RESEARCH ARTICLE

Assessment of heavy metals in the particulate matter of two Brazilian metropolitan areas by using *Tillandsia usneoides* as atmospheric biomonitor

Nelzair A. Vianna · Daniel Gonçalves · Flavia Brandão · Roberta P. de Barros · Gilberto M. Amado Filho · Rodrigo O. Meire · João Paulo M. Torres · Olaf Malm · Argemiro D'Oliveira Júnior · Leonardo R. Andrade

Abstract

Purpose The aims of this paper were to quantify the heavy metals (HM) in the air of different sites in Rio de Janeiro (RJ) and Salvador (SA) using *Tillandsia usneoides* (Bromeliaceae) as a biomonitor, and to study the morphology and elemental composition of the air particulate matter (PM) retained on the *Tillandsia* surface.

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N. A. Vianna · A. D. Júnior
Faculdade de Medicina, Universidade Federal da Bahia,
Salvador, Bahia, Brazil

D. Gonçalves · F. Brandão · R. P. de Barros · L. R. Andrade (✉)
Laboratório de Biomineralização,
Instituto de Ciências Biomédicas,
Universidade Federal do Rio de Janeiro (UFRJ),
Rio de Janeiro 21941-590, Brazil
e-mail: andrade@histo.ufrj.br

G. M. A. Filho
Programa Zona Costeira,
Instituto de Pesquisas Jardim Botânico do Rio de Janeiro,
Jardim Botânico, Rio de Janeiro, RJ, Brazil

R. O. Meire · J. P. M. Torres · O. Malm
Laboratório de Radioisótopos,
Instituto de Biofísica Carlos Chagas Filho, UFRJ,
Rio de Janeiro, RJ 21941-590, Brazil

Present Address:
L. R. Andrade
Section on Structural Cell Biology,
Laboratory of Cell Structure and Dynamics, NIDCD,
National Institutes of Health,
50 South Drive, Rm. 4249,
Bethesda, MD 20892-8027, USA

Methods Tillandsia samples were collected in a noncontaminated area and exposed to the air of five sites in RJ State and seven in SA for 45 days, in two seasons. Samples were prepared to HM quantification by flame atomic absorption spectrophotometry, while morphological and elemental characterizations were studied by using scanning electron microscopy.

Results HM concentrations were significantly higher when compared to control sites. We found an increasing metal concentration as follows: Cd < Cr < Pb < Cu < Zn. PM exhibited a morphology varying from amorphous- to polygonal-shaped particles. Size measurements indicated that more than 80% of particles were less than 10 μm. PM contained aluminosilicates iron-rich particles, but Zn, Cu, Cr, and Ba were also detected.

Conclusion HM input in the atmosphere was mainly associated with anthropogenic sources such as vehicle exhaust. Elemental analysis detected HM in the inhalable particles, indicating that those HMs may intensify the toxic effects of PM on human health. Our results indicated *T. usneoides* as an adequate biomonitor of HM in the PM belonging to the inhalable fraction.

Keywords Air particulate matter · Atmospheric pollution · Biomonitoring · Electron microscopy · Heavy metals · *Tillandsia*

1 Background, aim, and scope

Atmospheric pollution is a critical problem that affects the health and well-being of people living principally in urban areas. The World Health Organization (WHO) estimates that more than 2 million premature deaths occur annually worldwide, and these can be attributed to the effects of

outdoor and indoor air pollution (WHO 2002). The main outdoor air pollutants are as follows: particulate matter (PM), carbon monoxide, nitrogen oxides, sulfur oxides, ozone, and volatile organic compounds. Most of the mortality/morbidity caused by atmospheric pollution in urban areas is caused by PM with aerodynamic diameters less than 10 μm, known as the inhalable fraction (MacNee and Donaldson 2000). These particles can be separated into coarse fraction (PM 10, diameters between 2.5 and 10 μm), fine fraction (PM 2.5, diameters between 0.1 and 2.5 μm), and ultrafine fraction (PM 0.1, diameter less than 0.1 μm). The main source of PM in urban areas originates from combustion of fossil-based fuels. Other common sources include construction and demolition sites, paved and unpaved roads, industrial activities, vehicle component wearing (e.g., brakes, tires, paint), bare ground sites, and incineration plants (Fernandez et al. 2000; Chow and Watson 2002). The PM atmospheric residence times depend on their aerodynamic dimension, weather and topographic conditions (fine particles have the longest), and they maybe transported over thousands of kilometers from their original source (Grantza et al. 2003).

Heavy metals (HM) are toxic elements that can be introduced into the atmosphere by different sources such as industrial activities, combustion of fossil-based fuels, fuel and lubricant additives, and erosion from distinct sources. PM-containing HM may induce the oxidative stress mediated by reactive oxygen, with potential mutagenic effects (Gonzalez-Flecha 2004).

PM can be quantified using automatic sampling instruments integrated in an atmospheric monitoring station, but these instruments are expensive and usually demand substantial government investments. Since the classical work of Nylander in Paris (1886), different biomonitors such as tree barks, mosses (bryophytes), lichens, and Spanish mosses (bromeliad genus) have been widely used in estimating atmospheric pollution, in special HM, during a specified period and in different sites simultaneously as an inexpensive and effective alternative (Ng et al. 2005; Tye et al. 2006; Wannaz et al. 2006). *Tillandsia* is a common biomonitor of choice in studying HM in the air due to its particular physiological features (Schrimpff 1984; Brighigna et al. 1998, 2002; Bastos et al. 2004; Pignata et al. 2004). *Tillandsia* has the crassulacean acid metabolism that allows its adaptation to extremely dry air and high temperature conditions. It has also an epiphytic habit (no soil contact), and its stems and leaves are covered by highly hygroscopic thricomes (or scales) that absorb water and nutrients directly from the atmosphere.

In Brazil, *T. aeranthos* and *T. recurvata* were primarily used to evaluate the atmospheric concentrations of sulfur and HM from industrial and residential areas in Porto Alegre City (Flores 1987). Malm et al. (1998) estimated the atmospheric Hg concentrations originated from mining activities in the Amazon region using *Tillandsia* samples. Calasans and Malm (1997), using *T. usneoides* as biomonitor, detected high concentrations of Hg inside and a distance gradient adjacent to a chlor-alcali industry in Rio de Janeiro City. In 2002, Amado Filho and coworkers showed through analytical scanning electron microscopy (SEM) that part of the atmospheric Hg originating from that chlor-alcali unit was adsorbed on the particles entrapped on *Tillandsia* scales. A detailed characterization of the individual atmospheric particles based on morphology and elemental composition can provide useful information about the source, reactivity, transport, and removal of atmospheric chemical species (Post and Buseck 1984).

The aim of this work was to use *Tillandsia usneoides* as an atmospheric monitor to assess HM in two metropolitan areas in Brazil. In Rio de Janeiro, the State Institute of Environment (Instituto Estadual do Ambiente [INEA]) provides daily information about the air quality in several areas using automated and manual sampling methods (INEA 2010); however, HM concentration is not a measured parameter. In Salvador, to date there has only been one report on HM in the air using automatic sampling (Pereira et al. 2007). Here, *Tillandsia* samples were collected in a noncontaminated area, transplanted to the studied sites, and exposed to the air for 45 days. The HM were quantified by flame atomic absorption spectrophotometry (AAS), and the PM analyzed by conventional and analytical SEM. The morphology and elemental analyses of the PM can provide evidence on the possible contamination sources, and help in establishing new regulatory policies to protect the urban air and, consequently, the population.

2 Material and methods

2.1 Studied areas

The HM concentration in the atmosphere was quantified in two Brazilian urban areas using *Tillandsia* as a biomonitor. In Rio de Janeiro State, we exposed the biomonitor in three sites in Rio de Janeiro City: Centro (CT), Jacarepaguá (JP), Jardim Botânico (JB); and also in São Gonçalo City (SG) and Nova Iguaçu City (NI). In Bahia State, we studied seven sites inside Salvador City: Dique do Tororó (DT), Comércio (CM), Barra (BR), Beira Mangue (BM), Brotas (BT), Pituba (PT), and Stella Mares (SM) (Supporting Information Online S1). All studied areas are highly populated, especially Rio de Janeiro City, which is the second most populous city in Brazil with approximately 10 million people, and Salvador, the third biggest city with 3 million people. Itatiaia National Park (IT, control 1) and Cordeiro City (CO, control 2) located

in Rio de Janeiro State were used as control areas because these cities are located far from populated and industrialized cities (Supporting Information Online S1). The climate in Rio de Janeiro City is tropical Atlantic with a daily mean temperature of 22°C in winter and 27°C in summer, while the monthly mean precipitation is 4 cm during winter and 13 cm in summer. Salvador has a daily mean temperature of 27°C in summer and 25°C in winter. The precipitation mean value is 12 cm for summer and 20 cm for winter.

2.2 Transplant experiments

Samples of *T. usneoides* were collected at Itatiaia National Park, washed with deionized water, air-dried, and transferred to web plastic baskets with an acrylic plate on the top, to protect against them intense rain (Supporting Information Online S2). In Rio de Janeiro sites, all the baskets with the plants were hung at a height of 3 m on the top of containers (20 ft, dry standard) used by INEA for housing the air quality equipment. In Salvador, the samples were hung at 2.5–3.5 m on trees at parking lots or gardens of public buildings.

The samples were exposed to the air in Rio de Janeiro sites during the winter of 2004 (June–August) and summer of 2006 (January–February), while in Salvador, this was done during the summer of 2005 and 2006 (December–January) and winter of 2006 (June–August). All samples were exposed for 45 days, and further analyzed by AAS, and conventional and analytical SEM.

2.3 Metals quantification

After the exposure period, the samples were carefully removed from the baskets, dried for 2 days at 50°C, weighted in triplicates (500 mg each replica), calcinated for 2 days at 400°C, and digested with concentrated HNO_3 and HCl (Merck P.A.) for 4 days on a hot plate at 70°C. We repeated this procedure (twice) for each analyzed site. The final solutions were evaporated and 10 ml of 0.1 N HCl was added. The metals Cu, Zn, Cr, Cd, and Pb were quantified by flame AAS (Varian Spectra AA240FS), and results were expressed in terms μg/g of dry weight. Analytical quality control was done by routine analysis of International Atomic Energy Agency certified reference material IAEA-140 (seaweed homogenate, *Fucus*). The results obtained from the certified material presented 97.5% of confiability for the method.

2.4 Conventional and analytical SEM

Conventional SEM was performed in order to study the morphology and dimension of the particles deposited on *Tillandsia* during the air exposure in the analyzed areas. Small fragments (3 mm long) were randomly cut from *Tillandsia* samples, placed on conductive-carbon-tape covering aluminum stubs, and gold-sputtered (Bal-Tec CPD 030). Samples were observed in a Jeol 5310 SEM (Tokyo, Japan) operated at 15 kV. We used magnifications ranging from 20,000 to 40,000× to study PM_{10} in more detail. Digital images of secondary electrons were obtained at 1,024×770 pixels of resolution by using the SEMAfore system (Jeol Inc.).

We applied qualitative energy dispersive X-ray analysis (EDXA) to identify the elements in PM_{10} using backscattered electrons that form a brighter image, in comparison with secondary electrons, when heavy atoms are present. At least 30 particles ≤10 μm in diameter were analyzed per sample. We selected representative spectra for the analyzed particles found in the studied areas. We also analyzed particles deposited on cellulose acetate membrane (Whatman, 0.45-μm filter) removed from automated sampling instruments, distributed at the Rio de Janeiro State, to compare with the X-ray signals originating from particles on *Tillandsia* surface. This comparison was aimed to exclude natural ions from the plant from those collected exclusively in the air. All samples were covered with carbon (Bal-Tec CED 030) and the elemental composition of the particles was acquired in a Jeol 6460 SEM equipped with a Noran-Voyager system. The parameters used for the EDXA were: accelerating voltage, 30 kV; spot size, 50 nm; beam current, 50 pA; live-time, 100 s; dead-time, 20%; magnification, 10,000–25,000×; working distance, 30 mm.

2.5 Size distribution of the PM adsorbed on the biomonitor

SEM images obtained from all analyzed areas were used to measure the particles adsorbed on the biomonitor. A total of 250 particles from each site were measured (total of 3,000). The scale bar of each image was used to calibrate the software (ImageJ, NIH) before particle measurements. For every particle, two values equivalent to the orthogonal axes were measured and a mean value was obtained for that particle. The final values of all particles were distributed by the size ranges (in micrometers) as follows: <2.5, 2.5–10, 10–50, and 50–100.

2.6 Statistical analysis

Statistical analysis of the data was performed with the Statistical Package R (Development Core Team 2007). The concentrations were expressed as arithmetic means and standard deviation, and the differences were considered significant when $p<0.05$ based on one-way analysis of variance (ANOVA) and post-hoc Tukey's test.

3 Results

3.1 Heavy metals quantification

Results of the heavy metal concentrations in *T. usneoides* exposed for 45 days during the two seasons (summer and winter) in the sites studied in Rio de Janeiro State and Salvador are presented in Tables 1 and 2, respectively. Table 1 shows that for the element Cd, only JP (0.32±0.09, summer) and SG (0.66±0.19, winter) had significant differences compared with the values found for the control sites (IT=0.18±0.09, winter). For Pb (e.g., CT=8.6±1.1, winter) and Cu (e.g., JP=13.6±2.1, winter), the values obtained in all studied areas were significantly higher than those for the controls (CO=1.8±0.3, winter for Pb; IT=2.4 ±0.3, winter for Cu). For Cr and Zn, only JB presented no difference compared with both control areas. Zn presented the highest absolute values compared with the other metals, whereas SG samples (385±42.3) accumulated 17.5-fold of Zn more than CO (22±4.7). The sum of the heavy metal values of all sites showed higher mean levels during the winter than summer, except for Cr; however, only Zn had a significant difference (Table 1).

Table 2 shows that for Cd, BR (0.8±0.08) in Salvador presented a significantly different value compared with the control areas only during the summer. All the concentrations found for Pb (e.g., PT=11.2±1.6, summer) and Cu (e.g., CM=10.8±1.5, summer) were higher than those in control areas. Interestingly, Cr values were higher principally during winter (e.g., CM=4.4±0.7), whereas Zn values were higher in summer (e.g., Zn=53.1±3.5). Similar circumstances occurred in Rio de Janeiro sites, where Zn

presented the highest absolute concentrations. However, the higher difference was 2.4-fold (BR=53.1±3.5) more compared with the control (CO=22±4.7). Heavy metal concentrations in Salvador showed that only the values for Cd and Cu in the summer seasons of 2005–2006 were significantly different than those in winter of 2006 (Table 2).

Comparing the overall values, the metals Cd, Cr, and Pb were similar in Rio de Janeiro and Salvador studied sites, except for Cu and Zn, which were significantly higher in Rio de Janeiro samples.

3.2 Morphology and elemental analyses of the PM

SEM images of *T. usneoides* samples collected in control sites, Itatiaia (Fig. 1a) and Cordeiro (Fig. 1b), revealed that atmospheric particles covered the scales and the epidermal surface. Distribution of the scales on thallus surface facilitates the retention of particles on the biomonitor. High-magnification images of particles in the range of inhalable fraction showed polygonal (Fig. 1a, inset), round, and amorphous forms (Fig. 1b, inset). Spectra generated from PM_{10} found in the samples collected in control areas IT (Supporting Information Online S3a,b and S4a) and CD (Supporting Information Online S3b and S4c,d) showed that the majority of them were basically composed of C, O, Al, Si, and Fe. The elements K, Ca, Mn, and Ti were also detected in several PM_{10} from IT and CD (Supporting Information Online S4b,d). Given that these sites were considered noncontaminated by HM, we assumed that the detected atoms in PM originated from the local soil. The spectra performed in particles on the filter (data not shown)

Table 1 Mean and standard deviation of heavy metal concentrations in *T. usneoides* during winter of 2004 (first line) and summer of 2006 (second line) in the studied sites in Rio de Janeiro state

Last row (Total) shows the overall sum

[a] Significant difference in comparison with the control areas

[b] Significant difference between total values of winter and summer. Numbers in bold are the highest values of each metal. Results are expressed in μg/g of dry weight

	Cd	Cr	Pb	Cu	Zn
Centro	0.28±0.08	3.1±0.6[a]	**8.6±1.1**[a]	11.5±1.8[a]	175±22.9[a]
	0.23±0.05	**4.4±1.4**[a]	7.8±0.9[a]	9.8±1.4[a]	106±48.1[a]
Jacarepaguá	0.25±0.06	2.6±0.7[a]	7.7±0.5[a]	**13.6±2.1**[a]	335±24.7[a]
	0.32±0.09[a]	4.1±0.2[a]	7.7±0.7[a]	10.3±0.4[a]	182±19.1[a]
J. Botânico	0.1±0.03	0.8±0.5	5.8±0.4[a]	7.8±0.9[a]	43.58±4.2
	0.07±0.04	0.9±0.5	4.2±0.3[a]	6.7±1.1[a]	27.6±2.9
N. Iguaçu	0.23±0.05	2.5±0.9[a]	6.9±0.5[a]	9.6±0.6[a]	354±43.8[a]
	0.3±0.08	2.9±1.6[a]	4.3±0.4[a]	7.6±1.3[a]	103±14.3[a]
S. Gonçalo	**0.66±0.19**[a]	2.6±0.2[a]	6.8±0.6[a]	7.8±1.2[a]	**385±42.3**[a]
	0.3±0.07	3.2±0.9[a]	5.2±0.6[a]	6.3±1.1[a]	101±27.7[a]
Itatiaia (control 1)	0.18±0.09	1.4±0.3	1.7±0.2	2.4±0.3	20.54±4.3
	0.1±0.04	1.9±0.3	1.3±0.2	1.1±0.6	16.4±1.6
Cordeiro (control 2)	0.08±0.02	0.7±0.7	1.8±0.3	1.6±0.7	22±4.7
	0.11±0.03	1.6±0.4	1.5±0.2	2.4±0.3	17±2.9
Total	0.3±0.08	2.3±0.6	7.2±0.6	10.1±1.3	258±27.5[b]
	0.24±0.06	3.1±0.9	5.8±0.6	8.1±1.04	103±22.4

Table 2 Mean and standard deviation of heavy metal concentrations in *Tillandsia usneoides* during the summers of 2005–2006 (first line) and winter of 2006 (second line) in Salvador City

	Cd	Cr	Pb	Cu	Zn
Barra	**0.8±0.08**[a]	2.1±0.5	7.3±1.1[a]	9.6±1.4[a]	22.8±4.5
	0.3±0.02	5.5±1.7[a]	6.6±1.7[a]	6.2±1.1[a]	**53.1±3.5**[a]
Beira-Mangue	0.2±0.06	2.6±0.2[a]	7.1±1.8[a]	9.5±0.1[a]	30.7±5.7[a]
	0.17±0.03	3.2±0.2[a]	6.1±1.4[a]	5.7±0.4[a]	30±3.5[a]
Brotas	0.2±0.05	1.2±0.3	6.6±0.7[a]	6.7±0.2[a]	28.4±2.1
	0.1±0.03	4.4±1.2[a]	7.1±1.8[a]	4.1±0.2[a]	49.1±3.6[a]
Comercio	0.3±0.05	**4.4±0.7**[a]	8.2±1.5[a]	**10.8±1.5**[a]	46.3±5.1[a]
	0.08±0.01	3.2±0.1[a]	6.3±0.2[a]	6.5±1.1[a]	23.5±5.3
Dique	0.2±0.09	2.6±1[a]	6.1±0.5[a]	5.9±0.5[a]	33.5±0.6[a]
	0.17±0.02	1.9±0.1	8±2.4[a]	5.5±0.8[a]	39.9±7.4[a]
Stella Mares	0.3±0.07	1.1±0.2	7.1±0.9[a]	8.6±0.6[a]	39.8±2.7[a]
	0.2±0.01	2.9±0.7[a]	5.1±1.1[a]	5.3±0.07[a]	24.8±3.1
Pituba	0.3±0.09	1.9±0.7	**11.2±1.6**[a]	10.2±0.6[a]	39.1±4.6[a]
	0.1±0.03	3.3±0.7[a]	5.5±0.9[a]	6.3±0.5[a]	20.2±5.1
Total	0.3±0.07[b]	2.2±0.5	7.6±1.2	8.8±0.7[b]	34.3±3.6
	0.15±0.02	3.5±0.7	6.4±1.4	5.6±0.6	34.3±4.5

Last row (Total) shows the overall sum.

[a] Significant difference in comparison with the control areas. Itatiaia and Cordeiro values are shown in Table 1

[b] Significant difference between total values of winter and summer. Numbers in bold are the highest values of each metal. Results are expressed in μg/g of dry weight

were very similar to those analyzed on *Tillandsia* surface, indicating that the atoms detected in PM_{10} did not originate from *Tillandsia* cells.

Although we did not estimate the particle number on the biomonitor, the overall difference regarding the quantity of particles covering the samples of monitored sites was greater when compared with that in controls (Fig. 1c, g, i). In general, the morphology of the particles varied from amorphous to polygonal shapes (Fig. 1d–m). In NI City, relatively large polygonal particles with amorphous precipitations on its surface (Fig. 1e) and round small particles (Fig. 1f) were observed. Figure 1g shows the variety of particles regarding size and shape on *T. usneoides* exposed to air in CT (downtown of Rio de Janeiro City), a region with intense vehicle traffic. Several large particles (exceeding 10 μm) were constituted by aggregates forming a single unit (Fig. 1h). Samples exposed to JP air, which is an area surrounded by several industries, revealed more amorphous particles than polygonal (Fig. 1i, j). Spectra from PM collected in the monitored sites in Rio de Janeiro State, such as SG, showed Ba, Cr, Cu, and Zn in several analyzed particles (Supporting Information Online S3c and S4e,f), whereas in CT, we found particles with Zn (Supporting Information Online S3d and S4g) and some particles with high concentrations of S and Ba (Supporting Information Online S3d and S4h). In JP and NI, PMs were composed basically by C, O, Al, Si, and Fe, with Zn in JP (Supporting Information Online S4a) and Zn plus Cu in NI (Supporting Information Online S4b). In JB, which presented the lowest levels of the studied metals in the biomonitor (*Tillandsia*), we did not detect metals in the analyzed PM_{10}, which basically contained aluminum silicates with Ti and Fe (Supporting Information Online S4c).

In Salvador, we observed the same pattern regarding size and morphology that was observed for RJ samples. We did not find any particular feature in all analyzed samples from the studied sites. In SM, the biomonitor was placed in an area in front of the sea, but close to the Salvador International Airport. We observed that SM samples exhibited visually less particle concentration (Fig. 2a,b) compared with other sites such as DT (Fig. 2d), BR (Fig. 2f), and CM (Fig. 2i). However, we did not measure the overall particles on the thallus. Figure 2c shows a SM polygonal particle in detail. Figure 2e shows a DT particle composed of an agglomeration of amorphous and polygonal minor particles. DT is an area with intense vehicular traffic. In BR, the samples were exposed to air in an area close to the sea, but with an intense number of circulating vehicles. We observed round (Fig. 2g) and polygonal (Fig. 2h) particles in BR as well. Figure 2j shows *T. usneoides* scales covered with round particles originating from air in CM, whereas Fig. 2k shows some particles with different morphologies found in BM and Fig. 2l in PT. In BT, high-magnification SEM images showed the details of a polygonal (Fig. 2m) and round (Fig. 2n) particle about 1 μm in size. The PM originating at SM presented the basic elements already described (C, O, Al, Si, Fe), but Na and Cl were constantly detected (Supporting Information Online S3e and S5d). HM were not detected in the analyzed particles from SM. However, we found metals in the other studied sites in Salvador. In BR, Cr and Cu were frequently detected, as well as Na and Cl (Supporting Information Online S3f and S5e). In BT, Cu was detected in many particles (Supporting Information Online S3g and S5f), whereas in CM, we found Cr and Cu in particles belonging to the inhalable fraction (Supporting Information Online
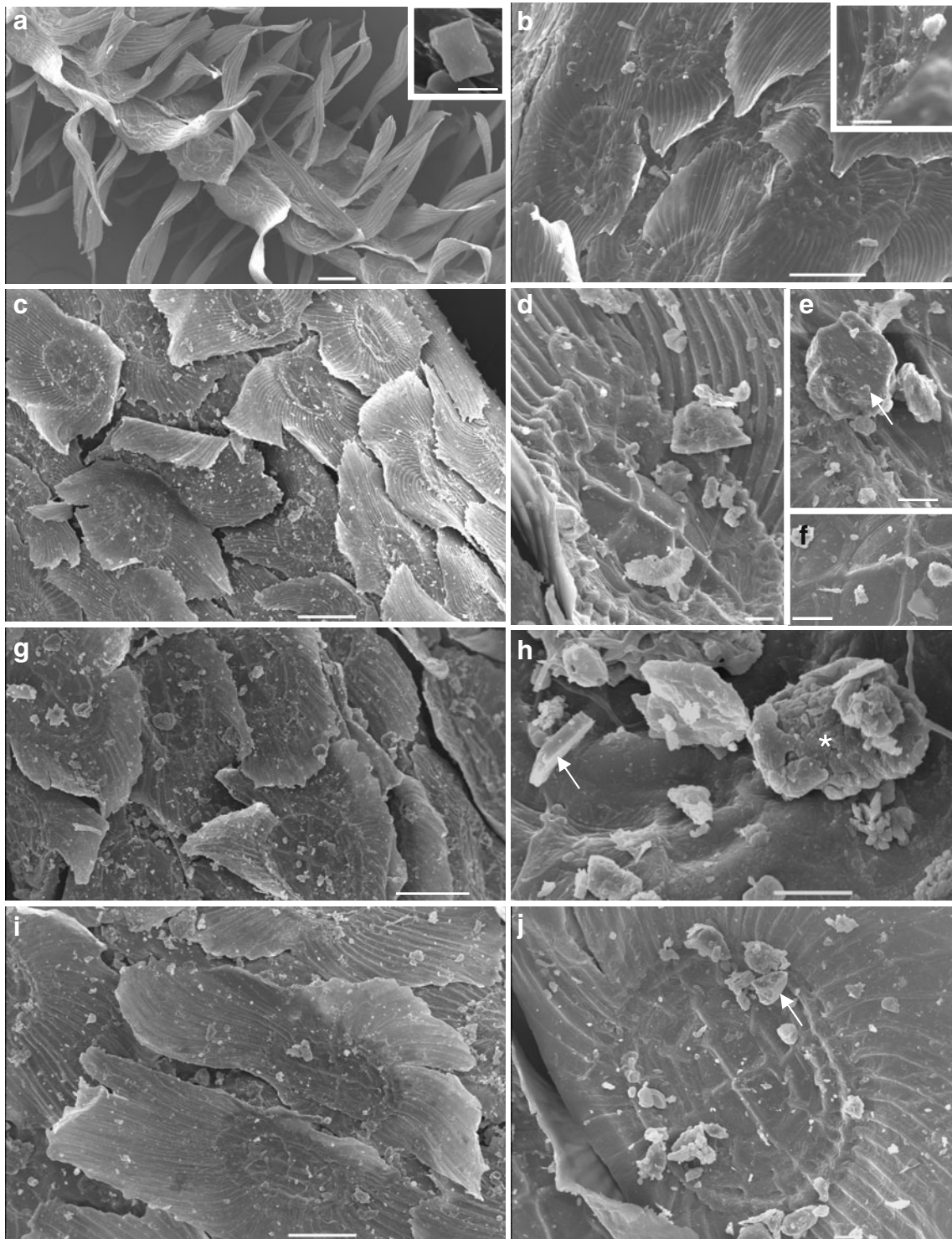
Fig. 1 SEM of *T. usneoides* exposed to the air in Rio de Janeiro for 45 days. **a** *T. usneoides* sample harvested from Itatiaia National Park with few particulates on the scales (bar=100 μm). *Inset* (*top right*): Detail of a polygonal shape particle (bar=5 μm). **b** *T. usneoides* exposed to air in Cordeiro City (bar=100 μm). *Inset* (*top right*): Amorphous particles with different sizes (bar=5 μm). **c** Numerous particles on the biomonitor surface exposed to the air in Nova Iguacu City (bar=100 μm). **d–f** High-magnification images showing the diversity of particles found in NI City. **d** Particulate matter with different morphologies (bar=10 μm). **e** Detail of a polygonal particle with amorphous material on its surface (*arrow*) (bar=1 μm). **f** Round particles seen on the biomonitor surface (bar=10 μm). **g** Different morphologies of the particles found in CT (downtown of Rio de Janeiro City) (bar=50 μm). **h** Particles from CT were mainly constituted by aggregates (*) or polygonal shape materials (*arrow*) (bar=10 μm). **i, j** Particles found in the air of JP with amorphous and polygonal forms (*arrow*) (bar=50 μm (**i**), 10 μm (**j**))

S3h and S5g). We did not detect Pb and Cd in any of the analyzed PM originating from all the studied sites by EDXA, probably because the SEM used a voltage that produces a weak X-ray signal of these elements.

3.3 PM size distribution

We measured the size of the particles adsorbed on *T. usneoides* exposed to air in all the studied sites. Figure 3a shows the distribution of particle sizes in Rio de Janeiro, and Fig. 3b shows the distribution in Salvador. In general, the size distribution percentage for both areas was decreasing as follows: 2.5–10> below 2.5>10–50>50–100 μm. Interestingly, when we combined the results of particle sizes below 10 μm, it was clear that the great majority of particles (>80%) were in that range. On the other hand, the particles with diameters exceeding 100 μm amounted to less than 1%.

4 Discussion

4.1 Biomonitoring of heavy metals

The atmospheric monitoring of two metropolitan areas (Rio de Janeiro and Salvador) using *T. usneoides* indicated that all studied sites presented some degree of heavy metal contamination compared with control sites (Itatiaia National Park and Cordeiro). Although Rio de Janeiro and Salvador cities are among the most important economic and populated regions in Brazil, to date, few data on HM in the air are available in the literature (de Andrade et al. 1996; Quiterio et al. 2004, 2005; Pereira et al. 2007).

The biomonitoring approach using *Tillandsia* was successfully applied in the metropolitan region of São Paulo, the most populated area in Brazil. Figueiredo et al. (2007) performed transplant experiments in ten sites in São Paulo, exposing the biomonitor for 56 days (five different time-points between April 2002 and May 2003). These authors found Zn values in São Paulo varying from 41 ± 1 μg/g (Santana) to 189 ± 12 μg/g (Sto Andre), while we measured the mean values of 258 ± 27.5 μg/g for Rio de Janeiro sites and 34.3 ± 4.5 μg/g for Salvador. In São Paulo, Cu varied from 4.3 μg/g (Congonhas) to 56 μg/g (Sto Andre), around 5-fold higher than the mean values found in Rio de Janeiro State (10.1 ± 1.3 μg/g) and Salvador (8.8 ± 0.7 μg/g). The values found for Cr were similar in São Paulo (maximum value of 3.7 ± 0.2 μg/g), Rio de Janeiro (3.1 ± 0.9 μg/g), and Salvador (3.5 ± 0.7 μg/g). Interestingly, PM_{10} concentration in São Paulo is often higher during the winter than summer (Artaxo and Orsini 1987), and probably in association with this datum, we also observed the same thing for Zn in Rio de Janeiro, due to the climate

feature where precipitation is higher during the summer and washes down the PM from the air.

Quiterio et al. (2005) measured heavy metal levels in airborne particulate using the filters of high-volume samplers in NI and the other six districts located in Baixada Fluminense (Rio de Janeiro State) between February 2002 and July 2003. They showed that Zn, Cu, Cd, and Pb contamination in NI was 60-fold higher compared with other analyzed areas, and the contamination was related with anthropogenic emissions. Here, the biomonitoring in NI showed that Cr, Pb, Cu, and especially Zn were significantly higher than those found in the control sites, and we also consider that as a consequence of anthropogenic emissions from the high level of vehicular traffic around the analyzed area.

Pereira et al. (2007) studied the atmospheric concentrations of Cu and Zn, beyond other metals, in PM_{10} collected on filters of automatic samplers in three different sites in Salvador region. Regarding the site inside Salvador, the authors concluded that Zn and Cu values were higher than the Brazilian standards, and their origins were mainly from anthropic contributions, vehicular emissions, and soil dust suspension (Pereira et al. 2007).

Zn is a naturally occurring metal, but it is often associated with anthropogenic activities and can be considered a key element to indicate contaminated urban areas. Zn reaches the atmosphere by the exhaustion of petroleum refining plants, incinerators, extraction and manufacturing industrial processes, and other sources (EPA 2005). Zn is also released by combustion of gasoline- and diesel-powered vehicles, fluid leakage, tires, vehicle paint, and component detachment (Ball et al. 1991; Monaci et al. 2000). Although Rio de Janeiro has Zn-related industrial activities (described above), the main source of Zn may likely be related with vehicles, given that a vehicular fleet with approximately 2.2 millions of units in 2009 (DETRAN-RJ 2009) contribute to a chaotic and heavy traffic flow, especially around the analyzed areas. The JB site in Rio de Janeiro showed the lowest Zn value because the plants were exposed to the air inside the Botanical Garden, a large green space inside the city. On the other hand, Salvador has an estimated fleet of 650,000 vehicles (DETRAN-BA 2009), but the traffic intensity is considered as the third highest in the country. Mean concentrations of Zn found in Salvador were 7.5-fold lower than in Rio de Janeiro, but were still significantly higher than control sites and should be monitored in the future.

According to our AAS data, Cd in JP and SG (Rio de Janeiro State) and in BR and PT (Salvador) presented significant differences from those in control areas. Cd is extremely toxic to humans even in very low concentrations, because is a possible carcinogenic agent, and the exposure can result in respiratory diseases (EPA 1999b). Industrial
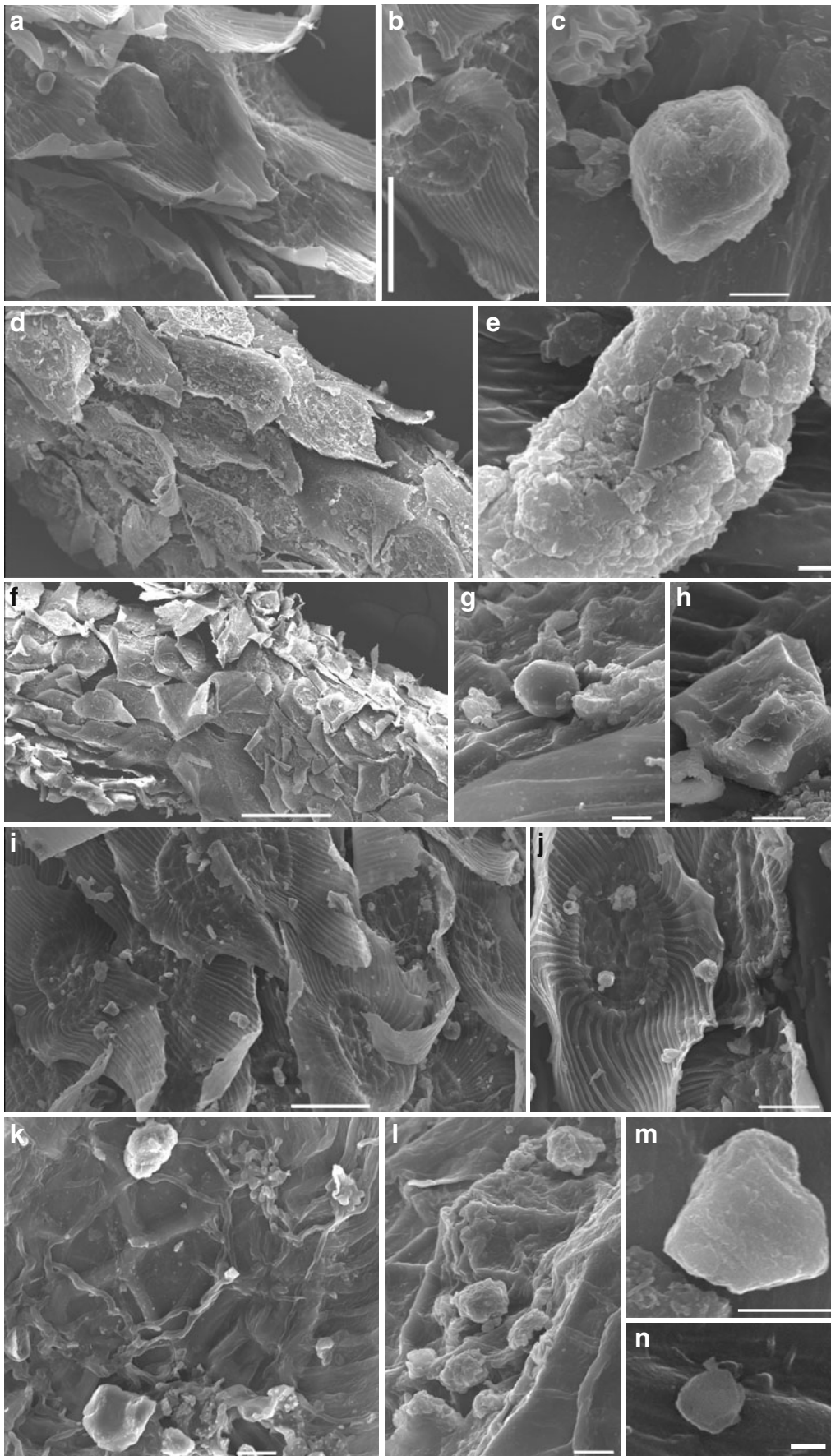
Fig. 2 SEM of *Tillandsia usneoides* exposed to the air in Salvador for 45 days. **a,b** SM samples exhibited few particles adhered on the scales (bar=50 μm (**a**), 100 μm (**b**)). **c** Detail of a polygonal particle found in SM (bar=5 μm). **d** Biomonitor covered by numerous particles in DT (bar=100 μm). **e** DT particle composed by an agglomeration of amorphous and polygonal minor elements (bar=1 μm). **f** Low-magnification of the biomonitor exposed to the air in BR (bar= 500 μm). **g** Round particle found in BR (bar=10 μm). **h** Polygonal particle observed in a BR sample (bar=10 μm). **i** Overview of the particulate matter found on *Tillandsia* in CM (bar=100 μm). **j** *T. usneoides* scales covered with round particles originating from air in CM (bar=50 μm). **k** Particles with different morphologies found in BM (bar=10 μm). **l** Some particulates found in a PT sample (bar=10 μm). **m** High-magnification image of a polygonal particle found in BT (bar= 5 μm). **n** Small round particle found in a BT sample (bar=1 μm)

activities that process Cd as anticorrosive agent, pigments in plastics, electric batteries, and electronic components are the main source for atmospheric Cd (EPA 1999b; Kelleher et al. 2000). Since JP and SG in Rio de Janeiro are considered industrial–residential areas, this may explain the presence of Cd in the atmosphere.

The concentration data for Pb in all analyzed sites (Rio de Janeiro State and Salvador) were significantly higher than those in control areas. The highest value for Pb (11.2±

Fig. 3 Size measures of the PM adsorbed on *T. usneoides* surface exposed to the air in all studied sites. **a** Size distribution in Rio de Janeiro sites (CT, IT, JP, NI, SG, and Total). **b** Size distribution in Salvador sites (BA, BM, BT, CM, DT, PT, SM, and Total). Numbers inside the bars represent the size range percentage. Both graphics show that particles below 10 μm comprised the majority found on the biomonitor

1.6 μg/g) was found in PT (Salvador), a residential area. Even in lower levels, Pb can cause severe effects on the central nervous system, kidney, and blood cells, especially among children (EPA 1999a; WHO 2001). Pb can reach the atmosphere by dust derived from lead-based paint, contaminated soils, water pipe debris, incinerators, smelters, power plants, refineries, lead-based gasoline and additives, and other sources (EPA 1999a). Brazilian vehicles no longer use leaded gasoline after it was banned in 1992. However, aviation fuels still have lead in their composition. Many countries have restricted or banned the use of leaded gasoline; however, Pb is still present in the environment due to past emissions. In Salvador, SM presented significantly higher Pb values compared with control areas, which surprised us. We were expecting lower values there since this site is located far from the downtown area. The international airport, which handles approximately 100,000 flights annually (Infraero 2009), is relatively close to the site where *Tillandsia* samples were exposed in SM, which could explain the levels of Pb contamination due to the prevailing winds.

The main sources of atmospheric emission of Cu are the use of fossil fuels and the industrial activities related to this metal, such as smelter plants (Nriagu and Pacyna 1988). Cu and Cr (except JB in Rio de Janeiro) levels in atmosphere were significantly higher than CTs in all Rio de Janeiro and Salvador analyzed sites. The Camaçari industrial complex, located 150 km north of Salvador, contributes to Cu pollution in soil and vegetation in nearby areas (Klumpp et al. 2003). However, the mechanism by which Cu is transported to Salvador's atmosphere remains unknown. We suggest that Cu and Cr pollution in Rio de Janeiro and Salvador maybe attributed to vehicular exhaust fumes. Cu is an essential metal for cellular metabolism as enzyme cofactor, but its imbalance may cause severe neurological disorders including Menkes, Wilson, and Alzheimer's diseases, as well as liver and kidney failures (Quinn et al. 2009). Cr dust can induce shortness of breath, coughing, wheezing, septum, bronchitis, decreased pulmonary function, pneumonia, and other respiratory disorders (ATSDR 1998).
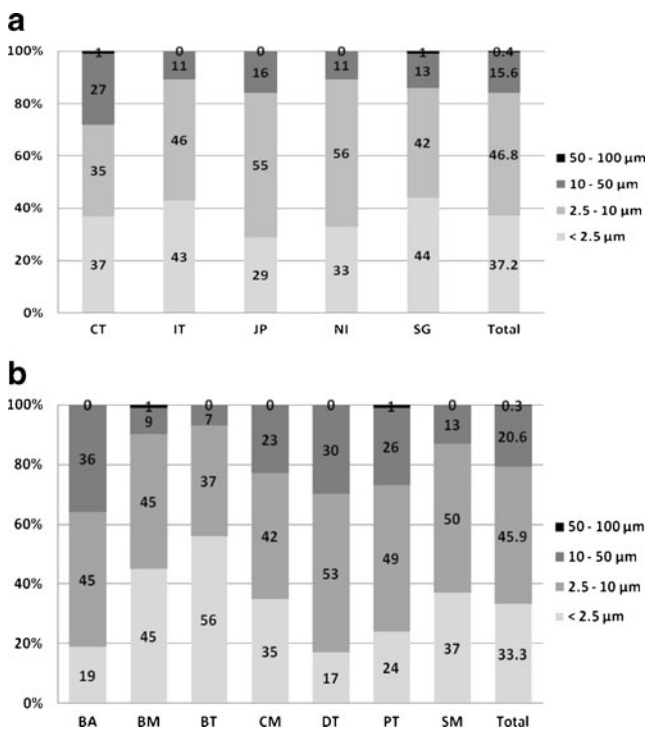
4.2 PM morphology and dimensions

The morphological diversity of the PM found in *Tillandsia* surface suggests multiple and complex sources for the atmospheric particles. In addition, particles formed by agglomerates also indicate that small particles with relatively bigger surface area can interact and form secondary particles. We found particles ranging in size from less than 1 μm to about 100 μm. However, as described earlier, more than 80% of the measured particles were in the range below 10 μm, showing that the biomonitor can mostly retain the inhalable fraction of PM.

Post and Buseck ([1984](#)) postulated that the particle size reflects its origin and formation—wherein larger particles usually have crustal or mineral origins, while smaller ones come from combustion processes or conversion reactions of gas to particles in the atmosphere. As reviewed by Valavanidis et al. ([2008](#)), coarse PM fraction (90–95% of total PM) is a material of noncombustible sources that consists mainly of insoluble crust-derived minerals, sea salt, and material of biologic origin; meanwhile, fine and ultrafine fractions (1–8% of total PM) are mainly carbonaceous aggregates with metals and organic species adsorbed on their surface. However, in urban areas with heavy traffic, particles originating from fuel combustion can range from 30 to 80% of the total PM found in the atmosphere (Shi et al. [2003](#)). By SEM and EDS, Perrone et al. ([2006](#)) examined samples of total PM collected on filters (300 particles) originating from coastal site in southeastern, Italy, and they found that 90% had a diameter less than 5 μm, while 50% of the analyzed PM were smaller than 2.5 μm.

In urban areas with multiple sources of PM and numerous forms of particle interactions, it is extremely complicated based on SEM images to determine a specific profile for PM or associate it with a unique source. However, it is possible to determine the appropriate features of a specific particle profile if one can collect particles from a unique source, and perform imaging and elemental analyses. Carbon particle is a classic example in that its spherical shape and size (diameters below 100 nm) can help in the identification of the PM by conventional and analytical electron microscopy (Kocbach et al. [2005](#)).

4.3 Heavy metals associated with the fine particles

We showed by SEM that the thalli of the biomonitor in the studied sites were abundantly covered with particles originating from the atmosphere. We also demonstrated by EDXA that the fine fraction (particles >10 μm) contained HM such as Cr, Cu, and Zn, which likely originated from anthropogenic activities and not from mineral or soil sources. The metals' absorption in breathable particles can increase health risks since metals can be extremely toxic to humans.

Amado Filho et al. ([2002](#)) demonstrated that part of the Hg^0, quantified previously by Calasans and Malm ([1997](#)) using *Tillandsia*, released in the atmosphere by a chlor-alkali plant was adsorbed in PM found in plant surfaces. Hg was also detected in the epidermal cells, but not in parenchymal and sclerenchymal cells. Based on these results, elemental Hg originating from an anthropogenic source was found in PM, indicating that other toxic metals can also be adsorbed in PM. The analyses of metal concentrations in *T. capillaris* collected in Cordoba (Wannaz et al. [2006](#)) allowed the authors to separate the studied

elements in two groups: natural origin (V, Fe, Co, Mn, Br, and Cu) and anthropogenic origin (Ni, Zn, and Pb). Early on, Pignata et al. ([2002](#)) studied the accumulation of Fe, Mn, and Co in some *Tillandsia* species also collected in Cordoba, and they associated the accumulation of these elements to their presence in the soil, while Pb, Zn, and Cu were identified as tracers of anthropogenic pollution.

In this study, we regularly detected Al, Si, and Fe as the main components of the PM_{10}, indicating the presence of aluminosilicate iron-rich particles. The elements Na, Mg, S, Cl, K, Ca, and Ti were also present in the analyzed particles, and these elements could have natural origins. On the other hand, the metals Cr, Ba, Zn, Cr, and Cu were detected in PM_{10} by EDXA, and we suggest an anthropogenic origin rather than a natural origin (soil) for these elements. Barium is a component of tires (Ball et al. [1991](#)) and may explain the presence of this element in some analyzed particles. Perrone et al. ([2006](#)) showed that PM collected in southeastern Italy were composed by at least C, O, Na, Mg, Al, Si, S, Cl, K, Ca, and Fe, with a predominance of Al, Fe, and Ca. They also indicated that besides Ti and Mn, Fe and Ca had a significant crustal origin, while Cr, V, Cu, Pb, and Zn were mainly associated with an anthropogenic origin (Perrone et al. [2006](#)). Kocbach et al. ([2005](#)) studied the morphology and composition of particles generated by vehicle exhaust, and they frequently observed aluminosilicates, Si particles (pure silicates), and Fe particles (iron oxides). The aluminosilicates contained Fe and some amounts of Mg, K, or Ca, which primarily originate from the erosion of soil and road material (Kocbach et al. [2005](#)).

Further experiments should address if HM in the PM are bioavailable for human body and cell absorption, since our results suggest that Cr, Zn, and Cu probably have an anthropogenic origin, and they are not part of the mineral composition of the aluminum silicate nature of the PM. If so, we could consider air pollution as an important source of toxic metals in the analyzed areas, and specific studies should address the effects of these pollutants in human health.

5 Conclusions

The biomonitoring showed that the atmosphere in the studied areas had significantly higher metal concentrations than the control sites. The morphology of inhalable particles was very diverse in the urban air of the analyzed areas, varying from amorphous to polygonal shapes. We did not associate a particular shape of particle in PM_{10} fraction with its composition. HM such as Cr, Cu, and Zn were detected in PM_{10} disseminated in the air of important populated cities in Brazil. The particles found adsorbed on

Tillandsia were in the size range of the inhalable fraction, and can contribute to health risks. Atmospheric biomonitors studied by analytical techniques used in this work can provide information on heavy metal concentrations in air, and also help in the detection and identification of sources of metal pollution.

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