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Determination of trace elements in lichens by instrumental neutron activation analysis

M. Saiki,* C. G. Chaparro,* M. B. A. Vasconcellos,* M. P. Marcelli**

**Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN/SP, Radiochemistry Division, P.O. Box 11 049, CEP 05422-970, São Paulo, SP, Brazil*
***Instituto de Botânica, P.O. Box 4005, CEP 01061-970, São Paulo, SP, Brazil*

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Instrumental neutron activation analysis has been applied to analyze epiphytic specimens of the lichen *Canoparmelia texana*. Samples collected from the barks of the trees were previously cleaned, lyophilized and ground to be irradiated under a thermal neutron flux in the IEA-R1 nuclear reactor. Elements Al, As, Br, Ca, Cd, Cl, Co, Cr, Cs, Fe, Hf, K, La, Ce, Nd, Sm, Eu, Tb, Yb, Lu, Mg, Mn, Na, Rb, Sb, Sc, Se, Th, Ti, U, V and Zn were determined quantitatively by using short and long irradiations. Comparisons were made between the results for washed and unwashed samples as well as those collected in two different years, from individual palm trees and also from different sites of São Paulo State. The precision and the accuracy of the method were evaluated by analyzing NIST 1572a Citrus Leaves, IAEA 336 Lichen and USGS W-1 Rock reference materials.

Introduction

Among the great number of biological materials, lichens have been widely analyzed for monitoring atmospheric particulate matter and deposition because of their high capacity of absorbing and accumulating many inorganic pollutants. Lichens are very sensitive to anthropogenic changes of the environment and they have provided relevant information in biomonitoring programmes.

The use of lichens as a biomonitor presents advantages compared to air filters and deposition collectors because of their easy sampling and by using less expensive equipments. Besides, their high degree of trace element accumulation enables the determination of several elements with a high precision and accuracy of the results. Also, lichens can be used in continuous and retrospective monitoring and samples can be collected at the same time in several places and furthermore a comparative study of analytical data can be made.

With the increase of the environmental problems originated from the industrial development, automobile pollution and waste incineration, in several European and North American countries, lichens have been analyzed in order to get maps of geographical distributions of the pollutant concentrations.[1–5]

In Brazil, analytical data of trace elements in lichens are very scarce and the purpose of the present work was to establish adequate conditions for instrumental neutron activation analysis of this material for their further use in the monitoring of the environmental pollution.

Experimental

Samples

Samples of *Canoparmelia texana* (Tuck.) Elix & Hale were carefully collected from the bark of trees at a height of about 1.5 m from the ground and stored in paper bags. In this process a titanium knife was used. In the laboratory the samples were firstly cleaned by examining them under a stereoscopic microscope to remove eventual bark substrates or extraneous materials. Then, they were washed in distilled water to remove dust and sand. Next, samples were lyophilized for about 16 hours and in this drying process a mean weight loss of about 13% was found. To verify the washing effect one sample was divided into two parts: one of them was washed into water before drying and the other was directly dried. To avoid contamination of the samples a series of precautions were taken and the handling of the samples was performed inside a class 100 laminar flow hood.

The *Canoparmelia texana* specimens analyzed in this work were collected in the following sites:

Site 1: Four samples were collected on the same day (April 6, 1993, Autumn) from four individual palm trees in the surroundings of the Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP) located at the Campus of the University of São Paulo, SP, within an area of about 350 m^2. In this site, other two samples were also collected from palm trees during Summer (February 2, 1994);

Site 2: Samples were also collected from near the University amphitheatre situated at the Campus of the University of São Paulo;

Site 3: One sample was collected in a countryside town called Ibiuna, SP, considered as a clean region.

Standards

Synthetic standards were prepared by pipeting aliquots of 50 or 100 µl of multielement and single element standard solutions onto a small sheet of Whatman No. 41 filter paper. After drying at room temperature these sheets were placed into clean polyethylene bags and irradiated together with the samples.

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Table 1. Results obtained in reference materials

Element	Reference material*	This work**		Refs 6–8	Element	Reference material*	This work**		Refs 6–8
Al (μg/g)	1	791 ± 49	(6.1)	655 (583–728)	Tb (μg/kg)	1	13.1 ± 1.6	(12.2)	13.2 ± 0.4
	2	120 ± 12	(10.0)	92 ± 14					
As (μg/kg)	1	614 ± 59	(9.6)	634 (597–671)	Yb (μg/kg)	1	34.8 ± 3.7	(10.7)	43.5 ± 3.6
	2	2.71 ± 0.19	(7.0)	3.1 ± 0.3					
Br (μg/g)	1	11.4 ± 0.5	(4.8)	12.3 ± 0.1	Lu (μg/kg)	1	6.3 ± 0.5	(7.9)	5.15 ± 0.72
	2	8.1 ± 0.8	(9.9)	(8.2)					
Ca (μg/g)	1	2122 ± 171	(8.1)	2560 ± 82	Mg (μg/g)	1	773 ± 115	(14.8)	616 ± 68
	2	28 624 ± 1497	(5.2)	31 500 ± 1009		2	6077 ± 279	(4.6)	5800 ± 302
Cl (μg/g)	1	1889 ± 92	(4.9)	1980 ± 32	Mn (μg/g)	1	64.7 ± 2.3	(3.6)	63.1(59.9–66.4)
	2	392 ± 22	(5.6)	(414)		2	22.3 ± 1.1	(4.9)	23 ± 2
Co (μg/kg)	1	282 ± 24	(8.6)	268 ± 12	Na (μg/g)	1	287 ± 30	(10.4)	350 ± 7
	2	37 ± 4	(10.8)	(20)	2	160 ± 21	(13.1)	160 ± 21	
Cr (μg/kg)	1	999 ± 42	(4.2)	1040 (940–1140)	Rb (μg/g)	1	1.60 ± 0.16	(10.0)	1.57 ± 0.19
	2	710 ± 40	(5.6)			2	4.85 ± 0.28	(5.8)	4.84 ± 0.06
Cs (μg/kg)	1	114 ± 7	(6.2)	123 ± 21	Sb (μg/kg)	1	78.6 ± 9.5	(12.1)	70.1 ± 7.7
	2	94 ± 8	(8.5)	(98)					
Fe (μg/g)	1	394 ± 37	(9.6)	441 (418–464)	Sc (μg/kg)	1	182 ± 6	(3.3)	188 ± 6
	2	82 ± 5	(5.9)	90 + 10					
Hf (μg/kg)	1	57.3 ± 4.6	(8.0)	58.3 ± 4.2	Se (μg/kg)	1	232 ± 28	(12.1)	218 (195–240)
K (μg/g)	1	1646 ± 105	(6.4)	1840 (1780–1900)	Th (μg/kg)	1	136 ± 4	(2.9)	137 ± 11
	2	18 429 ± 1847	(10.0)	18 200 ± 601					
La (μg/kg)	1	559 ± 20	(3.6)	625 ± 7	Ti (μg/g)	3	6557 ± 275	(2.9)	6410 ± 380
	2	150 ± 10	(6.7)	190 ± 10					
Ce (μg/g)	1	1.14 ± 0.6	(5.7)	1.31 ± 0.04	U (μg/kg)	1	81.8 ± 10.1	(12.4)	
Nd (μg/kg)	1	622 ± 60	(9.7)	894 ± 232	V (μg/g)	1	1.60 ± 0.10	(6.2)	1.47 (1.33–1.61)
						3	261 ± 5	(1.9)	260 ± 25
Sm (μg/kg)	1	106 ± 11	(10.6)	117 ± 3	Zn (μg/g)	1	29.9 ± 1.8	(6.0)	30.9 (29.4 ± 32.5)
						2	27.9 ± 0.4	(1.4)	29 ± 2
Eu (μg/kg)	1	23.4 ± 2.3	(9.8)	27.2 ± 1.3					

* 1 – IAEA 336 Lichen; 2 – NIST 1572a Citrus Leaves; 3 – USGS W-1 Rock.

** Values in parentheses are relative standard deviation.

Instrumental neutron activation analysis (INAA)

Aliquots of about 100 mg of lichen samples weighed and heat-sealed in polyethylene bags were irradiated at the IEA-R1 research nuclear reactor at the IPEN-CNEN/SP. Short irradiations of 5 minutes for the determination of Al, Cl, Mg, Mn, Na, Ti and V were carried out by using a pneumatic transfer system facility under a thermal neutron flux of $4 \cdot 10^{11}$ n · cm^{-2} · s^{-1}. Long irradiations of 16 hours under a thermal neutron flux of about 10^{13} n · cm^{-2} · s^{-1} were for As, Br, Ca, Cd, Co, Cr, Cs, Fe, Hf, K, lanthanides, Rb, Sb, Sc, Se, Th, U and Zn determinations. In the case of long irradiations the plastic bags with samples and standards were wrapped in Al foils which then were removed for the counting. After adequate decay times γ-ray measurements were performed using an EG&G Ortec Model GMX20190 Ge detector coupled to an EG&G Ortec 918A Multichannel Buffer connected to a microcomputer. Samples and standards were counted at least twice after different decay times. Analyses of γ-spectra were carried out by using an appropriate computer program and the concentrations of the elements were calculated by comparative method.

Results and discussion

For quality assurance INAA was applied in the analyses of the IAEA 336 Lichen, NIST 1572a Citrus Leaves and USGS W-1 Rock reference materials.[6–8] Results obtained in these determinations, presented in Table 1, show a good precision for most elements with relative standard deviations lower than 10%. The accuracy of the results was also good. There was a good agreement between our results and the certified values or with those obtained by another laboratory.

Table 2 shows the results obtained from washed and unwashed lichens collected from the same palm tree. The unwashed sample presented concentrations of most elements of the same magnitude or slightly higher than the washed ones. However, within the experimental errors these results show no significant difference, with the exception of the elements Al, K, Na, Se and Ti. These results indicate that the washing procedure can be used to eliminate adhering materials since most of the elements retained by the lichens and of interest for environmental contamination were not removed.

To establish the variation in the elemental concentrations within one sampling site, lichen samples

Table 2. Results of washed and unwashed lichens

Element	Lichen sample		Variation between washed and unwashed, %	Analytical reproducibility, %
	Washed	Unwashed		
Al (μg/g)	1629 ± 68	1947 ± 84	16.3	6.1
As (μg/kg)	375 ± 5	408 ± 4	8.0	9.6
Br (μg/g)	3.15 ± 0.01	2.91 ± 0.01	8.2	4.8
Ca (%)	4.70 ± 0.03	4.54 ± 0.03	3.5	8.1
Cd (μg/kg)	1099 ± 32	1120 ± 42	1.9	13.7
Cl (μg/g)	428 ± 17	510 ± 20	1.6	4.9
Co (μg/kg)	304 ± 3	284 ± 4	7.0	8.6
Cr (μg/g)	24.1 ± 0.1	23.6 ± 0.1	2.1	4.2
Cs (μg/kg)	270 ± 4	272 ± 5	0.7	6.2
Fe (μg/g)	938 ± 4	877 ± 7	6.9	9.6
Hf (μg/kg)	345 ± 3	322 ± 3	7.1	8.0
K (μg/g)	1353 ± 14	3216 ± 16	57.9	6.4
La (μg/g)	2.667 ± 0.008	2.602 ± 0.008	2.5	3.6
Ce (μg/g)	4.71 ± 0.04	4.74 ± 0.04	0.6	5.7
Nd (μg/g)	1.88 ± 0.10	1.68 ± 0.09	11.9	9.7
Sm (μg/kg)	268.4 ± 0.5	272.3 ± 0.4	1.4	10.6
Eu (μg/kg)	46.2 ± 0.9	45.9 ± 0.9	0.6	9.8
Tb (μg/kg)	23.9 ± 2.1	25.5 ± 2.0	6.3	12.2
Yb (μg/kg)	56.6 ± 5.0	59.3 ± 5.8	4.5	10.7
Lu (μg/kg)	13.6 ± 0.4	14.1 ± 0.4	3.5	7.9
Mg (μg/g)	864 ± 88	988 ± 103	12.5	14.8
Mn (μg/g)	35.4 ± 0.5	37.4 ± 0.5	5.3	3.6
Na (μg/g)	106 ± 6	148 ± 7	28.4	10.4
Rb (μg/g)	14.0 ± 0.1	13.4 ± 0.1	4.5	10.0
Sb (μg/kg)	410.5 ± 1.4	430.3 ± 1.6	4.8	12.1
Sc (μg/kg)	289.5 ± 0.6	289.2 ± 0.8	0.1	3.3
Se (μg/kg)	178 ± 18	229 ± 20	22.3	12.1
Th (μg/kg)	311 ± 2	337 ± 2	7.7	2.9
Ti (μg/g)	158 ± 27	130 ± 28	21.5	2.9
U (μg/kg)	144 ± 2	154 ± 2	6.5	12.4
V (μg/g)	4.3 ± 0.3	4.2 ± 0.3	2.3	6.2
Zn (μg/g)	95.9 ± 0.4	99.9 ± 3.7	4.0	6.0

Table 3. Results obtained for lichens collected from same site in different periods

Element	Samples from Site 1 February 3, 1994			Samples from Site 1 April 6, 1993		
	Mean ± s (s_r)		Range	Mean ± s (s_r)		Range
Al (μg/g)	4763 ± 531	(11)	4388–5139	2360 ± 598	(25)	1687–2936
As (μg/kg)	873 ± 227	(26)	712–1034	737 ± 194	(26)	436–826
Br (μg/g)	34 ± 13	(39)	24–44	14 ± 6	(42)	8.7–21.5
Ca (%)	2.2 ± 0.5	(21)	1.85 ± 2.52	2.5 ± 0.5	(18)	1.86–2.92
Cd (μg/kg)	2037 ± 846	(41)	1439–2636	1779 ± 576	(32)	1023–2426
Cl (μg/g)	668 ± 202	(30)	525–811	605 ± 135	(22)	462–761
Co (μg/kg)	692 ± 171	(25)	571–813	409 ± 123	(30)	262–560
Cr (μg/g)	10.0 ± 1.3	(13)	9.1–11.0	5.0 ± 1.8	(36)	3.2–7.5
Cs (μg/kg)	514 ± 35	(6.8)	489–539	325 ± 185	(57)	131–575
Fe (μg/g)	2278 ± 222	(9.7)	2121–2435	1197 ± 789	(41)	740–1870
Hf (μg/kg)	576 ± 76	(13)	522–629	342 ± 167	(49)	239–590
K (μg/g)	1706 ± 563	(33)	1308–2105	1412 ± 362	(26)	1064–1785
La (μg/g)	5.0 ± 1.2	(24)	4.2–5.9	3.7 ± 2.3	(63)	1.93–7.22
Ce (μg/g)	9.1 ± 3.1	(34)	7.0–11.4	6.1 ± 2.5	(41)	3.5–9.6
Nd (μg/g)	3.5 ± 0.8	(23)	4.11–2.96	2.36 ± 1.40	(59)	1.24–4.41
Sm (μg/kg)	566 ± 155	(27)	456–675	372 ± 158	(42)	229–596
Eu (μg/kg)	119 ± 29	(24)	140–98	76 ± 48	(48)	36–145
Tb (μg/kg)	60 ± 14	(23)	50–70	40 ± 25	(62)	18–76
Yb (μg/kg)	191 ± 52	(27)	154–229	106 ± 53	(50)	66–184
Lu (μg/kg)	40 ± 16	(41)	28–51	19 ± 10	(51)	12.2–33.8
Mg (μg/g)	2966 ± 826	(28)	2382–3551	1870 ± 440	(23)	1363–2239
Mn (μg/g)	103 ± 53	(52)	65–140	97.3 ± 50.2	(51)	49.1–165.1

Table 3 (con'd)

Na (μg/g)	214 ± 26	(12)	195–232	143 ± 56	(39)	81–216
Rb (μg/g)	8.5 ± 1.3	(16)	7.6–9.5	6.9 ± 1.3	(20)	4.99–8.20
Sb (μg/kg)	992 ± 138	(14)	895–1090	560 ± 202	(36)	392–851
Sc (μg/kg)	647 ± 61	(9.5)	604–691	365 ± 153	(42)	228–574
Se (μg/kg)	413 ± 14	(3.4)	403–423	271 ± 79	(42)	162–343
Th (μg/kg)	1047 ± 192	(18)	911–1183	605 ± 258	(43)	419–985
Ti (μg/g)	421 ± 112	(26)	342–500	197 ± 94	(47)	110–331
U (μg/kg)	433 ± 154	(35)	324–542	167 ± 58		38–270
V (μg/g)	13.3 ± 0.4	(3.0)	13.0–13.6	8.1 ± 2.2	(27)	5.4–10.8
Zn (μg/g)	106 ± 15	(14)	96.2–116.9	94.7 ± 24.1	(25)	60.5–113.5

s – standard deviation.

s_r – relative standard deviation.

Table 4. Results obtained for lichens collected from different sites of the State of São Paulo

Element	Ibiuna, SP	São Paulo, SP (Site 1)*	São Paulo, SP (Site 2)**
Al (μg/g)	2747 ± 44	4388 ± 239	7129 ± 137
As (μg/kg)	411 ± 14	1034 ± 9	1057 ± 14
Br (μg/g)	39.40 ± 0.07	24.92 ± 0.07	24.85 ± 0.05
Ca (%)	4.67 ± 0.08	2.52 ± 0.03	4.13 ± 0.07
Cd (μg/kg)	456 ± 59	2636 ± 67	3917 ± 209
Cl (μg/g)	639 ± 14	525 ± 24	284 ± 39
Co (μg/kg)	219 ± 4	571 ± 20	1063 ± 14
Cr (μg/g)	2.85 ± 0.05	9.14 ± 0.06	16.4 ± 0.1
Cs (μg/kg)	117.5 ± 4.1	489 ± 9	1016.4 ± 9.4
Fe (μg/g)	1033 ± 6	2121 ± 9	4135 ± 21
Hf (μg/kg)	378 ± 3	522.1 ± 3.5	1464 ± 5
K (μg/g)	1892 ± 116	1308 ± 21	3849 ± 233
La (μg/g)	1.454 ± 0.006	4.19 ± 0.01	7.05 ± 0.05
Ce (μg/g)	3.30 ± 0.02	6.98 ± 0.02	16.58 ± 0.04
Nd (μg/g)	1.62 ± 1.09	2.96 ± 0.17	6.52 ± 0.21
Sm (μg/kg)	180.7 ± 0.4	456.5 ± 0.4	1055 ± 1
Eu (μg/kg)	39.4 ± 2.4	98.5 ± 1.7	181 ± 2
Tb (μg/kg)	20.7 ± 1.9	50.2 ± 2.3	103.6 ± 3.2
Yb (μg/kg)	53.1 ± 4.1	154.4 ± 5.4	346.6 ± 6.7
Lu (μg/kg)	10.8 ± 0.4	28.2 ± 1.5	60.1 ± 0.5
Mg (μg/g)	2513 ± 138	3551 ± 305	3540 ± 437
Mn (μg/g)	37.8 ± 0.9	140.4 ± 1.3	164.4 ± 1.2
Na (μg/g)	77.2 ± 0.1	195.8 ± 0.5	422.9 ± 0.5
Rb (μg/g)	6.0 ± 0.1	7.6 ± 0.1	20.2 ± 0.2
Sb (μg/kg)	280 ± 6	895 ± 5	2000 ± 10
Sc (μg/kg)	315 ± 1	604 ± 2	1190 ± 3
Se (μg/kg)	201 ± 18	403 ± 21	665 ± 24
Th (μg/kg)	327 ± 2	911 ± 4	1933 ± 5
Ti (μg/g)	195 ± 39	342 ± 77	510 ± 89
U (μg/kg)	55.4 ± 5.8	324 ± 2	190.2 ± 8.7
V (μg/g)	1.53 ± 0.27	13.6 ± 0.7	14.0 ± 0.9
Zn (μg/g)	137.0 ± 0.5	116.9 ± 0.4	145.7 ± 0.5

* IPEN-CNEN/SP, Campus of São Paulo University.

** University Amphitheatre, Campus of São Paulo University.

from individual palm trees were prepared separately. For this study *Canoparmelia texana* samples were collected at the IPEN-CNEN/SP (site 1) in two different years. Table 3 shows the means and the ranges of the elemental concentrations obtained for samples from different trees. In general, the relative standard deviations of these results varied from 3.0 to about 50%. The comparison between the results obtained for samples collected in different dates indicates, for some elements, a slight increase of the elemental concentrations depending on the length of exposure.

Table 4 shows the results obtained in lichens collected in 1994 from three sites (two sites in São Paulo city and one in Ibiuna). As expected, the highest concentrations of the elements were found in samples from São Paulo when compared to the one collected in Ibiuna which is considered a clean region of São Paulo State.

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