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Suitability of *Tillandsia usneoides* and *Aechmea fasciata* for biomonitoring toxic elements under tropical seasonal climate

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HIGHLIGHTS

- The biomonitoring ability of epiphytic bromeliads were analyzed under seasonal climate.
- *Tillandsia usneoides* and *Aechmea fasciata* are adequate qualitative bioindicators of toxic elements.
- Both species indicate adequately seasonal differences in the pollution levels.
- *A. fasciata* is indicated for biomonitoring the atmospheric levels of trace metals.
- *T. usneoides* revealed a higher ability for biomonitoring spatial variations.

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ABSTRACT

Aechmea fasciata was evaluated for the first time as a biomonitor of toxic elements, in comparison to the biomonitoring capacity of *Tillandsia usneoides*, a well-established biomonitor bromeliad species. Plants of both species were exposed to air pollutants from industrial, urban, and agricultural sources, under the tropical seasonal climate, from June/2011 to April/2013, in five sites of São Paulo State, Brazil, for 8 consecutive exposure periods of 12 weeks each. The levels of essential and non-essential elements, including trace metals, were quantified at the end of each exposure. *T. usneoides* and *A. fasciata* indicated N, Fe, Zn, Co, Cr, and V as air contaminants in the studied sites, during wet and dry seasons and both species were recommended for qualitative biomonitoring. Concentration levels of N, Ca, S, Fe, Zn, Cu, B, Co, and Ni were significantly higher in *T. usneoides* than in *A. fasciata*. However, *A. fasciata* showed a higher effective retention capacity of Ni, Pb, V, Cu, Fe, Cr, and Co during field exposure, as indicated by the estimate of enrichment factor relative to basal concentrations. This species is more suitable for detecting the atmospheric pollution level of those metals than the *T. usneoides*. Both species indicated adequately the seasonal differences in the pollution levels of several elements, but *T. usneoides* presented higher ability for biomonitoring the spatial variations and for indicating more properly the sources of each element in the studied region than the *A. fasciata*.

1. Introduction

Air pollutants, such as SO_2, NOx, and PM_{10} enriched by toxic organic and inorganic constituents, may affect directly or indirectly plants, animals or the environment in general (Considine and Foyer, 2015; Peel et al., 2013; Pope et al., 2009). Even essential nutrients for plants, such as N, S, Fe, Cu, and Zn, when above the optimum concentrations for their growth, may cause noxious effects on sensitive species. Both trace metals, the essential ones (Fe, Cu, and Zn) and non-essential ones (Cd, Cr, Co, Hg, Mo, Ni, Pb, Al, As, and Se) for plants, may be particularly toxic and have been targets of eco-toxicological studies (Nagajyoti et al., 2010; Nriagu and Pacyna, 1988), which highlights the importance of establishing an efficient system of risk monitoring caused by such toxic elements.

Standardized grass culture (*Lolium multiflorum* ssp. *italicum* cv. Lema) and many tropical tree species, among them *Psidium guajava* cv. Paluma and *Tibouchina pulchra*, have been studied for their

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potential as bioaccumulators (Bulbovas et al., 2015; Domingos et al., 2003; Klumpp et al., 2009; Moraes et al., 2002; Nakazato et al., 2015). Currently, several epiphytic bromeliads have been used as biomonitors of trace metals, due to their high ability to accumulate toxic elements, mostly without showing visible damage, with the advantages of absorbing these elements only from the air and showing higher capacity of accumulation. Biological monitoring using bromeliads allows assessment of hundreds of sites at the same time; it integrates exposure over longer periods of time; very low cost, and giving an idea of bioavailability (Amado Filho et al., 2002).

Bromeliads of *Tillandsia* genus, among them *Tillandsia usneoides* have been commonly used as biomonitors of toxic elements in Brazil, Germany, and Argentina (Amado Filho et al., 2002; Calasans and Malm 1977; Fonseca et al., 2007; Malm et al., 1998; Pereira et al., 2007; Rodriguez et al., 2010; Vianna et al., 2011; Wannaz et al., 2012). For example, increasing concentrations of As, Cr, Fe, V, and Zn were measured in *T. usneoides* exposed in urban areas of São Paulo, when compared to a control site (Figueiredo et al., 2007). High accumulation of Cd, Cr, Pb, Cu, and Zn was also observed in *T. usneoides* maintained in populated and industrialized Brazilian cities (Vianna et al., 2011). However, the low growth rate, no commercial production of the species, and the non-acclimation of *T. usneoides* plants in certain monitoring areas highlight the necessity of searching for new epiphytic bromeliads for biomonitoring.

Studies have already detached the bioaccumulation potential of *Aechmea* species, such as *Aechmea coelestis* (Elias et al., 2006) and *Aechmea blanchetiana* (Giampaoli et al., 2012). *Aechmea fasciata* (silver vase plant) is a new potential alternative for biomonitoring purposes under tropical seasonal climate because it occurs naturally in tropical and subtropical regions (Central and South Americas) and is able to grow and adapt in different habitats (Smith and Downs, 1974). Its tank-shaped leaves, which store water and nutrients effectively, can control or reduce water stress. It is an ornamental plant species with epiphytic behavior, easily cultivated *in vivo* or *in vitro*, and it is a fast-growing species when compared to *Tillandsia* species (Cueva et al., 2006; Vinterhalter and Vinterhalter, 1994).

The present study aimed to evaluate comparatively the biomonitoring capacity of *T. usneoides* and *A. fasciata* for evaluating atmospheric levels of anthropogenic toxic elements, when exposed in polluted regions under tropical seasonal climate and to determine which of them would be recommended for qualitative biomonitoring and for discriminating the seasonal and spatial variations of atmospheric pollution levels associated with distinct emission sources of these elements.

2. Material and methods

2.1. Plant material

T. usneoides plants were acquired from a commercial producer located in Cordeirópolis City, São Paulo, Brazil, and were cultivated for six months before the beginning of the field experiments in a greenhouse for acclimation. Each sample unity used in the experimental period was composed by 50 g of fresh plants tied by a nylon thread.

Plants of *A. fasciata* were acquired from a producer located at Holambra City, São Paulo, Brazil. Plants were transferred to individual vessels containing *Pinus* composted bark as substrate and cultivated in a greenhouse for three months before field exposure, irrigated daily and fertilized weekly with 2.0 g L^{-1} of Peters® (N:P:K 20:20:20) until they achieved a minimum weight of 30 g, and with the tank totally formed.

2.2. Experimental sites

The study was carried out in the Metropolitan Region of Campinas (MRC), São Paulo State (22°30′23°15′S and 46°30′47°00′W). The region is composed of 20 municipalities, with agricultural activities (sugarcane, corn and citrus crops), an industrial park and intense vehicular traffic (CETESB, 2006). The original Semidecidual forest in the MRC, included in the Atlantic Vegetation Domain, was highly fragmented by the diverse anthropic land uses. It is located in an intertropical zone plateau, characterized by pronounced wet or dry seasons (Vicente, 2005).

Five exposure sites were selected in an area of 314 km^2 of the MRC with industrial, urban and agricultural pollution sources (Fig. 1). The exposure sites were located in the cities of Campinas (CA - 22°49′22.65″S-47°06′17.38″W), Paulínia (PA – 22°41′52.19″S-47° 6′10.27″W), Jaguariúna (JA – 22°43′3.78″S-47°01′50.71″W), Paulínia (PC – 22°46′13.4″S-47°09′25.3″W) and, Holambra (HO - 22°39′48.25″S-47°06′26.71″W). PA is next to the major petrochemical industry of the country; PC is located in Paulínia downtown and next to chemical and agrochemical industries; CA is located in the most urbanized portion of the study area, with lower proximity to agriculture activities than the other sites, and JA and HO are rural sites, mainly characterized by their proximity to agriculture activities, which are overspread in all the study region. CA also receives urban pollutants coming from the Metropolitan Region of São Paulo City (Boian and Andrade, 2012) (Fig. 1). PC is located near a monitoring station of air quality, managed by the Environmental Company of São Paulo State (CETESB).

Eight exposure periods were performed from June/2011 to April/2013 in all sites. Each exposure period lasted 12 weeks and started with a new group of five samples of *T. usneoides* and five of *A. fasciata* per site. The plants were maintained in the exposure sites under polyethylene 50% shade cover and were weekly irrigated with deionized water. At the end of each exposure period, the plants were collected for analysis. Three additional sample unities or individuals of both species were separated immediately before starting each exposure period for determining start point of the concentrations of all elements analyzed (time zero – T0).

Three exposure periods (E1, E5, E6) were conducted during the dry seasons (defined by total rainfall < 200 mm throughout the exposure) and five exposure periods (E2, E3, E4, E7, E8) during wet seasons (total rainfall $\geq$ 200 mm throughout the exposure) (Table 1; data from the meteorological station located in the petrochemical refinery area). Data from the monitoring station of air quality located near to PC site were used to describe the climatic conditions (air temperature and relative humidity) and PM_{10}, SO_2 and NO_2 concentrations during the exposure periods (Table 1; CETESB, www.ar.cetesb.sp.gov.br/qualar/).

2.3. Chemical analysis

Unwashed samples of both species were oven dried (Marconi M035, Piracicaba, Brazil) at 60 °C and were powdered in agate ball micro-mill (Pulverisette 0, Fritsch, Germany). The concentrations of essential and non-essential elements to plants (N, K, P, S, Ca, Mg, B, Cu, Mn, Zn, Fe, Pb, Co, Ni, Sr, V and Cr) were analyzed in the powdered samples. The N contents were measured by the Kjeldajhl method (Sarruge and Haag, 1974) after digestion with a solution mixture containing 30% hydrogen peroxide, lithium sulfate, selenium powder and sulfuric acid in a digester block that was gradually heated up to 350 °C. Aliquots of powdered samples were digested with nitric acid at room temperature for 12 h and then were gradually heated up to 160 °C. After partial evaporation, perchloric acid was added and the samples were heated up to 210 °C. The resulting extract was diluted with deionized water and

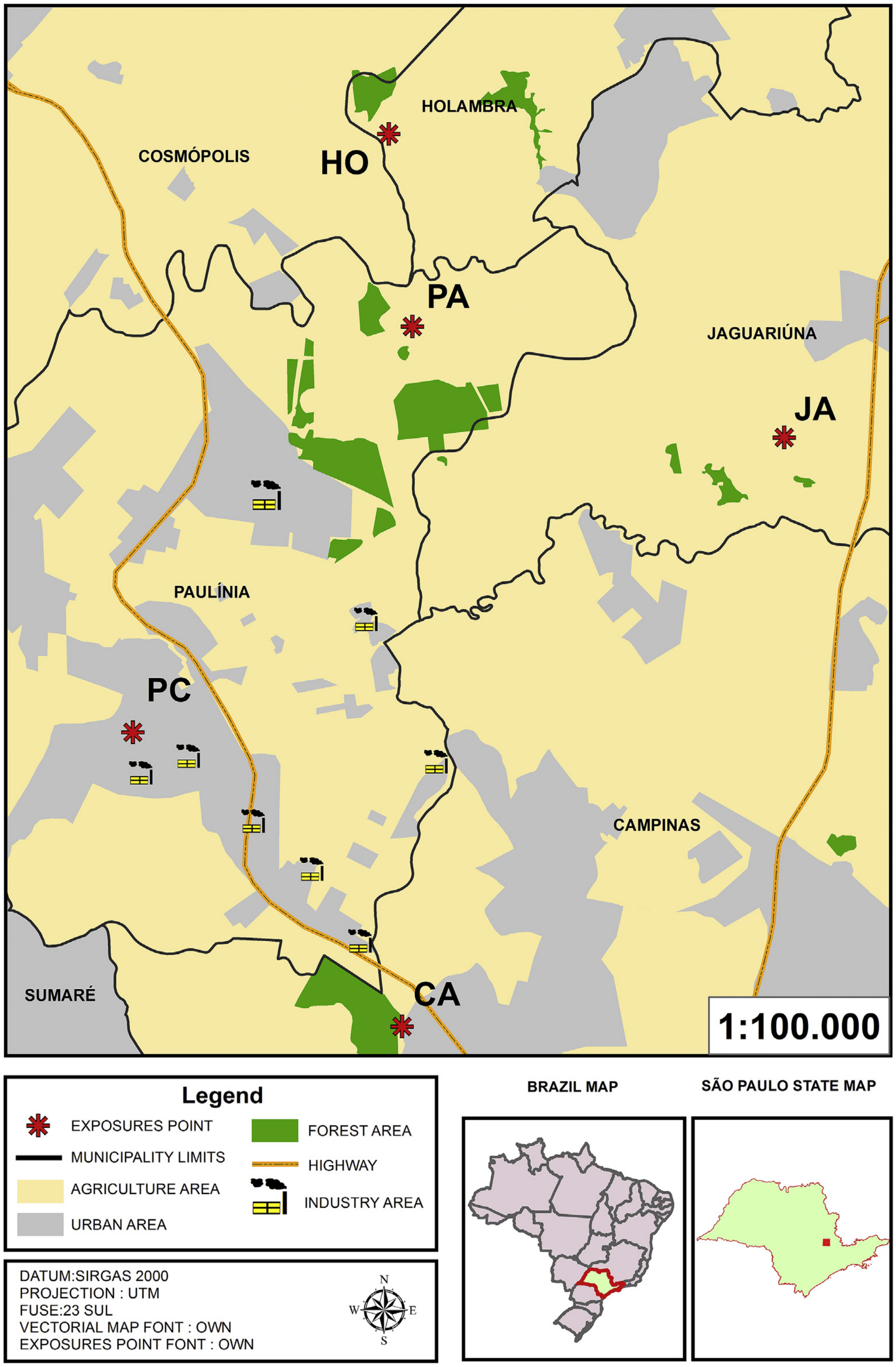

Fig. 1. Map of the study area and surroundings locating the exposure sites and the main sources of toxic elements analyzed in the plant samples: HO (Holambra), PA (Paulínia), JA (Jaguariúna), PC (Paulínia downtown), and CA (Campinas).

K, Ca, Mg, B, Cu, Fe, Mn, and Zn were evaluated by atomic absorption spectrophotometry. P and S were determined in the same extracts by colorimetric and turbidimetric methods, respectively (Malavolta et al., 1997).

Pb, Co, Ni, Sr, V, and Cr contents were analyzed by Total Reflection X-Ray Fluorescence (TXRF) using Synchrotron Radiation. The powdered material was reduced to ashes at 400 °C for 4 h, and then digested with HNO_3 (20%) at 25 ± 2 °C, at least for 24 h. The solid residues were separated by centrifugation and 450 μl of supernatant was separated and kept at room temperature until 50 μl of Ga 100 ppm be added for internal standardization. In transparent acrylic plates 5 μl of the solution with Ga were deposited and kept in closed boxes for drying. Therefore, standard solutions with known concentrations of different elements and Ga as an internal

Table 1
Characterization of the environmental conditions near to PC site during the experiment period of air quality located near to PC. Dry exposure periods – E1, E5 and E6; Wet exposure periods – E2, E3, E4, E7 and E8.

Exposure periods	PM_{10} (ppm)	NO_2 (ppm)	SO_2 (ppm)	RH (%)	Temp. (°C)	Rainfall. (mm)
E1	52.3	31.2	7.3	65	19.4	36
E2	32.5	19.2	2.6	68	22.5	592
E3	34.5	18.7	3.4	69	24.3	227
E4	27.1	22.5	3.8	–	–	349
E5	37.3	36.0	6.6	77	31.9	68
E6	41.5	30.6	8.0	66	23.3	155
E7	24.4	18.4	7.0	82	24.2	378
E8	22.9	20.7	5.8	91	23.7	509

standard were used for system calibration. Samples were measured for 100 s using the Total Reflection Fluorescence x-rays (TXRF) at Brazilian Synchrotron Light Laboratory (LNLS) at Campinas, SP, Brazil, with a white ray with approximately 0.3 mm wide and 2 mm high used for excitation. HPGe detector was used with an energy resolution of 148 eV at 5.9 keV for X-ray detection. The blanks and the standard reference materials "Soybean flour (INCT-sbf-4), Oriental Tobacco Leaves (CTA-OTL-1, ICTJ) and CRM 281 (ryegrass, European Commission/BCR)" were prepared with the same procedures described previously and were performed five times to instrument calibration. The coefficient of variation of the replicate analysis was calculated to the different determinations. The variations were found were less than 10%.

2.4. Enrichment factor

Enrichment factors were calculated according to the following equation, aiming to exclude the basal concentrations of elements analyzed and inferring about their retention capacities during the experimental period: $EF_x = C_s/C_{T0}$; EF_x is the enrichment factor of the element x; C_s is the element concentration (ppm) in each site and C_{T0} is the concentration of the same element in the plant evaluated before being exposed - time zero (T0). Samples of both species were considered enriched by elements whose EF rates have exceeded 1.0.

2.5. Statistical analyses

The concentrations of all elements analyzed were initially described by average, maximum, minimum and median values. The non-parametric Mann–Whitney test compared the accumulation capacity of the species, based on concentrations, and the EF estimated for all elements analyzed. Significant differences between dry and wet periods for both concentrations and EF for each species were also identified by the Mann–Whitney test. Spatial variations in estimated EF ratios were undertaken by the non-parametric Kruskal–Wallis test, followed by multiple comparisons Dunn test. All the analyses were performed using the Sigma Plot software 11.0.

Only elements enriched (EF > 1) in 50% or more samples of each species were considered for locating significant spatial variations (by means of Kruskal–Wallis and Dunn tests) and discriminating the groups of elements coming from the same sources with exploratory hierarchical cluster analyses. The cluster analysis was performed with standardized matrices of EF ratios using the STATISTICA software (Statsoft™). The Ward's Method was applied as the joining rule and 1-Pearson r as the metric distance.

3. Results and discussion

The average air temperatures and the relative humidity did not vary characteristically in function of wet and dry seasons. The lowest and highest average temperatures were measured during E1 (19.4 °C) and E5 (31.9 °C) exposure periods respectively. Lower mean values of relative humidity (ranging from 60 to 70%) were verified during E1, E2, E3, and E6 periods. The air pollution concentrations were seasonally marked, following the rainfall patterns. The dry exposure periods were characterized by higher levels of PM_{10}, NO_2, and SO_2 than those measured during the wet exposure periods.

Average concentrations of the elements measured in plants of *T. usneoides* and *A. fasciata* decreased in the following order: N > Ca > K > S > Fe > Mg > P > Zn > Sr > Mn > Pb > Cu > B > Co > V > Cr > Ni, with little variation among species. As expected, this decreasing order of magnitude was mainly due to macronutrients, followed by micronutrients and other trace metals (Table 2).

Levels of N, Ca, S, Fe, Zn, Cu, B, Co, and Ni in *T. usneoides* were significantly higher than in *A. fasciata* during wet and dry periods, and levels of Sr only in the wet season and Pb only in the dry season. K, Mg, P, and Mn were measured in higher concentrations in *A. fasciata* than in *T. usneoides* (Table 2).

Samples of *T. usneoides* presented seasonal differences in the concentrations of seven elements (Mg and Zn were more concentrated in plants exposed during wet periods than during the dry periods; opposite findings were obtained for N, Fe, P, Mn, and B). These results were in accordance to those obtained by Ferreira (2014), who also exposed plants of *T. usneoides* in the same region and found higher levels of trace metals in the dry season, coinciding with higher deposition of particulate matter. The contents of a small number of elements varied seasonally in *A. fasciata* plants (N contents were higher during wet periods and Ca, Mg, and B were higher during the dry seasons) (Table 2).

The average values of many trace metals (Fe, Zn, Sr, Pb, Cu, Co, and Ni) in T0 plants of *T. usneoides* were higher in comparison to the levels detected in the other bromeliad species, indicating a higher basal concentration than observed in T0 plants of *A. fasciata*. Additionally, *A. fasciata* started the exposure experiments with higher levels of K, N, P, Mg, and Mn (T0 plants) than the other species. Consequently, plants of *A. fasciata* exposed in MRC presented average levels of N, K, Mg, P, and Mn (essential elements) below those observed in T0 plants, probably due to the initial fertilization of T0 plants and the use of these elements for plant growth and development.

However, the evaluation of elements concentration in plants at the end of the field exposure doesn't exclude the basal concentrations, i.e., the level naturally presented in the plants. So, this approach does not indicate the effective element retention in plants during the field exposure. This goal can be achieved by calculating the enrichment factor (EF) of each element (Fig. 2). The enrichment ordination has differed from the one previously proposed for element concentrations (Fe > V > Co > Zn > N > Mn > P > Cu > Ni > Mg > Ca > K > S > Pb > B > Cr > Sr for *T. usneoides* and Cr > Fe > Pb > Ni > Co > V > Cu > Zn > Sr > Mn > B > N > Mg > S > Ca > P > K for *A. fasciata*). The ordinations of EF

Table 2
Average, median, maximum and minimum concentrations of essential and non-essential elements of *T. usneoides* and *A. fasciata* plants exposed during dry and wet seasons in MRC and before plant exposure (T0 values).

Element	Season	*Tillandsia usneoides*							*Aechmea fasciata*						
		Exposed plants				T0 plants			Exposed plants				T0 plants		
		Mean	Median	Max	Mín	Mean	Max	Mín	Mean	Median	Max	Mín	Mean	Max	Mín
N (mg g^{-1})	Wet	11.6 b[a]	11.8	15.3	7.6	11.6	16.3	7.5	11.3 a	9.7	23.1	7.1	14.6	18.5	8.7
	Dry	12.7 a[a]	12.4	17.5	9.1	12.7	14.0	10.4	6.7 b	6.6	8.7	4.9	13.2	22.6	7.5
Ca (mg g^{-1})	Wet	7.8 a[a]	7.5	14.6	4.0	10.8	15.5	2.5	4.8 b	4.6	10.1	2.0	7.8	12.2	4.9
	Dry	8.2 a[a]	7.9	12.6	4.5	11.1	14.5	8.5	6.8 a	6.7	9.9	4.1	9.6	11.5	7.8
K (mg g^{-1})	Wet	4.9 a	4.8	8.7	2.6	7.8	29.1	3.8	15.3 a[a]	11.2	35.2	4.8	20.8	29.1	5.4
	Dry	4.9 a	4.7	7.7	2.0	5.6	6.6	3.8	13.7 a[a]	12.8	22.2	3.8	20.7	34.4	12.2
S (mg g^{-1})	Wet	1.2 a[a]	1.1	2.9	0.7	1.9	4.1	0.5	0.5 a	0.5	0.8	0.3	0.8	1.3	0.6
	Dry	1.2 a[a]	1.2	2.2	0.7	1.4	2.4	0.7	0.5 a	0.6	0.9	0.3	0.9	1.0	0.6
Fe (μg g^{-1})	Wet	979.1b[a]	947.5	2320	488	719.2	1231	115	739.0 a	770	1305	241	356.8	733.3	139.0
	Dry	1305.5a[a]	1308.3	1958	682	723.8	926.5	98	853.4 a	780	1828	448	475.5	871.0	179.9
Mg (mg g^{-1})	Wet	1.0 a	1.0	1.7	0.4	1.3	1.8	0.8	1.3 b[a]	1.1	3.0	0.4	2.0	2.5	1.4
	Dry	0.9 b	0.9	1.4	0.5	1.1	1.5	0.8	1.8 a[a]	1.5	5.0	0.7	2.2	3.0	1.7
P (mg g^{-1})	Wet	0.6 b	0.5	2.1	0.2	0.7	3.3	0.1	2.6 a[a]	2.5	4.9	1.5	4.6	5.5	3.4
	Dry	0.9 a	0.7	1.9	0.2	0.7	1	0.3	2.8 a[a]	2.8	4.2	1.2	4.5	5.7	3.4
Zn (μg g^{-1})	Wet	141.5 a[a]	39.2	282.7	23.5	111.9	250	20.5	25.9 a	23.0	54.5	13.0	24.9	33.5	20.5
	Dry	117.3 b[a]	112.0	280.5	51.0	143.6	221	115	27.2 a	27.2	48.9	11.5	28.4	31.2	24.0
Sr (μg g^{-1})	Wet	70.9 a[a]	58.0	279.6	16.8	105.5	243.4	63.0	55.8 a	54.3	143.4	12.4	63.2	117.3	17.1
	Dry	81.1 a	60.7	234.4	28.3	174.9	276.1	21.9	64.2 a	57.7	185.9	22.9	77.4	108.0	25.6
Mn (μg g^{-1})	Wet	40.9 b	39.2	76.2	23.5	56.0	187.5	20.5	123.8 a[a]	115.0	357.5	40.0	158.4	231.5	84.0
	Dry	44.0 a	44.2	62.5	19.5	36.8	44.5	28	143.5 a[a]	124.0	319.6	44.5	127.6	182.3	79.0
Pb (μg g^{-1})	Wet	28.4 a	18.2	201.1	0.8	29.3	90.4	0.8	25.1 a	17.3	133.0	3.2	15.4	38.4	6.1
	Dry	45.6 a[a]	23.9	238.8	8.6	36.6	221.5	14.5	21.2 a	16.8	73.7	3.0	15.3	34.2	0.9
Cu (μg g^{-1})	Wet	17.9 a[a]	16.5	46.0	9.5	18.9	30	5.5	6.5 a	6.0	16.5	3.0	8.5	20.0	3.5
	Dry	20.0 a[a]	18.6	53.1	9.0	20.9	21,5	15.5	9.0 a	7.0	28.1	4.0	7.4	8.3	6.0
B (μg g^{-1})	Wet	11.2 b[a]	11.3	21.1	4.8	18.0	28.1	10.1	11.8 b	11.6	19.4	5.7	18.3	34.7	12.7
	Dry	18.7 a[a]	18.4	31.6	12.0	22.0	31.1	18.3	16.2 a	15.3	29.0	4.8	17.0	21.4	12.6
Co (μg g^{-1})	Wet	9.6 a[a]	8.4	32.5	2.0	9.9	22	1.4	3.2 a	0.7	1.9	0.0	1.1	5.4	0.2
	Dry	9.6 a[a]	8.4	36.9	2.0	5.12	9.2	1.5	0.7 a	0.8	22.9	0.1	0.6	1.1	0.3
V (μg g^{-1})	Wet	9.2 a	6.6	78.8	0.8	5.7	21.5	1.8	8.6 a	5.8	40.2	1.4	2.5	4.3	0.6
	Dry	7.6 a	6.4	24.4	1.2	6.3	6.3	1.9	12.8 a	7.7	74.1	1.0	13.4	32.5	2.4
Cr (μg g^{-1})	Wet	6.7 a	6.6	21.6	0.1	5.5	23.1	1.6	7.3 a	5.3	31.5	0.3	3.1	6.1	0.3
	Dry	5.8 a	5.7	17.3	0.3	9.3	21.5	1.2	8.0 a	4.0	74.6	0.5	12.5	34.2	0.3
Ni (μg g^{-1})	Wet	3.5 a[a]	2.8	14.6	0.1	2.5	4.4	0.6	2.1 a	1.0	21.2	0.0	0.8	1.8	0.05
	Dry	2.9 a[a]	2.3	8.0	0.7	3.3	4.7	0.0	1.5 a	1.1	5.5	0.0	1.2	2.2	0.7

Distinct letters indicate significant differences between the seasons for each species.
[a] It indicates significantly higher average values in one species compared to the other one, during the same season.

showed minor variations between wet and dry seasons.

Significant variations in the enrichment levels between wet and dry seasons were observed in *T. usneoides* (higher EF of Sr, Ni, Zn during wet periods and B, S, K, P, Mn, Co, and Fe during the dry periods) and *A. fasciata* (higher EF of K, N, V, and Co during wet periods and of Ca, Mg, B and Mn during the dry periods). Values of EF were significantly higher and mostly estimated for nutrients (N, P, Ca, K, and Zn) in *T. usneoides*, compared to *A. fasciata* during wet and/or dry seasons. *A. fasciata* plants were predominantly more enriched than *T. usneoides* with trace metals (V, Co, Pb, Fe, Cr, Sr, and Ni). EF values of Cr, Fe, Pb, Ni and V were five times higher for *A. fasciata* than for *T. usneoides* plants (Fig. 2).

The differential enrichment capacity of both species was related to the distinct basal concentrations at the beginning of the exposure periods in the field, as discussed before. Also, the enrichment capacity of both species can be attributed to the presence of overlapping scales on their surfaces, which are essential for water and nutrient absorption and for restraining the water loss due to wind action. The distribution of scales along the leaf surfaces of the species facilitates retention of atmospheric particles in the epidermis (Vianna et al., 2011). This constitutive adaptation might increase the efficiency of bromeliad plants to retain metals within their tissues (Billings, 1904; Sanches, 2009; Scatena and Segecin, 2005). Amado Filho et al. (2002), which exposed plants of *T. usneoides* to oxygen-enriched air with mercury (Hg) in a controlled study, found that particles of Hg were found in scales, stems and leaf surfaces of.

The higher enrichment capacity verified for *A. fasciata*, regardless if the exposure occurred during the dry or wet seasons, can be associated with the presence of rosette-shaped cisterns in plants, which allow the water accumulation for long periods and ensure more effective absorption of nutrients and water when compared to *T. usneoides*.

Independently of the level of enrichment factor, 50% or more of samples of the two species, from both seasonal periods, were more concentrated in N, Fe, Zn, Co, Cr, V, and Ni than the T0 plants (EF > 1). In contrast, less than 50% of the samples were enriched by Ca, P, S, Mg, Sr, Cu, and B. Most of the samples of both species were enriched by Fe during both seasons (80% and 98% of *T. usneoides* samples and 80% and 95% of *A. fasciata* samples, during wet and dry seasons respectively). During the dry exposure periods, 78% and 95% of *T. usneoides* samples were enriched by Mn and Co, respectively, and 80% and 73% of *A. fasciata* samples were enriched by Pb and Ni.

The higher concentrations of Fe in the species at the end of the exposure periods, relative to the basal concentrations, might be mainly attributed to the soil re-suspension, since the soils in the study region are Oxisols that are characterized by high contents of Fe (Fadigas et al., 2006; Rieuwerts, 2007). Fe was also found in high concentrations in rainwater along with Al and Zn in Campinas and Paulínia cities (Dafré-Martinelli, 2015).

High concentrations of Cr, Ni and Pb are normally associated to urban and industrial sources (De Paula et al., 2015). However, in the present study, these elements, along with Zn, seemed to have been

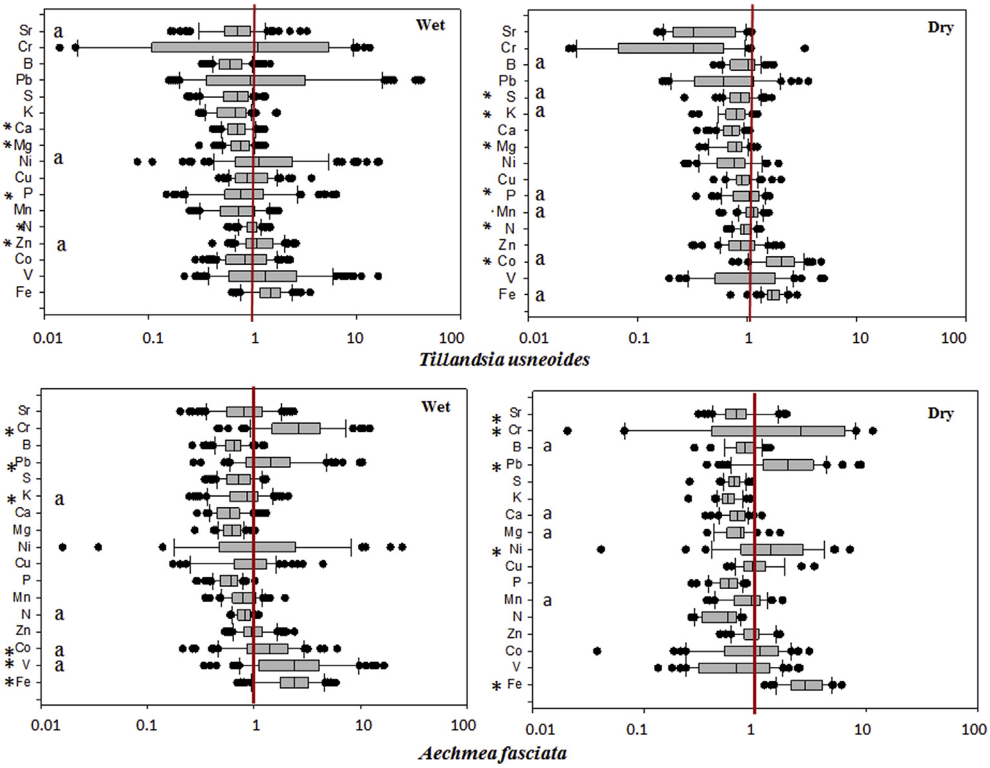

Fig. 2. Box plot representation of enrichment factor of the elements evaluated in plants of *T. usneoides* and *A. fasciata* exposed in the MRC sites, during wet and dry exposure periods. The line that divides the rectangles indicates the median; the rectangles delimit 25% of data below and above the median (25 and 75 percentiles); the error bars show values between percentiles of 10 and 25 and 75 and 90; the circles indicate the extreme values (below 10 percentile or above 90). Values above 1.0 (highlighted by red line) represent the accumulation of the element relative to the plant initial state (T0). (a) Signalizes the statically significant higher values in one season compared to the other, for each species; (*) Signalizes the statically significant higher values in one species, compared to the other, in the same season. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

also originated from agricultural sources, such as vinasse sprays, which is the main waste product from the ethanol production and has been commonly applied as fertilizer for sugarcane crops in the MRC region (Christofoletti et al., 2013; Ribeiro et al., 2010).

The presence of V and Ni in high concentrations in the environment is a characteristic of regions nearby petrochemical industries (Calvo et al., 2013; Nakazato et al., 2015), as observed in the present study. However, several authors also associate Ni with heavy traffic of cars, buses and trucks, along with Zn, Pb, Cu, V, and Cr (Markert et al., 2011; Nagajyoti et al., 2010; Rajšić et al., 2008). These trace metals are mostly originated from oil combustion (Pb, V, and Ni) and from wear debris of tire/brake (Zn and Cu) contributing to atmospheric contamination of large urban centers (Hillenbrand et al., 2005; López et al., 2011). All these emission sources are overspread in the MRC, helping to explain the origin of the preponderant enrichment of samples with these elements in both species. In fact, Dafré-Martinelli (2015), by estimated enrichment ratios, showed that Cu, Pb and Zn adsorbed in PM_{10} sampled in the MRC were from anthropogenic sources.

The analysis of spatial variations associated with distinct emission sources in the monitored region were performed with basis on the elements enriched in 50% or more samples of each species and were presented as boxplots (Figs. 3 and 4). A higher capacity to discriminate spatial variations was shown by *T. usneoides* plants, in particular for N, during the wet season and for P, Fe, and Mn, during the dry season (Fig. 3), which differed significantly between the exposure sites. The largest enrichment values of N were observed in HO, followed by JA and PA, all agricultural sites. The lowest values were observed in plants exposed in the most urbanized sites (PC and CA). The highest enrichment values of P, Fe and Mn were estimated in plants from PC. In contrast, *A. fasciata* only detected significant differences among sites concerning the Zn enrichment, which was significantly higher in PC than in the other sites, during both dry and wet seasons (Fig. 4). These results are in accordance to those found by Dafré-Martinelli (2015) in rainwater and PM_{10} samples collected in the same region.

High values of N may be associated with traffic of motor vehicles and burning of fossil fuels, since 96% of NO_x originate from vehicular emissions (CETESB, 2007). However, N, when associated with the presence of phosphate, could also indicates the influence of agricultural sources, in particular fertilizers, explaining the high values of those elements in the PA, HO and JA sites, nearby locations with extensive agricultural activity. P can also originate from combustion sources, such as fires for agriculture purposes (Wang et al., 2015). The higher values of Mn and Fe, especially during the dry season, may be directly associated to total suspended particles (Forbes et al., 2004), possibly of natural origin, as discussed before.

The cluster analyses performed with the same elements enriched in 50% or more samples of each species indicated that *T. usneoides* separated more properly the sources of each element than *A. fasciata* (Fig. 5). The analysis of data set of *T. usneoides* extracted three main groups of elements: Cr, V, Ni, and Zn; P and N; Co, Mn, and Fe (Fig. 5A). They indicate the presence of industrial/urban, agricultural and terrestrial/dust sources, respectively, in the MRC. The analysis of data set of *A. fasciata* also extracted three groups (Pb, Zn, and Fe; Cr and Ni; V, Co, and N; Fig. 5B). However, the markers of distinct pollution sources present in the region were mixed in the three groups.

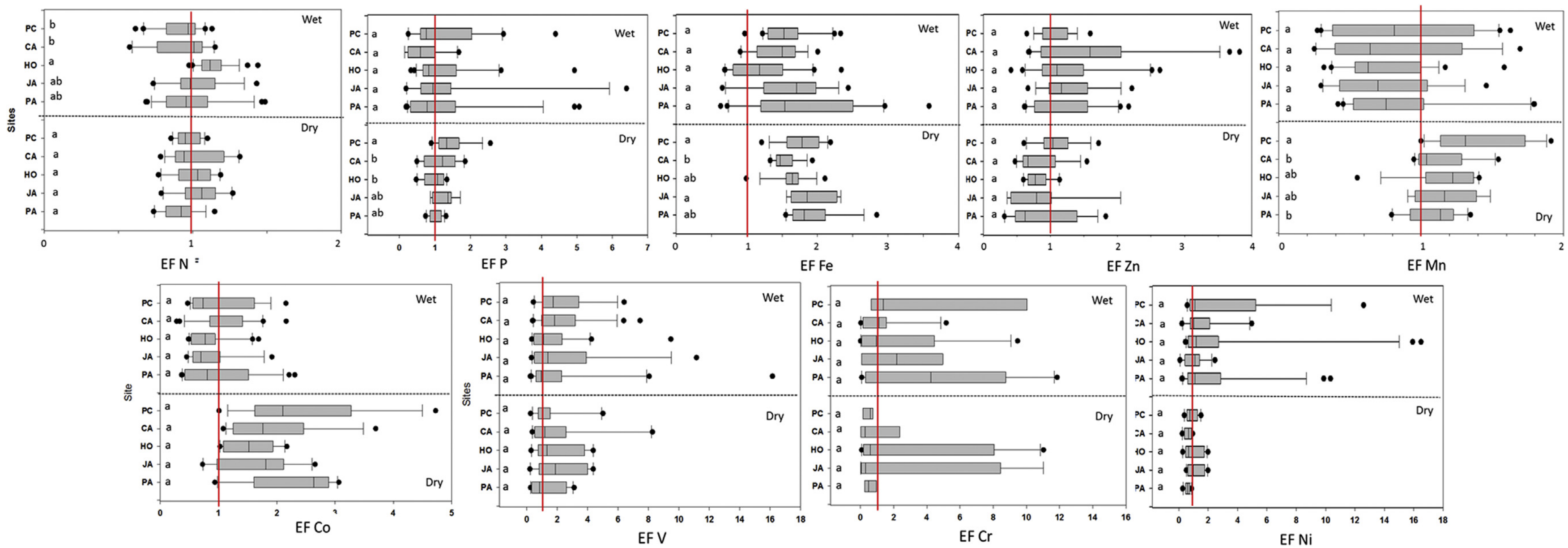

Fig. 3. Box plot representation of enrichment factor (EF), relative to T0 values, of essential and non-essential elements enriched in 50% or more samples of *T. usneoides* in each MRC site, during dry and wet exposure periods. Values above 1.0 (highlighted by red line) represent the accumulation of the element relative to the plant initial state (T0). Distinct letters indicate significant differences among exposure sites in each season. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

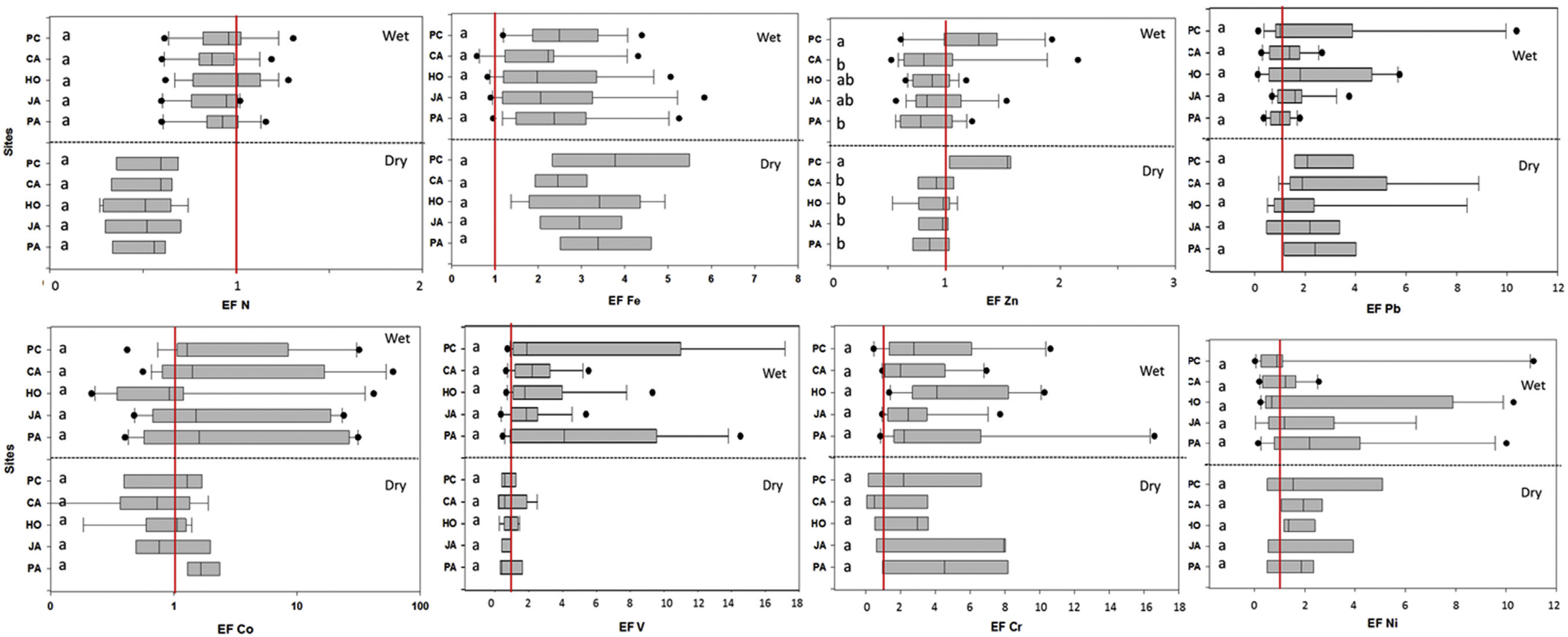

Fig. 4. Box plot representation of enrichment factor (EF), relative to T0 values, of essential and non-essential elements enriched in 50% or more samples of *A. fasciata* in each MRC site, during dry and wet exposure periods. Values above 1.0 (highlighted by red line) represent the accumulation of the element relative to the plant initial state (T0). Distinct letters indicate significant differences among exposure sites in each season. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

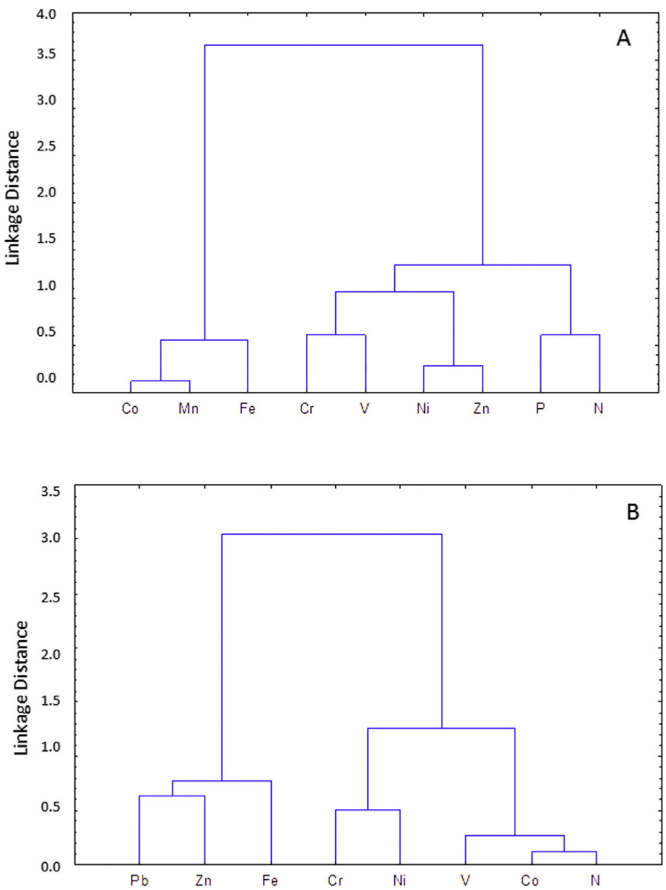

Fig. 5. Cluster analysis of the chemical elements with 50% or more of enriched values in plants of *T. usneoides* (A) and *A. fasciata* (B).

4. Conclusions

T. usneoides and *A. fasciata* indicated similarly N, Fe, Zn, Co, Cr, and V as air contaminants in the studied sites, during the wet and dry seasons. Therefore, both bromeliad species are recommended for qualitative biomonitoring of those elements under tropical seasonal climate.

Higher concentrations of most of the elements analyzed (N, Ca, S, Fe, Zn, Cu, B, Co, Ni, Pb, and Sr) were measured in sample unities of *T. usneoides* than in samples of *A. fasciata* during wet and/or dry exposure seasons. However, higher retention capacity of trace metals (V, Co, Pb, Fe, Cr, Sr, and Ni) was observed in *A. fasciata* plants during the field exposures, as shown by the calculation of enrichment factors. This ability could be attributed to the rosette-shaped cisterns in these plants, indicating that the species is able to accumulate metals of anthropogenic origin in the region (Zn and Pb).

Both species showed seasonal differences in the pollution levels of several elements studied, either by their tissue concentrations or enrichment capacities. An enhanced retention efficiency of elements was observed during the wet exposure periods than in dry season for both species due to their morphological characteristics (direct contact with the atmosphere and high density of scales in their surfaces).

T. usneoides presented a higher ability for biomonitoring spatial variations in the studied region than *A. fasciata* and indicated more properly the presence of industrial/urban (Cr, V, Ni and Zn), agricultural (P and N) and terrestrial/dust sources (Co, Mn and Fe), by means of three groups extracted by cluster analysis.

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