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COMPARISON BETWEEN ATMOSPHERIC POLLUTANTS FROM URBAN AND RURAL AREAS EMPLOYING THE TRANSPLANTED *Usnea Amblyoclada* LICHEN SPECIES

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ABSTRACT

Over the last decades, lichens have been used as biomonitors in studies related to atmospheric pollution of several elements. The capability of absorbing and accumulating aerial pollutants, their longevity and resistance to environmental stresses have made lichens suitable for studies on air quality evaluation. In this study, a preliminary investigation employing *Usnea amblyoclata* lichen species and instrumental neutron activation analysis was performed to compare the levels of elements in the air of an urban and rural area. Samples of *Usnea amblyoclada* (Mull. Arg) collected in a clean area were exposed in a polluted area by vehicular emissions in São Paulo city and in a rural area of Caucaia do Alto Municipality, Cotia, SP. After 6 months of exposure the lichens were collected, cleaned, freeze-dried and ground for analyses. Samples and elemental standards were irradiated at the IEA-R1 nuclear research reactor and their induced activities were measured using a gamma ray spectrometer. Results indicated that lichens exposed in the polluted urban area presented higher levels of Ba, Br, Ca, Co, Cr, La, Sb, Sc, Se, Th, V and Zn than those from the rural area. Besides that ,concentrations of As, Ba, Br, Ca, Co, Cr, Fe, Hf, La, Mg, Th, Sc and V in lichens exposed in the rural and polluted urban area were higher than those that were not exposed. Quality control of analytical results was achieved by the analyses of certified reference material. Lichen species used in this study proved to be very useful for active monitoring of a polluted urban environment

1. INTRODUCTION

Air pollution is today considered one of the greatest problems affecting mankind, with significant consequences on the environment and health of the population. In megacities such as São Paulo, the industrial processes, fuel combustion, transportation and urbanization are major causes of contamination sources. Consequently, air pollutant determinations have attracted great concern over the last decades because of evidence that they are associated with respiratory and cardiovascular diseases in humans [1-3].

Among several methods for determining elemental pollutants, the use of lichens as biomonitors is a well established technique which is widely used in European and North American countries. However, in Brazil biomonitoring data are very scarce. In a previous study [4] we performed passive monitoring, analyzing lichens collected in the São Paulo

Metropolitan area and an association between accumulation of trace elements in lichens and cardiopulmonary diseases was observed.

Within this scenario, it becomes important to investigate the suitability of using active biomonitoring since the use of lichen transplants sustains the possibility of additional advantages to sample in areas devoid of lichens, standardization of experimental materials in terms of its physiological condition and its capacity for biocentration [5]. However the question of the vitality of lichens to accumulate pollutants during the exposition period can be a limiting factor to use active biomonitoring

Thus the aim of this preliminary study was to investigate the possibility of using *Usnea amblyoclada* (Mull. Arg.) lichen species in active biomonitoring by comparing the elements accumulated in samples exposed in urban and rural sites with different levels of pollution.

In this study instrumental neutron activation analysis (INAA) was applied to the determination of 22 elements in transplanted lichen samples. INAA constitutes an advantageous method for environmental sample analysis enabling simultaneous multi-element determinations and does not require the step of sample dissolution.

2. MATERIALS AND METHODS

2.1. Sites for Lichen Exposure

The lichens were exposed in two sites, being one in a polluted urban area, near a heavy traffic street of the Cerqueira Cesar neighborhood in São Paulo city. The second site was in the Caucaia do Alto Municipality, Cotia, SP located about 50 km from São Paulo downtown in a rural area near a region of native vegetation and considered clean.

2.2. Lichen Sample Exposure and Preparation

Samples of thalli of the fructicose *Usnea amblyoclata* (Mull. Arg) Zahlbr species collected in an area with low pollution levels in Cordoba city, Argentina, were obtained from the National University of Cordoba. For transplantation, lichens were packed in fine tulle bags. Three lichen bags (each sample consisting of several thalli) were tied on three different supports at a height of 1.5 m above ground and exposed during the period from March to August 2009. When few some lichen thalli were found damaged after exposure they were discarded. For the analysis, lichen from each of the supports was mixed constituting one sample. These two exposed samples and an unexposed sample were ground to a powder using a vibratory micro mill for the analysis.

2.3. Neutron Activation Analysis Procedure

2.3.1. Preparation of synthetic elemental standards.

The synthetic standards were prepared by pippeting 50 or 100 µL of the elemental standard solutions onto sheets of Whatman N^o. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep

Chemical, USA. All the pippetors and volumetric flasks were calibrated before use. These filter sheets were dried at room temperature inside a desiccator with fresh silica and then placed into clean polyethylene bags which were heat sealed. In these standards the quantities of each element, in μg (in parentheses) were the following: As (1.50), Ba (499.0), Br (5.2), Ca (1,000), Ce (4.0), Cl (500.0), Co (0.150), Cr (2.0), Cs (12.0), Eu (0.10), Fe (280.0), Hf (1.00), K (1,000.0), La (0.998), Mg (997.9), Mn (4.0), Na (500.0), Rb (10.0), Sb (0.600), Sc (0.080), Se (8.0), Sm (0.500), Th (0.999), V (24.0) and Zn (35.0)

2.3.2. Irradiation, Counting and Calculation

Aliquots of about 190 mg of each sample weighed in polyethylene bags were irradiated in the IEA-R1 nuclear research reactor along with the synthetic element standards for 12 s and 16 h under a thermal flux of about 5×10^{12} n cm^{-2} s^{-1}. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GC2018 from Canberra coupled to a DSA-1000 Multichannel Analyzer. The resolution (FWHM) of the system was 1.0 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma ray peak of ^{60}Co. Each sample and standards were measured at least twice for different decay times. Counting times from 200 to 50,000 seconds were used, depending on the half-lives or activities of the radionuclides considered. Spectra were collected and processed using Canberra Genie 2000 Version 3.1 software. The radionuclides measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by a comparative method. The short lived radionuclides used were ^{38}Cl, ^{42}K, ^{27}Mg, ^{56}Mn and ^{24}Na. Long lived radionuclides were: ^{76}As, ^{131}Ba, ^{82}Br, ^{47}Ca, ^{141}Ce, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{152}Eu, ^{59}Fe, ^{182}Hf, ^{42}K, ^{140}La, ^{147}Nd, ^{233}Pa (Th), ^{86}Rb, ^{122}Sb, ^{46}Sc, ^{75}Se, ^{153}Sm, and ^{65}Zn.

The quality of the analytical results was evaluated by analyzing the certified reference material CTA-VTL-2 Virginia Tobacco Leaves provided by the Institute of Nuclear Chemistry and Technology, Poland. The elemental concentrations of the reference material were evaluated on a dry weight basis, as recommended in the certificate. Moisture weight loss of 5.8 % obtained for CTA-VTL-2 Virginia Tobacco Leaves was used to correct the final results.

3. RESULTS AND DISCUSSION

3.1. Quality Control of the Analytical Results

Results obtained in the analyses of certified reference material CTA-VTL-2 Virginia Tobacco Leaves are presented in Table 1 together with values of their certificate. These results indicated good accuracy and precision with relative standard deviations ranging from 1.9 to 11.4 % and relative errors lower than 13.5 %. The standardized difference or Z-score values [7] obtained for elements quantified in this reference material were |Z-score| <2, indicating that our results are satisfactory and are within the ranges of certified data at the 95 % confidence level.

3.2. Elemental Concentrations Obtained in *Usnea amblyoclada* Lichen

Results obtained in lichen samples exposed in the polluted urban area (PUA), in the rural area (RA) and that not exposed sample (NE) are presented in Fig. 1 to 4. Elements Ca, Fe, K, Mg and Na presented concentrations at the mg g^{-1} levels (Fig. 1), As, Ba, Br, Cl, Cr, La, Mn, Rb, Th, V and Zn, at the levels of mg kg^{-1} (Fig 2 and 3) and the elements Co, Cs, Hf, Sb, Sc and Se presented the lowest concentrations at the µg kg^{-1} levels (Fig.4).

Table 1. Element concentrations (in mg kg^{-1}) in certified reference material CTA-VTL-2 Virginia Tobacco Leaves

Elements	This study				Values of the certificate [6]
	M ± SD[a]	RSD[b], %	ER[c], %	Z-score	
As	0.980 ± 0.066	6.7	1.2	0.12	0.969 ± 0.072
Br	14.2 ± 1.3	9.1	0.7	-0.04	14.3 ± 1.4
Ca	36131± 1078	3.0	0.4	0.07	36000 ± 1500
Cd	1.63 ± 0.06	3.7	7.2	0.63	1.52 ± 0.17
Cl	7416 ± 365	4.9	0.2	-0.03	7430 ± 280
Co	0.459 ± 0.049	10.7	7.0	0.55	0.429 ± 0.026
Cr	2.044 ± 0.118	5.8	9.1	0.87	1.87 ± 0.16
Cs	0.513 ± 0.020	3.9	0.4	-0.03	0.515 ± 0.046
Fe	1129 ± 21	1.9	4.2	1.17	1083 ± 33
Hf	0.102 ± 0.010	9.8	13.5	-0.65	0.118 ± 0.022
K	10089 ± 308	3.1	2.1	-0.42	10300 ±400
Mg	5349 ± 325	6.1	4.9	0.63	5100 ± 230
Mn	83.3 ± 2.6	3.1	4.5	1.0	79.7 ± 2.6
Na	478 ± 11	2.3	-	-	(312)[d]
Rb	47.5 ± 1.0	2.1	2.2	0.43	48.6 ± 2.3
Sc	0.307 ± 0.010	3.3	-	-	(0.268)
Th	0.350 ± 0.040	11.4	7.5	-0.56	0.378 ± 0.031
V	3.83 ± 0.22	5.8	4.2	-0.35	4.00 ± 0.42
Zn	45.9 ± 1.0	2.1	6.0	1.1	43.3 ± 2.1
La	0.898 ± 0.068	7.5	11.1	-0.93	1.01 ± 0.10
Ce	1.84 ± 0.16	8.6	3.6	-0.22	1.91 ± 0.29
Sm	0.155 ± 0.015	9.7	1.2	0.07	0.157 ± 0.022
Eu	0.031±0.002	6.4	-	-	(0.033)

a. Unweighted mean and standard deviation of at least three determinations; b. relative standard deviation; c. Relative error; d. Numbers between parenthesis are informative values.

Comparisons made between the results obtained in samples exposed in the urban and rural sites (PUA and RA) and that not exposed (NE) showed that there was an accumulation of several elements (As, Ba, Br, Ca, Cr, Co, Fe, Hf, La, Mg, Mn, Sb, Sc, Se, Th, V and Zn) determined in this study (Fig. 1 to 4). Besides this, the sample exposed in the polluted urban site presented higher concentrations of Br, Cr, V, Co, Sb, Ba, Cl and Zn than those found in lichens exposed in the rural area.

The exception was K. The lower concentration of this element was obtained in the sample exposed in the polluted urban site than those found for sample from rural site and not exposed sample. K is an essential element for lichens but in polluted area this element is present in low level due to the stress caused by pollutants affecting its uptake. According Garty et al [8], a decrease of K is expected in lichen exposed to heavy pollution. There was also a decrease of Cl concentration with the exposure indicating that the lichen could have been affected by the transplantation. In addition, the exposed and unexposed lichen samples presented concentrations of the same levels for the elements Cs, Hf and Na.

The high levels of the elements Ba, V and Zn obtained in transplanted *U. amblyoclada* exposed in the polluted urban area may be associated to vehicular emissions. The origin of Ba can be attributed to the use of diesel as a fuel and V of gasoline. Zn may be associated to industrial origins and to a lesser extend by motor vehicle and tire rubber wear emissions [9, 10]. The content of Sb in transplanted lichens can be associated to the emission of plastic material or waste incineration. Brazilian plastic materials contain Sb, since the compound of this element is used in plastic processing [11-13].

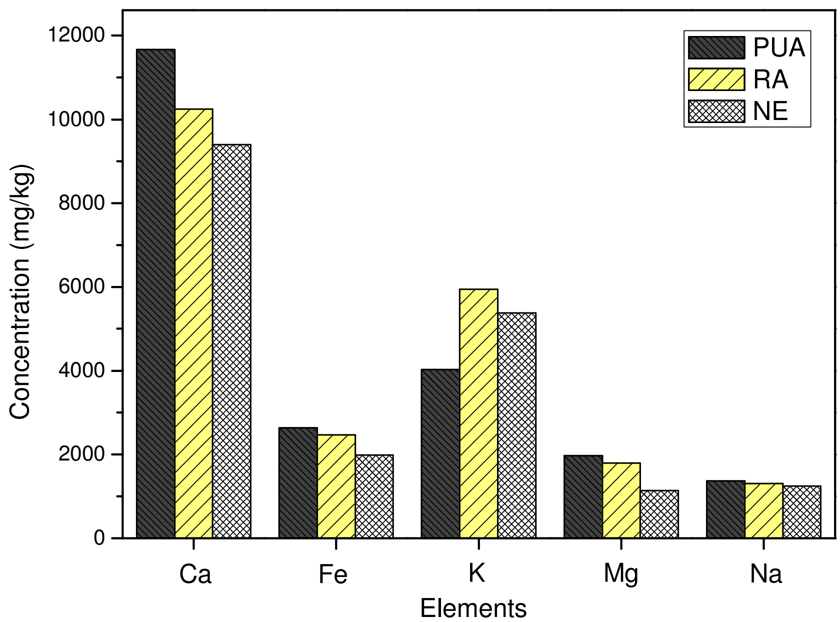

Figure 1. Concentrations of Ca, Fe, K, Mg and Na in lichens exposed in urban polluted area (PUA), urban area (UA) and not exposed (NE)

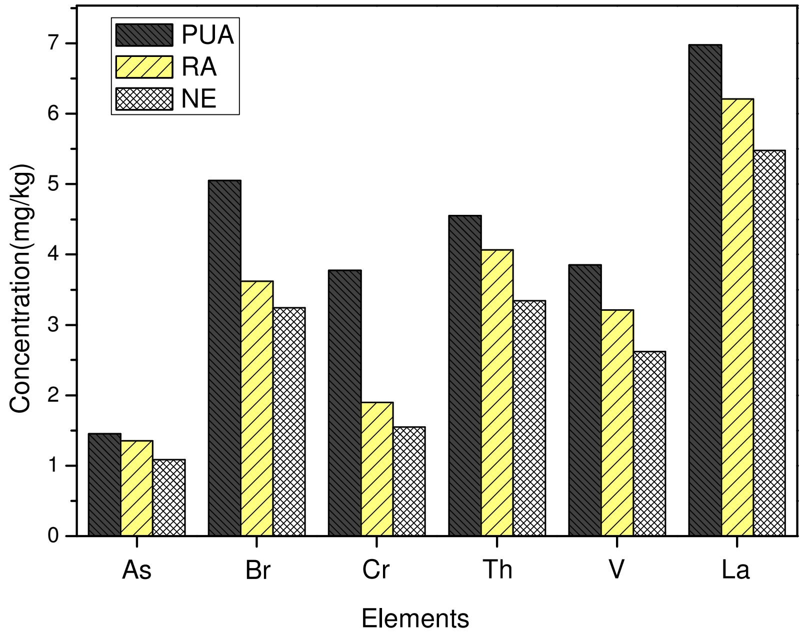

Figure 2. Concentrations of As, Br, Cr, Th, V and La in lichens exposed in urban polluted area (PUA), urban area (UA) and not exposed (NE)

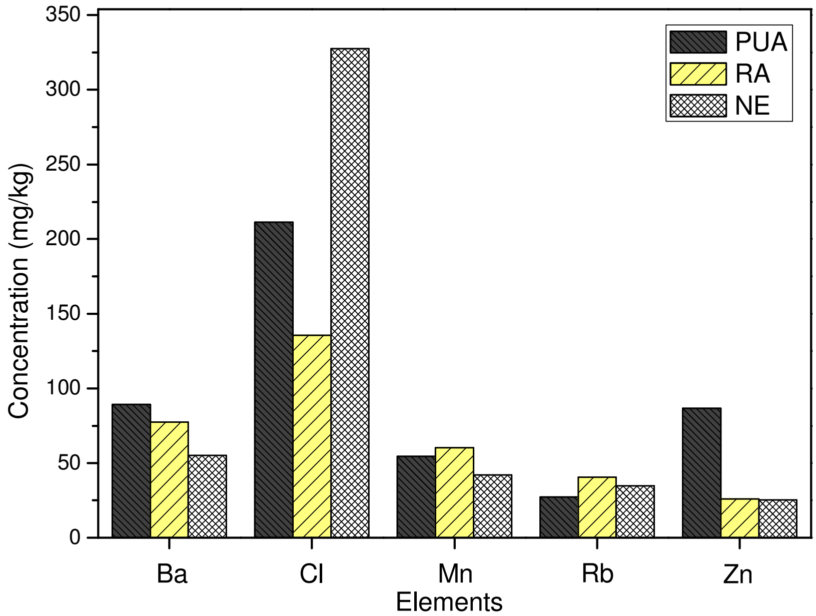

Figure 3. Concentrations of Ba, Cl, Mn, Rb and Zn in lichens exposed in urban polluted area (PUA), urban area (UA) and not exposed (NE)

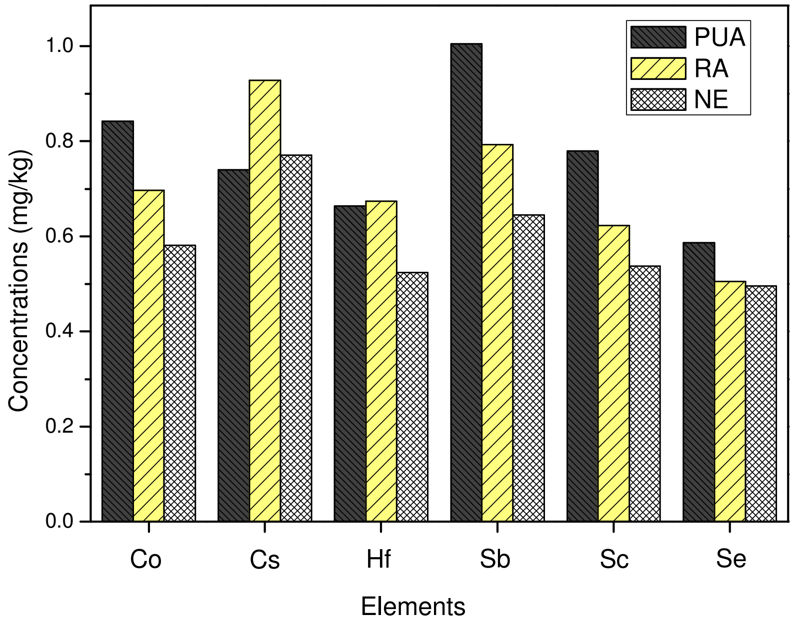

Figure 4. Concentrations of Co, Cs, Hf, Sb, Sc and Se in lichens exposed in urban polluted area (PUA), urban area (UA) and not exposed (NE)

The lichen analysis results were also submitted to cluster analysis for exposure site classification. The resulting dendrogram from this treatment revealed two groups of sites, as can be seen in Figure 5. The first group 1 is formed by the Cerqueira César exposure site (PUA) that is in a polluted urban area. The second group 2 is formed by the exposed site of a rural area of Caucaia do Alto (RA) and a not exposed sample (NE). The cluster analysis substantially showed coherent groups of pollution levels. The data indicate that there was a difference in the element accumulation depending on the sites of lichen exposure.

3. CONCLUSIONS

Based on the lichen analysis results we can conclude that *U. Amblyoclada* can be used in active biomonitoring of emissions where there is a scarcity of native lichens. The analyses indicate the element accumulation in lichens exposed in both sites of rural and urban areas. Further studies are required to ascertain the effect caused to the lichen by transplantation since element accumulation depends on vitality which in turn is related to climatic conditions and exposure period.

Comparison made between the results of lichens exposed in sites of different levels of pollution revealed, as expected, that urban area of São Paulo city is more polluted than rural area located 50 km from the São Paul downtown area. The elements were identified as emitted by some anthropogenic sources such as vehicular sources (Ba, V and Zn) as well as industrial sources (Sb and Zn)

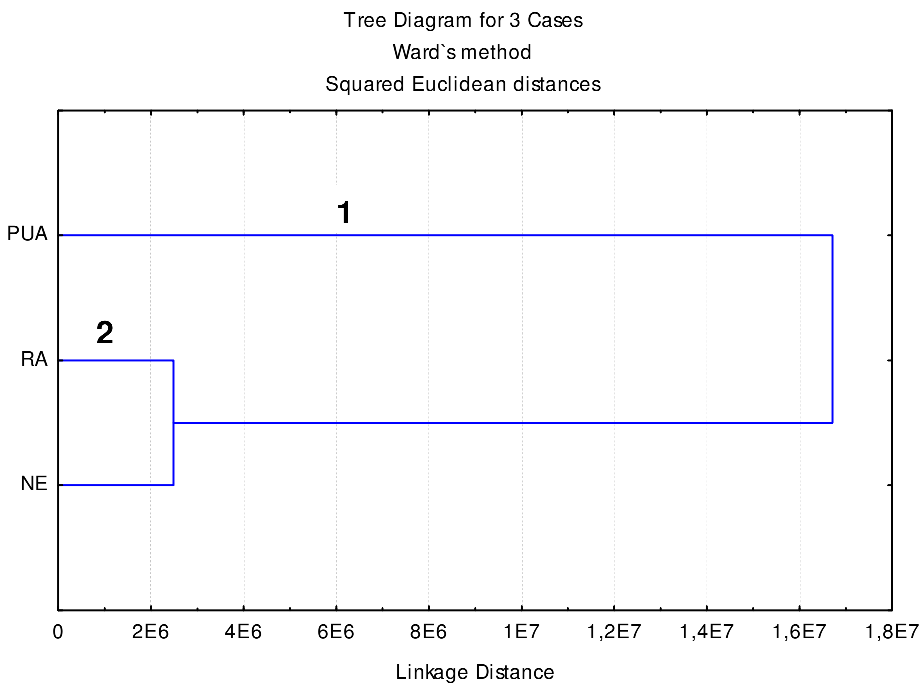

Figure 5. Dendrogram obtained in cluster analysis for lichens exposed in different sites PUA – Polluted urban area of São Paulo city, RA – Rural area of Caucaia do Alto, NE = Not exposed

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