660	0.36	3.0	44.4
	6 months	0.33	3.9	6335	0.28	2.0	7.1	514	1654	13.0	7.3	65.0	1010	0.47	8.9	54.3
	12 months	0.63	3.0	6510	0.43	2.7	7.5	658	1780	14.7	11.0	66.0	966	0.38	20.1	70.4
RO-2	2 months	0.39	3.7	20041	0.27	2.4	4.3	444	1557	16.5	4.9	33.0	570	0.40	1.4	41.1
	4 months	0.45	3.9	5620	0.36	3.8	3.5	703	1676	20.7	7.4	28.0	680	0.62	2.3	42.5
	6 months	0.60	5.0	4480	0.35	8.0	6.2	725	2121	29.4	6.4	32.0	1190	1.50	2.6	53.1
	12 months	0.74	2.3	7235	0.44	10.4	6.8	831	1798	29.6	9.2	50.0	890	1.29	3.0	59.0

Table 4
Element contents of *Evernia prunastri* in mg/kg

Site	Exposure time	As	Br	Ca	Co	Cr	Cu	Fe	K	Mn	Ni	Pb	S	Sb	V	Zn
All	"0" months	0.32	8.2	8200	0.18	1.1	4.6	280	2150	53.0	4.6	13.9	770	0.28	1.4	26.7
D-1	2 months	0.33	6.3	6974	0.27	1.4	4.6	282	2213	44.8	5.4	13.0	810	0.34	1.1	31.0
	4 months	0.39	9.8	13560	0.35	1.9	6.8	436	1911	52.7	3.7	20.5	1120	0.51	2.1	42.8
	6 months	0.42	8.7	9410	0.33	1.8	8.6	379	2278	50.3	7.4	18.0	1220	0.56	1.5	48.1
	12 months	0.42	6.8	9210	0.42	2.5	9.2	534	1735	43.8	6.8	17.0	1190	0.64	2.2	62.4
D-2	2 months	0.34	7.0	7300	0.42	2.1	8.5	525	2227	58.0	6.6	15.0	820	0.59	1.4	42.9
	4 months	0.49	6.8	8560	0.47	5.2	12.7	937	2154	50.3	4.1	15.5	920	1.07	1.5	71.9
	6 months	0.52	9.3	10295	0.50	5.5	18.2	1150	1969	64.6	8.1	14.3	1190	1.30	2.3	87.3
	12 months	0.57	5.5	11720	0.66	7.9	31.3	1960	1810	45.0	7.4	29.0	1220	1.90	2.4	125.0
I-1	2 months	0.36	7.9	8986	0.26	1.5	5.0	361	2095	51.4	6.7	20.0	940	0.46	1.4	95.6
	4 months	0.67	8.0	10858	0.29	2.3	8.2	439	2175	45.7	5.7	30.0	1010	0.81	1.8	97.5
	6 months	0.85	18.8	13940	0.67	5.9	20.6	1403	1452	53.8	8.7	74.0	1390	2.30	4.9	171.0
	12 months	1.00	18.7	19775	1.00	11.1	31.1	2225	1665	73.5	10.9	113.0	1510	3.30	7.8	269.0
I-2	2 months	0.42	9.3	12583	0.29	2.2	7.9	392	2144	49.7	6.3	25.3	1170	0.43	3.2	41.5
	4 months	0.62	14.5	8440	0.35	3.2	9.1	479	2374	49.0	8.4	17.7	1590	0.42	4.1	64.5
	6 months	0.78	23.0	9510	0.53	3.7	10.3	790	1438	38.8	12.2	25.0	1670	0.67	10.0	89.9
	12 months	1.00	33.3	14520	0.90	10.0	17.7	1315	1200	65.4	20.1	49.0	1950	0.79	14.9	126.5
RO-1	2 months	0.41	7.6	8325	0.28	1.7	7.4	415	2075	58.6	5.1	18.0	900	0.37	2.4	37.5
	4 months	0.55	7.7	8865	0.38	2.5	7.0	636	2382	56.9	7.5	40.0	1070	0.52	3.4	60.0
	6 months	0.69	9.7	8690	0.46	3.0	9.4	720	2051	54.3	9.0	55.0	1420	0.67	9.3	71.7
	12 months	0.91	11.3	12870	1.30	5.7	13.1	1440	1510	62.6	17.1	110.0	1465	0.62	38.5	121.5
RO-2	2 months	0.65	8.3	7553	0.38	4.2	5.8	578	1926	61.0	6.0	34.0	890	1.05	1.8	57.2
	4 months	0.89	9.0	11248	0.53	6.9	7.1	947	2304	72.6	5.8	45.0	1200	0.93	3.2	61.4
	6 months	0.96	10.1	12520	0.59	12.1	8.9	1161	2323	74.0	7.3	58.5	1560	1.60	4.4	74.4
	12 months	1.12	7.8	12440	0.90	21.7	10.9	1655	1780	75.1	11.6	89.0	1140	2.40	6.6	101.8

typical measuring conditions were: 40 kV, 0.1 mA, and 4000 s. The energy spectra, obtained by means of a multichannel analyser, were corrected for absorption and enhancement effects with the aid of self-written software (Cercasov and Schreiber, 1988).

For INAA, short and long irradiations of the samples, together with appropriate reference materials, were performed in the VVR-S research reactor in Bucharest-Măgurele (neutron fluence rate: 2.5×10^{12} cm^{-2} s^{-1}). The gamma ray spectra were measured using a HPGe detector (EG&G Ortec) of 160 cm^3 with 2.1 keV resolution for ^{60}Co. For the short-lived nuclides (Ca, Cu, Mn, and V), an irradiation time of 60 s, a decay time of 3–30 min, and a counting time of 2–10 min were chosen. For the medium and long-lived nuclides, an irradiation of 4 h was carried out. After a decay time of 4–5 days, measurements of 1–4 h made it possible to determine As, Br, K, and Sb. A second counting of 10–20 h, after 20–30 days, was necessary to analyse Co, Cr, Fe, Ni, and Zn.

The analytical errors for the measured elements lie between 3 and 30%. The quality control of the used analytical methods is assured by periodical participation in IAEA intercomparison runs. The last one was IAEA-0390 "Trace Elements in Algae".

A statistical intercomparison of all the results obtained on lichen samples made it possible to draw practical conclusions about the suitability of these analytical techniques for this particular matrix and at the present concentration ranges (Cercasov et al., 1999).

To study the distribution of the trace elements among the lichens and the lichen throughfall water, two polyethylene bottles of 1 l with funnels of 5 cm upper diameter were mounted at a distance of about 1 cm under the lichen support for every lichen species and exposure site (Fig. 1). One of the bottles was removed after 6 months together with the corresponding quarter of the exposed lichen sample and the other one after 12 months. If a bottle was almost full prior to this, it was replaced by an empty one and afterwards they were

Table 5
Element contents of *Ramalina farinacea* in mg/kg

Site	Exposure time	As	Br	Ca	Co	Cr	Cu	Fe	K	Mn	Ni	Pb	S	Sb	V	Zn
All	"0" months	0.35	3.7	6100	0.18	1.4	5.8	260	1670	28.8	4.8	12.4	1000	0.38	1.8	26.8
D-1	2 months	0.30	3.4	5792	0.21	1.1	4.5	249	1626	23.9	5.7	6.0	970	0.28	1.7	33.4
	4 months	0.39	3.1	5500	0.24	1.4	6.4	289	1377	21.4	4.9	11.8	1120	0.54	2.0	32.4
	6 months	0.53	3.8	5035	0.25	2.1	8.9	427	1700	16.1	5.1	11.0	1620	0.71	2.3	44.3
	12 months	0.50	2.3	6800	0.37	2.5	11.1	571	1440	19.3	6.7	25.0	1390	0.71	3.4	59.0
D-2	2 months	0.39	3.4	4782	0.22	1.6	7.1	510	1636	24.2	5.6	12.5	980	0.49	1.5	42.6
	4 months	0.49	3.8	8013	0.38	4.0	12.8	941	1655	36.4	6.7	23.5	1200	1.10	2.5	75.5
	6 months	0.45	3.4	6130	0.37	3.8	21.4	1013	1500	28.3	5.6	13.5	1550	1.20	2.1	85.1
	12 months	0.56	3.6	8465	0.56	6.9	30.4	1820	1535	34.1	8.0	27.0	1380	1.70	3.7	109.5
I-1	2 months	0.38	3.0	5748	0.16	1.2	6.3	251	1384	20.8	4.3	17.0	1030	0.37	1.8	180.0
	4 months	0.48	3.6	6315	0.22	2.0	9.3	411	1624	21.8	6.1	29.0	1290	0.81	2.5	240.8
	6 months	0.77	11.9	12420	0.55	4.7	19.5	1147	1480	36.6	6.9	66.1	1210	1.95	5.2	362.5
	12 months	0.83	9.6	14290	0.61	5.8	26.1	1240	1550	36.9	8.1	84.6	1550	2.60	6.1	643.5
I-2	2 months	0.38	3.8	7103	0.20	1.3	4.9	276	1425	22.6	5.2	19.0	1230	0.30	3.5	33.7
	4 months	0.49	6.6	4203	0.24	1.7	6.4	341	1617	15.5	7.2	9.0	1560	0.37	5.7	43.8
	6 months	0.89	10.3	7620	0.45	3.7	14.0	803	1727	22.2	10.2	29.0	1980	0.63	12.6	61.5
	12 months	1.02	14.0	6135	0.77	6.4	21.2	977	1250	22.8	15.0	38.0	1870	0.91	16.2	86.1
RO-1	2 months	0.48	3.7	5311	0.24	1.3	6.0	318	1835	29.5	5.7	22.1	1090	0.28	2.3	34.0
	4 months	0.69	5.3	8160	0.33	2.5	7.2	600	1829	30.4	4.3	40.0	1410	0.67	3.9	47.4
	6 months	0.70	3.7	6350	0.30	2.5	8.9	580	1696	23.7	8.9	64.5	1730	0.62	13.9	53.7
	12 months	0.82	4.2	6285	0.44	3.9	13.2	964	1960	26.8	11.5	101.0	1540	0.57	30.2	76.7
RO-2	2 months	0.51	3.2	6497	0.27	2.4	4.2	384	1525	30.4	6.6	25.0	1000	0.55	1.8	45.9
	4 months	0.82	5.0	6093	0.36	4.7	6.0	688	1563	35.9	5.9	32.0	1320	1.30	3.5	49.8
	6 months	1.00	4.7	7450	0.42	10.8	9.0	934	1794	42.0	7.4	57.7	1960	2.00	4.6	74.0
	12 months	0.77	3.7	7025	0.50	11.3	9.7	983	1500	37.3	8.9	77.0	1390	1.40	5.3	78.4

combined to give a single water sample. All the bottles were wrapped up in aluminium foil to prevent the development of algae.

Additionally, the bulk (wet and dry) deposition was collected using the same equipment as in Fig. 1, but without lichens, at the sites D-1 and RO-1. For the same purpose, a larger vessel with a funnel of 29 cm diameter was employed at the site I-2.

The volume of the water samples collected during 6 months ranges between 0.04 and 2.11 l for the lichen throughfall water and between 0.25 and 1.05 l for the precipitations.

In view of the analysis, the water samples were first concentrated at 50 °C and 0.15 bar by means of a rotating evaporator VV2000 (Heidolph Co.) to a volume of about 50 cm^3 and then dried on polyethylene foils under light bulbs. Residua with a mass of 5.5–97 mg (mean value of about 50 mg/l) were obtained.

The residua were analysed by INAA at Bucharest for the same elements as the lichens. They were irradiated together with the polyethylene foils firstly for 30 s and then for 2.75 h at the same neutron fluence rate as the lichens. The decay and the counting times were also similar to those used for the lichens.

3. Results and discussion

Based on the conclusions of the statistical intercomparison of the results obtained by the two analytical techniques (Cercasov et al., 1999), arithmetical mean values for Br, Ca, Fe, K, Mn, and Zn, INAA values for As, Co, Cr, Sb, and V, and XRFA values for Cu, Ni, Pb, and S are considered for the lichen samples. The water samples were measured by INAA only.

3.1. Initial element contents

The element contents before exposure could be considered as a criterion for the suitability of a lichen species to be used as a transplant. Table 2 presents the arithmetical means and the standard deviations for the measurements of 10 different portions for each of the three lichen species investigated. Additionally, some values of other authors (Sloof, 1993, 1995; Stone et al., 1995; Freitas et al., 2000) are listed in the same table for comparison.

A comparative evaluation of the initial values was done by calculating ratios between species for eleven elements, which show a considerable enrichment during exposure (see below). The mean values of these ratios are 0.82 for *Cetraria/Evernia* and 1.05 for *Ramalina/Evernia*. The lowest contents were found for seven elements (As, Br, Cu, Fe, Pb, Sb, and V) in *Cetraria islandica* and for two elements (Ni and Zn) in *Evernia prunastri*.

3.2. Enrichment effects

The element contents determined in all exposed lichen samples are presented in Tables 3, 4, and 5. Since the lichens were not washed before analysis, the enrichment represents the sum of the element amount accumulated in the lichen thalli by uptake plus that adhered to the surface. For practical application it is no reason to distinguish between these two components.

The ratios of the element contents of the exposed samples to those of the unexposed ones are named in this paper "enrichment factors".

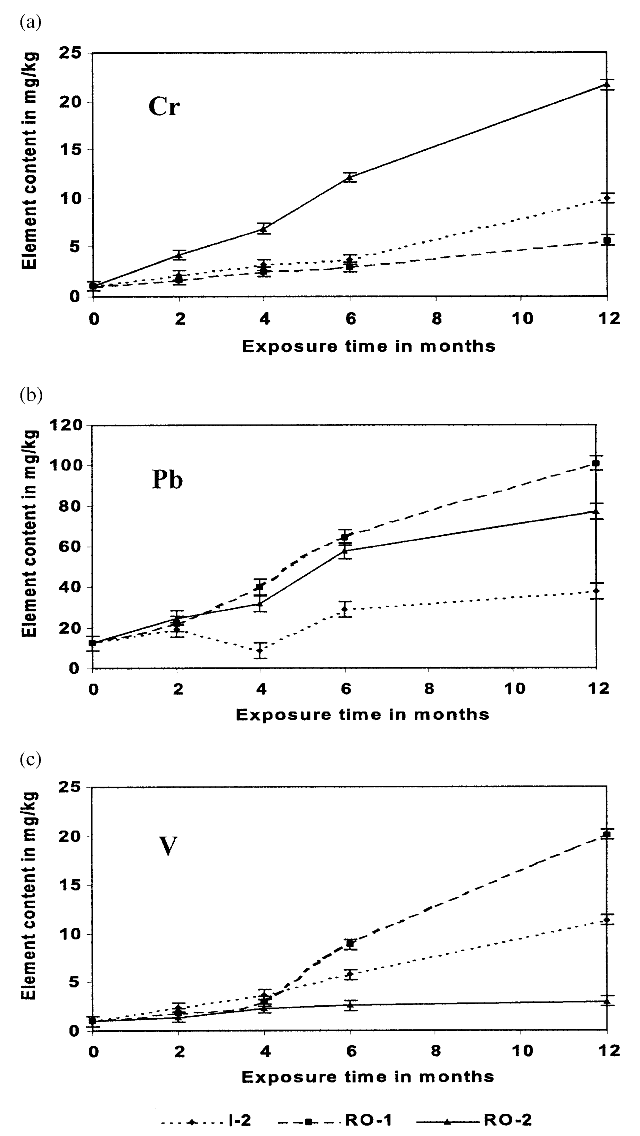

Fig. 2. Enrichment of Cr in *Evernia prunastri* (a), Pb in *Ramalina farinacea* (b), and V in *Cetraria islandica* (c).

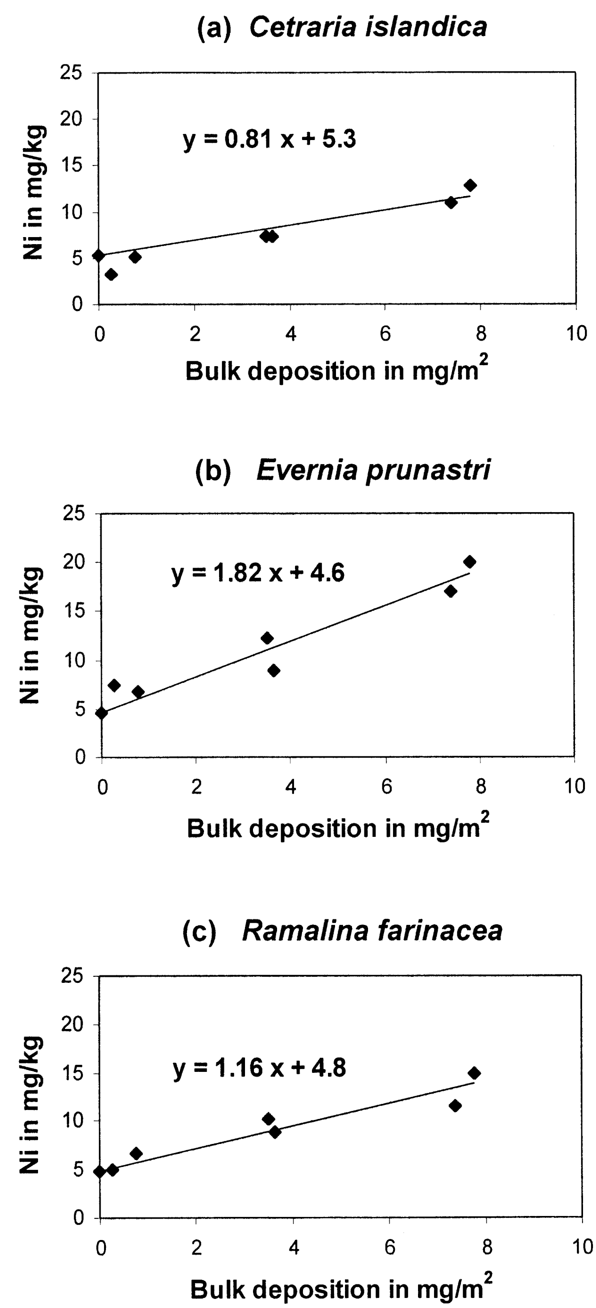

Fig. 3. Relationship between the Ni content in lichens and the bulk deposition for *Cetraria islandica* (a), *Evernia prunastri* (b), and *Ramalina farinacea* (c).

Table 6
"Accumulation Factors (AF)" according to Sloof (1993) in m^2/kg

Element	*Cetraria islandica*	*Evernia prunastri*	*Ramalina farinacea*
As	0.77	1.46	1.37
Br	0.51	2.75	1.09
Co	0.34	1.38	0.72
Cr	1.15	3.1	1.79
Cu	0.76	1.42	1.4
Fe	1.38	3.46	2.37
Ni	0.81	1.82	1.16
Sb	1.51	1.75	1.41
V	0.78	1.37	1.2
Zn	0.52	1.58	0.94
Mean	0.85	2.01	1.35

Considerable enrichment effects during the exposure time (i.e. enrichment factors $\geqslant 3$ for at least two lichen species) were registered for the following eleven elements: As, Br, Co, Cr, Cu, Fe, Ni, Pb, Sb, V, and Zn. As an example, Fig. 2 shows the contents of Cr in *Evernia prunastri* (Fig. 2a), of Pb in *Ramalina farinacea* (Fig. 2b), and of V in *Cetraria islandica* (Fig. 2c) at three exposure sites (I-2, RO-1, and RO-2), after 2, 4, 6, and 12 months of exposure. The maximum value of all enrichment factors is 27.5 for V in *Evernia prunastri* (RO-1, after 12 months).

Systematic leakage effects could be observed only for K (the enrichment factors are decreasing in some cases up to 0.3).

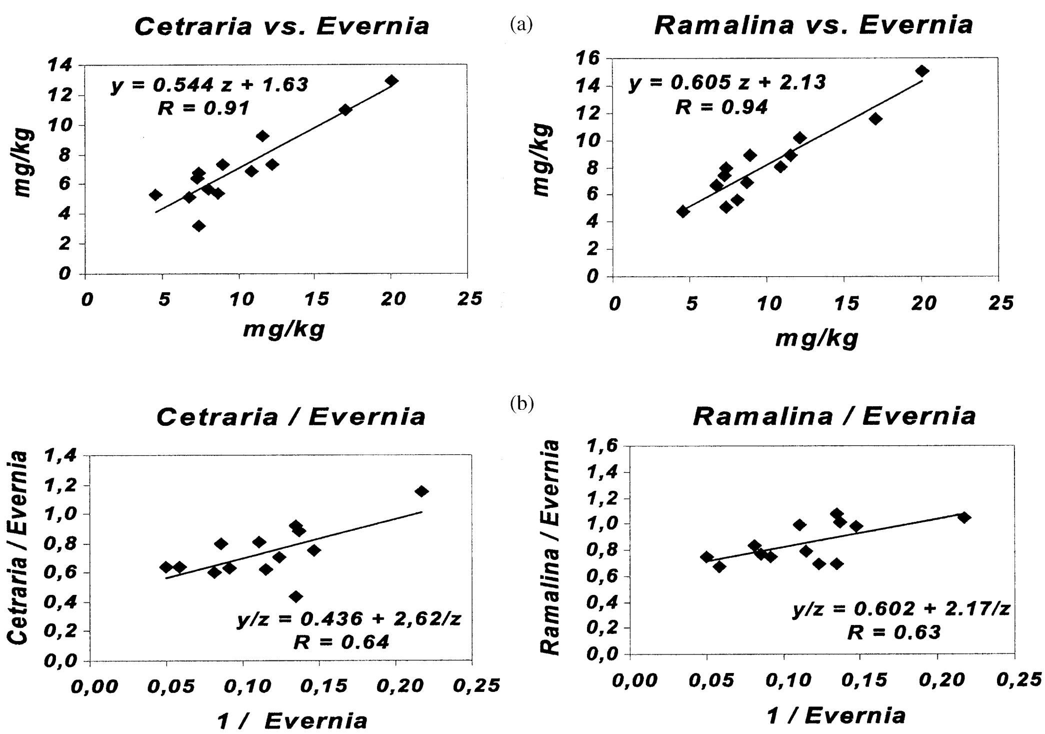

Fig. 4. Interspecies calibration for Ni: by the function $y = az + b$ (a) and by the function $y/z = a + b/z$ (b). R is the correlation coefficient.

Table 7
Interspecies calibration factors (a) and intercept values determined statistically ($b_{stat.}$) and calculated ($b_{calc.}$)

Element	*Cetraria / Evernia*			*Ramalina / Evernia*		
	Calibration factor (a)	$b_{stat.}$	$b_{calc.}$	Calibration factor (a)	$b_{stat.}$	$b_{calc.}$
As	0.54	0.061	0.057	0.71	0.135	0.123
Br	0.181	1.89	1.52	0.46	0.091	−0.07
Co	0.225	0.174	0.19	0.353	0.085	0.116
Cr	0.453	0.601	0.602	0.514	0.81	0.835
Cu	0.508	1.21	1.36	0.865	1.42	1.82
Fe	0.414	114	134	0.593	116	94
Ni	0.544	2.62	2.8	0.605	2.17	2.02
Pb	0.482	4	3.8	0.814	1.73	1.09
Sb	0.439	−0.07	−0.04	0.734	0.16	0.174
V	0.539	0.192	0.245	0.787	0.684	0.698
Zn	0.231	17.7	21.6	0.619	9.34	10.27
Mean	0.414			0.641		

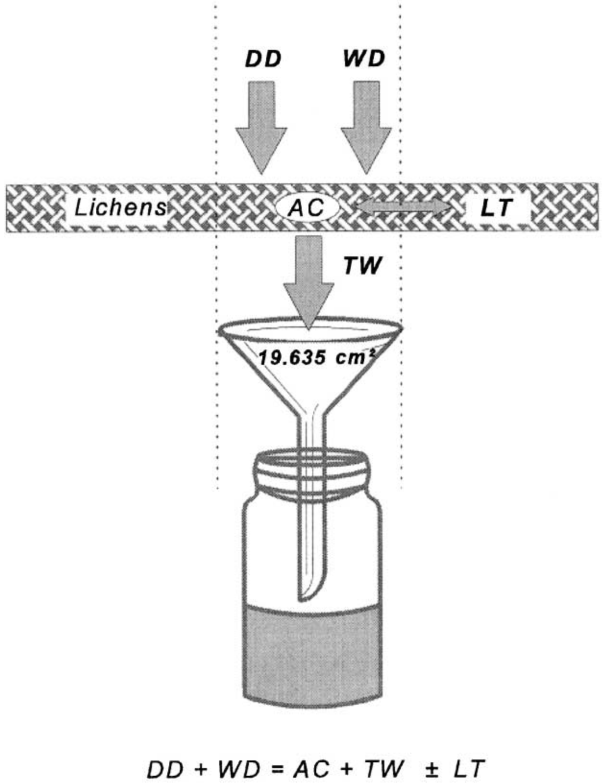

Fig. 5. Elemental balance inside a virtual column (DD, dry deposition; WD, wet deposition; AC, enrichment in lichens; TW, content in lichen throughfall water; LT, lateral transport).

3.3. Accumulation factors

Looking for quantitative criteria to evaluate the accumulating capacity, "**A**ccumulation **F**actors (AF)" according to Sloof (1993, 1995) were calculated for 10 elements as the slopes of the linear relationships between the element content of lichens (in mg/kg) and the cumulative bulk deposition (in mg/m^2). Consequently, the AF values result in m^2/kg. For this calculation, we could use only the data obtained after 6 and 12 months at the three exposure sites where the bulk deposition was measured (D-1, I-2, and RO-1).

Fig. 3 illustrates this relationship in the case of Ni.Table 6 summarises all the calculated AF values. The interspecies ratios of the mean values are 0.423 for *Cetraria*/*Evernia* and 0.672 for *Ramalina*/*Evernia*. This fact cannot be explained by differences in the thallus area per mass unit, as this parameter is rather uniform: 120 cm^2/g for *Cetraria islandica*, 145 cm^2/g for *Evernia prunastri*, and 155 cm^2/g for *Ramalina farinacea*.

3.4. Interspecies comparison

Another possibility for a comparative evaluation of the accumulating capacity is a direct interspecies comparison based on a simple linear model (Folkeson, 1979; Sloof and Wolterbeek, 1993, Sloof, 1993). One assumes

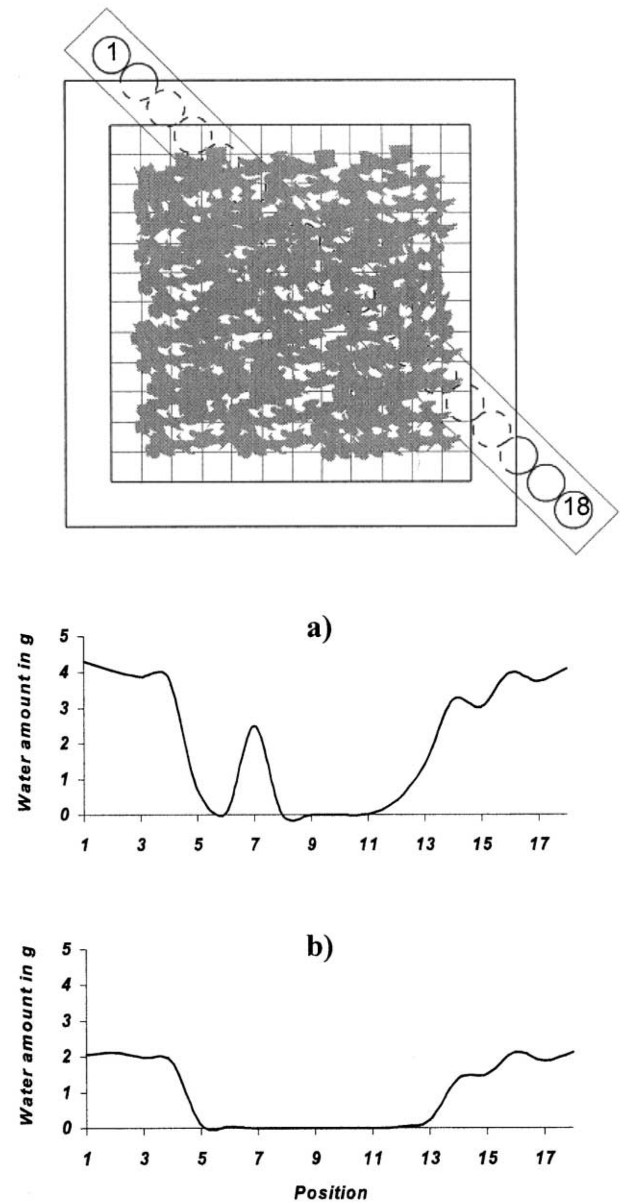

Fig. 6. Distribution of the throughfall water under a lichen sample (*Evernia prunastri*), considering all rainfall (a) and only light rainfall (b).

that the element content of each lichen species (y, z, ...) is a linear function of the atmospheric concentration or of the bulk deposition (x):

$$y = a_1x + b_{1,} z = a_2x + b_2, \ldots$$

Thereby results a linear interspecies relationship: $y = az + b$ with the slope $a = a_1/a_2$ (so-called "calibration factor") and the intercept $b = b_1 - ab_2$. For the transplants, this intercept can be calculated, as b_1 and b_2 are measured as initial element contents. To find out possible saturation effects, it is also useful to investigate the relation: $y/z = a + b/z$ with the slope b and the intercept a.

Table 8
"Retention Efficiencies": RE = AC/(AC + TW) calculated in %

Site	Time	As	Br	Co	Cr	Cu	Fe	Ni	Sb	V	Zn	General mean
Cetraria islandica												
D-1	6 months				48.6	45.6			84.2			48.6
	12 months	38.4		26.7	47.9	37.7	46.1		57.0	41.5	35.1	
D-2	6 months	35.4		11.0	61.7	70.8	74.3	32.5	77.8	42.4	70.6	
	12 months							35.6	50.2	45.9		
I-1	6 months											51.2
	12 months	49.9		34.3	54.6		61.8	58.1	67.9	64.4		
I-2	6 months											
	12 months	31.1	12.5	42.0	69.3		66.2	54.9		62.8	37.4	
RO-1	6 months	44.9	24.8	40.7	69.1	65.9	78.1	77.8	79.9	84.8	78.5	62.1
	12 months	55.4		56.3	65.9		69.8	70.3	60.1	65.2	63.1	
RO-2	6 months	55.4	31.3	34.0	78.9	67.6	72.9	59.4	86.1	67.0	63.1	
	12 months	47.5		47.6	67.3		60.8	70.3	78.5	43.8	53.4	
Mean		44.7	22.9	36.6	62.6	57.5	66.3	57.4	71.3	57.5	57.3	53.4
Evernia prunastri												
D-1	6 months											52.0
	12 months											
D-2	6 months		33.5					75.2				
	12 months	42.6		46.1	58.4	54.2	64.6	49.6	48.8	35.9	63.5	
I-1	6 months											63.6
	12 months	61.2	42.5	74.6	77.2	66.4	74.2	70.5	73.8	68.5	77.9	
I-2	6 months											
	12 months	42.6	35.9	71.3	82.0	38.5	82.0	67.9	45.9	57.3	61.6	
RO-1	6 months	51.7	27.5	64.4	74.0	65.5	77.2	76.8	73.2	69.8	81.0	71.2
	12 months	65.8	51.7	91.1	87.7	78.9	89.7	84.1	65.1	84.0	82.7	
RO-2	6 months	51.4	20.6	70.1	85.2		81.5	77.5	75.7	74.4	76.3	
	12 months	58.3		74.4	84.0		79.8	82.3	64.7	69.5	67.6	
Mean		53.4	35.3	70.3	78.4	60.7	78.4	73.0	63.9	65.6	72.9	65.2
Ramalina farinacea												
D-1	6 months	51.6		14.6	48.3	43.0	58.1		54.3	38.5	46.6	41.9
	12 months	26.1		15.3	30.5		45.4		27.3	48.8	38.0	
D-2	6 months											
	12 months	32.8		27.8	41.1	50.8	75.2	42.3	46.6	52.1	50.8	
I-1	6 months											54.8
	12 months	46.5	19.8	52.6	52.5	57.4	55.6	58.3	56.7	60.2	77.2	
I-2	6 months											
	12 months	51.5	20.7	56.6	68.1		68.1	63.3	56.8	68.8	50.9	
RO-1	6 months	75.1		68.5	81.7	77.6	88.0	86.5	62.5	93.5	75.9	64.2
	12 months	45.4		46.5	65.1	43.4	62.6	53.8	35.7	62.7		
RO-2	6 months	56.8	20.7		69.6	48.8	62.9	47.5	79.5	72.0	65.3	
	12 months	49.9		64.0	76.9	63.7	73.0	74.5	65.8	67.1	65.7	
Mean		48.4	20.4	43.2	59.3	55.0	65.4	60.9	53.9	62.6	58.8	52.8

This test was applied for the 11 elements listed above, comparing *Cetraria islandica* and *Ramalina farinacea* (y) with *Evernia prunastri* (z). Fig. 4 shows, for instance, the interspecies calibration for Ni. We observed no saturation effect for these elements. All the results for the parameters a and b are presented in Table 7. The values for the parameter b obtained by this statistical method ($b_{\text{stat.}}$) are compared with the values calculated from the initial contents ($b_{\text{calc.}}$). The generally good agreement supports the reliability of this model. The mean values of the calibration factors for *Cetraria*/*Evernia* (0.414) and for *Ramalina*/*Evernia* (0.641) are very close to the ratios of the average values of the "Accumulation Factors" (0.423 and 0.672). This fact suggests that the two criteria are equivalent, but both are suitable for an interspecies comparison.

3.5. Lichen throughfall water and "Retention Efficiencies"

Additionally, an attempt was made to investigate the elemental balance inside of a virtual column with the cross-section of 19.635 cm^2 (Fig. 5) by collecting and analysing the lichen throughfall water. The input fluxes are the dry deposition (DD) and the wet deposition (WD). The result is the accumulation in the lichens

(AC) and in the lichen throughfall water (TW). Besides these, the lateral transport (LT) also plays an important role. This explains why in two bottles put side by side under the same lichen sample, we found after 6 months 0.04 and, respectively 0.42 l water. To better understand this fact, a detailed investigation was carried out, mounting for about 12 weeks under a *Evernia prunastri* sample, a diagonal support with 18 small test tubes (Fig. 6), which were weighed individually after each rainfall. By integration over this time, it was possible to get the distribution of the throughfall water collected under the lichen sample (Fig. 6a). If we do not consider two heavy rainfalls which occurred during this period, we obtain the distribution in Fig. 6b. This illustrates the complexity of the problem with water collected under the lichen samples. The interpretation of such data requires, therefore, a high degree of caution.

We tried to elude this difficulty by normalising all the element contents of the water samples on the amount of precipitations during the collecting time at each exposure site. To avoid hazardous extrapolations, we did not consider for further interpretation the water samples whose amount was very different from those of the precipitations. This explains the incompleteness of Table 8.

To interpret the elemental distribution between lichens and lichen throughfall water in terms of bioaccumulation, we defined the "Retention Efficiency (RE)" as the per cent ratio between the lichen enrichment (AC) and the sum of lichen enrichment and lichen throughfall water content (AC+TW) inside a virtual column (Fig. 5). The values calculated in this way for ten elements, after 6 and 12 months, for all the exposure sites and the investigated lichen species, with the exception of the samples mentioned above, are presented in Table 8. The majority of these values are higher then 50%, which confirms the accumulating capacity of the lichens. The overall mean is the highest for *Evernia prunastri* (65.2%) and approximately equal for *Cetraria islandica* and *Ramalina farinacea* (53.4 and 52.8%).

An interesting tendency can be noted, if one compares the country means of the "Retention Efficiencies", in the last column of Table 8. For all the lichen species investigated, the values are the lowest for Germany and the highest for Romania, which could indicate the influence of climatic conditions as well as of type and level of the air pollution.

4. Conclusions

To estimate the suitability of a lichen species to be used as a transplant for trace-element air monitoring, some quantitative criteria can be applied: the initial element contents, the "Accumulation Factors" relative to the bulk deposition, the interspecies "Calibration Factors", and the "Retention Efficiencies".

Although, among the three investigated species, *Cetraria islandica* has on an average the lowest initial element contents, all the other criteria indicate *Evernia prunastri* as the most suitable for the transplantation. In second place follow *Ramalina farinacea*, regarding the accumulating capacity, and *Cetraria islandica*, concerning the "Retention Efficiencies".

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