Chemosphere 127 (2015) 27–34

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil

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HIGHLIGHTS

- No morphoanatomical damage was found in species exposed to iron particles.
- *A. schaueriana* and *L. racemosa* showed the highest leaf concentration of iron.
- X-ray diffraction (EDS) rendered a good method for evaluating iron pollution on leaves.

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form 30 December 2014
Accepted 8 January 2015

Handling Editor: X. Cao

Keywords:
Dust
Leaf anatomy
Mangrove
Mining
Particulate iron

ABSTRACT

In Brazil, some mangrove areas are subjected to air pollution by particulate iron from mining activities. However, the effect of this pollutant on mangrove plants is not well known. This study aimed to comparatively analyze the morphoanatomy, histochemistry, and iron accumulation in leaves of *Avicennia schaueriana*, *Laguncularia racemosa*, and *Rhizophora mangle*. Samples were collected from five mangrove sites of Espírito Santo state, each of which is exposed to different levels of particulate iron pollution. The amount of particulate material settled on the leaf surface was greater in *A. schaueriana* and *L. racemosa*, which contain salt glands. High iron concentrations were found in leaves of this species, collected from mangrove areas with high particulate iron pollution, which suggests the foliar absorption of this element. None of the samples from any of the sites showed morphological or structural damage on the leaves. Scanning electron microscopy (SEM) coupled to X-ray diffraction rendered a good method for evaluating iron on leaves surfaces. A histochemical test using Prussian blue showed to be an appropriate method to detect iron in plant tissue, however, proved to be an unsuitable method for the assessment of the iron bioaccumulation in leaves of *A. schaueriana* and *R. mangle*. So far, this study demonstrates the need of evaluating the pathway used by plants exposed to contaminated particulate matter to uptake atmospheric pollutants.

1. Introduction

Mangroves are often exposed to various pollution sources, including particulate material due to its coastal distribution and proximity to urban centers (Silva Filho et al., 1998; Bayen, 2012). The particulate air pollution consists of a mixture of solid particles and/or liquids that vary in size, shape, and composition, and are the result of anthropogenic or natural processes (Salgado, 2003). The first studies on the effects of particulate matter deposits in plants date back to the early twentieth century (Farmer, 1993); however, the effects of these deposits on mangrove species are not well elucidated, due to the low number of studies (Paling et al., 2001; Naidoo and Chirkoot, 2004; Naidoo and Naidoo, 2005).

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http://dx.doi.org/10.1016/j.chemosphere.2015.01.011
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The consequences of particulate matter deposit on leaves include chlorosis, necrosis (Ali, 1993), decrease in photosynthetic pigments content (Prusty et al., 2005), and reduction in photosynthesis and gas exchange (Naidoo and Chirkoot, 2004). Silva et al. (2006) also reported foliar absorption of iron from particulate matter deposited on the surface of restinga vegetation. Furthermore, particulate material may indirectly affect the vegetation due to its accumulation in soil, which would increase the concentration of some elements to phytotoxic levels (Grantz et al., 2003).

Mangrove species have a relative tolerance to heavy metals present in sediment (Macfarlane and Burchett, 2002), which are largely retained in root (Macfarlane et al., 2003; Liu et al., 2009); for this reason, the amount of metals present in aerial part of plants is lower compared to the concentration in sediment (Machado et al., 2002; Sarangi et al., 2002). However, in disturbed environments, where the dynamics and balance can be affected, the accumulated metals are mobilized and, consequently, end up in the flora and coastal food chains (Vannucci, 2001).

Iron is an essential trace element for plants and it presents important roles in metabolic functions, such as the biosynthesis of chlorophyll, catalase, peroxidase, and superoxide dismutase (Marschner, 1999). However, at high levels, this element can be toxic to plants (Kampfenkel et al., 1995; Connolly and Guerinot, 2002).

In the state of Espírito Santo, Brazil, some mangroves are exposed to air pollution by iron particulate matter (PMFe) from mining activities, since this state has the largest production of iron pellets and constitutes the largest mining export harbor in Brazil (IBEF, 2011). Given this perspective, the aim of the present study was to comparatively analyze the morphoanatomy, histochemistry, and iron accumulation in the leaves of *Avicennia schaueriana* Stapf & Leechm. ex Moldenke, *Laguncularia racemosa* (L.) C.F. Gaertn., and *Rhizophora mangle* L. from five mangrove areas of Espírito Santo in Brazil that are exposed to different iron particulate pollution conditions. Thus, we look to elucidate the effects of foliar iron absorbtion by these plants, exposed to different levels of atmospheric particulate iron, which could exceed levels of this metal absorbed through roots, posing phytotoxic risk.

2. Materials and methods

2.1. Location of sample collection

Harbor of Tubarão (20°17′03.8″S and 40°14′24.9″W), which is located in Vitória, Espírito Santo state, Brazil, was considered the main source of iron particulate pollution in the state for the purposes of this study (Fig. 1).

The study was conducted in five areas located at different distances from Tubarão Harbor in three mangrove estuaries in Espírito Santo state (Fig. 1): site 1, Passage Channel (20°16′55.7″S and 40°18′57.8″W), at 7.2 km from the source of the iron particles; site 2, Lameirão Island (20°14′60.6″S and 40°18′68.6″W), at 8.0 km; site 3, Cariacica (20°18′95.8″S and 40°22′13.0″W), at 13.5 km; site 4, Santa Cruz (19°56′26.2″S and 40°12′87.0″W), at 38.4 km and site 5, Conceição da Barra (18°33′55.2″S and 39°43′98.1″W), at 198 km from the source of iron particles. The sites 1–3 were chosen because they are near to the pollutant source of iron particles (Harbor of Tubarão). Additionally, this estuary had also suffered strong environmental degradation; thus, these three sampling sites were also under influence of industrial and domestic effluents (Souza et al., 2014a,b). Unlike sites 1 and 2, the site 3 is exposed to particles from a railroad located near to the site and was also in direct contact with sewage, which means that this site was under greater human impact. Because the direction of the prevailing winds was northeastern, the mangroves located in Santa Cruz (site 4) and Conceição da Barra (site 5), which lie north of the particulate iron emission source, were considered reference areas for this study. Six samples of atmospheric suspended particles were collected in pre-cleanes petri dishes. Sample site for atmospheric particulate is located between the Harbor of Tubarão and site 2. Six samples were collected along 4 days at a height of about 20 m (building balconies), avoiding the collection of particles arising from soil resuspension. Particulate matter was then transferred to pre-cleaned plastic tubes, caped and stored until analysis. Further details of study sites are found in Arrivabene et al. (2014).

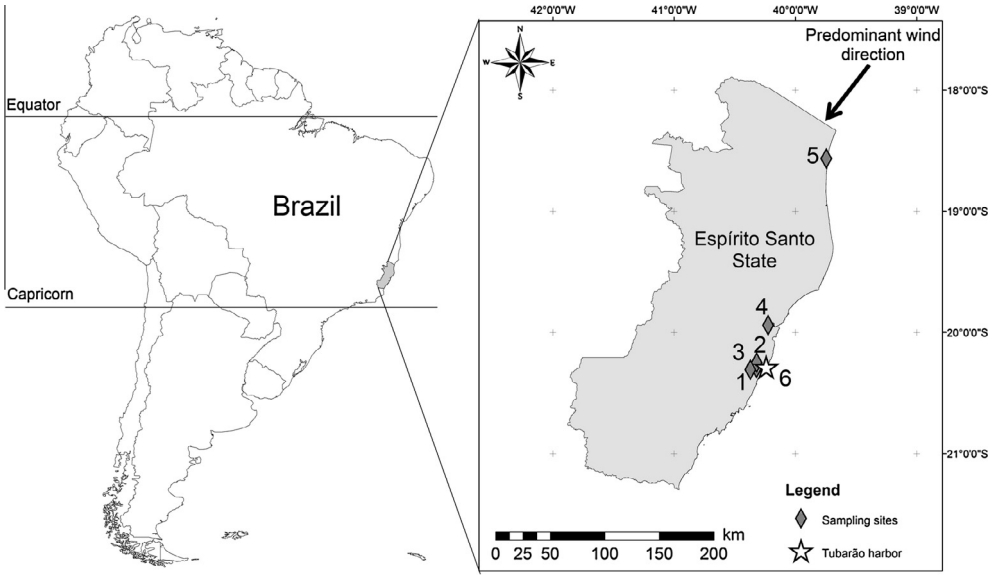

Fig. 1. Map of Brazil and State of Espírito Santo with the location of the sampling areas (1 = Passage Channel, 2 = Lameirão Island, 3 = Cariacica, 4 = Aracruz, 5 = Conceição da Barra, and 6 = Tubarão Harbor).

2.2. Botanical samples

Plant samples consisted of fully expanded leaves from the third node below the apical bud and were collected from adult plants of *A. schaueriana* (Acanthaceae), *L. racemosa* (Combretaceae) and *R. mangle* (Rhizophoraceae). Exsiccates of studied species were deposited at the VIES herbarium at the Federal University of Espírito Santo, identified with numbers 19649, 19650, and 19651. The samples were collected in late February 2010, after two months with little rain recorded (Supplementary data, Fig. S1).

Table 1
Chemical analysis of atmospheric particulate material, sampled between the Harbor of Tubarão (main particulate iron source in Vitória area) and site 2. Values are reported as mean ± standard deviation.

Element	Values ($\mu g\ g^{-1}$)
B	<LOD
Al	18 950 ± 1305
V	92 ± 7
Cr	173 ± 7
Mn	2908 ± 72
Fe	149 386 ± 3916
Ni	100 ± 3
Cu	57.2 ± 0.7
Zn	1471 ± 764
As	8.6 ± 0.9
Se	<LOD
Rb	21 ± 3
Sr	219 ± 13
Ag	<LOD
Cd	<LOD
Hg	<LOD
Pb	52 ± 4

<LOD (below detection limit). LODs: B (17.70 $\mu g\ g^{-1}$); Se (0.46 $\mu g\ g^{-1}$), Ag (0.13 $\mu g\ g^{-1}$), Cd (0.14 $\mu g\ g^{-1}$), and Hg (0.37 $\mu g\ g^{-1}$).

2.3. Chemical analysis of atmospheric particulate matter

Samples were digested in according to Chappaz et al. (2012) with less modifications, using nitric and chloride acid (ultra pure, sub boiling grade) in pre-cleaned Teflon tubes (Sevillenex) at constant temperature for 24 h. Controls were prepared using the same protocol without sample (only reagents). Digested samples were stored at 4 °C until analysis. The analysis of metals and metalloids (B, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Hg and Pb) was performed with a Mass Spectrometer Inductively Coupled Plasma (ICP–MS), Agilent 7500cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE).

2.4. Chemical analysis of settled particulate matter on the leaf surface and leaf micromorphology

Samples of two leaves of each species were dehydrated in an oven at 60 °C, fixed in copper plates with the aid of a carbon ribbon and coated with gold to a thickness of approximately 20 nm in a sputter coater (Emitech K550X, Kent, UK). The third median of the interveinal region was analyzed with a low-vacuum scanning electron microscope (Zeiss EVO 40, Jena, Germany) around 5 Pa. The percentage of chemical elements contained in each particle was determined through energy dispersive X-ray spectrometry (EDS) point analysis from an average of 15 tests per sample. During analysis, the accelerating voltage was 20 kV, data collection time 50 s, working distance approx. 12 mm, and magnification up to 800×.

2.5. Physical and chemical analysis of sediment, interstitial water and leaves

Three sediment samples (approx. 20 cm depth from the sediment–water interface) were collected in pre-cleaned plastic

Table 2
Inorganic fractions, textural classification and organic matter (OM) of sediment and salinity and pH of interstitial water in each mangrove area studied, iron concentration in sediment, interstitial water and leaves, and iron bioconcentration factor (BCF) in *A. schaueriana, L. racemosa*, and *R. mangle* from each mangrove area studied. The values represent the average ± standard deviation. The significant differences between the study areas are indicated with different letters within the same column. For iron concentrations, significant differences within species between different areas of study are indicated with lowercase letters, and the significant differences between the three species in the same area of study are indicated with capital letters (Kruskal–Wallis test, $P < 0.05$).

Sites	Sediment						Interstitial water	
	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture classification	OM ($g\ dm^{-3}$)	Salinity (psu)	pH
Site 1	54 ± 7 a	24.6 ± 5.4 ab	13 ± 4 bc	8 ± 2 bc	Sandy loam	69 ± 10 abc	25.2 ± 0.4 b	4.2 ± 0.2 c
Site 2	40 ± 2 ab	6.5 ± 2.0 bc	46 ± 5 ab	7 ± 1 bc	Sandy loam	168 ± 24 a	22.8 ± 2.4 b	4.4 ± 0.2 bc
Site 3	25 ± 15 bc	7.8 ± 5.1 bc	43 ± 13 ab	25 ± 6 ab	Loam	63 ± 5 bc	29.9 ± 3.3 ab	6.0 ± 0.4 abc
Site 4	6 ± 2 c	1.7 ± 0.5 c	51 ± 6 a	41 ± 2 a	Silty clay	111 ± 8 ab	34.7 ± 4.8 a	6.7 ± 0.4 ab
Site 5	32 ± 6 abc	56.9 ± 4.3 a	6 ± 1 c	5 ± 1 c	Sand	7 ± 3 c	46.8 ± 15.4 a	8.1 ± 0.2 a

	Fe sediment ($\mu g\ g^{-1}$ dry mass)	Fe Interstitial water ($\mu g\ L^{-1}$)	Fe Leaves ($\mu g\ g^{-1}$ dry mass)	Fe BCF
Site 1				
A. schaueriana	21 434 ± 206 ab AB	7933.8 ± 663.7 abc A	162.2 ± 4.8 abc AB	0.010 ± 0.00019 abc AB
L. racemosa	17 124 ± 801 bc B	1626.3 ± 81.5 c B	200.3 ± 9.0 ab A	0.010 ± 0.00009 ab A
R. mangle	25 355 ± 690 a A	2020.1 ± 48.9 c AB	113.0 ± 18.7 ab B	0.004 ± 0.00067 ab B
Site 2				
A. schaueriana	23 810 ± 713 a A	7308.8 ± 64.5 bc AB	104.3 ± 0.7 bc A	0.004 ± 0.00011 bc A
L. racemosa	22 349 ± 521 a B	5763.6 ± 347.4 abc B	107.4 ± 7.5 bc A	0.005 ± 0.00042 bc A
R. mangle	21 935 ± 491 a B	8164.4 ± 0.1 abc A	71.2 ± 2.2 bc B	0.003 ± 0.00017 bc B
Site 3				
A. schaueriana	21 380 ± 243 ab A	2012.6 ± 15.4 c B	332.7 ± 6.9 a A	0.020 ± 0.00048 a A
L. racemosa	21 015 ± 176 ab A	2780.0 ± 107.5 bc AB	300.9 ± 6.0 a AB	0.010 ± 0.00034 a AB
R. mangle	21 469 ± 2630 a A	7242.5 ± 460.0 bc A	91.2 ± 1.3 abc B	0.004 ± 0.00045 ab B
Site 4				
A. schaueriana	19 220 ± 250 b B	12863.6 ± 445.0 ab AB	282.9 ± 15.9 ab A	0.010 ± 0.00098 ab A
L. racemosa	20 719 ± 729 abc AB	15867.1 ± 2040.1 ab A	180.1 ± 16.2 abc AB	0.010 ± 0.00050 abc AB
R. mangle	22 339 ± 1106 a A	10841.8 ± 784.3 ab B	131.7 ± 15.6 a B	0.010 ± 0.00081 a B
Site 5				
A. schaueriana	24 099 ± 386 a A	16745.3 ± 878.4 a AB	81.4 ± 1.2 c A	0.003 ± 0.00002 c A
L. racemosa	15 825 ± 403 c B	56781.6 ± 3161.2 a A	26.8 ± 2.7 c B	0.002 ± 0.00016 c B
R. mangle	23 072 ± 1583 a A	11520.3 ± 65.1 a B	64.9 ± 5.2 c AB	0.003 ± 0.00037 c AB

containers during low tide, close to the rhizosphere of the species. Samples of leaves were collected from four individuals of *A. schaueriana*, *L. racemosa* and *R. mangle* from each site and washed with distilled water. The leaf samples were dried at 37 °C to constant weight, homogenized with a mortar and pestle and stored in sealed container until analysis. Interstitial water was extracted from sediment samples by centrifugation (3000 rpm; 40 min) and the supernatant was filtered using 0.45 μm nitrocellulose filters (Millipore, USA). Interstitial water was submitted for analysis of pH and salinity and further acidified with ultrapure HNO_3 (sub-boiling grade), and stored at 4 °C until multi-element analysis. The sediment was subsequently air-dried and sieved through nylon mesh (63 μm) with an acrylic frame to avoid the transfer of metals. The granulometry, texture classification and organic matter content (OM) of sediment, pH and salinity of interstitial water (measured through the electrical conductivity – EC) were performed in the Agronomic Analysis and Consulting Lab – Fullin (Linhares, Espírito Santo, Brazil) according to methods described by Arrivabene et al. (2014).

Dried sediment and leaf samples (0.5 g each) were digested with nitric and hydrochloric acids (ultrapure, sub-boiling grade) in pre-cleaned quartz close-vessel using a microwave oven (Anton Paar Multiwave 3000, Austria). Controls were prepared using the same protocol without sample (only reagents). All glassware, plastic bottles/containers and ICP–MS (Mass Spectrometer Inductively Coupled Plasma) PTFE probes and pipes were acid-washed. The iron concentration in sediment, interstitial water and leaves was analyzed in triplicate using a mass spectrometer with induced coupled plasma (ICP–MS) (Agilent 7500cx, USA), following the method described by USEPA (2009) (reference material EnviroMAT Sewage Sludge BE-1 and NIT1547 for sediment and leaves, respectively). All of the samples showed a recovery of at least 80%, as recommended by the USEPA. To determine the degree of iron accumulation in each plant species, the bio-concentration factor (BCF) was

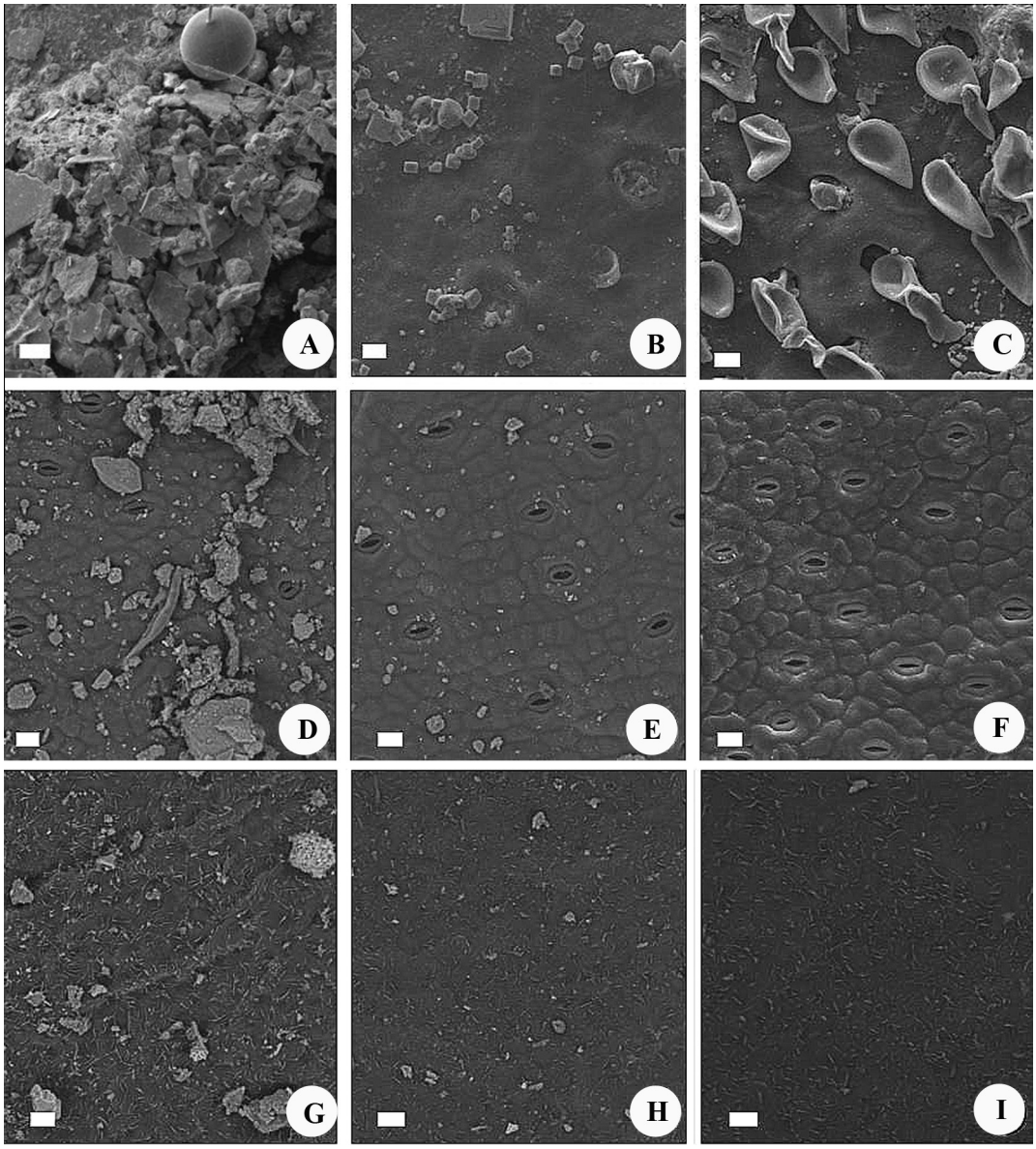

Fig. 2. Settled particulate material in leaf surface detected through scanning electron microscopy. *A. schaueriana* (A–C) *L. racemosa* (D–F), and *R. mangle* (G–I) show a high deposit in site 3 (A, D, and G), an intermediate deposit in sites 1 and 2 (B, E, and H), and a low deposit in sites 4 and 5 (C, F, and I). Bars = 50 μm (A) and 20 μm (B–I).

calculated as the ratio of the iron concentration in leaf tissue to the iron concentration in sediment.

2.6. Histochemical detection of iron in leaves

Leaf samples were washed in distilled water to remove any particulate deposited on the surface. The median cross sections of fresh leaves, obtained by freehand using a razor blade, were incubated in Perl's reagent (1% potassium ferrocyanide and 2% hydrochloric acid; Bancroft et al., 2008). After 24 h of incubation, the sections were washed in water and mounted on a slide with a coverslip. Positive reaction is indicated by Prussian blue color. The slides were analyzed using a light microscope (Nikon E200, Tokyo, Japan) and results were documented using photomicrographs.

2.7. Statistical analysis

Data are reported as mean ± standard deviation. The statistical package InfoStat (Di Rienzo et al., 2010) was used for the analysis. All data were tested for normal distribution and, since data were not normally distributed, Kruskal–Wallis analysis of variance was applied with significance $P < 0.05$.

3. Results and discussion

3.1. Particulate matter (PM) and leaf micromorphology

Chemical analysis of atmospheric particulate matter, taken close to the particulate iron source (Harbor of Tubarão), revealed that Fe presented the highest concentration among studied elements, but also high levels of Al, with less amount of Mn, Zn, Sr, Cr, Ni, V, Cu, Pb, Rb and As were found (Table 1). Regarding the particulate matter (PM) settled on leaf surfaces, the particle size ranged from 8 μm to 130 μm. The chemical analysis by EDS showed the predominance of Si, Al, Mg, Fe, and, to a lesser extent, Ca and Cl (both of which were from soil resuspension). We also detected iron ore particles (composed of Fe and Oxygen), salt (consisting of Na and Cl), and organic particles (containing C and O). Despite the absence of a quantitative study of the settled PM, leaf samples from site 3 showed a significant accumulation of particles (Fig. 2), particularly PMFe. Lower loads of settled material were found on leaves from sites 4 and 5 (Fig. 2), where PMFe were rarely observed (data not shown).

It was possible to observe a higher deposit of particulate matter in plants from areas that are more exposed to this pollutant. When we compare these data with the data of total suspended particulates (TSP) from site 1–3 and 4 (data obtained from the State Institute for the Environment (IEMA), the National Institute of Meteorology – INMET, and the Automatic Weather Station of Company Fibria – Supplementary data, Fig. S2), we can observe a positive correlation between the amount of particulate matter on the leaf surface within the study areas and the monitored TSP data (which followed the order of values: site 3 > sites 1 and 2 > site 4). This correlation also explains the lower deposition of particles on leaf samples collected from sites 4 and 5. Although there are no monitoring stations in site 5, the pollutant levels are considered minimal, mainly because of the lack of industrial activity in this area.

PM accumulation on the leaf surface differed among the analyzed species, with higher levels of PM in *A. schaueriana*, followed by *L. racemosa* and *R. mangle* (Fig. 2), which appeared to be related to the morphological differences in the leaf surfaces (Fig. 3). In *R. mangle*, the glabrous nature of the leaves and the waxy cuticle could be responsible for the lower deposit of particulate matter, whereas the large amount of salt glands in *A. schaueriana*, which release a saline solution to the leaf surface, could result in a greater adhesion of particles. *L. racemosa*, a species that has sparse salt glands, showed an intermediate level of PM accumulation compared to the other two species. These results are in agreement with other studies that also attributed the particle adhesion to the characteristics of the epidermis and the cuticle (Naidoo and Chirkoot, 2004; Naidoo and Naidoo, 2005; Prusty et al., 2005; Silva et al., 2006; Kuki et al., 2008).

3.2. Leaf morphoanatomy

In all mangrove areas studied, the leaf samples did not show visual damage on the leaves, such as chlorosis and necrosis, or anatomical changes, such as collapsed cells and recently divided. Although these injuries are commonly reported for plants sensitive to particulate materials (Dixit, 1988; Ali, 1993; Lopes et al., 2000; Silva et al., 2006), field observations evidenced that particles remain on the leaf surface for a short time, and are easily washed

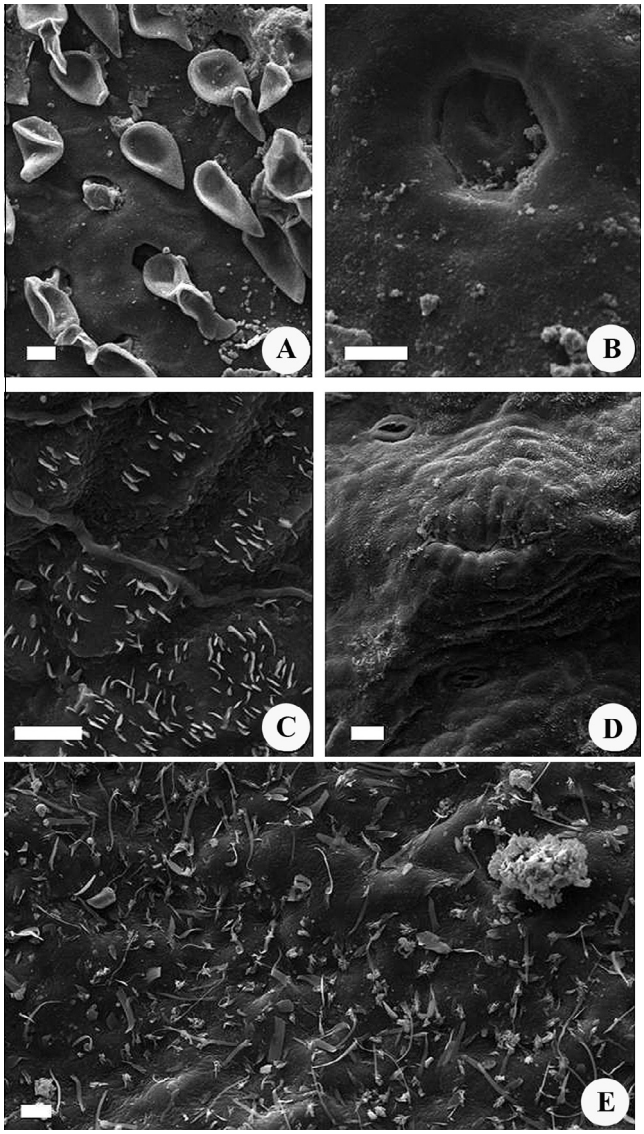

Fig. 3. Aspect of the adaxial leaf surface. (A–B) *A. schaueriana.* A: General appearance, showing salt trichomes. B: Detail of salt gland. (C–D) *L. racemosa.* C: General appearance, showing epicuticular wax. D: Detail of the salt gland. (E) *R. mangle*, showing abundant epicuticular wax. Bars = 20 μm (A, B and D), 10 μm (C and E).

out by small rainfall events. This may contribute to the absence of symptoms because the effect of this pollutant also depends on the extent of exposure (Grantz et al., 2003).

In future works, the gas exchange and transpiration of these species will be assessed to determine if they are influenced by pollutants, considering that the particles have a diameter that is sufficiently small to block the stomatal pore, as observed for *L. racemosa* (Fig. 2E).

3.3. Physical and chemical analysis of sediment, interstitial water and leaves

The evaluated variables showed a wide variation between the mangrove areas. Coarsest fractions were found at site 5, while finest fractions were found at site 4. The salinity was lowest at site 2 and highest at site 5. The pH ranged from acidic at sites 1 and 2, to basic at site 5. The organic matter content was lowest at site 5 and highest at site 2 (Table 1).

The iron concentration in the sediment sampled close to *A. schaueriana* was higher at sites 2 and 5, while for *L. racemosa* was higher at site 2, with no significant differences for *R. mangle* (Table 1). These values did not appear to show any correlation with the other physico-chemical parameters evaluated, including organic matter content, as was previously observed by Aragon et al. (1986). In general, the content of organic matter is correlated with the accumulation of heavy metals in sediments, due to high adsorption capacity and the activity of microorganism that change the microenvironment, causing a lower oxidation state (Lacerda and Abrão, 1984; Zhou et al., 2010). However, unlike other metals, Fe has been found to be less sensitive to these conditions (Aragon et al., 1986).

The three species studied showed highest iron concentrations at site 5, whereas the lowest concentrations were found at site 1 for *L. racemosa* and *R. mangle*, but at site 3 for *A. schaueriana* (Table 1). The highest concentrations at site 5 likely are due to the sandy characteristics of the sediment, which contributes to the availability of this metal (Machado et al., 2005). In contrast, the lowest concentrations at sites 1–3 indicates that an even smaller proportion would be bioavailable. In swamp soils, where domestic sewage is released, most of the added metals are adsorbed onto cation exchange sites or precipitated as insoluble sulfide (Tam and Wong, 1993, 1995) which explains the low bioavailability of iron in these areas. The iron concentration in interstitial water was very low compared to the concentration in sediment, which indicates that most of iron was strongly bound to the sediment fraction (Silva et al., 1990; Souza et al., 2014a,b).

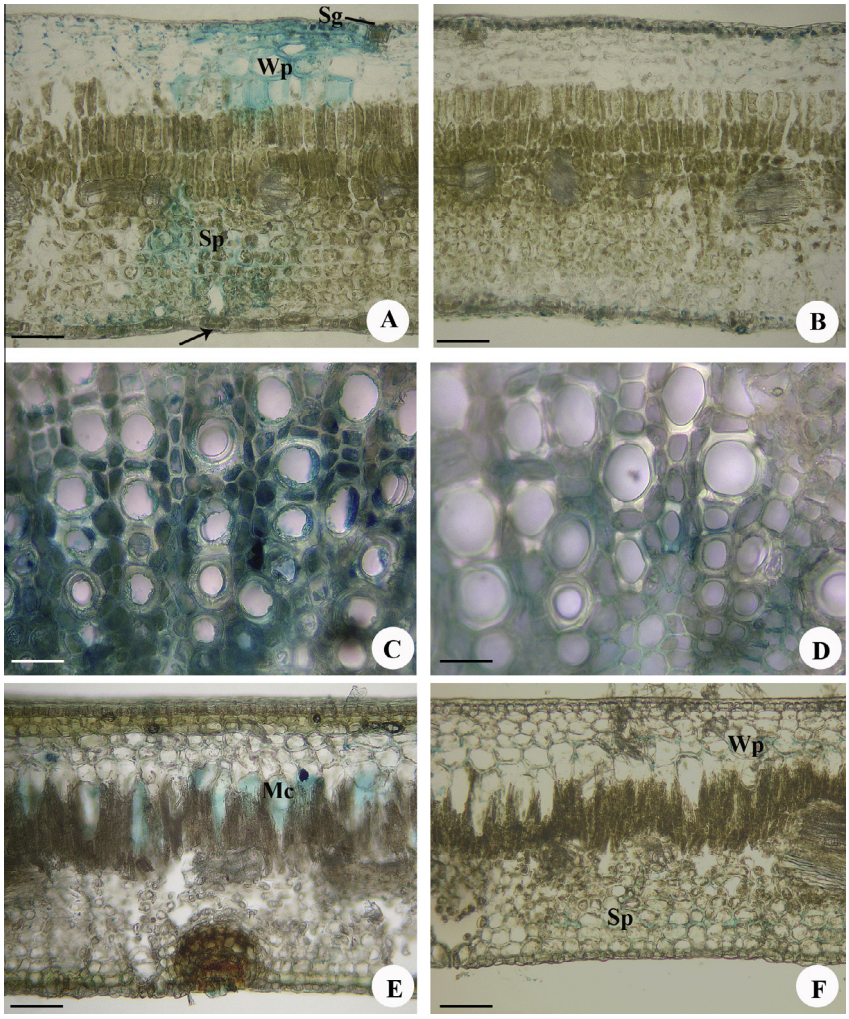

Fig. 4. Histochemical detection of iron in cross sections of leaves through the Prussian blue test. The blue color indicates a positive reaction to the test. (A–B) Internerval region of *A. schaueriana* leaves from site 4. (C–D) Midrib region of *L. racemosa* leaves from site 3 (C) and 5 (D). (E–F) Internerval region of *R. mangle* leaves from site 5 (E) and 2 (F). (Arrow = stomata, sg = salt gland, mc = mucilage cells, sp = spongy parenchyma, ws = water-storage parenchyma). Bars = 50 (A, B, E, F), 10 μm (C and D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

All studied species showed lower iron levels in leaves from site 5 (Table 1). In general, the iron concentration in the leaf tissue of the species did not reflect the concentration of this element in the sediment nor in the interstitial water of the sites. On sandy sediments, such as those found in site 5, the majority of metals, particularly Fe, are potentially bioavailable (Machado et al., 2005). However, despite the higher concentration of this element in interstitial water, Fe and Mn become insoluble oxides under oxidative conditions (Shaw et al., 1990), which explains the low foliar accumulation in this area. Moreover, the iron absorption is extremely low under alkaline conditions (Epstein and Bloom, 2006), as is found at site 5 (Table 1).

In relation to the highest levels of foliar iron, these values were found in *A. schaueriana* and *L. racemosa* at site 3, and in *R. mangle* at site 4 (Table 1), likely due to the pH conditions, since values between 5.5 and 7.0 are more favorable for nutrients absorption (Epstein and Bloom, 2006). For *A. schaueriana* and *L. racemosa*, is likely that foliar absorption is at least partially responsible for the high levels of iron found in the samples from site 3 as compared with *R. mangle*. Iron oxide has a low solubility (Fan et al., 2006); however, in the Fe^{3+} state, it can be absorbed by the leaves (Epstein and Bloom, 2006).

Overall, *A. schaueriana* and *L. racemosa* exhibited a higher iron concentration in leaf tissue compared to that found in *R. mangle*. Salt-secreting species, such as *A. schaueriana* and *L. racemosa* have higher salt concentrations in xylem sap than species that lack salt glands (Scholander et al., 1962) due to the ultrafiltration of salts present in roots (Werner and Stelzer, 1990). This mechanism also appears to be responsible for the higher concentration of metal in tissues of salt-secreting species, as has been described in previous studies (Lacerda et al., 1985; Cuzzuol and Campos, 2001; Sarangi et al., 2002; Bernini et al., 2006). Moreover, the effects of iron plaque are different among mangrove species that co-exist in the same environmental conditions (Machado et al., 2005), which can result in a differential absorption of metals.

All of the three species evaluated showed a low iron bioconcentration factor in leaf tissue, as have been reported for mangrove species (Sarangi et al., 2002; Bernini et al., 2006; Nath et al., 2014; Souza et al., 2014a,b). The reductive redox environment and the high content of organic matter decrease the bioavailability of this element. In addition, retention of this metal in root (Silva et al., 1990; Machado et al., 2005) suppresses its translocation to the leaves.

3.4. Histochemical detection of iron

All three studied species showed a positive reaction to the Prussian blue test. However, positive reaction did not always reflect the leaf iron contents (Fig. 4 and Table 1).

Some cross sections of *A. schaueriana* leaves from site 4 were found to be positive through the Prussian blue test on the aquiferous and spongy parenchyma cells, including those surrounding the substomatal chamber, and salt gland trichomes (Fig. 4A). Other sections did not show positive reaction (Fig. 4B).

In *L. racemosa*, cross sections of the midrib showed a positive Prussian blue reaction in the main vein elements and parenchyma cells of xylem throughout five studied sites. A higher intensity was observed in xylem cells of samples collected from site 3 (Fig. 4C), while lower intensity was observed in xylem cells of samples from site 5 (Fig. 4D).

In *R. mangle,* iron accumulation in mucilage cells of samples from site 5 was observed (Fig. 4E), while samples from site 2 exhibited a positive reaction in cell middle lamella of water-storage parenchyma and spongy parenchyma (Fig. 4F).

The histochemical test using Prussian blue proved to be a satisfactory method to detect iron in plant tissue, however showed to be an unsuitable method for the assessment of iron concentration in leaves. Although Silva et al. (2006) have founded correlation between intensity of reaction to the Prussian blue test and iron content in leaves of three species of restinga, our results did not show this correlation for *A. schaueriana* and *R. mangle*, once individuals from sites 4 and 5, which contained lower iron leaf content, exhibited more intense reaction to the test. The lack of correlation between the histochemical test and iron concentration emphasizes the importance of this test as a qualitative indicator of iron location (see Table 2 and Fig. 4).

4. Conclusions

Using the test methods described, *A. schaueriana, L. racemosa*, and *R. mangle* were found to be tolerant to iron particles, since no evident morphological or structural damage was found, even on leaves that were highly exposed to this pollutant.

The highest concentration of iron in leaves of *A. schaueriana* and *L. racemosa* was found by chemical analysis in samples collected from sites with highest levels of particulate iron, which suggests the foliar absorption of this element.

A histochemical test using Prussian blue proved to be an unsuitable method for the assessment of the iron bioaccumulation in leaves of *A. schaueriana* and *R. mangle*. Conversely, scanning electron microscopy coupled to X-ray diffraction (EDS) rendered a good method for evaluating iron on leaves surfaces.

Acknowledgments

The authors thank the Support Fund for Science and Technology of the City of Vitória (FACITEC) for the fellowship provided to the first author (Proc. 3310/2009), and to the National Council of Technological and Scientific Development (CNPq) and to the São Paulo Research Foundation (FAPESP) for the fellowship provided to I.C. Souza (Proc. 2014/04832-3).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.01.011.

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