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COMPARATIVE STUDY OF THE SUITABILITY OF TWO LICHEN SPECIES FOR TRACE ELEMENT ATMOSPHERIC MONITORING

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ABSTRACT

Lichens have been widely used in monitoring studies. Consequently, it is very useful to study the suitability of lichen species to monitor pollutants allowing in this way the best choice. The aim of this study was to compare the accumulation of trace elements by two epiphytic lichen species *Canoparmelia texana* (Tuck.) Elix & Hale and *Usnea amblyoclada* (Müll. Arg.) Zahlbr. Five samples of each species were collected during the period from November 2010 to November 2012 in a same site far from downtown São Paulo city. Lichens collected from tree barks were cleaned, freeze-dried, ground and analyzed by neutron activation analysis. Aliquots of lichen samples and synthetic elemental standards were irradiated at the IEA-R1 nuclear research reactor. The induced gamma activities were measured using a hyperpure Ge detector coupled to a digital spectrum analyzer. Concentrations of As, Ba, Cd, Cr, Cs, Fe, Mg, Mn, Na, Rb, V and Zn were determined in both lichen species. The results demonstrated that both species can be used for evaluating air quality. The element concentrations showed difference between lichen species and also among their sampling periods. These differences may be attributed to the distinct mechanisms of element absorption by lichens as well as various other factors that affect their element accumulation. The comparative evaluation made calculating the ratios between *C. texana* species sample and that in *Usnea amblyoclada* for elemental concentrations indicated that, in general, foliose *C. texana* presented similar or higher concentrations than those presented by fruticose *Usnea*.

1. INTRODUCTION

Lichens have been widely used as trace element air monitors since they are widespread and capable of absorbing elements directly from the atmosphere and accumulating these in their tissues. Besides this, the use of lichens as biomonitors presents advantages because of their easy sampling, their wide geographical distribution and the use of less expensive technical equipment for sample collecting.

In the last decades, numerous researchers have successfully used epiphytic lichens as indicators of pollutants, especially for the impact of air pollution [1-4]. Moreover, lichens are not used only to detect concentrations of chemical elements but also radionuclides [5] and persistent organic compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) [6-7].

Consequently the analyses of different lichen 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 sampling and sample treatment, tolerance to pollution and background concentrations of the elements should be considered for the choice of lichen species [8]. The occurrence of about 2,800 lichen species in Brazilian territory [9] has been published. However, data concerning their use in monitoring studies and their elemental composition are very scarce.

The aim of the present study was to compare the accumulation of trace elements by two epiphytic lichen species *Canoparmelia texana* (Tuck.) Elix & Hale and *Usnea amblyoclada* (Müll. Arg.) Zahlbr. For practical purposes, it is very useful to study the suitability of lichen species to monitor pollutants thus allowing in this way the best choice for monitoring.

Canoparmelia texana (Tuck.) Elix& Hale is an epiphytic lichen species of the Parmeliaceae family. It is one of the most widely spread lichenized fungi species in open spaces of the natural primary vegetable formations as well inside cities all over the Brazilian territory except in the coastal cities. It is foliose lichen with large thallus (5 to 10 cm in diameter) (Figure 1) with radial growth found on tree barks or, more rarely, on rocks.

Usnea amblyoclada (Mull. Arg.) Zahlbr is also of the Parmeliaceae family. It is a fruticolous lichen and most abundant saxicolous species in Argentina. It is used in popular medicine and as biomarker of air quality and is often refered as the beard lichen. Its thallus is shrubby, compact with 2-10 cm long branching (Figure 2).

Figure 1. *Canoparmelia texana* species.
Reference:
http://www.sharnoffphotos.com/lichensH/usnea_amblyoclada.html

Figure 2. *Usnea amblyoclada* species.
Reference:
http://www.sharnoffphotos.com/lichensH/usnea_amblyoclada.html

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2. MATERIALS AND METHODS

2.1. Lichen Sample Collection and Treatment

Samples of both lichen species were collected on the same dates and same location of Parelheiros sub prefecture, located far from São Paulo downtown in a rural area near a region of native vegetation with geographical coordinates of S23°49.221´ and W46°43.992´. Five samples of each lichen species were collected during the period from November 2010 to November 2012. They were carefully collected from the bark 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 first cleaned by immersing in purified water for about 3-5 minutes. In the case of *C. texana* species samples they were examined under an Olympus zoom stereomicroscope Model SZ4045 to remove foreign materials and bark substrates. The cleaned samples were freeze-dried and then ground to a powder using a vibratory micro mill.

2.2. Neutron Activation Analysis (NAA) Procedure

Firstly, synthetic standards of elements were prepared by pipetting 50 μL of the elemental standard solutions onto sheets of Whatman No. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep Chemical, USA. All the pipettes and volumetric flasks were calibrated before use. These filter sheets were dried at room temperature inside a desiccator and then placed into clean polyethylene bags and sealed. In these standards the quantities of each element, in μg (in parentheses) were the following**:** As(1.5), Ba(200.2), Cd(10.0), Cr(2.0), Cs(0.60), Fe(360), Mg(998), Mn(4.0), Na(200), Rb(10.0), V(24.0) and Zn(36.0).

For NAA, about 180 mg of the sample weighed in clean polyethylene bags were irradiated at the IEA-R1 nuclear research reactor with synthetic standards of elements. Short irradiations of 10 s under a thermal flux of about 6.6×10^{12} n cm^{-2} s^{-1} were carried out for the determination of Mg, Mn, Na and V. Sixteen-hour irradiations under a thermal neutron flux of about 5×10^{12} n cm^{-2} s^{-1} were performed for As, Ba, Br, Cr, Cs, Fe, Na, and Zn determinations. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GX1820 coupled to a Digital Spectrum Processor DSA1000, both from Canberra. The resolution (FWHM) of the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1,332 keV gamma ray of ^{60}Co. Counting times from 300 to 50,000 seconds were used, based on the half-lives or activities of the radioisotopes considered. Spectra were collected and processed using Canberra Genie 2000 Version 3.1 software. Every samples and standards were measured at least twice for different decay times. The radionuclides measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by comparative method. The short lived radionuclides used were ^{27}Mg, ^{56}Mn, ^{24}Na and ^{52}V. Long lived radionuclides were ^{76}As, ^{131}Ba, ^{115}Cd, ^{51}Cr, ^{134}Cs, ^{59}Fe, ^{24}Na, ^{86}Rb and^{65}Zn.

The quality control of the analytical results was evaluated by analyzing certified reference materials, IAEA 336 Lichen provided by the International Atomic Energy Agency and CTA-VTL-2 Virginia Tobacco Leaves from the Institute of Nuclear Chemistry and Technology, Poland. These results indicated good accuracy and precision with relative standard deviations and relative errors lower than 11.5 %. The standardized difference or Z-score values [10] obtained for elements quantified in these references materials were |Z-score|<2, indicating that our results are satisfactory and are within the ranges of certified data at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Elemental Concentrations Obtained in Lichen Samples

Elemental concentrations obtained in the analyses of *C. texana* and *Usnea amblyoclada* samples in different periods of sampling are presented in Figures 3 and 4. From the point of element accumulation, our data demonstrated that both species can be used for detection of air quality.

The results obtained for each species show variations in elemental concentrations among different period of sample collection. As can be seen in the case of *C. texana* for the elements As Ba, Cr, Fe and Rb there are tendencies of concentration increase of these elements with exposure period but this fact does not occur for Cd concentrations. In *C. texana*, there was an increase of concentrations for Cr, Cs, Fe, Na, V and Zn in last sample collection (November, 2012) however in other periods these element accumulations were of the same order the magnitude.

For *Usnea amblyoclada* samples, it was observed that As, Cd, Cs, Fe, Na and V concentrations found are of the same order of magnitude for samples collected in different periods (Figure 3 and 4) and the highest concentrations for Cr, Mg and Zn were found in samples collected in the second campaign on August 14, 2011. Results of Figures 3 and 4 indicate differences between species, among elements and sampling periods. These differences can be attributed to various factors that affect element accumulation by lichens. Three mechanisms have been proposed with regards to the metal absorption in lichens: intracellular absorption through an exchange process, intracellular accumulation and entrapment of particles that contain metals [11]. On the other hand other several studies have revealed that lichens may selectively accumulate elements and metabolize or eliminate those elements that enter the cell wall [12, 13]. Bergamaschi et al [14] compared the capacity to accumulate trace elements from the atmosphere by lichens transplanted to urban sites. Their results showed that, in general, elements do not exhibit well defined trends, but rather fluctuations.

3.2. Comparison between element concentrations obtained in *C. texana* and *Usnea amblyoclada* species

This comparative evaluation was made calculating the ratios between species *C. texana* sample and that in *Usnea amblyoclada* for elemental concentration found. These ratio values presented in Table 1 indicate that *C. texana* lichens presented similar or higher concentrations than those presented by *Usnea* for most of elements analyzed. According to Di Lella et al [15] the capacity of particle trapping of lichens can be influenced to a great extent by their morphology. Besides, Bosserman and Hagner [16] showed that fruticose lichen, *Usnea,* are

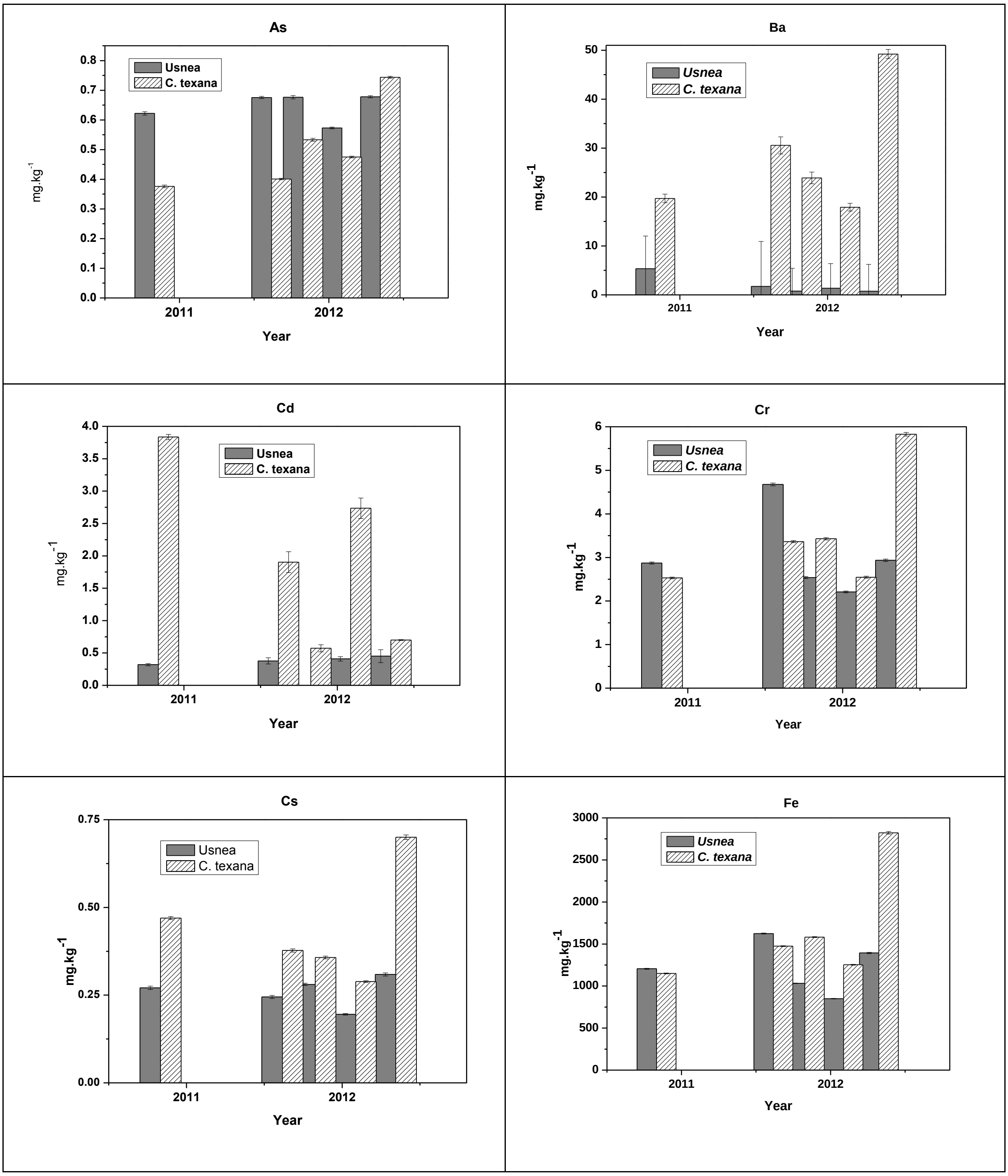

Figure 3. Concentrations of As, Ba, Cd, Cr, Cs and Fe in *C. texana* and *Usnea amblyoclada* lichen samples collected during the period from November 2010 to November 2012.

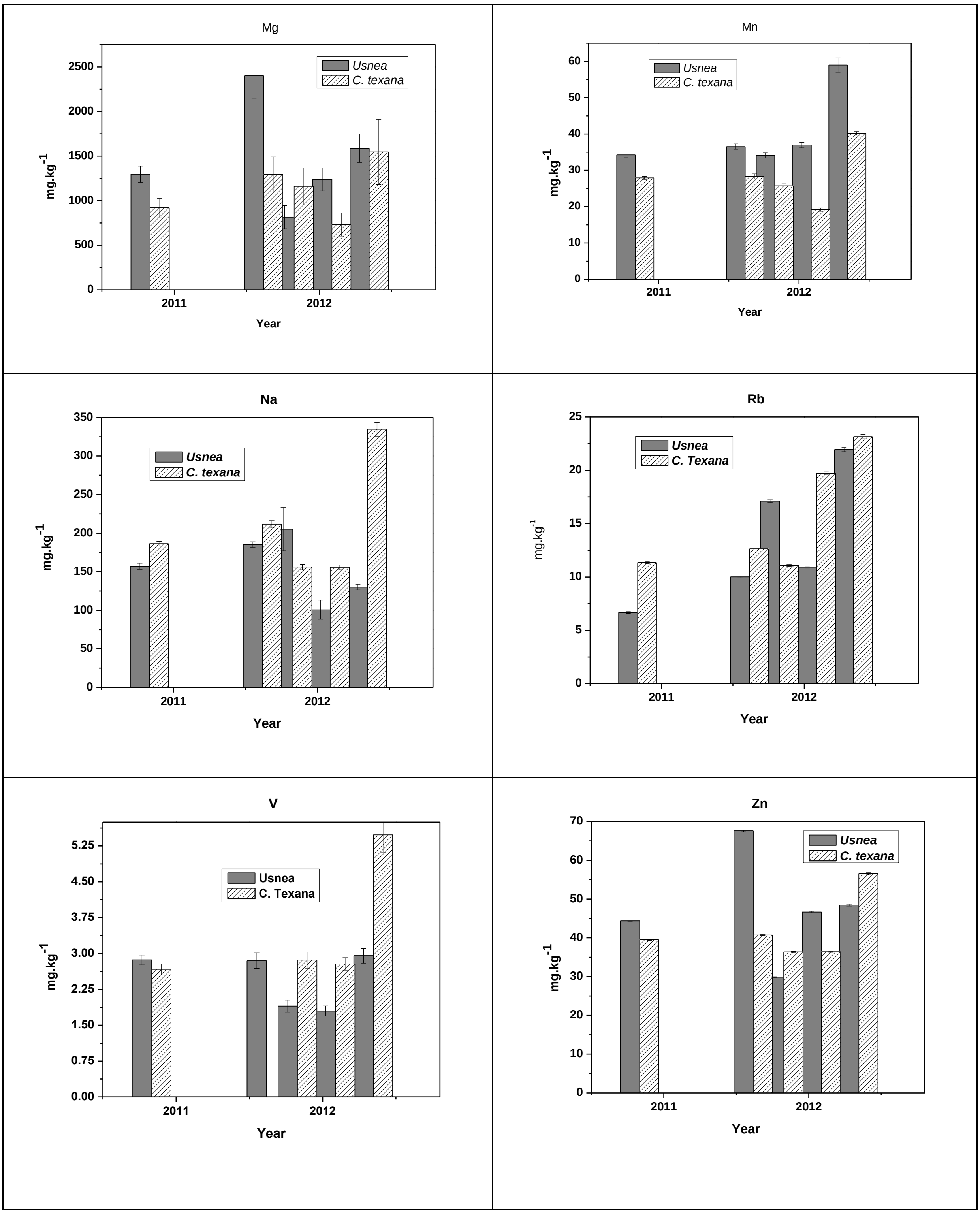

Figure 4. Concentrations of Mg, Mn, Na, Rb, V and Zn in *C. texana* and *Usnea amblyoclada* lichen samples collected during the period from November 2010 to November 2012.

more dependent on wet deposition while foliose Parmelia species accumulate more from dry deposition.

Table 1. Ratio values between elemental concentrations obtained in *C. texana* and *Usnea amblyoclada*

Elements	Ratio between element concentrations in *C. texana* and *Usnea amblyoclada* collected in different periods				
	November, 1' 2010	August, 14, 2011	October, 30, 2011	May, 6, 2012	November, 2, 2012
As	0.604 ± 0.009	0.593 ± 0.005	0.789 ±0.010	0.829 ± 0.007	***1.096±0.007***
Ba	*0.98 ± 0.27*	0.66 ± 0.05	***1.45 ± 0.01***	***1.03 ± 0.09***	***2.07 ± 0.08***
Cd	***11.99 ± 0.66***[b]	***5.04 ± 0.77***	n.d[a]	***1.55 ± 0.34***	***5.00 ± 3.60***
Cr	0.88 ± 0.01	0.719 ± 0.007	***1.35 ± 0.02***	***1.15 ± 0.02***	***1.98 ± 0.02***
Cs	***1.73 ± 0.04***	***1.54 ± 0.03***	***1.27 ± 0.02***	***1.48 ± 0.02***	***2.27 ± 0.04***
Fe	***0.954 ± 0.007***	***0.908 ± 0.004***	***1.533 ± 0.009***	***1.474 ± 0.009***	***2.03 ± 0.02***
Mg	0.71 ± 0.09	0.54 ± 0.10	***1.42 ± 0.34***	0.59 ± 0.12	***0.97 ± 0.25***
Mn	0.81 ± 0.02	0.77 ± 0.03	0.75 ± 0.02	0.52 ± 0.02	0.68 ± 0.02
Na	***1.19 ± 0.04***	***1.14 ± 0.03***	0.76 ± 0.11	***1.55 ± 0.19***	***2.57 ±0.10***
Rb	***1.70 ± 0.02***	***1.26 ± 0.01***	0.648 ± 0.007	***1.80 ± 0.02***	***1.06 ± 0.01***
V	***0.93 ± 0.05***	n.d	***1.50 ± 0.13***	***1.55 ± 0.12***	***1.86 ± 0.15***
Zn	0.890 ± 0.005	0.602 ± 0.003	***1.217 ± 0.007***	0.780 ± 0.004	***1.167± 0.008***

a. Concentration was not determined. b. Result in italic bold indicates that the *C. texana* lichen concentration was similar or higher than that presented by *Usnea* sample

3. CONCLUSIONS

The results obtained indicated that *C. texana* and *Usnea amblyoclada* lichen species analyzed can be used to monitor air quality pollution. Various elements of interest from the environmental point of view were found in both species. However *C. texana* showed higher accumulation capacity of several elements than *Usnea amblyoclada.* From the laboratory work point of view it can be stated that *Usnea* that is fruticose lichen presents easier cleaning for the analysis when compared with foliose *C. texana.*

The results obtained in different sampling periods showed that, in general, elements did not exhibit well defined trends, but rather showed fluctuations. These findings of this study suggest further researchon the vitality of both species to be used as a transplant for trace element monitoring.

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