

Characterization of *Tibouchina granulosa* (Desr.) Cong. (Melastomataceae) as a biomonitor of air pollution and quantification of particulate matter adsorbed by leaves

Maria Cristina T. Zampieri^a, Jorge E.S. Sarkis^{a,*}, Rafael C.B. Pestana^a, Armando R. Tavares^b, Gladys F.A. Melo-de-Pinna^c

^a Laboratório de Caracterização Química e Isotópica, Instituto de Pesquisas Energéticas e Nucleares, Centro de Química e Meio Ambiente, Universidade de São Paulo, Avenida Lineus Prestes 2242, 05508-000 São Paulo, SP, Brazil

^b Núcleo de Plantas Ornamentais, Instituto de Botânica, Av. Miguel Stéfano 3687, Água Funda, 04045-972 São Paulo, SP, Brazil

^c Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão 321, Travessa 14, 05508-090 São Paulo, SP, Brazil



ARTICLE INFO

Article history:

Received 13 May 2013

Received in revised form 3 August 2013

Accepted 20 September 2013

Available online 31 October 2013

Keywords:

Tibouchina granulosa

Morphoanatomy

Stomatal index

Trichomes

Particulate matter

Scanning electron microscopy–energy

depressive X-ray spectroscopy ;

ABSTRACT

The main anatomical features of *Tibouchina granulosa* (Desr.) Cong. (Melastomataceae) have been investigated in order to assess the potential of the species as a possible biomonitor of air pollution in the state of São Paulo, Brazil. Various types of trichomes located on the adaxial and abaxial surfaces of the leaves of this ornamental tree were able to adsorb particulate matter (PM) within the size range 2.5–100 μm . Following dry or wet deposition, the particles remained adsorbed to the leaves and did not return to the environment under normal weather conditions. The numbers of particles adsorbed per unit area of leaf differed significantly depending on the location at which the samples were collected. Leaves from a relatively unpolluted site located in a remnant of the Atlantic Forest within the city of São Paulo showed the lowest particle density, while samples collected in the city centers of São Paulo and Cubatão presented the highest numbers of particles with aerodynamic sizes <2.5 and 2.5–10 μm . It is concluded that *T. granulosa* may be employed as a passive biomonitor, thereby offering a valuable alternative for monitoring air pollution and spatial–temporal evaluation of PM composition. Additionally, landscape cultivation of *T. granulosa* in inner-city areas may help to improve the quality of air by reducing the concentration of harmful PM_{2.5} and PM₁₀.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Air pollution represents a serious threat to the health and quality of life of urban populations in general (Fontenele et al., 2009; Sucur et al., 2010; Tao et al., 2012), and to children and individuals with lung or heart diseases in particular. Moreover, in the most vulnerable section of the population, high levels of air pollution can give rise to a reduction in life expectancy of more than one year (World Health Organization, 2011).

The particulate matter (PM) present in polluted air results primarily from anthropogenic activities and consists of a mixture of metals, carbon black from burning fuels, aromatic polycyclic hydrocarbons and other substances suspended in the atmosphere (Ignotti et al., 2010; Kardel et al., 2011; Saebo et al., 2012). PMs are

categorized according to their aerodynamic diameters, and are usually denoted as PM_{2.5}, PM₁₀ (with diameters <2.5 and 10 μm , respectively) (Fierro, 2000; Ignotti et al., 2010). Particles classified as PM_{2.5} are the most harmful because they can reach the inner parts of the lungs (bronchioles) and interfere with oxygen exchange (World Health Organization, 2011).

Atmospheric pollutants can be sedimented by wet deposition (precipitation), which does not depend on soil cover, or by dry deposition involving gravitational sedimentation, compaction, interception and diffusion depending on the size of the particles (McDonald et al., 2007). Thus, the presence of plants that are able to retain sedimented PMs and to resist the stress associated with air pollution can assist in the improvement of air quality in urban and suburban areas (Alves et al., 2008; Markert, 2007; Moraes et al., 2000; Saebo et al., 2012; Zampieri et al., 2012).

Networks designed to monitor air pollution in urban areas generally comprise a limited number of stations since the costs involved in the purchase of equipment and subsequent maintenance are high. Thus, air quality and PM concentration cannot

* Corresponding author. Tel.: +55 11 31339377; fax: +55 11 31339383.

E-mail addresses: crislessari@gmail.com, crislessari@usp.br (M.C.T. Zampieri), jesarkis@ipen.br (J.E.S. Sarkis).

Table 1

Geographical locations of sampling sites and dimensions of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) trees used as potential biomarkers of pollution at different locations in São Paulo State, Brazil.

Site	Address	Characteristics region	Geographical coordinates	Diameter at breast height (cm)	Diameter at base (cm)	Height (m)	Diameter of canopy (m)
IBT	Miguel Stéfano Av. 2535, São Paulo City	Urban area of environmental protection and conservation; with Atlantic rainforest	23° 38' 30.30" S; 46° 37' 20.61" W	80	97	4.3	2.8
DPA	Darcy Penteado Square, São Paulo City	Urban area near cultural centers, restaurants and governmental offices	23° 32' 44.57" S; 46° 38' 41.05" W	75	98	5.3	2.3
PRA	Ramos de Azevedo Square, São Paulo City	Same as DPA plus an intense commercial activity of precious metals	23° 32' 44.24" S; 46° 38' 15.22" W	15	Not determined	3.5	4.0
JAB	Jabaquara Av. 1632, São Paulo City	Urban residential area, with intense commerce and public transportation (bus and subway). Circa 2.3 km from Congonhas Airport	23° 37' 08.57" S; 46° 38' 22.18" W	33	61	4.5	4.0
IPE	Lineu Prestes Av. 2242, São Paulo City	Urban region located in the campus of São Paulo State University, with areas of native forest	23° 37' 08.57" S; 46° 38' 22.18" W	19	55	2.2	4.2
ISE	Itapeperica da Serra Av. 62, São Paulo City	Urban region near the Guarapiranga reservoir (5.8 km) and Billings (3.7 km), with green areas and industries (galvanization industries for example)	23° 40' 00.07" S; 46° 39' 27.55" W	98	24	3.5	2.5
APA	Paulista Av. 960, São Paulo City	Urban region located in the largest business center in Latin America	23° 33' 55.04" S; 46° 39' 05.91" W	25	45	3.5	3.0
SCB	Washington Luiz Highway (SP310) Km 235 São Carlos City, State of São Paulo	Rural region of the São Paulo State, inside a large area of green forest, next to the São Carlos Federal University (UFSCar)	21° 59' 00.32" S; 47° 52' 58.46" W	84	79	7.5	5.5
SCS	Washington Luiz Highway (SP310) Km 235 São Carlos City, State of São Paulo	Rural region of the São Paulo State, on the border of a large area of green forest, next to the São Carlos Federal University (UFSCar)	21° 58' 57.59" S; 47° 53' 05.27" W	120	95	4.0	3.0
CUB	9 de Abril Av. 1910, Cubatão City, State of São Paulo	Industrial area with refineries and industries of fertilizers, located between two major highways (Anchieta Highway and Prof. Abraham de Morais Highway) and an access to the Port of Santos	23° 53' 12.30" S; 46° 25' 12.27" W	25	20	1.5	2.0

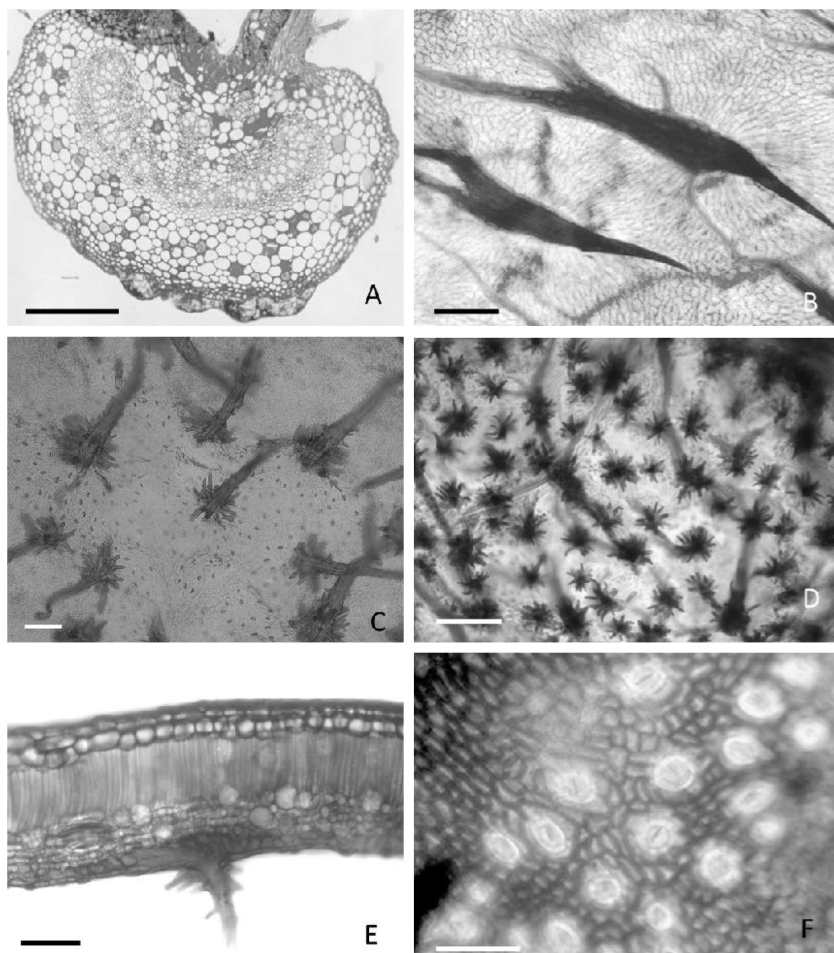


Fig. 1. Transversal section of leaf in *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae): midrib region (A), appressed scabrous trichomes in the adaxial surface (B), branched trichomes in abaxial surface (C and D), dorsiventral mesophyll (E), and stomata on the adaxial surface (F). Bar = 100 μ m.

normally be monitored with high spatial resolution (Kardel et al., 2011). Furthermore, conventional monitoring networks evaluate the concentrations of pollutants at a specific time but do not provide information regarding the integrated effects over a protracted period (Alves et al., 2008; Kardel et al., 2011). Since pollutants affect the survival of plants to a greater or lesser extent, much research effort has focused on the development of monitoring systems using plants as pollution biomarkers. Such systems can provide high spatial resolution because plants are relatively inexpensive and easy to cultivate (Alves et al., 2008; Kardel et al., 2011; Markert, 2007; Saebo et al., 2012).

The hypothesis tested in the present study is that *Tibouchina granulosa* (Desr.) Cong. (Melastomataceae), a semideciduous perennial tree originating from the Brazilian Atlantic Forest and commonly cultivated as an ornamental along streets in various cities in the state of São Paulo (Brazil), can be used as a biomonitor for PMs. Hence, the objectives were (i) to characterize the leaves of *T. granulosa*, and (ii) to analyze the accumulation of PMs on mature leaves collected from specimens of *T. granulosa* distributed in different urban areas within the São Paulo State.

2. Materials and methods

2.1. Sites of study

The state of São Paulo is the most populated of the Brazilian federation and comprises 41 million inhabitants (Instituto Brasileiro

de Geografia e Estatística, 2010). The climate is tropical-Atlantic in the central region and tropical-altitude in the mountainous region of the Serra da Mantiqueira and Serra do Mar. The average annual temperature ranges from 20 to 22 °C. Most parts of the State exhibit well defined humid and dry seasons, except for the coastal region of the Serra do Mar where the dry season is very short. The flora is characterized by mangroves in the Atlantic coast, preserved parts of Atlantic forest in the Serra do Mar, and tropical forest in the remaining parts of the territory (Biblioteca Virtual do Governo do Estado de São Paulo, 2013).

The study sites comprised industrial districts, urban areas (including densely populated city centers), green suburban areas (leisure parks) and rural areas (natural reserves, pasture and agricultural land). The exact locations of the sites and the characteristics of the *T. granulosa* trees studied in the present work are shown in Table 1.

2.2. Residence time of leaves

In order to determine the length of time that the leaves remained on the branches, three trees were selected in the location defined as IPE (Table 1) and ten branches at the apical region of each tree were tagged. Residence times were estimated for new leaves produced during a 12 month period categorized as distal leaves (those located in the more apical regions of the tagged branches)

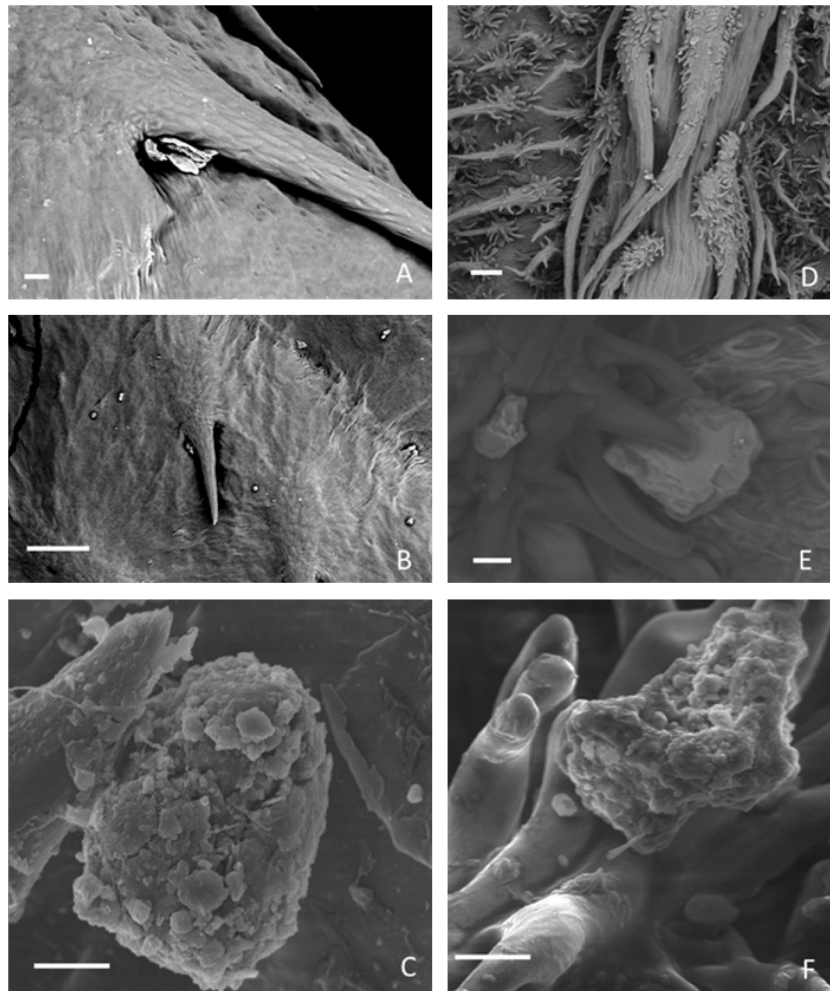


Fig. 2. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site JAB in São Paulo city showing particulate matter on the adaxial surface (A–C) and on the abaxial surface (D–F). Bar = 100 μm .

and proximal leaves (those located in the more basal regions of the tagged branches).

2.3. Sampling of leaves

Three samplings per tree were performed from June 2011 to September 2012. The dimensions of the tree were established, and branches (up to 25 cm in length) located in the most exposed part of the canopy at the point closest to the roadway and between 2 and 4 m above ground level (to avoid soil contamination) were cut using pruning shears. Branches and leaves were transferred to kraft paper bags and stored in polystyrene boxes in order to maintain the temperature and humidity of the specimens. Leaves from the fourth and fifth nodes were selected for the subsequent analyses. Samples collected at location IBT (Table 1) were employed as controls for dry deposition, since this site was situated in a remnant of the Atlantic Forest and was subjected to the lowest level of air pollution of all study sites.

2.4. Morphoanatomic evaluation of leaves

At each site, three leaves were collected from three different specimens and their lengths and widths at the midline were determined with the help of a digital pachymeter (Mitutoyo, Aurora, IL, USA). Morphometric analyses were performed using leaf

samples that had been fixed in FAA (formalin–glacial acetic acid–50% alcohol, 5:5:90) for 48 h and stored in 70% ethanol (Johansen, 1940). Leaves were treated according to the technique described by Strittmatter (1973), the epidermal layers were dissociated and the abaxial and adaxial surfaces separated, stained in safranin 1% and astra blue 1% (9:1; Kraus et al., 1998) and subsequently embedded in glycerin 50% (Johansen, 1940). The numbers of trichomes, stomata and epidermal cells, were analyzed using a Zeiss Standard 25 light microscope (Carl Zeiss, Oberkochen, Germany) and Zeiss KS100 software (version 3.0) from 10 mm² of surface area apical, median and basal region.

Detailed anatomical analyses of apical, median and basal portions of the leaves were performed on transverse sections that been stained with safranin 1% for 20 min, rinsed with ethanol 50% and subsequently stained with astra blue 1% for 20 min. Sections were examined under a Leica DM-LB photomicroscope (Leica Microsystems, Wetzlar, Germany) and the images digitalized using Leitz IM 50 software. For scanning electron microscopic studies, apical, median and basal portions of the leaves were fixed, dehydrated at 60 °C for 20 days in oven model 320F (Fanem, São Paulo, Brazil), critical point dried with liquid carbon dioxide (Balzers, Liechtenstein, Germany) model CPD-030 Drier, mounted on a specimen stub and coated with gold (Silveira, 1989). Digital images were acquired using a Hitachi Tabletop microscope model TM-3000 (High Technologies America, Schaumburg, IL, USA). The results were subjected

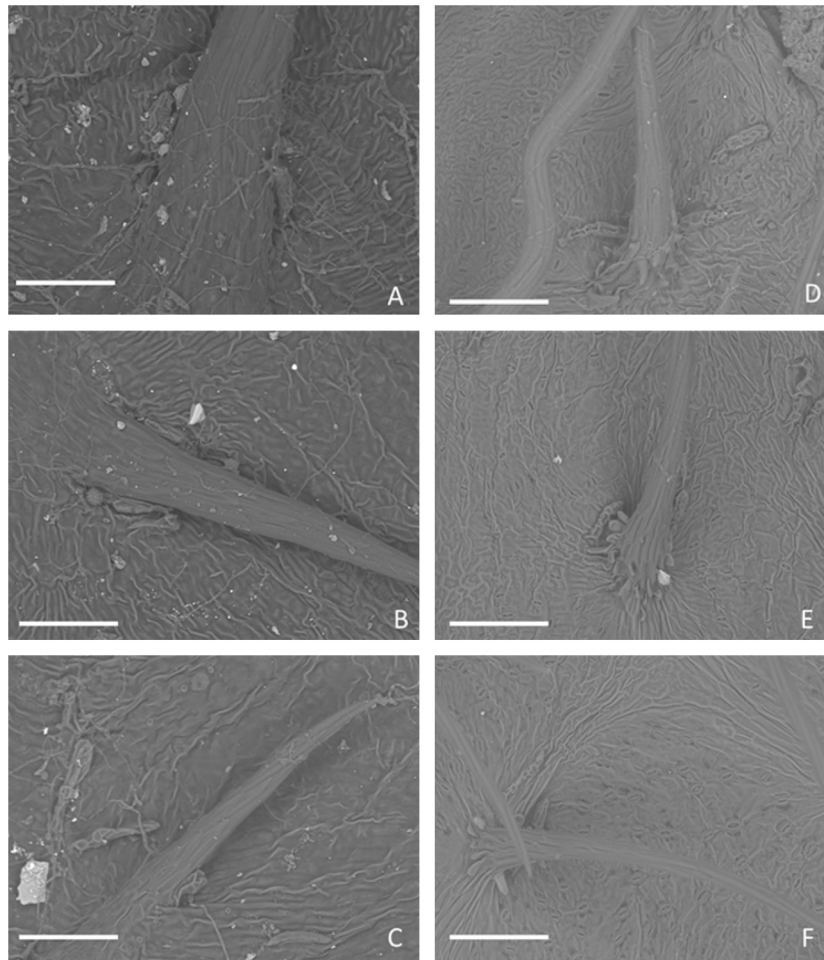


Fig. 3. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site IBT (dry deposition control) in São Paulo city showing particulate matter adsorbed at the apical, median and basal regions of the adaxial surface (A, B and C, respectively) and of the abaxial surface (D, E and F, respectively). Bar = 100 μm .

to ANOVA (F -test) followed by the Tukey test to check for significant differences between means ($P < 0.05$).

2.5. Analysis of particles adsorbed onto leaves

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM–EDS) provides qualitative and quantitative information concerning the elements present on the surface of a sample, and was applied in the present study to determine the distribution of particulate matter adsorbed onto leaves. Samples (two leaves) from each site were dehydrated at 60 °C during 8 days in an oven and divided into apical, median and basal portions. For each of the portions, two areas (1 cm \times 1 cm) of the adaxial and abaxial surfaces were mounted individually onto specimen stubs and held by double-sided conductive carbon tape. Digital images (600 \times magnification) were acquired using a Hitachi Tabletop microscope model TM-3000 (High Technologies America, Schaumburg, IL, USA) equipped with a Bulker Quantax 70 X-ray (Billerica, MA, USA) microanalysis system, and particles on the surfaces of the leaves were automatically counted with the help of ImageJ software version 1.44p with Java 1.6.0 (Ottel  et al., 2010). Finally, the particles were measured and classified according to size (<2.5, 2.5–10, 10–50, 50–100 and >100 μm).

3. Results

3.1. Time of abscission of leaves

Detailed observations of new leaves formed on tagged branches located near to the apex of *T. granulosa* trees growing at the IPE site revealed that those in the more apical regions (distal leaves) presented a residence time of 20–24 weeks, while for the proximal leaves (those in the more basal regions) the residence time varied between 14 and 16 weeks. Additionally, one internode, from which the lateral leaf buds protruded, developed each month.

3.2. Morphoanatomic aspects of leaves

T. granulosa presented simple opposite-decussate phyllotaxis and the leaves were leathery with grooved adaxial and convex abaxial surfaces. The venation pattern of the leaf blade was of the palmate-parallel type (Fig. 1A) and the leaf surfaces were smooth and hairy. On the adaxial surfaces, glandular and appressed scabrous trichomes (Fig. 1B) were present, while on the abaxial surfaces, glandular and strigose trichomes could be observed together with trichomes that were branched at the base (Fig. 1C and D). As displayed in Fig. 1E, both surfaces of

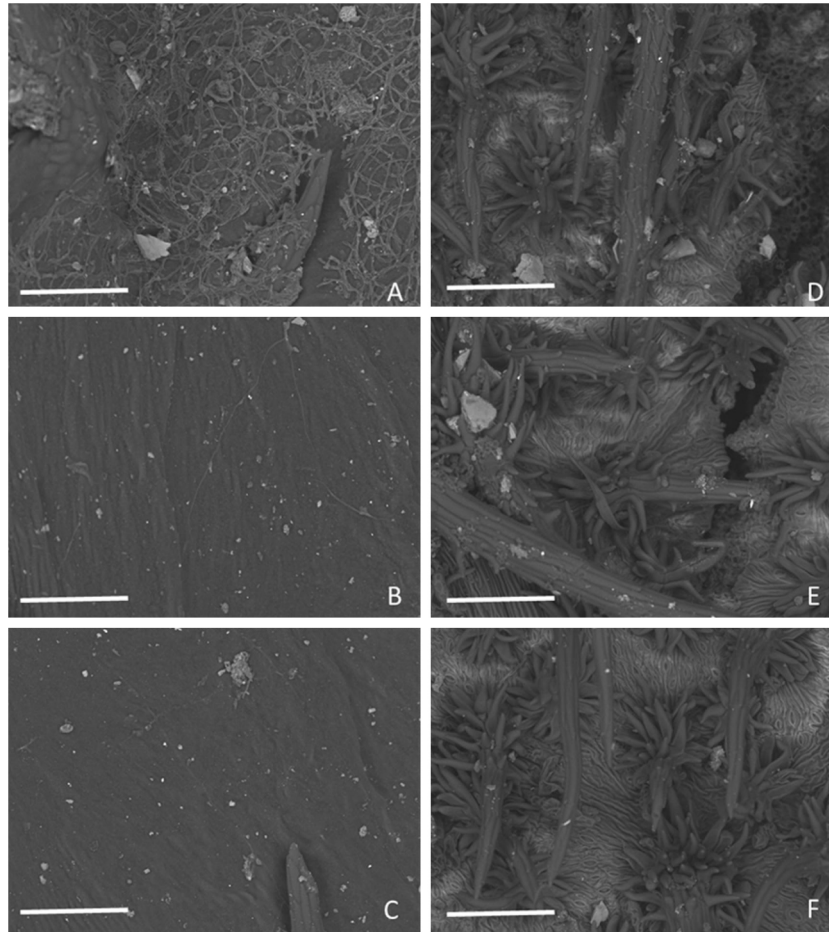


Fig. 4. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site IPE in São Paulo city showing particulate matter adsorbed at the apical, median and basal regions of the adaxial surface (A, B and C, respectively) and of the abaxial surface (D, E and F, respectively). Bar = 100 μm .

the leaf were coated with a thin cuticle. The numbers of stomata and branched trichomes per mm^2 on the apical, median and basal portions of the epidermis of the abaxial leaf surface differed significantly (Table 2). However, there were no significant differences between the densities of stomata present on the apical and median portions of leaves collected from trees located at different sites in São Paulo city, but the differences in stomatal indices between samples from trees located in São Carlos city and those located in São Paulo city were statistically significant (Table 2).

The leaves of *T. granulosa* were hypostomatic, but stomata were also assembled on the adaxial surface under the appressed scabrous trichomes all along the foliar margin (Fig. 1F). In the midrib region, the vascular system displayed the form of an open arch (Fig. 1A). The epidermis on the abaxial and adaxial surfaces was single-layered, while the hypodermis comprised three layers (Fig. 1E) containing sclerenchyma cells and idioblasts with druse type crystals. The dorsiventral mesophyll presented a *uniseriate* palisade parenchyma.

The SEM images showed that particulate material was distributed over the epidermis of both the abaxial and adaxial surfaces of the leaves. On the adaxial surface, PM was concentrated in the vicinity of the appressed scabrous and glandular trichomes (Fig. 2A–C), while on the abaxial surface, the particles were not only adsorbed onto the strigose and branched trichomes (Fig. 2D–F) but were also dispersed in the spaces between the trichomes.

3.3. Densities of particles on leaf surfaces

Detailed analysis of the SEM images revealed that PM was distributed abundantly on the adaxial and abaxial surfaces of the leaf blades of samples collected from the different sites (Figs. 3–8). Images of the apical, median and basal portions of the adaxial (Fig. 3A–C) and abaxial (Fig. 3D–F) surfaces of leaf blades of samples collected from the IBT site were taken as controls for dry deposition.

Tables 3–5 present the numbers of particles of various sizes (>2.5 to >100 μm) per 0.1 mm^2 of adaxial and abaxial surfaces of *T. granulosa* leaves collected at sites IBT, IPE, JAB, PRA, APA, SCB and CUB. Samples from sites APA and CUB presented the highest densities of $\text{PM}_{2.5}$, while the adaxial surfaces presented 4.5 times more particles than the abaxial surfaces. The total numbers of PM per 0.1 mm^2 present on the adaxial and abaxial surfaces of *T. granulosa* leaves collected from these sites are shown in Figs. 9 and 10.

SEM-EDS analysis indicated that Al, Au, Ba, Ca, Cr, Cu, Fe, Mg, Mn, Pb, Si and Ti were present in the dry deposition of the *T. granulosa* leaf samples for all sites. The element Au was found only in PRA site (Fig. 11).

4. Discussion

The pattern of development and growth of leaves represents an important trait that must be considered in the evaluation and management of a species as a spatial and regional biomarker of pollution. The residence time of the leaves on the branches depends

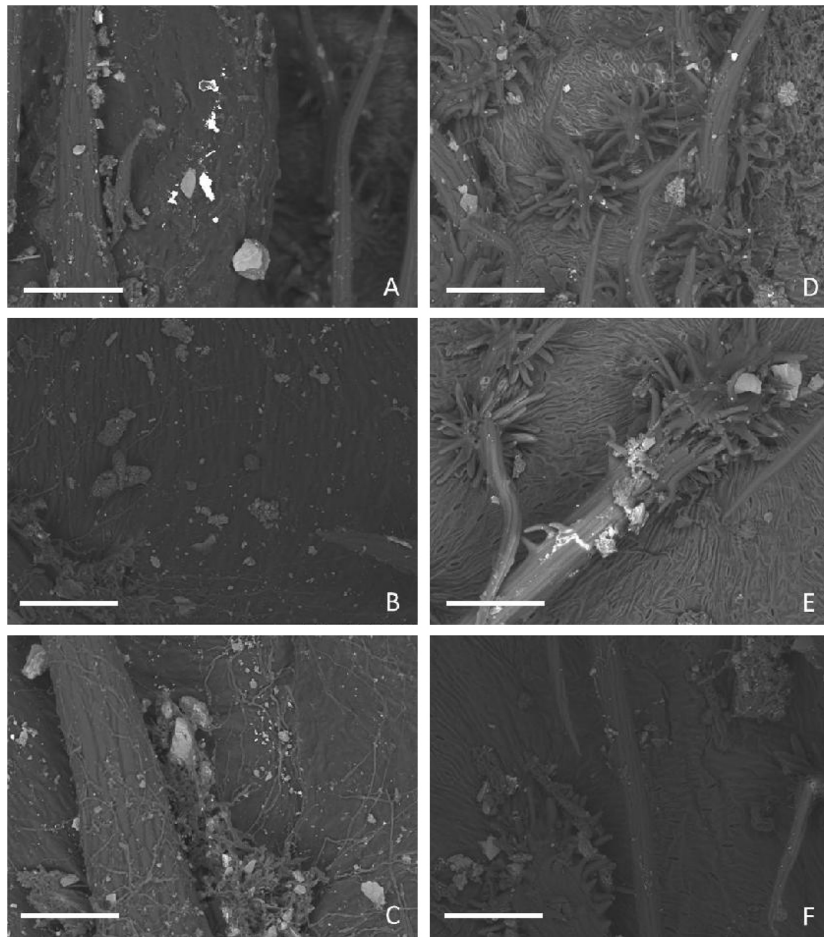


Fig. 5. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site PRA in São Paulo city showing particulate matter adsorbed at the apical, median and basal regions of the adaxial surface (A, B and C, respectively) and of the abaxial surface (D, E and F, respectively). Bar = 100 μm .

Table 2
Mean density (\pm standard deviation) of anatomical structures present in the epidermis of leaf samples from *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected at different sites in São Paulo State, Brazil.

Leaf surface	Site ^a	Leaf portions					
		Apical	Median	Basal	Apical	Median	Basal
		Number of glandular trichomes per 1 mm ²			Number of appressed scabrous trichomes per 1 mm ²		
Adaxial	IBT	5 \pm 1 ^A	5 \pm 1 ^A	5 \pm 1 ^A	2 \pm 1 ^A	2 \pm 1 ^A	2 \pm 1 ^A
	DPA	5 \pm 1 ^A	5 \pm 1 ^A	5 \pm 1 ^A	3 \pm 1 ^A	3 \pm 1 ^A	3 \pm 1 ^A
	PRA	3 \pm 1 ^{BC}	4 \pm 1 ^A	4 \pm 1 ^{AB}	2 \pm 1 ^{BC}	2 \pm 1 ^{ABC}	2 \pm 1 ^{AB}
	JAB	2 \pm 1 ^C	3 \pm 1 ^A	3 \pm 1 ^B	2 \pm 1 ^C	1 \pm 0.5 ^{CD}	2 \pm 1 ^B
	ISE	4 \pm 1 ^{AB}	4 \pm 1 ^A	5 \pm 1 ^A	3 \pm 1 ^{AB}	2 \pm 1 ^{AB}	2 \pm 1 ^A
	SCB	3 \pm 1 ^{BC}	4 \pm 1 ^A	3 \pm 1 ^B	2 \pm 1 ^{BC}	2 \pm 1 ^{BC}	2 \pm 1 ^B
	SCS	5 \pm 2 ^A	3 \pm 1 ^A	4 \pm 2 ^{AB}	3 \pm 1 ^A	2 \pm 1 ^{AB}	2 \pm 1 ^{AB}
Leaf surface	Site ^a	Leaf portions					
		Apical	Median	Basal	Apical	Median	Basal
		Number of stomata per 1 mm ²			Number of branched trichomes per 1 mm ²		
Abaxial	IBT	167 \pm 26 ^{ab}	191 \pm 57 ^{ab}	156 \pm 27 ^a	20 \pm 12 ^c	20 \pm 15 ^c	18 \pm 10 ^c
	DPA	137 \pm 21 ^b	190 \pm 53 ^{ab}	147 \pm 26 ^{ab}	25 \pm 9 ^{bc}	27 \pm 3 ^{bc}	27 \pm 4 ^{ab}
	PRA	170 \pm 14 ^{ab}	156 \pm 26 ^{bc}	148 \pm 23 ^{ab}	25 \pm 3 ^{bc}	27 \pm 3 ^{bc}	28 \pm 3 ^a
	JAB	162 \pm 53 ^{ab}	169 \pm 55 ^b	169 \pm 55 ^a	33 \pm 11 ^{ab}	34 \pm 12 ^{ab}	32 \pm 12 ^a
	ISE	184 \pm 6 ^a	220 \pm 39 ^a	126 \pm 24 ^{bc}	40 \pm 6 ^a	40 \pm 5 ^a	27 \pm 4 ^a
	SCB	101 \pm 28 ^c	111 \pm 22 ^d	114 \pm 23 ^c	23 \pm 8 ^c	24 \pm 3 ^c	18 \pm 8 ^c
	SCS	102 \pm 13 ^c	130 \pm 34 ^{cd}	112 \pm 17 ^c	31 \pm 1 ^b	33 \pm 7 ^{ab}	21 \pm 3 ^{bc}

Dissimilar superscript uppercase (adaxial surface) and lowercase (abaxial surface) letters indicate significant differences between study sites (LSD Student's *t*-test; $P < 0.05$).

^a For key to site location see Table 1.

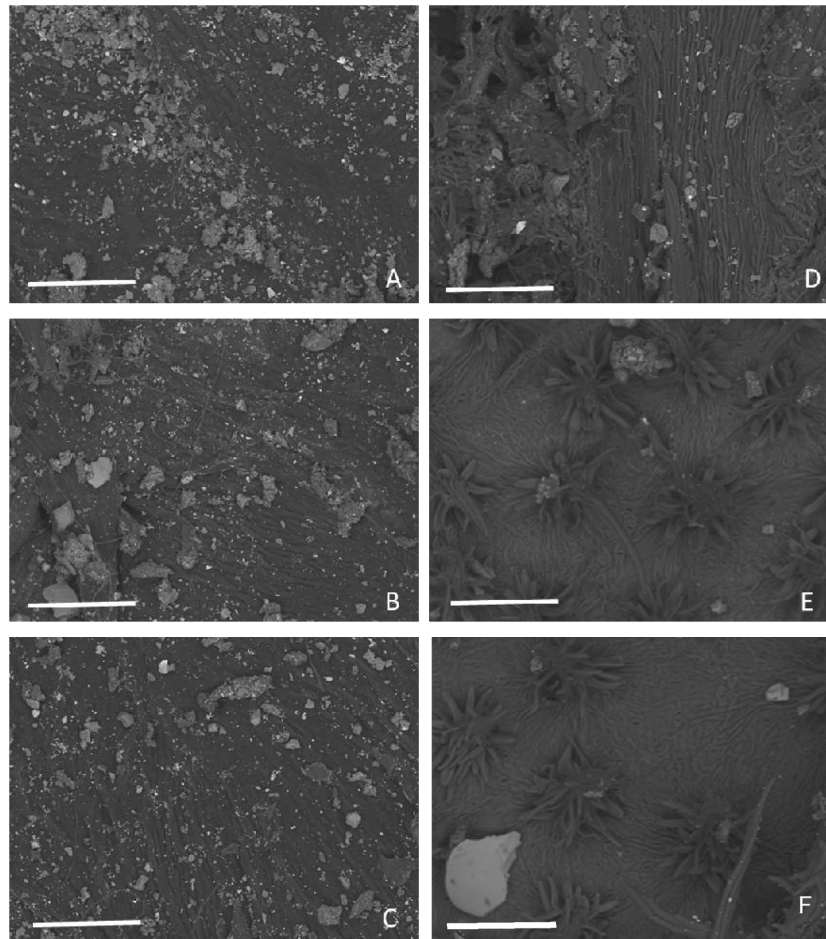


Fig. 6. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site APA in São Paulo city showing particulate matter adsorbed at the apical, median and basal regions of the adaxial surface (A, B and C, respectively) and of the abaxial surface (D, E and F, respectively). Bar = 100 μm .

on various environmental factors including climate, soil conditions and nutrient availability (França et al., 2008). In the case of *T. granulosa*, the leaves remained on the branches for up to 24 weeks. Moreover, leaves of the IPE samples presented the expected growth pattern in relation to the availability of water and minerals in the soil (Malavolta, 1974).

The most relevant anatomical characteristic of the leaves of *T. granulosa* was the variety of trichomes present, with appressed scabrous and glandular types on the adaxial surface, and branched, glandular and strigose trichomes on the abaxial surface. Such morphological diversity of trichomes has been described previously (Metcalf and Chalk, 1950) for other representatives of

the family Melastomataceae. Leaves bearing trichomes attract special attention since they tend to accumulate metals at levels that are up to 10-fold higher than those of glabrous leaves (Markert, 1993). The results presented herein indicate that after wet or dry deposition, the PM remains adsorbed to the hairy structures of the leaves and does not return to the environment even under dry windy conditions. When leaves are exposed to rainfall, however, the particles move along the surface of the leaf blade and concentrate in the apical parts. This behavior is apparent in Figs. 6–8, and was observed during a period of six months under the influence of natural elements including bad weather.

Table 3

Average number of particles per 0.1 mm² on the adaxial and abaxial surfaces of the apical portions of leaf samples from *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected at different sites in São Paulo State, Brazil.

Site ^a	Size of particulate matter (μm)									
	<2.5		2.5–10		10–50		50–100		>100	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
IBT	82	9	35	4	6	12	1	1	1	1
IPE	375	116	90	51	24	15	2	1	4	3
JAB	520	313	88	276	24	117	2	27	3	31
PRA	645	995	205	403	77	128	8	12	11	10
APA	1239	579	548	173	244	57	28	6	29	5
SCB	457	49	152	13	82	6	22	1	9	2
CUB	600	398	631	301	295	161	41	15	115	17

^a For key to site location see Table 1.

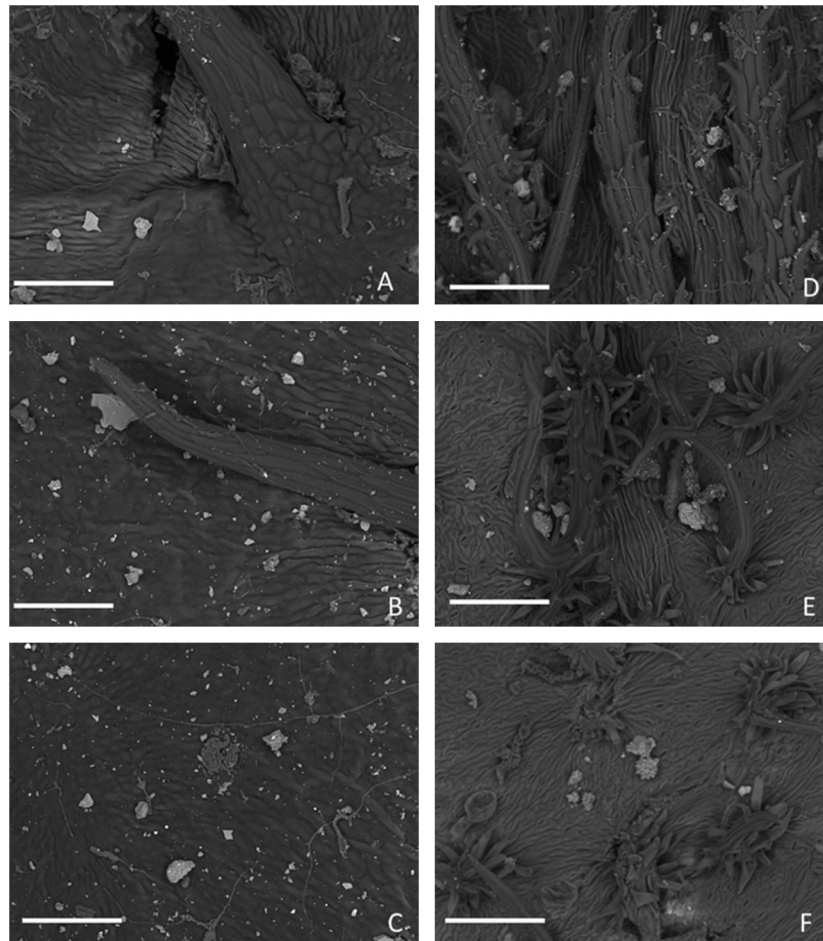


Fig. 7. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site SCB in São Carlos showing particulate matter adsorbed at the apical, median and basal regions of the adaxial surface (A, B and C, respectively) and of the abaxial surface (D, E and F, respectively). Bar = 100 μm .

The stomatal indices of leaf samples collected in São Carlos city (sites SCB and SCS) were significantly lower in comparison with those from sites in São Paulo city. The increased density of stomata in samples from the latter locations may be related to a higher physiological demand with respect to the influx and efflux of air in the inner tissues, in that the higher the stomatal index the more intense is the air exchange process. However, the observed structural changes are often associated with decreased transpiration and photosynthetic activity caused by the breakdown of leaf epicuticular waxes, which may obliterate the stomata thereby impeding gas exchange (Alves et al., 2001).

Leaves of *T. granulosa* collected at different sites within the state of São Paulo were also divergent with regard to the density of particles derived from natural and pollutant sources. Particles classified as $\text{PM}_{2.5}$ originate from anthropogenic sources, principally vehicular traffic (>80%), and are very harmful to human health (Ottel e et al., 2010). In urban areas, PM arise mainly from the incomplete combustion of fossil fuels, the friction between mechanical components such brakes and rubber tires, the exhaust emissions from diesel-driven heavy vehicles, and materials released from industrial and domestic combustion processes (Adamo et al., 2008). Comparison of the densities of particles on leaves collected from

Table 4
Average number of particles per 0.1 mm^2 on the adaxial and abaxial surfaces of the median portions of leaf samples from *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected at different sites in São Paulo State, Brazil.

Site ^a	Size of particulate matter (μm)									
	<2.5		2.5–10		10–50		50–100		>100	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
IBT	64	5	4	2	2	1	2	1	1	1
IPE	149	62	99	40	26	20	2	2	1	1
JAB	567	309	153	114	46	89	5	18	2	23
PRA	375	774	142	198	53	85	6	9	1	3
APA	1577	56	458	36	123	9	12	2	10	1
SCB	350	274	105	103	53	90	9	13	3	29
CUB	1155	297	473	173	220	94	47	13	40	52

^a For key to site location see Table 1.

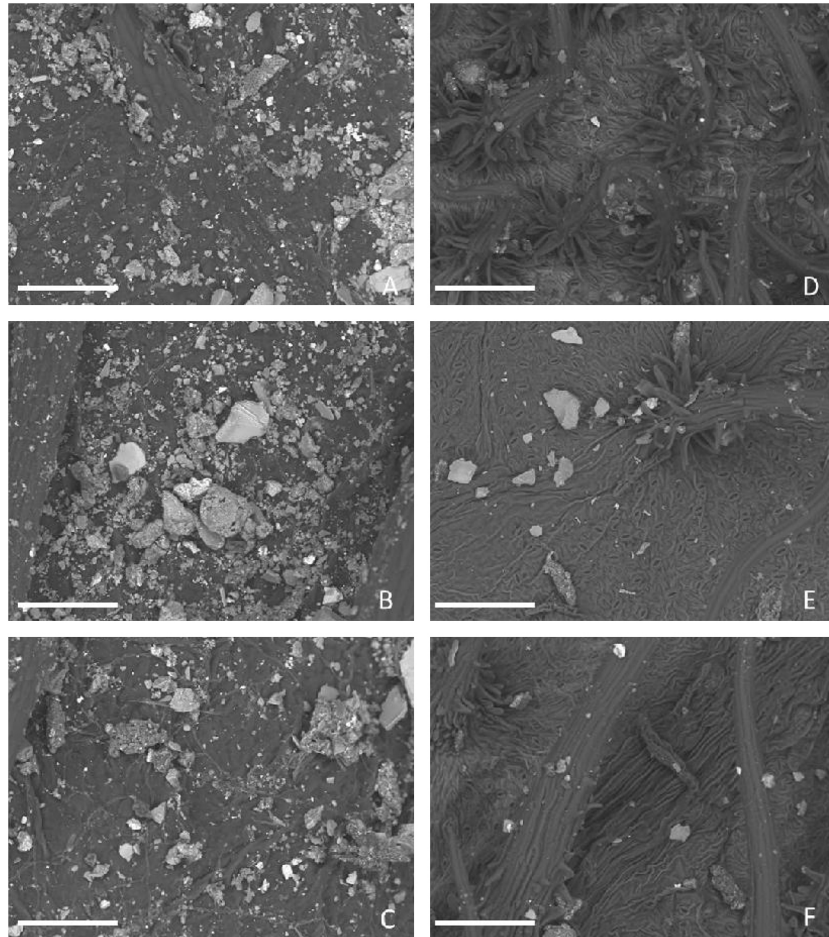


Fig. 8. Scanning electron micrographs of leaves of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected from site CUB in Cubatão showing particulate matter adsorbed at the apical, median and basal regions of the adaxial surface (A, B and C, respectively) and of the abaxial surface (D, E and F, respectively). Bar= 100 μm .

city sites with those on samples from the less polluted IBT site confirms that leaves of *T. granulosa* are able to adsorb particles emitted by polluting sources. Moreover, airflow vortices produced by road vehicles push some of these particles upwards and enables them to be deposited on adaxial and abaxial leaf surfaces as demonstrated, respectively, by the samples from APA and PRA, both of which are sites of intense vehicular and pedestrian traffic. For example, an average of 4200 vehicles per hour pass through the APA site located in the heart of São Paulo City, with 8365 and 6825 vehicles traveling from Consolação Avenue to Paraiso Avenue during the morning (7–10 am) and evening (5–8 pm) rush hours, respectively, while

11,844 and 9730 vehicles travel in the opposite direction during the same respective periods (Bornsztajn and Espel, 2011).

Among the samples collected in the urban areas, the sample APA showed the highest density of particles <2.5. This result was already expected thus this is a region of São Paulo City with intense vehicular traffic. The sample CUB (region of industries of São Paulo State), which despite the determination to control the total suspended particles (PM) by the standards (CONAMA 003/1990 and 008/1990) on industries and refineries in the region (São Paulo State Decree Law 8468 of 09/8/1876), was the second region with higher density of particles <2.5.

Table 5

Average number of particles per 0.1 mm² on the adaxial and abaxial surfaces of the basal portions of leaf samples from *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) collected at different sites in São Paulo State, Brazil.

Site ^a	Size of particulate matter (μm)									
	<2.5		2.5–10		10–50		50–100		>100	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
IBT	82	5	11	1	7	2	1	2	2	1
IPE	282	162	99	48	30	33	2	2	1	1
JAB	468	14	110	16	25	7	2	1	2	1
PRA	168	659	61	162	21	92	2	5	2	20
APA	1572	31	500	1	151	4	16	2	13	1
SCB	380	222	125	82	52	70	4	13	3	13
CUB	1770	559	685	210	300	93	39	9	29	4

^a For key to site location see Table 1.

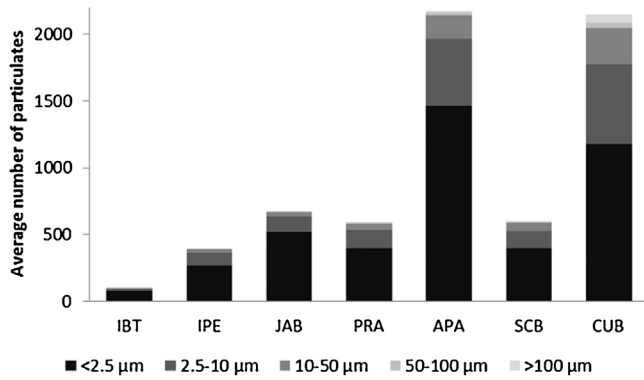


Fig. 9. Total numbers of particles per 0.1 mm² present on the adaxial surfaces of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) leaves collected from sites IBT, IPE, JAB, PRA, APA, SCB and CUB.

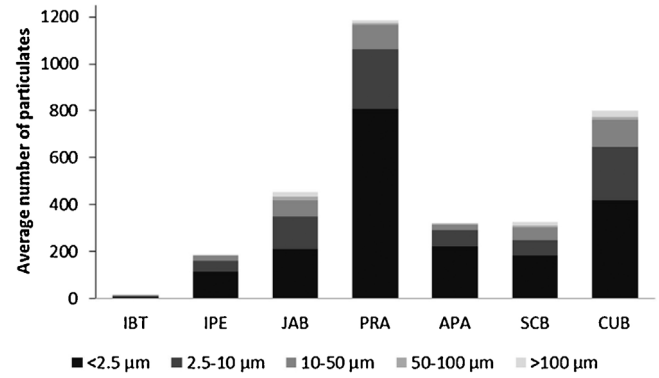


Fig. 10. Total numbers of particles per 0.1 mm² present on the abaxial surfaces of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) leaves collected from sites IBT, IPE, JAB, PRA, APA, SCB and CUB.

The amounts of PM present on the adaxial and abaxial surfaces of *T. granulosa* leaves were unexpectedly high. PMs containing metals such as Au, Ba, Fe, Pb and Ti probably derive from anthropogenic activities and not from natural sources considering the sites of collection and of the morphology of the particles. Since these toxic metals were present in inhalable particles such as PM₁₀, the possibility of adverse health effects is increased.

In order to evaluate the efficacy of measures implemented by the authorities with the aim of protecting public health, it is necessary to monitor the chemical composition of polluting particulates. The current methods of analysis involve the use of expensive filters and complicated techniques, factors that place severe restrictions on the resolution of the monitoring network. The results presented herein demonstrate that *T. granulosa* trees may be used as passive

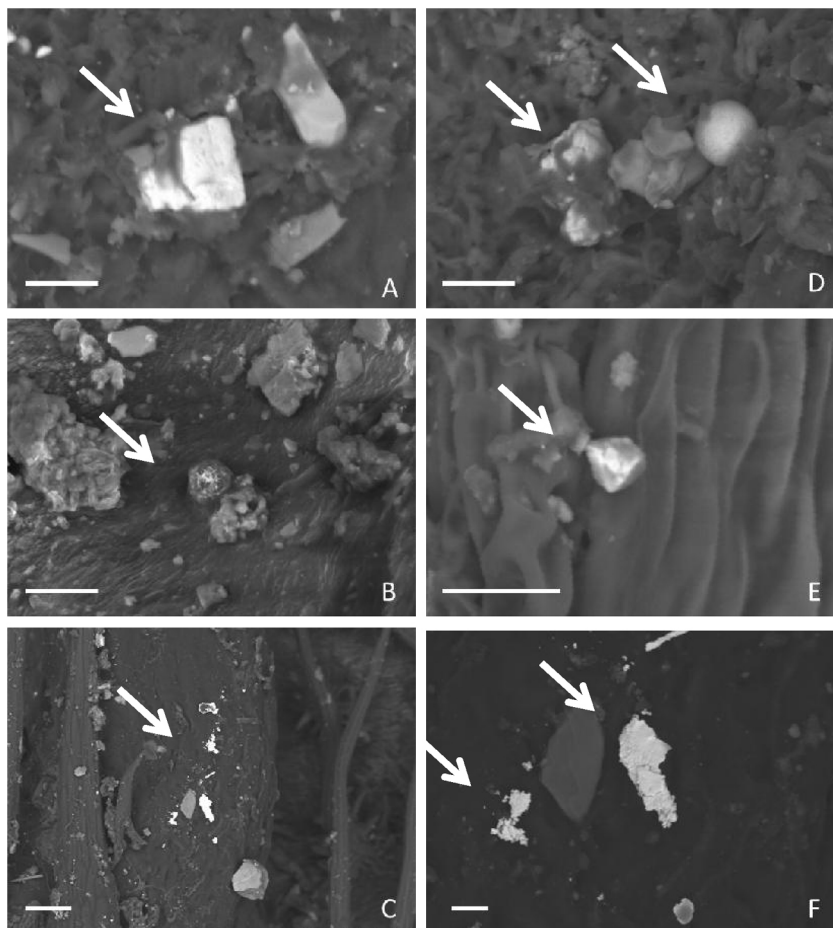


Fig. 11. SEM-EDS images of the adaxial surfaces of *Tibouchina granulosa* (Ders.) Cong. (Melastomataceae) leaf samples from various locations showing: CUB (A) – Ba particles (arrow; 42.64% Ba, 28.63% C, 19.24% O, 7.65% S, 1.07% Si, 0.76% Al; 50 µm); IPE (B) – Pb particle (arrow; 42.27% Pb, 36.29% Ti, 13.01% Si, 8.43% Al; 50 µm); PRA (C) – Au particle (arrow; 55.91% Au, 24.99% C, 6.50% O; Bar 50 µm); CUB (D) – K and Fe particles (arrows 1 and 2, respectively; 50 µm); PRA (E) – Ti particle (arrow; 40.40% Ti, 23.17% C, 21.13% Fe, 2.13% Mn; 50 µm); PRA (F) – Au particle (arrows; Bar 10 µm).

biomonitor for the spatial and temporal assessment of polluted air through the examination of the dry deposition on the leaves.

5. Conclusions

Trichomes located on the adaxial and abaxial surfaces of leaves of *T. granulosa* (Ders.) Cong. (Melastomataceae) trees were able to adsorb particulate materials within the size range 2.5–100 μm . Following deposition, the particles remained adsorbed to the leaves and did not return to the environment under normal weather conditions. SEM-EDS analysis of leaf material collected at different sites provided comparative information regarding both the size distribution and the chemical nature of the adsorbed PM. *T. granulosa* is particularly appropriate for urban landscaping, producing exuberant flowers in colors that vary from pink to purple, and cultivation in inner-city areas may help to improve the quality of air by reducing the concentration of harmful PM_{2.5} and PM₁₀. Additionally, *T. granulosa* may be employed as a passive biomonitor, thereby offering a valuable alternative for monitoring air pollution and spatial–temporal evaluation of PM composition.

Acknowledgments

The authors are grateful to Mr. Armando Tessari and Mrs. Rutilde Vitor Tessari for assistance with the sampling of plant material and for providing information regarding the geographical location of the trees. The authors wish to thank the Instituto de Pesquisas Energéticas e Nucleares and the Comissão Nacional de Energia Nuclear for scholarships awarded to MCTZ and RCBP.

References

- Adamo, P., Giordano, S., Naimo, D., Bargagli, R., 2008. Geochemical properties of air-borne particulate matter (PM₁₀) collected by automatic device and biomonitors in a Mediterranean urban environment. *Atmos. Environ.* 42, 346–357.
- Alves, E.S., Giusti, P.M., Domingos, M., Saldiva, P.H.N., Guimaraes, E.T., Lobo, D.J.A., 2001. Estudo anatômico foliar do clone híbrido 4430 de *Tradescantia*: alterações decorrentes da poluição aérea urbana. *Rev. Bras. Bot.* 24, 567–576.
- Alves, E.S., Tresmondi, F., Longui, E.L., 2008. Análise estrutural de folhas de *Eugenia uniflora* L. (Myrtaceae) coletadas em ambientes rural e urbano, SP, Brasil. *Acta Bot. Bras.* 22, 241–248.
- Biblioteca Virtual do Governo do Estado de São Paulo, 2013. Geografia do Estado. Subsecretaria de Comunicação da Casa Civil, São Paulo, Available at: <http://www.bibliotecavirtual.sp.gov.br/saopaulo-geografia.php> (accessed in March 2013).
- Bornsztajn, L.L., Espel, M., 2011. Nova Paulista: Uma quebra de paradigmas no tratamento das travessias de pedestres. *Boletim Técnico* 48. Companhia de Engenharia de Tráfego, São Paulo.
- CONAMA – National Council for the Environment – Ministry of the Environment. Resolution 003 of June 28, 1990. Available at: <http://www.mma.gov.br/port/conama/res/res90/res0390.html>
- CONAMA – National Council for the Environment – Ministry of the Environment. Resolution 008 of December 28, 1990. Available at: <http://www.mma.gov.br/port/conama/res/res90/res0890.html>
- França, M.G.C., Araújo, A.P., Rossiello, R.O.P., Ramos, F.T., 2008. Relações entre crescimento vegetativo e acúmulo de nitrogênio em duas cultivares de arroz com arquiteturas contrastantes. *Acta Bot. Bras.* 22, 43–49.
- Fierro, M., 2000. Particulate Matter, Available at: <http://www.airinforow.org/pdf/Particulate.Matter.pdf> (accessed in March 2013).
- Fontenele, A.P.G., Pedroti, J.J., Fornaro, A., 2009. Avaliação de metais traços e íons majoritários em águas de chuva na cidade de São Paulo. *Quím. Nova* 32, 839–844.
- Ignotti, E., Valente, J.G., Longo, K.M., Freitas, S.R., Hacon, S.S., Netto, P.A., 2010. Impact on human health of particulate matter emitted from burnings in Brazilian Amazon region. *Rev. Saude Publica* 44, 121–130.
- Instituto Brasileiro de Geografia e Estatística, 2010. Censo Demográfico 2010, Available at: <http://www.ibge.gov.br/> (accessed in March 2013).
- Johansen, D.A., 1940. *Plant Microtechnique*. Mc Graw Hill, New York.
- Kardel, F., Wuyts, K., Maher, B.A., Hansard, R., Samson, R., 2011. Leaf saturation isothermal remanent magnetization (SIRM) as a proxy for particulate matter monitoring: inter-species differences and in-season variation. *Atmos. Environ.* 45, 5164–5171.
- Kraus, J.E., Sousa, H.C., Rezende, M.H., Castro, N.M., Vecchi, C., Luque, R., 1998. Astra blue and basic fuchsin double staining of plant materials. *Biotechnic. Histochem.* 73, 235–243.
- Malavolta, E., 1974. *Nutrição Mineral e Adubação de Plantas Cultivadas*. Pioneira, São Paulo.
- Markert, B., 1993. Plants as biomonitors: indicators for heavy metals in the terrestrial environment. Wiley-Blackwell, Hoboken, pp. 645.
- Markert, B., 2007. Definitions and principles for bioindication and biomonitoring of trace metals in the environment. *J. Trace Elem. Med. Biol.* 21, 77–82.
- McDonald, A.G., Bealey, W.J., Fowler, D., Dragosits, U., Skiba, U., Smith, R.I., Donovan, R.G., Brett, H.E., Hewitt, C.N., Nemitz, E., 2007. Quantifying the effect of urban tree planting on concentrations and depositions of PM₁₀ in two UK conurbations. *Atmos. Environ.* 41, 8455–8467.
- Metcalfe, C.R., Chalk, L., 1950. *Anatomy of the Dicotyledons*, vol. 2. Clarendon Press, Oxford.
- Moraes, R.M., Delitti, W.B.C., Moraes, J.A.P.V., 2000. Respostas de indivíduos jovens de *Tibouchina pulchra* Cong. à poluição aérea de Cubatão, SP: fotossíntese líquida, crescimento e química foliar. *Rev. Bras. Bot.* 23, 443–449.
- Ottel, M., van Bohemen, H., Fraaij, A.L.A., 2010. Quantifying the deposition of particulate matter on climber vegetation on living walls. *Ecol. Eng.* 36, 154–162.
- Saebo, A., Popek, R., Nawrot, B., Hanslin, H.M., Gawronska, H., Gawronski, S.W., 2012. Plant species differences in particulate matter accumulation on leaf surfaces. *Sci. Total Environ.* 427–428, 347–354.
- Silveira, M., 1989. Preparação de amostras biológicas para microscopia eletrônica de varredura. *Manual Sobre Técnicas Básicas em Microscopia Eletrônica*, vol. 1. Universidade de São Paulo, São Paulo, pp. 71–79.
- State Law 8468, of 08 September 1976. It provides for the prevention and control the environment air pollution. Available at: <http://www.cetesb.sp.gov.br/Institucional/documentos/Dec8468.pdf>
- Strittmatter, C.G.D., 1973. Nueva técnica de diafanización. *Bol. Soc. Argent. Bot.* 15, 126–129.
- Sucur, K.M., Anicic, M.P., Tomsevic, M.N., Antanastjevic, D.Z., Peric-Grujic, A.A., Ristic, M.D.J., 2010. Urban deciduous tree leaves as biomonitors of trace element (As, V and Cd) atmospheric pollution in Belgrade, Serbia. *J. Serb. Chem. Soc.* 75, 1453–1461.
- Tao, J., Shen, Z., Zhu, G., Yue, J., Cao, J., Liu, S., Zhu, L., Zhang, R., 2012. Seasonal variations and chemical characteristics of sub-micrometer particles (PM₁) in Guangzhou, China. *Atmos. Res.* 118, 222–231.
- World Health Organization, 2011. Air Quality and Health. Fact sheet No 313. WHO, Geneva, Available at: <http://www.who.int/mediacentre/factsheets/fs313/en/index.html> (accessed in March 2013).
- Zampieri, M.C.T., Saiki, M., Tavares, A.R., Pinna, G.F.A.M., 2012. Acúmulo de minerais em *Aechmea blanchetiana* (Baker) L.B. Smith (Bromeliaceae), contaminadas com zinco em cultivo in vitro. *Hoehnea* 39, 379–385.