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THE USE OF *CANOPARMELIA TEXANA* LICHENIZED FUNGI IN THE STUDY OF ATMOSPHERIC AIR POLLUTION

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ABSTRACT

The use of epiphytic lichens as passive biomonitors is well established and they have played an important role in studies on atmospheric pollution. There are several species of lichens in Brazil although data on their trace elements and their use in monitoring studies are practically non-existing.

This paper presents results obtained in the analysis of *Canoparmelia texana* species collected at Instituto de Pesquisas Energéticas (IPEN), area located inside the Campus of São Paulo University – SP, and at non-polluted areas. Thirty-six samples were collected at IPEN and four at non-polluted sites located at least 100 km far from São Paulo city. Lichen samples collected from bark trees were cleaned and analyzed by instrumental neutron activation analysis. Concentrations of the elements As, Ca, Cl, Cr, Cs, Co, Fe, K, La, Mn, Na, Rb, Sb, Sc, Se, U, V and Zn were determined in these samples. Concentrations of several elements were significantly higher in lichens from IPEN than those from non-polluted areas. These results indicated that pollutants from hospital incinerators, pilot plants and traffic are leading to element accumulation by lichens. The higher uranium content was also observed in lichens collected at sampling sites located near a laboratory used in the past for uranium processing. These findings showed viability of using *C. texana* species as an accumulator for air pollution biomonitoring.

1 INTRODUCTION

In the past years, epiphytic lichens have long been regarded as suitable materials for biomonitoring the relative levels of metal atmospheric pollution and their role as biomonitors has been demonstrated for several elements [1,2].

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The use of lichens as pollution biomonitors presents several advantages when compared to conventional instruments that are very expensive and can be used at only a limited number of sites so that comparative data cannot be achieved. Lichens are perennial and can be accumulate elements from natural and anthropogenic sources over long periods of time. Their high degree of trace element accumulation enables highly precise and accurate determination of several elements.

The use of lichens for atmospheric pollutant biomonitoring of big cities like São Paulo is of great interest. The occurrence of about 2,800 lichen species has been identified[3]; however, data concerning their use for environmental pollution monitoring are very scarce.

Among several lichen species that can be studied for biomonitoring, *Canoparmelia texana* (Tuck.) Elix & Hale species of the Parmeliaceae family was chosen in this study due to its availability in Brazil and its tolerance. It is a foliose lichen with a large thallus and, in non-polluted ecosystems, this species is limited to twigs and branches whereas in polluted sites it frequently covers almost the complete tree trunks.

This paper presents results obtained in the analysis of *Canoparmelia texana* species collected at both Instituto de Pesquisas Energéticas (IPEN) located inside Campus of São Paulo University, SP and non-polluted sites. Thirty-six samples were collected at IPEN and four at clean sites located at least 100 km far from São Paulo city. Lichen samples collected from tree barks were cleaned and analyzed by instrumental neutron activation analysis.

2 EXPERIMENTAL

2.1 Lichen Sample Collection and Sampling sites

The lichen samples were collected from tree barks at about 1.5 m high from the ground, by using a titanium knife and were stored in paper bags. Plastic bags were not adequate for storing lichen samples because of their humidity and mould formation. A picture of *C. texana* species is presented in Fig 1.



Figure 1: *Canoparmelia texana* species

C. texana species were collected at the following sampling sites of non-polluted areas:

- Site of Ibiuna city, SP, part of the green ring (horticultural and tourism region) located in the countryside at about 100 km far from São Paulo city, and considered non-polluted.

- Sites of Itapetininga region, SP, located in the Atlantic Forest, considered a non-polluted area São Paulo State. In Itapetininga lichens were collected in two sites in July 2001.
- Site of Manuel Botelho Park, considered a non- polluted area of São Paulo State.
- Sites of IPEN. Thirty-six samples were collected at Instituto de Pesquisas Energéticas e Nucleares, IPEN area located inside the Campus of São Paulo University, at 650 meters above sea level and covering an area of 500,000 square meters, which includes laboratories, and buildings that occupy an area of about 120,000 square meters. This area was chosen for biomonitoring because it is located near a hospital incinerator, cement industry and IPEN, and there was a laboratory for uranium processing in the past. These samples were collected from July to December 2001. Fig.2 shows sampling sites of this area.

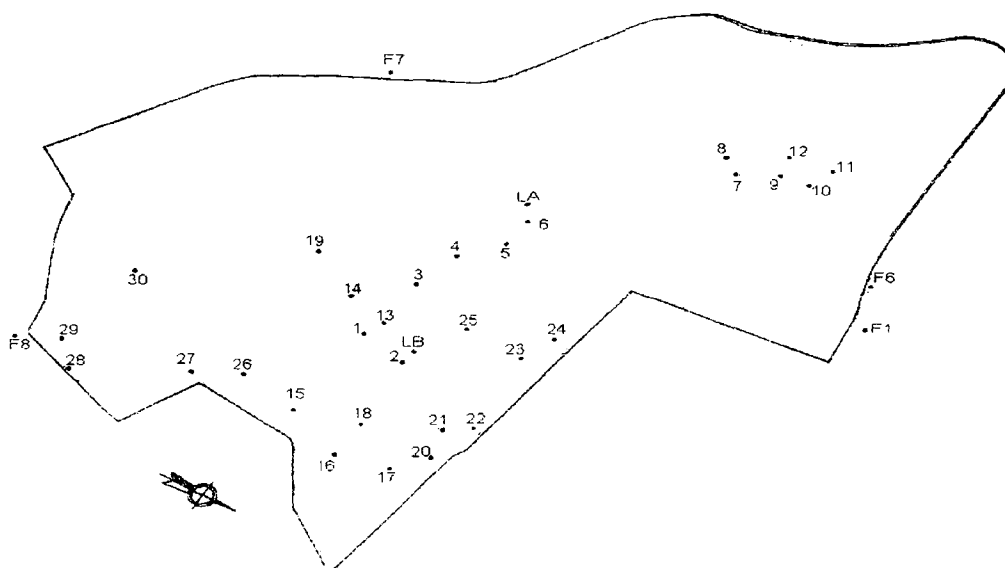


Figure 2: Sampling sites of IPEN (Scale 1:1,000)

2.2 Treatment of Lichen Samples

In the laboratory the samples were firstly examined under an Olympus zoom stereomicroscope model SZ4045 and cleaned in order to remove eventual bark substrates or extraneous materials. Then, they were washed in purified milliQ water to remove dust and sand. In the washing procedure the lichen samples were immersed in water for about 3-5 minutes and then placed on filter papers to be freeze dried for 16 h under a pressure of about $4 \cdot 10^{-2}$ mbar. The fine powder of lichen was obtained by manual grinding in an agate mortar.

2.3 Procedure for Neutron Activation Analysis

Standardised solutions of elements were either supplied by from SpexCertiprep or they were prepared by dissolving high-purity metals, oxides or salts of elements using appropriate

reagents. Diluted solutions containing one or more elements were prepared from these stock solutions. Aliquots of these diluted solutions were pipetted onto sheets of Whatman No. 41 filter paper using an Eppendorf pipette. After dried at room temperature inside a desiccator, these sheets were placed into clean polyethylene bags and irradiated with the samples. In these standards the quantities of each element, in μg (in parentheses) were the following: As (1.5), Ca (1000), Cd (10.0), Cl (200), Cr (1.5), Cs(530.0), Co(0.10), Fe (271), K (1000), La (0.50), Mn (1.5), Na (130), Rb (4.0), Sb (0.6), Sc (0.060), Se (40), V (2.0), U (5.0) and Zn (35.0).

About 150 mg of sample weighed in clean polyethylene bags were irradiated at the IEA-R1 research nuclear reactor together with the standards. Five-minute irradiations were carried out under a thermal neutron flux of $4 \times 10^{11} \text{ n cm}^{-2} \text{ s}^{-1}$ to determine of Cl, K, Mn, Na, and V. Sixteen-hour irradiations, under thermal neutron flux of about $10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ were carried out for As, Ca, Cr, Co, K, La, Mn, Na, Rb, Sb, Sc, U and Zn determinations. After adequate decay times, gamma ray measurements were performed by a GX2020 hyperpure Ge detector which was coupled to Model 1510 Integrated Signal Processor and System 100 MCA Card, both by Canberra. The detector used had a resolution (FWHM) of 0.90 keV for 122 keV gamma rays of ^{57}Co and 1.78 keV for 1332 keV gamma rays of ^{60}Co . Samples and standards were measured at least twice for different decay times. Counting times from 200 to 50 000 seconds were used, depending on either the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and gamma - ray energies. The concentrations of elements were calculated by a comparative method.

2.4 Quality control of the results

Two certified reference materials, IAEA 336 Lichen and NIST 1547 Peach Leaves were analyzed to check the accuracy and precision of the method. They were analyzed by applying the same experimental conditions used in the lichen material analyses. In order to express the concentrations of elements on a dry-mass basis, moisture was determined in a separate sub-sample (not taken for analysis) that was dried at 85°C during 5 hours. The following values (in percent) of weight loss were obtained: 4.8 % for Lichen and 4.2 for Peach Leaves. These values were used to correct the final results.

Most results were in good agreement with certified values with relative errors and relative standard deviations lower than 11% [4].

3 RESULTS AND DISCUSSION

Firstly, *C. texana* was collected in non-polluted sites and then analysed to determine baseline levels of the elements found in this species. These results are presented in Table 1. They showed variability in some element concentrations. Variability is an inherent problem to all biological investigations and in this study it may be attributed to the element accumulation from soil dust that can occur in some sites but not in all.

Results obtained in the analysis of lichen samples collected at IPEN are presented in Table 2. The element concentration means obtained for lichens collected at IPEN were compared with those from clean places. The mean values for Co, Cr, Fe, La, Na, Sb and U were significantly high in samples from IPEN whereas for the elements As, Ca, Cl, K, Rb and Se and Zn, there were no differences.

Elements Co, Cr, Fe and La found in high concentrations in samples from IPEN may derive from the diesel used in the hospital incinerator located near this institute. The analysis of residual oil fly ash from this incinerator showed high concentrations of these elements[5].

Table 1. Element concentrations in *Canoparmelia texana* from non-polluted sites

Element	Samples from non-polluted sites:				
	Itapetiniga (1)	Itapetininga (2)	Carlos B. Park	Ibiuna	Mean \pm s
As, $\mu\text{g kg}^{-1}$	471 \pm 5 ^a	493 \pm 4	686 \pm 9	411 \pm 14	515 \pm 119
Ca, %	0.57 \pm 0.01	0.81 \pm 0.02	0.122 \pm 0.005	4.67 \pm 0.08	1.54 \pm 2.10
Cl, $\mu\text{g g}^{-1}$	301 \pm 7	333 \pm 9	1189 \pm 20	639 \pm 14	615 \pm 411
Co, $\mu\text{g kg}^{-1}$	178 \pm 6	107 \pm 4	142 \pm 3	219 \pm 4	161 \pm 48
Cr, $\mu\text{g kg}^{-1}$	763 \pm 26	767 \pm 26	1720 \pm 28	-	1083 \pm 551
Cs, $\mu\text{g kg}^{-1}$	31 \pm 3	29 \pm 4	188 \pm 4	117 \pm 4	91 \pm 76
Fe, $\mu\text{g g}^{-1}$	308 \pm 3	958 \pm 5	863 \pm 6	1033 \pm 6	790 \pm 329
K, $\mu\text{g g}^{-1}$	5011 \pm 28	1553 \pm 9	3044 \pm 9	1892 \pm 116	2875 \pm 1560
La, $\mu\text{g kg}^{-1}$	1027 \pm 8	1017 \pm 7	1186 \pm 14	1456 \pm 6	1171 \pm 204
Mn, $\mu\text{g g}^{-1}$	30.5 \pm 0.3	104.5 \pm 0.9	88.1 \pm 1.6	37.8 \pm 0.9	65.2 \pm 36.6
Na, $\mu\text{g g}^{-1}$	34.5 \pm 0.2	42.9 \pm 0.1	115.7 \pm 0.2	77.2 \pm 0.1	67.6 \pm 37.0
Rb, $\mu\text{g g}^{-1}$	6.5 \pm 0.2	3.72 \pm 0.05	8.68 \pm 0.09	6.0 \pm 0.1	6.2 \pm 2.0
Sb, $\mu\text{g kg}^{-1}$	66 \pm 1	146 \pm 2	70 \pm 4	280 \pm 6	140 \pm 100
Se, $\mu\text{g kg}^{-1}$	164 \pm 19	194 \pm 20	335 \pm 18	201 \pm 18	223 \pm 76
U, $\mu\text{g kg}^{-1}$	91 \pm 3	67 \pm 1	68 \pm 5	55 \pm 6	70 \pm 15
V, $\mu\text{g g}^{-1}$	3.2 \pm 0.4	3.1 \pm 0.2	2.6 \pm 0.2	1.5 \pm 0.3	2.6 \pm 0.8
Zn, $\mu\text{g g}^{-1}$	47.1 \pm 0.2	39.8 \pm 0.2	24.9 \pm 0.2	137.0 \pm 0.5	62.2 \pm 50.7

A - Uncertainties of the results were evaluated by statistical counting errors of the standards and samples.

Besides lichen samples collected at different sites from IPEN area presented a large variability in their elemental concentrations, as it can be seen in Table 2. Uranium results showed the highest variability: from 125 to 6338 $\mu\text{g kg}^{-1}$. The results obtained for this element in different sites are given in Table 3. U showed, in general, an increase in its concentrations in samples collected near a laboratory used in the past for uranium processing.

Table 2. Elemental concentrations obtained for lichen samples collected at IPEN sites

Elements	Mean \pm s	Range	Median
As, $\mu\text{g kg}^{-1}$	890 \pm 289	380 - 1540	910
Ca, %	3.73 \pm 2.54	0.59 - 10.5	3.34
Cl, $\mu\text{g g}^{-1}$	492 \pm 122	278 - 750	487
Co, $\mu\text{g kg}^{-1}$	839 \pm 582	387 - 3556	690
Cr, $\mu\text{g kg}^{-1}$	7347 \pm 4928	1244 - 25576	7181
Cs, $\mu\text{g kg}^{-1}$	475 \pm 234	188 - 1147	439
Fe, $\mu\text{g g}^{-1}$	2759 \pm 1318	1020 - 5935	2592
K, $\mu\text{g g}^{-1}$	2814 \pm 853	564 - 4603	2923
La, $\mu\text{g kg}^{-1}$	5060 \pm 1878	2255 - 8693	4935
Mn, $\mu\text{g g}^{-1}$	42 \pm 25	4.9 - 85.7	50
Na, $\mu\text{g g}^{-1}$	350 \pm 146	106 - 659	348
Rb, $\mu\text{g g}^{-1}$	14.9 \pm 5.7	7.3 - 30.9	13.7
Sb, $\mu\text{g kg}^{-1}$	930 \pm 370	403 - 1998	875
Sc, $\mu\text{g kg}^{-1}$	669 \pm 298	259 - 1390	658
Se, $\mu\text{g kg}^{-1}$	500 \pm 243	17.9 - 1073	461
U, $\mu\text{g kg}^{-1}$	661 \pm 1154	125 - 6338	289
Zn, $\mu\text{g g}^{-1}$	101 \pm 26	49.2 - 166.2	99.2

CONCLUSION

Results obtained indicated that the use of *C. texana* species for environmental passive biomonitoring is possible and the method of neutron activation analysis applied was very appropriate to analyze this kind of material due to its multielemental character, accuracy, precision and simplicity.

Results obtained in the present work illustrated how trace element biomonitoring is useful to evaluate relative air quality. This work motivated the use of *C. texana* in the future studies focusing on urban environmental biomonitoring of São Paulo city.

Table 3. Concentrations of U obtained in lichens from different sampling sites of IPEN

Sampling site	U, $\mu\text{g kg}^{-1}$	Sampling site	U, $\mu\text{g kg}^{-1}$	Sampling site	U, $\mu\text{g kg}^{-1}$
1	258 \pm 12	13	134 \pm 3	25	316 \pm 2
2	198 \pm 7	14	291 \pm 4	26	149 \pm
3	214 \pm 10	15	491 \pm 4	27	125 \pm 2
4	287 \pm 4	16	444 \pm 5	28	192 \pm 5
5	372 \pm 4	17	286 \pm 4	29	152 \pm 4
6	757 \pm 5	18	223 \pm 6	30	188 \pm 5
7	231 \pm 2	19	268 \pm 4	LA	534 \pm 3
8	1343 \pm 4	20	375 \pm 5	LB	263 \pm 3
9	2006 \pm 5	21	472 \pm 4	F1	311 \pm 3
10	1533 \pm 6	22	137 \pm 3	F6	310 \pm 4
11	3242 \pm 13	23	502 \pm 4	F7	476 \pm 2
12	6338 \pm 14	24	248 \pm 4	F8	145 \pm 2

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