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Evaluation of trace elements in different species of lichens by neutron activation analysis

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Instrumental neutron activation analysis has been applied for the determination of trace elements in five epiphytic lichens: *Canoparmelia texana*, *Canoparmelia caroliniana*, *Parmotrema tinctorum*, *Parmotrema sancti-angeli* and *Usnea* sp. that were collected at the same sampling area of the Botanic Institute, São Paulo, Brazil. The elemental concentration results showed that these five lichens can be chosen in the species intercalibration for biomonitoring studies. Also, some aspects related to the occurrence, tolerance to pollution, treatment and ease of sampling of the species are presented.

Introduction

In the past few decades, the use of biomonitors in environmental pollution assessment has become an alternative for the analysis of particulate matter and heavy metal deposition.[1–3]

Consequently the analyses of different lichens species are of interest since their choice depends on the purpose of the monitoring and on the biodiversity of the ecosystem under study. In trace element biomonitoring, factors such as occurrence, accumulation characteristics, ease of sampling and sample treatment, tolerance to pollution and background concentrations of the elements should be considered for the choice of lichen species.[4]

The selection of the species for pollution study is important since lichens are extremely susceptible to changes in the environment. Actually some species tend to vanish from polluted and/or urban areas. Also, there are species that only appear under those circumstances. The occurrence of about 2800 lichen species in Brazilian territory[5] has been published, however, data concerning their use in monitoring studies and their elemental composition are practically nonexistent.

Instrumental neutron activation analysis (INAA) was used to the trace element determinations in five lichens: *Canoparmelia texana*, *Canoparmelia caroliniana*, *Parmotrema tinctorum*, *Parmotrema sancti-angeli* and *Usnea* sp. The purpose of these analyses was to obtain preliminary data of trace elements accumulated in distinct species of lichens for their future selection in the biomonitoring studies.

Experimental

Sampling

The following samples of epiphytic lichens were collected: six specimens of *Canoparmelia texana* (Tuck.) Elix & Hale, three of *Canoparmelia caroliniana* (Nylander) Elix & Hale, four of *Parmotrema tinctorum* (Nylander) Hale, two of *Parmotrema sancti-angeli* (Lynge) Hale and three of *Usnea* sp. All these lichens are folioses except for *Usnea* sp., which is fruticose. These samples were collected from the bark of trees at about 1.5 m from the soil, and stored in paper bags. A titanium knife was used to remove the sample from the bark. The sampling was performed at the Botanic Institute, SP, Brazil, around an area of approximately 1 km^2.

Preparation of the samples for analysis

In order to remove eventual bark substrates or other extraneous materials, the lichens were cleaned by examining them in an Olympus zoom stereo microscope model SZ 4045. They were then washed with distilled water (immersed for about 5 minutes) and placed on filter papers for drying at room temperature. The samples were also freeze-dried for about 8 hours under a pressure of about $4\cdot10^{-2}$ mbar. The fine powder of lichen sample was obtained by grinding, manually, in an agate mortar.

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Procedure used for neutron activation analysis

About 150 mg of each sample were weighed in polyethylene bags and irradiated together with the standards. Two separate irradiations were performed to determine a large number of elements. Irradiations of 5 minutes under a thermal neutron flux of $4.25 \cdot 10^{11}$ $n \cdot cm^{-2} \cdot s^{-1}$ from the IEA-R1 nuclear reactor were carried out to determine Al, Br, Cl, K, Mg, Mn, Na, Ti and V. Longer irradiations of 16 hours with a neutron flux of 10^{13} $n \cdot cm^{-2} \cdot s^{-1}$ were done to determine As, Ca, Cd, Ce, Cr, Cs, Cu, Eu, Fe, La, Lu, Mo, Nd, Rb, Sb, Sc, Se, Sm, Tb, Th, Yb and Zn.

Irradiated samples and standards were placed on stainless steel planchets and, after adequate decay times, they were measured using a Canberra Model GM2020 Ge detector. The resolution FWHM of the system was 0.80 keV for the 121.97 keV gamma-ray of ^{57}Co and 1.80 keV for the 1331.49 keV gamma-ray of ^{60}Co. The detector was coupled to a Canberra Model 1510 Integrated Signal Processor, a microcomputer and associated electronic devices. Samples and standards were measured at least twice after different decay times. Analyses of gamma spectra were carried out by using VISPECT software[6] and the elemental concentrations were calculated by the comparative method.

In a previous paper the certified reference materials IAEA 336 Lichen, NIST 1572 Citrus Leaves and NIST 1575 Pine Needles were analyzed for the evaluation of the accuracy of the method.[7]

Table 1. Elemental concentrations in four lichen species collected at the Botanic Institute

Element		*Usnea sp.*			*Parmotrema tinctorum*	
	X ± s	Range	*n*	X ± s	Range	*n*
Al (μg/g)	876 ± 423	410–1236	3	748 ± 216	613 – 1070	4
As (μg/kg)	923 ± 261	624–1100	3	287 ± 91	189 – 369	3
Br (μg/g)	11 ± 7	5–19	3	10 ± 6	5 – 15	4
Ca (%)	0.8 ± 0.6	0.2–1.4	3	0.23 ± 0.06	0.2–0.3	3
Cd (μg/g)	0.83 ± 0.26	0.60–1.12	3	0.51 ± 0.05	0.45–0.55	3
Ce (μg/g)	3.1 ± 0.5	2.5–3.5	3	0.24 ± 0.06	1.4–2.6	3
Cl (μg/g)	139 ± 9	134–150	3	386 ± 183	181–584	4
Cr (μg/g)	4.7 ± 3.1	2.0–7.4	3	3.6 ± 1.3	2.7–5.1	3
Cs (μg/kg)	355 ± 163	240–470	3	339 ± 15	328–349	2
Cu (μg/g)	9 ± 5	4–14	3	*		
Eu (μg/kg)	34 ± 8	25–40	3	19 ± 2	17–20	2
Fe (μg/g)	1078 ± 787	274–1846	3	764 ± 333	481–1130	3
K (μg/g)	3452 ± 1013	2355–4350	3	2477 ± 681	584–2882	4
La (μg/g)	1.7 ± 0.3	1.3–1.9	3	1.1 ± 0.3	0.8–1.3	3
Lu (μg/kg)	7.5 ± 1.3	6.2–8.7	3	6.7 ± 3.7	4.5–10.9	3
Mg (μg/g)	882 ± 72	835–965	3	484 ± 117	369–645	4
Mn (μg/g)	25 ± 5	19–29	3	17 ± 8	11–29	4
Mo (μg/g)	1.35 ± 0.83	0.49–2.15	3	0.61 ± 0.16	0.48–0.79	3
Na (μg/g)	75 ± 33	38–100	3	48 ± 7	41–56	4
Nd (μg/kg)	1211 ± 318	844–1414	3	1063 ± 505	669–1633	3
Rb (μg/g)	15 ± 4	11–18	3	12 ± 2	11–14	2
Sb (μg/kg)	686 ± 307	345–942	3	365 ± 118	285–501	3
Sc (μg/kg)	221 ± 77	166–275	2	88 ± 24	71–104	2
Se (μg/kg)	376 ± 164	235–556	3	178 ± 52	124–228	3
Sm (μg/kg)	170 ± 34	132–196	3	223 ± 93	165–330	3
Tb (μg/kg)	23 ± 6	17–28	3	13 ± 6	9–18	3
Th (μg/kg)	278 ± 216	104–519	3	189 ± 76	120–270	3
Ti (μg/g)	103 ± 45	61–151	3	71 ± 23	55–104	4
V (μg/g)	3.2 ± 2.6	1.4–5.1	2	2.5 ± 0.9	1.9–3.8	4
Yb (μg/kg)	43 ± 18	24–59	3	38 ± 14	24–52	3
Zn (μg/g)	67 ± 13	55–80	3	62 ± 20	43–83	3

Table 1. (continued)

Element	*Canoparmelia caroliniana*			*Parmotrema sancti-angeli*		
	X ± s	Range	*n*	X ± s	Range	*n*
Al (μg/g)	1640 ± 362	1426–2058	3	981 ± 624	540 – 1422	2
As (μg/kg)	712 ± 103	604–809	3	455 ± 40	427 – 483	2
Br (μg/g)	15.6 ± 0.9	14.6–16.3	3	6 ± 2	4–7	2
Ca (%)	5.2 ± 1.7	3.2–6.6	3	1.1 ± 0.2	0.9–1.2	2
Cd (μg/g)	1.90 ± 0.69	1.94–2.56	3	0.44 ± 0.07	0.40–0.49	2
Ce (μg/g)	4.0 ± 0.9	3.5–5.0	3	1.9 ± 1.0	1.2–2.6	2
Cl (μg/g)	357 ± 37	315–381	3	718 ± 202	575–861	2
Cr (μg/g)	5.62 ± 0.05	5.6–5.7	2	5.1 ± 3.1	2.9–7.3	2
Cs (μg/kg)	533 ± 62	489–577	2	224 ± 64	178–269	2
Cu (μg/g)	*			10.0 ± 0.4	9.7–10.2	2
Eu (μg/kg)	38.0 ± 0.1	37.9–38.0	2	*		
Fe (μg/g)	1515 ± 357	1107–1770	3	1016 ± 565	617–1416	2
K (μg/g)	709 ± 315	365–983	3	2216 ± 638	1765–2667	2
La (μg/g)	2.4 ± 0.5	2.1–3.0	3	0.96 ± 0.35	0.71–1.24	
Lu (μg/kg)	24.5 ± 13.2	15.2–39.6	3	6.9 ± 2.9	3.5–8.6	2
Mg (μg/g)	696 ± 140	555–835	3	734 ± 166	844–868	2
Mn (μg/g)	43 ± 29	11–66	3	14 ± 3	11–16	2
Mo (μg/g)	1.23 ± 0.17	1.12–1.43	3	0.87 ± 0.21	0.72–1.01	2
Na (μg/g)	89 ± 20	74–112	3	58 ± 33	33–95	2
Nd (μg/kg)	1817 ± 288	1612–2146	3	964 ± 543	580–1348	2
Rb (μg/g)	5.1 ± 0.6	4.5–5.7	3	21.9 ± 2.2	20.4–23.5	
Sb (μg/kg)	436.7 ± 29.5	408.4–467.0	3	489 ± 183	360–619	2
Sc (μg/kg)	216 ± 5	212–220	2	163 ± 100	92–234	2
Se (μg/kg)	198 ± 62	127–255	3	244 ± 30	218–265	2
Sm (μg/kg)	230 ± 38	200–273	3	164 ± 62	207–218	2
Tb (μg/kg)	45 ± 20	31–59	2	*		
Th (μg/kg)	388 ± 47	355–421	2	245 ± 158	133–356	2
Ti (μg/g)	114 ± 49	63–161	3	105 ± 81	47–162	2
V (μg/g)	5.3 ± 2.4	2.5–6.8	3	3.2 ± 1.5	2.1–4.2	2
Yb (μg/kg)	131 ± 87	68–231	3	36 ± 19	22–49	2
Zn (μg/g)	94 ± 38	69–138	2	41 ± 2	39–42	2

* No detected value.
X ± s: Arithmetic mean and standard deviation.
n: Number of samples in which the elements were detected.

Results and discussion

The results obtained for the elemental concentrations of the five species analyzed are shown in Tables 1 and 2. In these tables the arithmetic means with their standard deviations are presented and the concentration ranges obtained for different samples of the same species. It was possible to detect the same elements, around thirty, for all the species studied. As it can be seen in these tables, higher concentration mean values at the percentage levels were obtained for Ca and concentration values, around μg/g, were found for Al, Br, Cd, Ce, Cl, Cr, Cu, Fe, K, La, Mg, Mn, Mo, Na, Rb, Ti, V and Zn. Lower concentration values, around μg/kg, were found for As, Cs, Eu, Lu, Nd, Sb, Sc, Se, Sm, Tb, Th and Yb. The concentration levels of the elements in different lichen species are, in general, of the same order of magnitude.

It can also be seen from the Tables 1 and 2 that the elements determined exhibit considerable variability among the samples of the same species. This variability for a same lichen species can probably be attributed to the lichen samples with different age or of its position in the trunk of the tree.

The "Statistical Package for the Social Sciences" (SPSS) computer program was applied for the statistical analysis of the analytical data related to different lichen species. Correlation matrices obtained by using elemental concentration data are presented in Table 3. The Pearson correlation coefficient[9] is 0.46, at 5% level of significance. Therefore, these data show a correlation among the elemental concentrations found in all the species studied. By analyzing the data from the Table 3, it can also be concluded that *C. caroliniana* presents strong correlation with all the other species and mainly with *C. texana*. The lichens *P. sancti-angeli and P. tinctorum* showed a low degree of correlation with *C. texana*. The *Usnea sp.*, in general, presented a stronger correlation with *P. sancti-angeli* and *P. tinctorum* than with *C. texana*.

The five species analyzed are considered as one tolerant to pollution and they are found in the urban area of the São Paulo city. In most of the cases, the first lichen that one can find is *C. texana*, widely spread in the Brazilian territory except coastal cities. *C. texana* is a foliose lichen, with a large thallus. In non-polluted ecosystems this species is limited to twigs and branches in well-lit woods or on the trunks of exposed trees at the sides of non-paved roads, and it does not occur frequently. However, in polluted or urban areas, where its competitors cannot be present, and in comparatively dry and illuminated environments, it occurs frequently, covering practically the whole tree trunks, as observed at the Botanic Institute. As the degree of urbanization decreases, species like *C. caroliniana, P. sancti-angeli, P. tinctorum*, among others, have been found. *Usnea* sp. appears in even less polluted areas.[5]

From the points of view of sample collection and treatment, our experimental work has shown that *Usnea* sp. is the easiest one to be collected and cleaned, since it is a fruticose lichen, not very attached to the substract, followed by *P. tinctorum, P. sancti-angeli, C. texana* and *C. caroliniana*. Although *C. texana* is not the easiest one for cleaning, it presents adequate characteristics for biomonitoring programs due to its abundance.

From the results obtained in this work, it can be concluded that the five lichens present the same characteristics to accumulate the elements and they can be used for interspecies calibration and monitoring studies.

Table 2. Concentrations of elements in *Canoparmelia texana* samples collected at the Botanic Institute

Element	X ± s	Range	*n*
Al (μg/g)	727 ± 365	426–1321	5
As (μg/kg)	339 ± 57	274–438	6
Br (μg/g)	4.1 ± 2.3	1.3–8.0	6
Ca (%)	7.1 ± 3.9	2.0–12.0	6
Cd (μg/g)	3513 ± 1829	799–6235	6
Ce (μg/g)	3.2 ± 1.0	1.7–4.8	6
Cl (μg/g)	479 ± 148	337–696	5
Cr (μg/g)	2.9 ± 1.5	1.7–2.9	5
Cs (μg/kg)	102 ± 60	52–214	6
Cu (μg/g)	12 ± 4	8–14	3
Eu (μg/kg)	30 ± 13	16–53	6
Fe (μg/g)	800 ± 437	366–1537	6
K (μg/g)	1104 ± 277	900–1491	4
La (μg/g)	2.1 ± 0.7	0.9–3.0	6
Lu (μg/kg)	8 ± 3	3.4–11.6	6
Mg (μg/g)	510 ± 102	336–597	5
Mn (μg/g)	30 ± 16	11.0–45.0	6
Mo (μg/g)	0.7 ± 0.1	0.6–0.8	6
Na (μg/g)	44 ± 12	34–60	6
Nd (μg/kg)	1321 ± 422	646–1606	6
Rb (μg/g)	4 ± 3	2.5–9.1	6
Sb (μg/kg)	248 ± 69	164–300	6
Sc (μg/kg)	108 ± 46	57–119	6
Se (μg/kg)	155 ± 61	104–267	6
Sm (μg/kg)	179 ± 79	113–328	6
Tb (μg/kg)	23 ± 9	11–37	6
Th (μg/kg)	163 ± 62	83–270	6
Ti (μg/g)	53 ± 25	23–90	5
V (μg/g)	2.0 ± 1	2.3–3.0	5
Yb (μg/kg)	48 ± 21	25–80	6
Zn (μg/g)	103 ± 28	66–140	6

Table 3. Pearson's correlation coefficients obtained from elemental concentrations in lichens

Variable	CCAR09	CCAR11	CCAR12	PSAN29	PSAN33
CCAR11	0.9623				
CCAR12	0.9524	0.9969			
PSAN29	0.4650	0.6324	0.6534		
PSAN33	0.8585	0.9105	0.9052	0.7803	
PTIN17	0.8263	0.8748	0.8834	0.7667	0.8869
PTIN22	0.9512	0.9311	0.9244	0.5423	0.8908
PTIN25	0.8845	0.9435	0.9494	0.7615	0.9552
TEXEST1	0.7816	0.7397	0.7345	0.2030	0.5172
TEXEST2	0.8866	0.8852	0.8829	0.3896	0.6866
TEXEST3	0.9714	0.9181	0.9087	0.3467	0.7635
TEXEST4	0.8504	0.8023	0.7944	0.2350	0.5935
TEXOR	0.9489	0.9099	0.9033	0.3128	0.7051
TEXPAL	0.9913	0.9584	0.9486	0.5137	0.9007
US20	0.8850	0.8191	0.7972	0.3242	0.7676
US21	0.9115	0.9277	0.9164	0.6900	0.9831
US27	0.7351	0.8129	0.8101	0.8022	0.9646
Variable	PTIN17	PTIN22	PTIN25	TEXEST1	TEXEST2
PTIN22	0.9278				
PTIN25	0.9578	0.9378			
TEXEST1	0.4087	0.5776	0.5290		
TEXEST2	0.6078	0.7340	0.7183	0.9645	
TEXEST3	0.6967	0.8635	0.7889	0.8993	0.9528
TEXEST4	0.4869	0.6650	0.6061	0.9915	0.9790
TEXOR	0.6785	0.8379	0.7632	0.9137	0.9676
TEXPAL	0.8291	0.9457	0.9046	0.7591	0.8669
US20	0.7306	0.8864	0.7577	0.6036	0.6993
US21	0.8955	0.9418	0.9437	0.5541	0.7131
US27	0.8592	0.8223	0.9192	0.2946	0.4985
Variable	TEXEST3	TEXEST4	TEXOR	TEXPAL	US20
TEXEST4	0.9464				
TEXOR	0.9875	0.9521			
TEXPAL	0.9583	0.8307	0.9203		
US20	0.8339	0.6604	0.8000	0.8739	
US21	0.8171	0.6366	0.7609	0.9376	0.8462
US27	0.6001	0.3846	0.5321	0.7894	0.6722
Variable	US21				
US27	0.9301				

C. caroliniana (CCAR), *C.texana* (TEXOR, TEXPAL, TEXEST), *P. sancti–angeli* (PSAN), *P.tinctorum* (PTIN), *Usnea* sp. (US).

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