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Determination of trace elements in *Tillandsia usneoides* by neutron activation analysis for environmental biomonitoring

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Neutron activation analysis was applied to the determination of the elements Al, As, Ba, Br, Cl, Co, Cr, Fe, K, Mg, Mn, Mo, Na, Rb, Sb, Sc, Ti, Th, V, Zn, and the rare earths La, Ce, Nd, Sm, Eu, Tb and Yb in the epiphytic bromeliad *Tillandsia usneoides*. The samples were collected at an unpolluted area and exposed in different sites of the city of São Paulo, Brazil, and in a control site outside São Paulo. The results obtained showed an accumulation of Al, As, Cr, Fe, Mo, Sb, Ti, V and Zn elements in *Tillandsia usneoides* exposed in polluted sites, indicating a promising potential of this species as a biomonitor of air pollution in São Paulo.

Introduction

Biomonitors present several advantages in comparison to conventionally used instruments for pollution monitoring, such as low costs, the possibility of integrating pollutants in longer time intervals, and monitoring of several sites simultaneously. Epiphytic plants are efficient biomonitors of air pollution, since they do not make contact with soil, taking nutrients from the atmosphere. Their tissue content largely reflects atmospheric contamination. In general, they are excellent accumulator biomonitors.

The epiphytic lower plants such as lichens and mosses have been widely used as indicators of regional air quality in several European countries.[1–3] The epiphytic bromeliad *Tillandsia usneoides* L. has also been used as monitor of air quality,[4,5] due to its morphological and physiological characteristics. This species is well adapted to dry and hot conditions, which makes it adequate for use as biomonitor in tropical areas. In Brazil, *Tillandsia usneoides* was demonstrated to be a suitable biomonitor for atmospheric mercury contamination, in a chlor-alkali plant.[6] Active biomonitoring, which consists of transferring plants collected in unpolluted sites to the area to be monitored, was employed by these authors. This methodological approach is especially useful when the biomonitor species does not occur naturally in the monitoring sites.

The objective of this work was to evaluate the potential usefulness of *Tillandsia usneoides* as an active accumulator biomonitor for environmental monitoring in São Paulo, a highly populated and industrialized city in Brazil. For this purpose, samples of *Tillandsia usneoides*, collected at an unpolluted area, were exposed in different sites of the city of São Paulo and in a control site outside São Paulo. Neutron activation analysis (NAA) was employed for the determination of trace element accumulation in the plants, and analytical conditions such as sample preparation, irradiation and counting time, detection limits and accuracy and precision of the method were studied. Preliminary results for trace element concentrations in *Tillandsia usneoides* exposed for 8 weeks in different sites of the city are presented and showed promising possibilities for using this species as a biomonitor in São Paulo.

Experimental

Tillandsia usneoides L.

Tillandsia usneoides L., popularly known as Spanish moss, is an epiphytic species widely distributed and representative of the tropical and subtropical family Bromeliaceae. It is considered an aerial epiphyte, since its survival is completely dependent on the ambient air. According to CALASANS and MALM,[6] plants of *T. usneoides* have leaf scales and large surface areas due to their reduced form and structure. Their ranked vegetative growth results in entangled masses of plants. Consequently, they are excellent accumulators of atmospheric elements. Slow growth and no contact with soil are other interesting characteristics of this species for air quality biomonitoring.

Study sites

São Paulo, one of the largest cities in the world, with a population of around 18 million, represents an important industrialized and economic center of Brazil. This city is situated in a lower region of South America Atlantic Planalt, occupying an area of about 5000 km^2.

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Table 1. Annual mean concentrations, obtained in 1998, of the principal air pollutants and their maximum value ranges measured in Congonhas and Cerqueira Cesar stations, in São Paulo city. Data from CETESB[8]

Station	PM_{10},[a] µg/m^3		CO,[b] ppm		SO_2,[a] µg/m^3		NO_2,[b] µg/m^3		O_3,[b] µg/m^3	
	Mean	Daily maximum	Mean	Hourly maximum	Mean	Daily maximum	Mean	Hourly maximum	Mean	Hourly maximum
Cerqueira Cesar	40	110	4	11	12	48	109	394	NM	NM
Congonhas	54	149	6	21	18	63	138	392	43	214

[a] Mean based on daily values.
[b] Mean based on maximum hourly event in each day.
NM – Not measured.
PM_{10} – Particulate material – 10 µm.

Table 2. General characterization of air quality in the rural and urban sites, based on instantaneous measurements of CO and O_3 concentrations performed at midday, in sunny days of November/1999 (Data extracted from GUIMARÃES et al.[9])

Sites	CO, ppm	O_3, µg/m^3
Caucaia do Alto	0	40
Cerqueira Cesar	5	140
Congonhas	8	100

According to Köppen system, its climate is Cwa type, characterized by hot and wet summer (mean temperature of the hottest month higher than 22 °C), dry winter (mean temperatures of the coldest month below to 18 °C) and annual rainfall around 1300 mm.[7] The city is subjected to frequent thermal inversions. According to the Environmental Protection Agency of the State of São Paulo (CETESB), the governmental agency of air quality control, emissions from about 4 million motor vehicles daily are the main sources of air pollution in the city.[8]

Two highly polluted sites in São Paulo city were chosen, both situated in avenues exposed to air pollutants from heavy traffic of passenger cars, buses and trucks: Bandeirantes Avenue, near to one of the air quality monitoring stations of CETESB, denominated Congonhas; and Dr. Arnaldo Avenue, near to the monitoring station Cerqueira Cesar. The air pollutant concentrations in both urban sites, hereafter referred as Congonhas and Cerqueira Cesar, are shown in Table 1.

Caucaia do Alto, with low influence of traffic and around 50 km from São Paulo city, was selected as the control site. The economy of this city is mainly based on agricultural activities. According to Köppen system, the climate of Caucaia do Alto is a Cfb type, temperate, without dry season (mean temperatures of the hottest month below 22 °C and of the coldest month below 18 °C) and with annual rainfall of approximately 1500 mm.[7] Air quality data is not available for this site, but CETESB considers the air as clean and non-polluted. Additionally, the better air quality in this control site in comparison with urban ones was confirmed by instantaneous measurements of CO and O_3 concentrations, performed by colorimetric techniques (Dräger – Lubeck, Germany) at midday, in sunny days of November/1999 (Table 2).

In situ monitoring

Plants of *T. usneoides* were taken in a public park in Paranapiacaba, situated about 50 km from São Paulo city. That region is predominantly occupied by portions of the Atlantic Forest and is not reached by air pollutants from São Paulo.

Each sample for exposure was composed of 5 g of entangled green mass of plants, packed in a polyethylene net bag (1 cm mesh). The biomonitoring started in January 1999. The samples were hanged in gyrator apparatus (6 bags per apparatus) which turned with the wind so that homogenous contact with air contaminants was guaranteed. Samples from each site were taken after 8 weeks. The *T. usneoides* plants in the polluted and control sites remained green during the monitoring period, even showing some growth. In this respect, the species is well suited to in situ monitoring studies in urban environments, such as São Paulo city.

After each exposure time, the samples were separately frozen without washing and stored at –20 °C until analyses.

Analytical procedure

Sample preparation: Samples were freeze dried for 20 hours at a pressure of $4\cdot10^{-2}$ mmHg. After this procedure, the samples were ground and homogenized manually in agate mortars. Two hundred mg of the samples were accurately weighed in polyethylene envelopes, previously cleaned with diluted nitric acid solution.

Standards: Standards of the elements of interest were prepared by mixing appropriate aliquots of solutions of these elements made from spectroscopically pure reagents or from SPEX standard solutions. Aliquots of these solutions were pipetted onto 1 cm^2 pieces of Whatman No. 40 filter paper, evaporated to dryness under an infrared lamp, and sealed in polyethylene envelopes, similar to those used in the preparation of the samples. About 200 mg of the biological standard reference material Orchard Leaves (NIST SRM 1571) and of the geological reference material Soil-7 (IAEA) were weighed and prepared similarly to the sample.

INAA: For the determination of Al, Ti, V, Cl, Mg and Mn, samples and standards were irradiated for 5 minutes at a thermal neutron flux of $4\cdot10^{11}$ $n\cdot cm^{-2}\cdot s^{-1}$, at the IEA-R1m nuclear reactor of Instituto de Pesquisas Energéticas e Nucleares. For the other analysed elements (As, Ba, Br, Co, Cr, Fe, K, Mo, Na, Rb, Sb, Sc, Th, Zn, and the rare earths La, Ce, Nd, Sm, Eu, Tb and Yb), samples and standards were irradiated for 16 hours at a thermal neutron flux of $1\cdot10^{13}$ $n\cdot cm^{-2}\cdot s^{-1}$. The measurements of the induced gamma-ray activity were carried out using a GMX20190 hyperpure Ge detector. The multichannel analyzer was a 8192 channel Canberra S-100 plug-in-card in a PC computer. The resolution (FWHM) of the system was 1.90 keV for the 1332 keV gamma-ray of ^{60}Co. For the 5 minute irradiation, decay and counting times were each 3 minutes. Two series of measurements were performed after the 16 hour irradiation; the first was done from 5th–7th days after irradiation and the second one after 15–20 days of decay. Counting times ranged from 3 to 10 hours. The gamma-ray spectra were processed by using an in-house gamma-ray software, VISPECT,[10] which locates peak position and calculates the energies and net areas.

Table 3. Trace elements (in $\mu g\cdot g^{-1}$) determined in different aliquots of the same sample to verify homogeneity

Element	Sample 1		Sample 2	
	Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2
Mo	2.25 ± 0.10*	2.36 ± 0.10	2.11 ± 0.96	2.27 ± 0.99
Mn	216 ± 7	223 ± 7	325 ± 10	322 ± 10
Na	827 ± 50	919 ± 39	613 ± 31	613 ± 37
V	6.3 ± 0.2	6.3 ± 0.3	4.2 ± 0.2	4.1 ± 0.2
Cl	991 ± 53	929 ± 41	522 ± 33	552 ± 38
Al	2684 ± 30	2828 ± 34	1209 ± 17	1284 ± 16

* Uncertainties calculated using statistical counting errors of samples and standards (1σ).

Table 4. Elemental concentrations and detection limits obtained for Orchard Leaves SRM 1571

Element	This work	Orchard leaves Reference[12]	Detection limits
As, $ng\cdot g^{-1}$	10.3 ± 0.3*	10 ± 2	0.17
Ba, $\mu g\cdot g^{-1}$	44 ± 3	(44)	3.5
Br, $\mu g\cdot g^{-1}$	9.1 ± 0.1	(10)	0.057
Cl, $\mu g\cdot g^{-1}$	675 ± 43	(690)	41
Co, $\mu g\cdot g^{-1}$	0.183 ± 0.02	(0.2)	0.002
Cr, $\mu g\cdot g^{-1}$	2.47 ± 0.02	2.6 ± 0.3	0.17
Fe, $\mu g\cdot g^{-1}$	277 ± 7	300 ± 20	8.5
K, %	1.46 ± 0.09	1.47 ± 0.03	787
Mg, %	0.63 ± 0.05	0.62 ± 0.02	72
Mn, $\mu g\cdot g^{-1}$	94 ± 4	91 ± 4	0.25
Mo, $\mu g\cdot g^{-1}$	0.43 ± 0.06	0.37 ± 0.08	0.06
Na, $\mu g\cdot g^{-1}$	76 ± 6	82 ± 6	1
Rb, $\mu g\cdot g^{-1}$	11.2 ± 0.4	12 ± 1	0.35
Sb, $\mu g\cdot g^{-1}$	2.87 ± 0.01	2.9 ± 0.3	0.023
Sc, $\mu g\cdot g^{-1}$	64 ± 1	–	1.2
Th, $ng\cdot g^{-1}$	42 ± 4	64 ± 6	0.006
Zn, $\mu g\cdot g^{-1}$	22.5 ± 0.1	25 ± 3	0.32
La, $\mu g\cdot g^{-1}$	1.11 ± 0.01	(1)	0.006
Ce, $\mu g\cdot g^{-1}$	1.24 ± 0.08	(0.9)	0.12
Nd, $\mu g\cdot g^{-1}$	0.62 ± 0.09	(0.57)	0.37
Sm, $\mu g\cdot g^{-1}$	0.12 ± 0.01	(0.1)	0.002
Eu, $\mu g\cdot g^{-1}$	0.015 ± 0.001	(0.021)	0.002
Yb, $\mu g\cdot g^{-1}$	0.020 ± 0.002	(0.025)	0.005

Figures in parentheses correspond to information values.
* (1σ).

Table 5. Trace element concentrations (in $\mu g \cdot g^{-1}$) in *Tillandsia usneoides* exposed for 8 weeks in different sites of São Paulo

Element	Caucaia	Congonhas	Cerqueira Cesar
Al	933 ± 20	1016 ± 14	3606 ± 42
As	0.31 ± 0.01	0.38 ± 0.02	0.65 ± 0.02
Ba	32 ± 3	49 ± 4	100 ± 9
Br	4.31 ± 0.07	6.43 ± 0.06	3.40 ± 0.06
Cl	534 ± 40	988 ± 39	617 ± 33
Co	1.1 ± 0.1	1.3 ± 0.2	1.6 ± 0.2
Cr	2.6 ± 0.1	4.5 ± 0.3	6.7 ± 0.2
Fe	2536 ± 61	2914 ± 117	3960 ± 95
K	4713 ± 221	4318 ± 156	4912 ± 230
Mg	2252 ± 105	1614 ± 78	2508 ± 106
Mn	300 ± 10	175 ± 6	273 ± 10
Mo	0.64 ± 0.05	0.93 ± 0.07	1.4 ± 0.1
Na	601 ± 40	1285 ± 43	1486 ± 79
Rb	89 ± 3	14 ± 1	23 ± 1
Sb	0.50 ± 0.02	0.52 ± 0.01	1.83 ± 0.01
Sc	0.18 ± 0.01	0.20 ± 0.01	0.44 ± 0.04
Ti	45 ± 17	91 ± 18	373 ± 37
Th	0.34 ± 0.01	0.62 ± 0.17	0.95 ± 0.03
V	3.3 ± 0.2	4.4 ± 0.2	9.4 ± 0.3
Zn	73 ± 2	80 ± 3	190 ± 35
La	2.7 ± 0.1	2.7 ± 0.1	5.61 ± 0.03
Ce	5.1 ± 0.1	5.8 ± 0.1	10.1 ± 0.1
Nd	2.7 ± 0.1	2.8 ± 0.1	3.9 ± 0.2
Sm	0.26 ± 0.01	0.31 ± 0.03	0.48 ± 0.04
Eu	0.06 ± 0.01	0.07 ± 0.01	0.11 ± 0.02
Yb	0.06 ± 0.01	0.06 ± 0.01	0.14 ± 0.01

Results and discussion

To verify the homogeneity of the samples, two samples of *Tillandsia usneoides* were prepared in two aliquots which were exposed separately to a neutron flux of $4 \cdot 10^{11}$ $n \cdot cm^{-2} \cdot s^{-1}$ for 5 minutes and then analyzed by gamma-ray spectrometry. The elements Mg, Mn, Na, V, Cl and Al were determined, and the results obtained are presented in Table 3. The reproducibility was better than 10% for the analyzed elements, which demonstrates the homogeneity of the sample.

To verify the accuracy and precision of the method, the biological reference material Orchard Leaves NIST SRM 1571 was analysed. The results obtained as well as detection limits are presented in Table 4. The results are the mean of three replicate analyses and the errors associated represent one standard deviation. The method showed accuracy and precision better than 15% for the majority of the elements. Detection limits were calculated by using Curie’s criteria.[11]

Preliminary results obtained for trace element concentrations in samples of *Tillandsia usneoides* submitted to exposure of 8 weeks in different sites are presented in Table 5. The results are the mean of the analyses of the samples exposed in the same device, and the errors represent one standard deviation. The data obtained, even though preliminary, show a tendency of increasing concentration of elements such as Al, As, Cr, Fe, Mo, Sb, Ti, V and Zn in samples of *Tillandsia usneoides* exposed in sites presenting increasing degrees of pollution. These results indicate a promising potentiality of this species as an accumulator biomonitor of air pollution in the city of São Paulo.

The well known profile of São Paulo, from the point of view of pollution, makes it very interesting to find a pollution control system, which is in balance economically and ecologically. According to the tremendous influence of uncontrolled pollution sources, a system of biomonitoring can help to establish and improve the air quality and to overcome especially the high costs of instrumental observation.

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