SUBSTRATE INFLUENCE ON ELEMENTAL COMPOSITION OF *CANOPARMELIA TEXANA* LICHENIZED FUNGI

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Abstract

In order to investigate the contribution of substrate derived elements to the elements present in lichens, *Canoparmelia texana* species and their respective tree bark substrates and xylem tissues (sapwoods) were analysed by instrumental neutron activation analysis. Concentrations of the elements Al, As, Br, Ca, Cl, Co, Fe, K, La, Mn, Mo, Na, Sb Se, and Zn were determined in these samples. Concentrations for most elements obtained in lichen were generally similar to or higher than those results found for bark substrates and xylem tissues. The exceptions were Mo and Br. The lowest concentrations of Mo were found in all lichens samples and concentrations of Br were the lowest in lichens sampled on bark substrates of the palm-tree and eucalyptus. These results indicated that the tree bark contribution for lichen elemental concentrations depends on the element and varies among the substrate types of trees but barks are not a significant source of metals to *C. texana* species. Also, the concentrations of most of elements present in xylem samples were of the same levels or lower than those obtained for bark substrates. *C. texana* is a foliose species that occurs well fixed on the surfaces of tree barks however the substrate influence on elemental composition of lichens was very small or absent for various elements, indicating that this species could be used in biomonitoring studies.

1. INTRODUCTION

Epiphytic lichens are able to concentrate various pollutants and thus the analyses of their metal contents have been studied to be used in the environmental monitoring. In these studies it is generally assumed that lichen species accumulate metals from air and precipitation, and only to a minor extent from the substrate.

However this assumption is questionable mainly for lichens growing on substrates rich in minerals or when the lichens are well fixed on the substrates. Besides the experimental data have indicated that element present in lichens can be originated from tree bark substrates. De Bruin and Hackenitz [1] obtained concentrations of Ba, Ca, Cd, Mn, and Zn in *Lecanora conizaeoides* similar to those found in barks and they concluded that there is a possibility of the influence of the substrate on the lichen element content. Sloof and Wolterbeek [2] analysed lichens, barks, and rings of the supporting trees and concluded that Cd, Mn, and Zn levels in lichens might have been originated from both wet and dry deposition and the bark substrate. On the other hand, lately bark materials have also been analysed as biomonitoring tools to indicate and characterise deposition of inorganic pollutants [3,5].

In this paper, in order to evaluate the possibility of lichen element uptake from bark substrates of supporting tree, comparisons were made between the elemental concentrations present in epiphytic lichens with those obtained in the bark substrates and in xylem tissues (sapwoods).

The biomonitor *Canoparmelia texana* chosen for this investigation is one of the most widely spread lichenized fungi species in open places of natural primary and secondary vegetal formation as well as inside cities all over the Brazilian territory except on the coastal cities. It is an epiphytic foliose lichen tolerant to pollution. In non polluted ecosystems, this species is

limited to twigs and branches in well–lit woods or on the trunks of exposed trees and in polluted or urban areas it occurs frequently covering almost the whole tree trunks.

The analytical method utilised was instrumental neutron activation analysis and during each series of analysis the quality of the results was checked by simultaneous analysis of standard reference materials. In the previous papers [6,7], results of the analysis of reference materials were presented and their accuracy was generally found to be within 10%.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

In the present work for each lichen sample, their respective bark substrate and xylem were analysed. The samples were collected at two considered low pollution sites situated at the Campus of São Paulo University, São Paulo, SP and on Vila Velha Park, Ponta Grossa, PR. Samples of *Canoparmelia texana* (Tuck.) Elix & Hale were collected together with the wood branches or trunks at a height of about 1.5 m above the ground and from different types of trees: palm tree (Sample 1), eucalyptus(Sample 2), rubber-tree (Sample 3), and the twigs of a wild bush (Sample 4). In this sample collection, the trunk or branch of trees were cut diametrically and wrapped up in a clean paper foil to bring to the laboratory. For the analyses the lichen samples were removed from the bark substrate using a titanium knife. The lichens were, firstly, cleaned using a pair of tweezers with teflon points and by examining them under an Olympus zoom stereoscopic microscope model SZ 4045 to remove eventual bark substrates or extraneous materials. Then, they remained immersed in distilled water for one minute to remove dust and sand. Next, lichens were placed on filter paper, freeze-dried, and ground manually in a small agate mortar to obtain a fine powder.

The 2–3 mm thick dark brown external layers of branches or trunks were defined as bark substrates. The barks were not separated in their outer and inner layers. After removing the substrates, a next thin layer of about 5 mm of xylem tissue was obtained for the analyses. Xylem tissue is the internal part or the sapwood of the tree, where the mobility of the elements from the roots to the leaves occurs. The bark substrates and the xylem tissues were obtained in small chips that were washed using distilled water. Next, they were also freeze dried for about 30 hours.

2.2. Preparation of elemental standards

Stock solutions of elements were provided from Spex Chemical or they were prepared by dissolving high purity metals or salts in pure reagents or distilled water. Single or multielement solutions were prepared by using appropriate amounts of these stock solutions and they were pipetted onto sheets of Whatman 42 filter paper. After drying these sheets in a desicator, they were placed in polyethylene envelopes that were heat sealed for irradiation with the samples.

2.3. Procedure used for neutron activation analysis

The samples, ranging in mass from 80–150 mg weighed in polyethylene envelopes were used for instrumental neutron activation analysis. Irradiation of 5 minutes were carried out using a pneumatic transfer system of the IEA-R1m nuclear reactor and under a thermal neutron flux of 4×10^{11} n cm^{-2} s^{-1} for the determination of Al, Cl, K, Mn, and Na. Longer irradiations of 16

h under thermal neutron flux of about 10^{12} n cm^{-2} s^{-1} were performed for the determinations of As, Br, Ca, Co, Cr, Fe, K, La, Mo, Na, Sb, Se, and Zn. Samples and standards were measured at least twice after adequate decay times using a Canberra GX2020 hyperpure Ge detector which was coupled to Model 1510 Integrated Signal Processor and System 100MCA Card also from 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. The gamma ray spectra were processed using VISPECT software[8] that evaluates peak areas (counting rates) and gamma ray energies of the photo peaks. The radioisotopes were identified by gamma ray energies and half lives and the concentrations of the elements were calculated by comparative method.

3. RESULTS AND DISCUSSION

Table I and II show the results obtained in the analysis of Al, As, Br, Ca, Cl, Co, Fe, K, La, Mn, Mo, Na, Sb, Se, and Zn in *C. texana* and their respective tree bark substrates and xylem tissues. These results were normalized in relation to the concentrations obtained in bark substrate and are presented in Fig1.To verify the substrate contribution to the element content in lichen, the elemental concentrations of barks were compared to those obtained in lichens. Lichens sampled from four different trees presented concentrations of As, Cl, Fe, K, La, and Mn higher than the data obtained for their respective tree bark substrates. For Al, Ca, Na, Sb, Se, and Zn , concentrations of these elements in lichens were the same levels or higher than those presented by bark substrates depending on the type of tree substrate. These results indicate that for this set of elements the bark substrates were not significant source of metal to *C. texana*. However for Mo, the concentrations of this element in lichens were of the same magnitude or lower than those found in barks and/or xylem tissues. Also the lichens collected from palm tree (Sample 1) and from eucalyptus (Sample 2) presented the lowest concentrations of Br. These results indicate that the uptake from the substrate by lichens varies among the substrates and elements.

In conclusion, the results obtained here show the possibility of using *C. texana* for evaluating air quality and as a biomonitor of trace element in the environment since most of elements analysed in lichens presented higher concentrations than those of bark substrates. *C. texana* is a foliose lichen very well fixed on the tree barks, however if this uptake from bark by lichen occurs, its quantity is very low when compared to element accumulations from both wet and dry deposition.

TABLE I. ELEMENTAL CONCENTRATIONS IN *CANOPARMELIA TEXANA* SPECIES AND THEIR RESPECTIVE TREE SUBSTRATES OF PALM-TREE AND EUCALYPTUS

Elements	Sample 1 from palm-tree			Sample 2 from eucalyptus		
	Lichen 1	Bark 1	Xylem 1	Lichen 2	Bark 2	Xylem 2
Al, $\mu g\ g^{-1}$	789 ± 24[a]	437 ± 11	432 ± 10	1398 ± 36	1502 ± 54	8.2 ± 2.6
As, $\mu g\ kg^{-1}$	469 ± 11	59 ± 7	46 ± 5	501 ± 2	95 ± 3	21.7 ± 5.9
Br, $\mu g\ g^{-1}$	23.0 ± 0.1	72.3 ± 0.3	40.0 ± 0.1	2.40 ± 0.03	5.41 ± 0.02	0.324 ± 0.003
Ca, %	0.27 ± 0.02	0.240 ± 0.005	0.114 ± 0.004	2.24 ± 0.03	0.357 ± 0.004	0.055 ± 0.006
Cl, $\mu g\ g^{-1}$	529 ± 21	188 ± 10	284 ± 15	886 ± 16	134 ± 6	5.2 ±2.2
Co, $\mu g\ kg^{-1}$	295 ± 4	404 ± 11	396 ± 12	307 ± 9	358 ± 5	8.7 ± 0.5
Fe, $\mu g\ g^{-1}$	540 ± 3	290 ± 7	149 ± 4	845 ± 9	365 ± 4	2.8 ± 0.2
K, $\mu g\ g^{-1}$	2516 ± 87	512 ± 9	233 ± 6	1076 ± 74	483 ±4	242 ± 2
La, $\mu g\ kg^{-1}$	1311 ± 6	1189 ± 5	1050 ± 4	1985 ± 2	720 ± 6	39.7 ± 0.6
Mn, $\mu g\ g^{-1}$	138 ± 2	106 ± 5	129± 2	743 ± 7	70.8 ± 0.9	14.9 ± 0.2
Mo, ,$\mu g\ kg^{-1}$	1250 ± 32	1337 ± 57	1112 ± 30	501.6 ± 0.3	1134.0 ± 0.7	594 ± 7
Na, $\mu g\ g^{-1}$	53.0 ± 0.5	21 ± 3	25 ± 3	63.2 ± 0.3	59.6 ± 1.4	52 ± 2
Sb, $\mu g\ kg^{-1}$	200 ± 2	184 ± 4	125 ± 2	217. ± 2	307 ± 2	7.3 ± 0.2
Se, $\mu g\ kg^{-1}$	141 ± 17	168 ± 21	168 ± 36	264 ± 27	320 ± 16	19 ± 2
Zn, $\mu g\ g^{-1}$	98.4 ± 0.4	95.9 ± 0.6	105 ± 2	122.9 ± 0.9	54.9 ± 0.6	3.1 ± 0.3

(a) - Uncertainties calculated using statistical counting errors of standards and samples.

TABLE II. ELEMENTAL CONCENTRATIONS IN *CANOPARMELIA TEXANA* SPECIES AND THEIR RESPECTIVE TREE SUBSTRATES OF RUBBER - TREE AND WILD BRUSH

Elements	Sample 3 from rubber-tree			Sample 4 from wild bush		
	Lichen 3	Bark 3	Xylem 3	Lichen 4	Bark 4	Xylem 4
Al, $\mu g\ g^{-1}$	1782 ± 45	128 ± 5	90 ± 2	801 ± 25	558 ± 13	19 ± 1
As, $\mu g\ kg^{-1}$	530 ± 2	127 ± 2	151 ± 1	343 ± 6	173 ± 2	13.6 ± 0.7
Br, $\mu g\ g^{-1}$	4.5 ± 0.1	1.14 ± 0.01	0.75 ± 0.01	3.30 ± 0.01	1.78 ± 0.01	0.386 ± 0.002
Ca, %	2.94 ± 0.08	1.55 ± 0.01	1.06 ± 0.02	5.74 ± 0.03	0.457 ± 0.003	0.073 ± 0.001
Cl, $\mu g\ g^{-1}$	612 ± 3	183 ± 10	111 ± 5	665 ± 15	12 ± 4	1.1 ± 0.5
Co, $\mu g\ kg^{-1}$	718 ± 17	152 ± 3	127 ± 3	110 ± 2	272 ± 12	67 ± 4
Fe, $\mu g\ g^{-1}$	1844 ± 16	108 ± 7	40 ± 7	626 ± 4	457 ± 7	14.0 ± 0.9
K, $\mu g\ g^{-1}$	5244 ± 12	790 ± 11	169 ± 10	296 ± 9	195 ± 9	45.1 ± 0.9
La, $\mu g\ kg^{-1}$	3075 ± 3	980 ± 8	1068 ± 7	663 ± 12	304 ± 6	25.3 ± 0.5
Mn, $\mu g\ g^{-1}$	33.5 ± 0.3	29.2 ± 0.1	6.29 ± 0.08	73.0 ± 0.4	59.8 ± 0.7	21.3 ± 0.2
Mo, ,$\mu g\ kg^{-1}$	882 ± 25	1299 ± 20	2746 ± 27	911 ± 24	795 ± 18	1271 ± 13
Na, $\mu g\ g^{-1}$	152.3 ± 0.7	107 ± 4	15.0 ± 0.1	20.6 ± 0.3	21.6 ± 0.4	13.7 ± 0.3
Sb, $\mu g\ kg^{-1}$	497 ± 5	89 ± 1	71 ± 1	67 ± 2	51 ± 1	26.2 ± 0.5
Se, $\mu g\ kg^{-1}$	335 ± 65	41 ± 5	53 ± 7	98 ± 8	92 ± 12	44 ± 2
Zn, $\mu g\ g^{-1}$	175 ± 2	28.4 ± 0.3	29.9 ± 0.8	32.2 ± 0.25	39.8 ± 0.2	9.1 ± 0.1

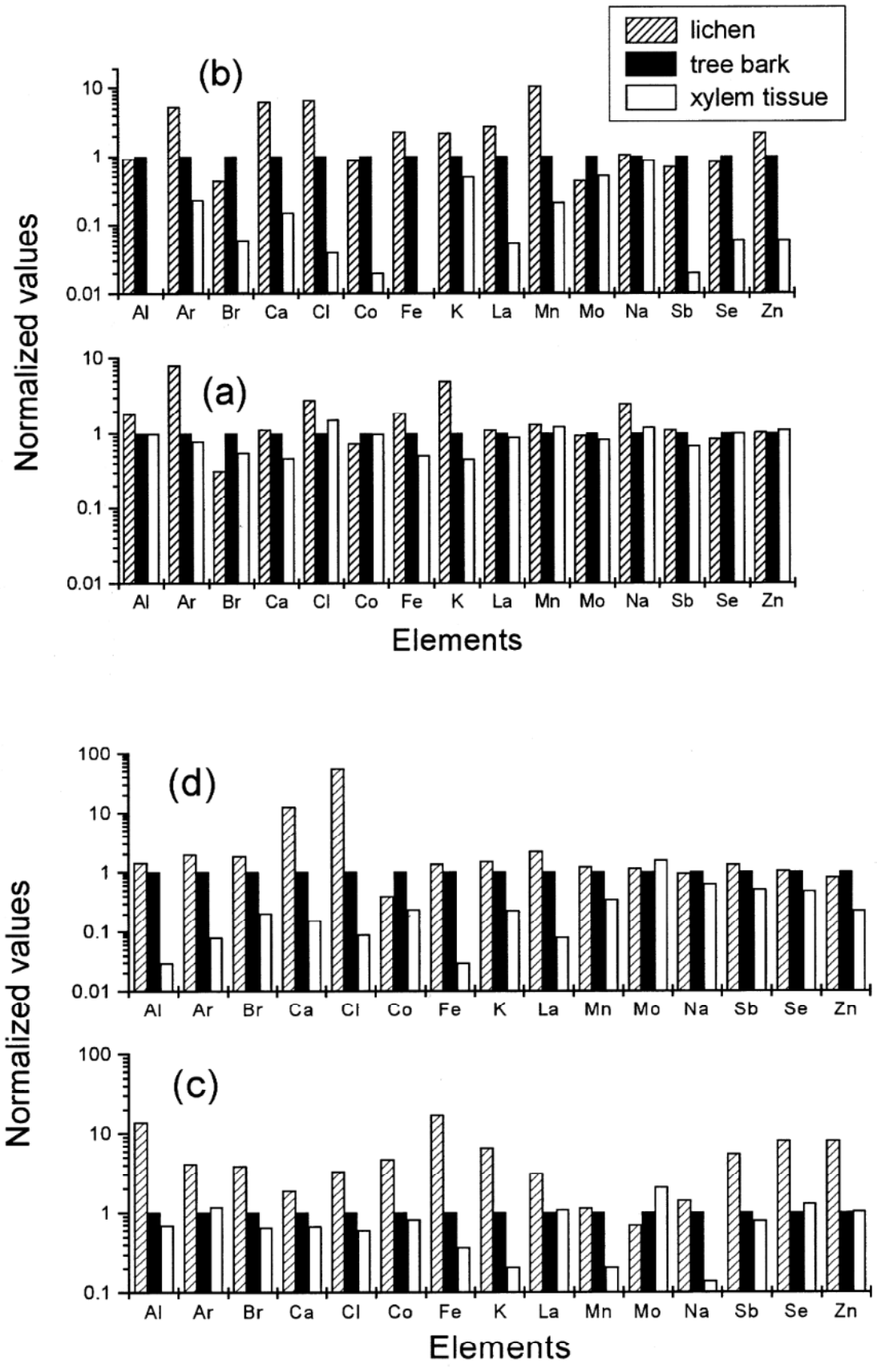

FIG. 1. Normalized values of elemental concentrations obtained for lichen, bark substrate and xylem tissue. (a): palm tree; (b): eucalyptus; (c): rubber-tree; (d): wild bush.

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