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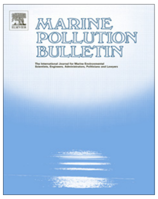

Changes in bioaccumulation and translocation patterns between root and leafs of *Avicennia schaueriana* as adaptive response to different levels of metals in mangrove system

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ABSTRACT

Espírito Santo estuaries (Brazil) are impacted by industrial activities, resulting in contamination of water and sediments. This raise questions on biological uptake, storage and consequences of metal contamination to mangrove plants. The goal of this work was evaluating accumulation and translocation of metals from sediment to roots and leaves of *Avicennia schaueriana*, growing in areas with different degrees of contamination, correlating bioaccumulation with changes in its root anatomy. Highest bioconcentration factors (BCFs) were observed in plants growing in less polluted areas. Conversely, highest translocation factors were found in plants from highest polluted area, evidencing an adaptive response of *A. schaueriana* to less favourable conditions. Namely, the absorption of metals by roots is diminished when facing highest levels of metals in the environment; alternatively, plants seem to enhance the translocation to diminish the concentration of toxic metals in roots. Root also responded to highly polluted scenarios with modifications of its anatomy.

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1. Introduction

Mangrove ecosystems, in tropical and subtropical intertidal zones, play a key role in maintaining the coastal ecological balance and species diversity (Du et al., 2013). However, industrialisation in many countries has resulted in coastal habitats being subject to metallic contaminant input (De Wolf and Rashid, 2008).

The anthropogenic contamination and bioaccumulation of metals in estuarine ecosystems are well documented (Borja et al., 2012; Maiti and Chowdhury, 2013) due to inadequate efforts to control dredging and discharge of wastewater (Nayar et al., 2004; Wu et al., 2014). This degree of contamination has resulted from industrial and urban expansion thus potentially contaminating the estuarine biotic and abiotic components (Bayen, 2012).

The bioavailability and toxicity of metals in sediment are associated with pH, salinity, redox potential, mineral and organic content, resident biota, and the synergistic interactions between these variables (Martin et al., 2006; Morrissey and Guerinot, 2009). Thus, the accumulation of metals in plant tissues is determined by the bioavailability of these elements in the sediment and the plant efficiency to absorb and translocate it in roots and vascular tissues (Pilon-Smit, 2005; Qian et al., 2012). Metal accumulation may produce different responses in plants, as the root anatomy appears sensitive to physical and chemical substratum changes, including contamination levels (Redjala et al., 2011; Stañová et al., 2012). These can induce adaptive changes, for example, root diameter and internal tissues thickness such as aerenchyma and vascular tissues (Vaculík et al., 2012).

Avicennia schaueriana Stapf & Leechm. ex Moldenke is a mangrove tree with a geographical distribution from south Brazil to west Venezuela, including some islands of Central America (Turks and Caicos Islands, Trinidad and Tobago and Lesser Antilles), occurring between 32°W and 51°W longitude (Spalding

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et al., 2010). Due to restricted distribution of this species, previous studies are scarce, and those that evaluated metals have focused on assessment of nutritional plant status (Lacerda et al., 1985; Cuzzuol and Campos, 2001; Bernini et al., 2006; Cuzzuol and Rocha, 2012) or the formation of iron plaque (Machado et al., 2005).

The study of metal accumulation in biotic and abiotic compartments of mangrove areas is needed to assess the conservation status of coastal areas. In addition, understanding metal absorption and translocation is necessary given that some of these elements can be highly toxic when accumulated in cells (Valko et al., 2005). Thus, the present study aimed to determine the process of metal accumulation in sediment, interstitial water, roots and leaves of *A. schaueriana* in relation to uptake, root anatomy and translocation to aerial parts of the plant in four sites of two estuaries of Espírito Santo (Brazil) with different degrees of pollution. To our knowledge, there are no previous studies of toxic metals present in sediments and their uptake to biological compartments of *A. schaueriana* as well as their influence on the root anatomy.

2. Materials and methods

This study was conducted in two neotropical estuaries located in the State of Espírito Santo, Brazil: Vitória Bay and Santa Cruz, which were selected because they represent areas affected by different pollution sources and marine processes (Fig. 1). Vitória Bay is an estuarine complex formed by five rivers that are sites of several environmental impacts caused by harbours, metallurgical activities, steel industry and iron mining but little urban infrastructure. Three sites were selected in this estuary: Santa Maria (S 20°14′31.5″ and W 40°19′84.7″), a wider area with greater influence of continental and marine waters, Serra (S 20°14′19.6″ and W 40°18′48.7″), an area under higher impact of the metallurgical activities, textile, paint industry and sanitary effluents, and Lameirão (S 20°14′60.6″ and W 40°18′68.6″), a legally protected mangrove conservation area. The second estuary, Santa Cruz (S 19°56′26.2″ and W 40°12′87″) is formed by two rivers and has an extensive and well-preserved mangrove area (Souza et al., 2013), without significant industries that could introduce metal contamination. Both studied areas were geo-referenced during field sampling, using a portable GPS 368 (Garmin Vista, USA).

2.1. Water, sediment and plant sampling

Sediment and roots samples were collected from all sites during two seasons (winter-2009 and summer-2010). Water dissolved oxygen (DO) and conductivity were determined *in situ* in the superficial water using a multiparametric probe (YSI model 85, USA). Sediment samples were taken simultaneously with plant sampling (see Monferrán et al., 2011, 2012). Sediment samples (approx. 20 cm depth from the sediment–water interface), close to areas of *A. schaueriana* rhizospheres, were collected and stored in plastic containers (1 L) without headspace, for metal analyses. In the laboratory, interstitial water was extracted from sediment samples by centrifugation (3000 rpm; 40 min) and the supernatant was filtered using 0.45 μm nitrocellulose filters, acidified with ultrapure HNO_3 (sub-boiling) and stored at 4 °C until analysis. The sediment was subsequently air-dried and sieved through nylon mesh (63 μm) with an acrylic frame to avoid the transfer of metals.

Samples of absorption roots, pneumatophores and leaves were collected from five individuals of *A. schaueriana* from each site and were immediately washed with distilled water. The samples were dried at 37 °C to constant dry weight, homogenised with a mortar and pestle and stored in sealed container until analysis. Sediment and plant 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). Sediment organic matter

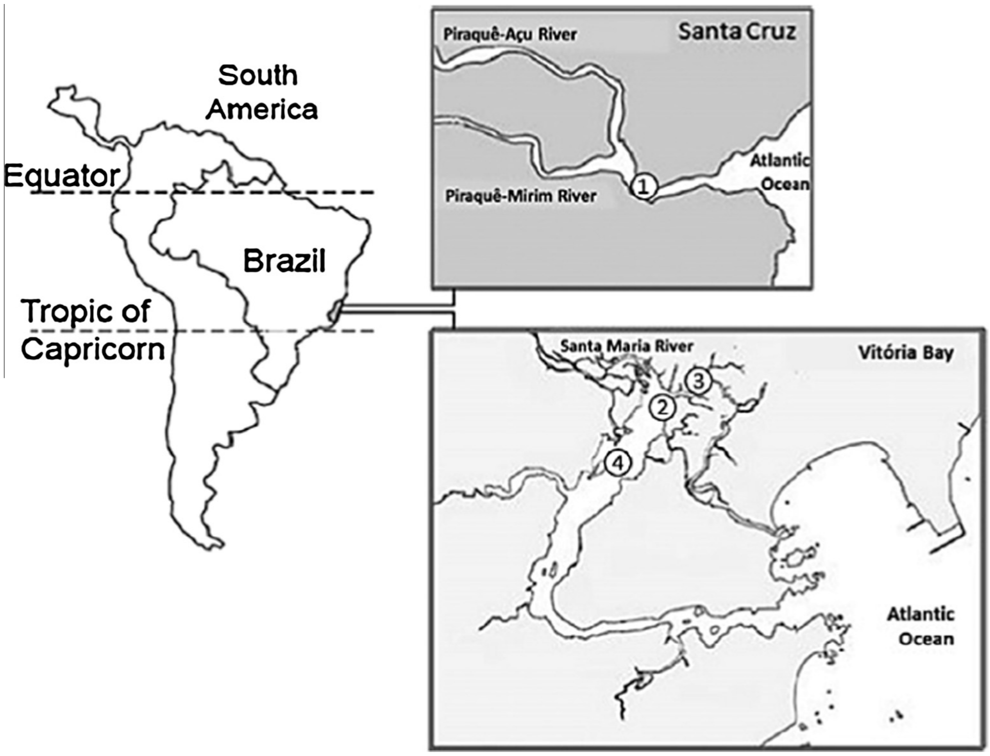

Fig. 1. Localization of the State of Espírito Santo (South America, Brazil), showing sampling sites. 1 – Santa Cruz (S 19°56′26.2″; W 40° 12′87″); 2 – Lameirão (S 20°14′60.6″; W 40°18′68.6″); 3 – Serra (S 20°14′19.6″; W 40°18′48.7″); and 4 – Santa Maria (S 20°14′31.5″; W 40°19′84.7″).

in the sediment was determined using the Walkey and Black (1934) method. All glassware, plastic bottles/containers and ICP-MS (Mass Spectrometer Inductively Coupled Plasma) PTFE probes and pipes were acid-washed.

2.2. Multielement analyses

Metals and metalloids (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb) in both digested abiotic and biotic samples were analysed by ICP-MS, Agilent 7500 cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE) in triplicate; the repeatability of ICP-MS measurements was generally $\geqslant 97\%$. Quality assurance (QA) and analytical quality control (AQC) were done using certified reference materials (CRMs): NIST SRM 1547 (peach leaves) and NIST 1573a (sediment sludge). Recoveries from CRMs were 87.00 ± 14.30% and 95.90 ± 15.46%, respectively. CRMs were selected according to the respective elements; spiked samples were also prepared. Variable amounts of mixed standard solutions, containing all the elements analysed in the samples, were added to 0.3–0.5 g of dried roots, leaves or sediment samples, doubling the starting concentration for each element. The remainder of the procedure was the same as used for non-spiked samples. The average recovery of these assays was 95.17 ± 12.62%.

The bioconcentration factor (BCF) was calculated to compare the levels of metals accumulated in roots and sediments (BCFs = [ ] metal in root/[ ] metal in sediment ratio). The translocation factor (Tf) evaluated the metal concentrations in leaves and its correlation to values found in roots ([ ] metal in leaf/[ ] metal in root ratio) (Ali et al., 2013).

2.3. Analysis of root anatomy

Roots samples for anatomical analyses were collected from all sites during winter (2009), considering that seasonal variations did not influence in root anatomy (Souza et al., 2014a,b). The cortex thickness, air gap area, vascular cylinder diameter and root diameter of pneumatophores and absorption roots were measured and the cortex/vascular cylinder ratio was calculated. In pneumatophores the periderm thickness was also measured. The root samples were then dehydrated in an ethanol series, embedded in historesin (Leica®, Germany), cross sectioned (8–10 μm in thickness) with a rotatory microtome and stained with 0.05% toluidine blue, pH 4.7 (O'Brien et al., 1964). The measurements were performed using the Nikon NIS-Elements software (Tokyo, Japan) and the photomicrographs were obtained using a Nikon Eclipse 50i microscope (Tokyo, Japan).

2.4. Statistical analysis

Data were reported as mean ± standard deviation. The statistical packages, STATISTICA 7.1 from StatSoft Inc. (2005) and Infostat (Di Rienzo et al., 2010) were used for the statistical analysis. All data were tested for normality. One-way analysis of variance (ANOVA) was applied to compare metal concentrations in interstitial water, sediment, root and leaf of *A. schaueriana* between the mangrove sites, followed by Tukey's post-test with significance $P < 0.05$. The Spearman rank (r_s) test was used to determine correlations within the biological, chemical and physical variables with significance $P < 0.05$.

The multivariate analysis was used to look for patterns and links between variables simultaneously and to look for relationships in biological variables according to differences in physical and chemical variables. As we have many parameters analysed within a sample, the use of both multivariate techniques and data reduction are almost mandatory to achieve satisfactory results. In the present work we have applied four multivariate techniques to evaluate both spatial and temporal variations in the estuarine areas: generalised procrustes analysis (GPA), principal components analysis (PCA), factor analysis (FA) and stepwise linear discriminant analysis (LDA).

GPA was used to see the spatial and temporal segregation, generating a consensus configuration of a group of datasets, matching interstitial water elemental data to the corresponding sediment and plant data (metals in root, leaves and anatomical responses). The PCA and FA are unsupervised analyzes that aims to eliminate overlapping data and to select the most representative forms of them from linear combinations original data variables. The PCA graphical representation shows which parameters are indications of each area segregation. We used the rotation of the axis defined by PCA producing a new groups of variables (varifactors), which are used for factor analysis (FA). Thus, the FA helps to reduce the data matrix, selecting those variables with high loadings, which can be further used for performing a supervised discrimination among areas by LDA. In stepwise LDA, a subset of variables is used to construct discriminant functions, which further enable distinguishing between studied areas, using a reduced dataset and obtaining 100% of correct classification. Thus, LDA was performed to verify differences between studied sites (spatial response), considering both global parameters and biological interaction (Arrivabene et al., 2014; Di Paola-Naranjo et al., 2011; Wunderlin et al., 2001). PCA were performed using SPSSv17 and PRIMERv6. Given that all the variables have had different units, these were standardised and normalised using the Brodgar formula before plotting the PCA (García-Alonso et al., 2011).

3. Results and discussion

3.1. Environmental physical and chemical variables

In both seasons, highest levels of conductivity and dissolved oxygen were found in Santa Cruz, while highest organic matter levels were found in three sites of Vitória Bay (Santa Maria, Serra and Lameirão) (Table 1). In mangroves, organic matter in sediment leads to oxygen depletion due to microbial consumption (Holmer, 1999), hence explaining the lower oxygen levels and higher organic matter levels found in Vitória Bay, although anoxic conditions were not found in any of sampling areas and periods, as indicated by DO values >0.5 mg L^{-1} (redox scale according to McMahon, 2012).

There was a significant positive correlation between DO and conductivity ($r_s = 0.58$). The increase of oxygen levels results in

Table 1
Conductivity and dissolved oxygen in water and organic matter in sediment samples ($n = 9$) collected in estuaries of Santa Cruz, Lameirão, Serra and Santa Maria (Espirito Santo, Brazil) in summer and winter. Values are expressed as mean ± SD. Equal letter in the same line data do not differ significantly (Tukey test; $P < 0.05$).

	Organic matter (mg g^{-1} sediment)	Conductivity (mS)	Dissolved oxygen (mg L^{-1})
Santa Cruz			
Winter	15.1 ± 0.1^a	72.2 ± 0.2^f	4.81 ± 0.10^c
Summer	16.0 ± 0.1^a	72.4 ± 0.1^f	4.71 ± 0.10^c
Lameirão			
Winter	27.6 ± 0.5^d	50.4 ± 0.5^c	1.22 ± 0.10^b
Summer	31.3 ± 0.3^f	$54.6 \pm 0.5^{d,e}$	1.21 ± 0.10^b
Serra			
Winter	21.5 ± 0.5^b	47.4 ± 0.5^b	0.85 ± 0.05^a
Summer	29.5 ± 0.5^e	56.0 ± 1.0^e	0.74 ± 0.05^a
Santa Maria			
Winter	24.5 ± 0.5^c	40.4 ± 0.5^a	0.76 ± 0.05^a
Summer	25.5 ± 0.5^c	53.6 ± 0.5^d	0.80 ± 0.05^a

lower pH values and higher oxidation–reduction potential (redox potential) that strongly influences diagenesis, modifying the distribution of trace metals in different chemical fractions (Khalid et al., 1978). Additionally, conductivity and organic matter levels showed seasonal variation in Vitória Bay. During rainy periods, organic matter pool is expected to contain a higher proportion of humic-like components derived from riverine discharge and runoff, resulting in high dissolved organic carbon and higher conductivity values (Khalid et al., 1978; Romigh et al., 2006; Costa et al., 2011), as observed during summer results in this study (Table 1).

3.2. Metals

Estuary of Vitória showed higher concentration of metals–metalloids in interstitial water (Al, Cr, Cu, Se, Ag, Pb) when compared to Santa Cruz, with highest levels of most metals–metalloids observed in Santa Maria. In sediment, highest concentrations of B, Cr, Ni, Cu and Se were found at Serra in winter, while Santa Maria showed highest concentrations of Pb and Fe, and Santa Cruz exhibited maximum levels of As, Al and Mn (Table 2). Values of As in Serra (winter) and Santa Cruz (both seasons) exceeded the Interim Sediment Quality Guidelines (ISQG) of Canada (ratio marine As/estuary As > 7.24) (CCME, 1995). The presence of As poses a potential threat to marine ecosystems, where the contamination can affect the quality of overlying seawater and the dominating sedimentary and detritus feeding organisms (Elliott and McLusky, 2002; Christophoridis et al., 2009).

In roots, highest concentrations of B, Al, Cr, Mn, Fe and As were found in Santa Cruz (winter), while highest concentrations of Ni, Zn and Hg were found in Vitória (Ni in Lameirão during summer; Zn in Lameirão and Serra during winter; and Hg in Lameirão and Santa Maria during summer). For Pb, highest concentrations were found in Santa Cruz and Serra (winter). Despite the high levels of metals–metalloids in sediments of Vitória Bay, lowest values of BCFs were found in this estuary (except for Al) (Table 2), i.e. greater concentration of metals–metalloids in the sediment is not accompanied by more concentration in the root.

Chromium in roots was positively correlated with chromium in sediment (r_s 0.79). Due to its structural similarity with several elements, chromium uptake occurs and can affect nutrient and water conduction to aerial parts, leading to impacts in cellular metabolism, such as reduction in photosynthetic pigments, disruption of nutrient and water balance, and negative impact on carbon assimilation, respiration and enzyme activities (Singh et al., 2013). Furthermore, chromium and iron in tissues of *A. schaueriana* were strongly interrelated (r_s 0.92 in roots and 0.98 leaves) and, in sediment, chromium was also correlated with nickel (r_s 0.80) and iron (r_s 0.83). Vitória harbour is the main place in Brazil for exporting iron and iron pellets to the world, being the presence of metal manufacture industries documented (Souza et al., 2013). The local iron and/or steel production industry uses ferrochrome alloys, mainly iron, chrome and nickel (HSDB, 2000), which may explain the higher metal levels in this site (Table 2).

Highest concentrations of B, Al, Cr, Mn, Fe, Ni and As were found in leaf samples of Santa Cruz (winter), while highest concentrations of Cu, Zn, Se, Ag, Cd and Pb were found in leaf samples of Vitória (Lameirão, Serra or Santa Maria during winter). High values of translocation factor (Tf) were found in Vitória Bay, especially in Santa Maria, which exhibited high Tf for Cu, Zn, As, Se, Ag, Cd and Pb mainly in the winter. Vitória bay has more anthropogenic contamination that results in a higher bioavailability thus giving higher translocation factors here.

In general, most metals levels found in sediment did not cause similar values in plant tissues (roots and leaves) (Table 2), which may indicate that *A. schaueriana* has a mechanism to prevent the metal input. In mangrove plants, as in other plants inhabiting wetlands and aquatics environments, roots receive oxygen from the shoots for respiration and lose some oxygen to the soil, a process known as radial oxygen loss (Wegner, 2010). As a result, oxidation of rhizosphere precipitates compounds rich in iron, including the root surface. The presence of iron plaque can prevent the uptake and accumulation of metals and acts as a reservoir to immobilize trace metallic contaminants (Machado et al., 2005). In addition, iron plaque formation largely depends of iron levels in sediment (Pi et al., 2010), which may have influenced negative correlations between iron present in sediment and most of contaminants analysed in roots, such as Al (r_s −0.53), As (r_s −0.65), Cr (r_s −0.55), Mn (r_s −0.56), Zn (r_s −0.56), and Pb (r_s −0.51).

In conclusion, about the analysed factors, highest bioconcentration factors (BCFs) were observed in plants growing in less polluted areas, while highest translocation factors were found in plants from highest polluted area. Thus, the absorption of metals by roots is diminished when facing highest levels of metals in the environment, furthermore plants seems to enhance the translocation to reduce even more the concentration of toxic metals in roots (Monferran and Wunderlin, 2013).

3.3. Root anatomy

Absorption roots showed lowest values of air gap area, cortex/vascular cylinder ratio and cortex thickness at Santa Cruz, site with the highest dissolved oxygen levels (Table 3 and Fig. 2) compared to other sites. In pneumatophores, highest values of air gaps area and lowest values of cortex thickness were found at Santa Cruz, while the opposite was observed in Santa Maria, site with lowest dissolved oxygen levels (Table 1). Aerenchyma promotes internal aeration and is typical in species that tolerate waterlogged and hypoxic soil conditions (Wegner, 2010). Aerenchyma development, mainly in roots, is related to the amount of oxygen available in the environment (Bona and Morretes, 2003; Rodrigues and Estelita, 2004; Batista et al., 2008). This is confirmed in the present study where the cortex/cylinder vascular ratio of absorption roots, cortex thickness of pneumatophores and root diameter of pneumatophores were negatively correlated with the dissolved oxygen (r_s −0.80, −0.84 and −0.69, respectively). Thus, lower levels of oxygen may imply further development of aerenchyma through stimulation of cell division, and the consequent formation of thicker cortex or through stimulation of ethylene production, inducing the formation of aerenchyma through cell disintegration (Taiz and Zeiger, 2006).

3.4. Integration of analyses

Factor analysis (FA) was carried out first using the entire data matrix, including 11 anatomical and 42 chemicals parameters. In FA, four factors components (F1 to F4) account for 81% of accumulated variance. The first factor (F1), accounted for 35% of the variance and includes 20 parameters from four analyses (root anatomy and metals in sediment, root and leaf), explaining almost a third of observed variance; the second principal component (F2), with 18% of retained information, presented high loadings for six metals–metalloids in sediment; the third principal component (F3), with 18% of retained information, presented high loadings for three metals–metalloids in interstitial water and three metals–metalloids in leaves from *A. schaueriana*; while the fourth principal component (F4), with 10% of retained information, presented high loadings for three anatomical parameters. Altogether, F1 to F4 considered 35 out of 53 studied parameters.

The FA/PCA showed a differentiation between the sites (Fig. 4A) in terms of metal concentration (sediment and interstitial water) and physical and chemical parameters. Santa Cruz differs from others sites due to its conductivity, dissolved oxygen and

Table 2
Metal concentrations in interstitial water, sediment in Santa Cruz, Lameirão, Serra and Santa Maria and in *A. schaueriana* roots and leaves ($n = 9$ in each site) from these sites. Values are expressed as mean ± SD. NA: not analysed, IW: interstitial water (μg L^{-1}), SE: sediment (μg g^{-1} dry mass), RO: root (μg g^{-1} dry mass), LE: leaf (μg g^{-1} dry mass), BCF: bioconcentration factor, T_f: translocation factor.

	Metals													
	B	Al	Cr	Mn	Fe	Ni	Cu	Zn	As	Se	Ag	Cd	Hg	Pb
Santa Cruz														
Winter														
IW	*NA	3584 ± 170^{a,b}	<LOD^{a}	1412 ± 13^{e}	18853 ± 179^{d}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}
SE	<LOD^{a}	28116 ± 633^{e}	29.7 ± 0.7^{c}	75.7 ± 1.3^{d}	23539 ± 310^{c,d}	8.02 ± 0.13^{c}	3.79 ± 0.11^{a,b}	<LOD^{a}	11.19 ± 0.27^{d}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	6.58 ± 0.04^{b}
RO	10.0 ± 1.2^{c}	6869 ± 272^{f}	7.26 ± 0.33^{f}	74.8 ± 2.2^{d}	8938 ± 454^{d}	0.91 ± 0.11^{c}	<LOQ^{a}	9.7 ± 0.8^{b,c}	7.36 ± 0.44^{d}	<LOQ^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	1.69 ± 0.01^{e}
LE	27.23 ± 0.95^{e}	964.1 ± 72.3^{e}	0.75 ± 0.07^{f}	169.6 ± 14.1^{d}	665.3 ± 57.9^{f}	0.27 ± 0.05^{c}	0.78 ± 0.07^{b}	17.71 ± 1.50^{d}	0.72 ± 0.05^{e}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	0.32 ± 0.01^{d}
BCF	199.73 ± 23.44^{c}	0.24 ± 0.01^{c}	0.24 ± 0.02^{e}	0.99 ± 0.04^{e}	0.38 ± 0.02^{d}	0.11 ± 0.01^{c}	–	194 ± 16^{b,c}	0.66 ± 0.05^{d}	–	–	–	–	0.26 ± 0.00^{f}
T*f*	2.8 ± 0.3^{a}	0.14 ± 0.01^{e}	0.10 ± 0.01^{b}	2.27 ± 0.14^{d}	0.07 ± 0.00^{b,c}	0.30 ± 0.05^{a,b}	52.3 ± 4.9^{b}	1.8 ± 0.1^{a}	0.10 ± 0.01^{a}	–	–	–	–	0.19 ± 0.01^{a,b}
Summer														
IW	*NA	2802 ± 96^{a}	<LOD^{a}	1398 ± 40^{e}	12864 ± 445^{c}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}
SE	<LOD^{a}	30936 ± 210^{f}	27.4 ± 0.3^{b,c}	64.6 ± 0.8^{c}	19220 ± 250^{a,b}	7.43 ± 0.18^{b,c}	3.25 ± 0.04^{a}	<LOD^{a}	7.39 ± 0.04^{c}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	6.06 ± 0.06^{a}
RO	1.8 ± 0.5^{a,b}	2220 ± 165^{c}	1.86 ± 0.12^{b,c}	35.6 ± 2.5^{b}	3781 ± 253^{c}	0.26 ± 0.15^{a,b}	<LOQ^{a}	8.2 ± 2.2^{b}	1.94 ± 0.41^{c}	<LOQ^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	0.34 ± 0.01^{b}
LE	25.13 ± 0.15^{d}	414.3 ± 25.1^{d}	0.30 ± 0.02^{c}	94.1 ± 5.1^{c}	282.9 ± 15.9^{c}	<LOQ^{a}	0.46 ± 0.03^{a}	10.76 ± 0.58^{a,b}	0.37 ± 0.02^{d}	0.04 ± 0.02^{a,b}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	0.10 ± 0.00^{b}
BCF	36.69 ± 10.59^{a,b}	0.07 ± 0.01^{a}	0.07 ± 0.01^{b,c}	0.55 ± 0.04^{b,c}	0.20 ± 0.02^{c}	0.04 ± 0.02^{a,b}	–	164 ± 44^{b}	0.26 ± 0.06^{c}	–	–	–	–	0.06 ± 0.00^{c}
T*f*	14.4 ± 3.9^{b}	0.19 ± 0.01^{f}	0.16 ± 0.01^{c}	2.65 ± 0.09^{e}	0.07 ± 0.00^{b,c}	0.14 ± 0.06^{a}	30.7 ± 2.3^{a}	1.4 ± 0.3^{a}	0.19 ± 0.03^{a,b}	0.43 ± 0.35^{a}	–	–	–	0.29 ± 0.00^{a,b,c}
Lameirão														
Winter														
IW	*NA	8966 ± 79^{d}	15.5 ± 0.6^{c}	201 ± 10^{c}	12222 ± 226^{c}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	48 ± 16^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	7.7 ± 1.5^{b}
SE	<LOD^{a}	25664 ± 348^{c,d}	28.3 ± 0.1^{b,c}	47.0 ± 0.2^{a}	22407 ± 232^{c}	5.92 ± 0,05^{a,b}	3.93 ± 0.04^{a,b}	<LOD^{a}	3.93 ± 0.18^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	12.13 ± 0.11^{g}
RO	<LOQ^{a}	3465 ± 32^{d}	2.61 ± 0.03^{d}	51.8 ± 0.8^{c}	2539 ± 31^{b}	<LOQ^{a}	<LOQ^{a}	13.6 ± 0.7^{c}	0.19 ± 0.06^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	0.63 ± 0.01^{c}
LE	19.52 ± 0.67^{b}	194.6 ± 14.2^{c}	0.39 ± 0.03^{d}	33.4 ± 2.4^{a}	430.8 ± 27.2^{d}	0.04 ± 0.03^{a}	1.69 ± 0.13^{e}	13.83 ± 1.14^{c}	0.09 ± 0.01^{b}	0.05 ± 0.02^{b}	0.02 ± 0.00^{e}	0.01 ± 0.00^{b}	<LOQ^{a}	0.32 ± 0.02^{d}
BCF	3.00 ± 0.00^{a}	0.14 ± 0.00^{b}	0.09 ± 0.00^{c,d}	1.10 ± 0.02^{e}	0.11 ± 0.00^{b}	0.01 ± 0.00^{a}	–	272 ± 13^{c}	0.05 ± 0.01^{a,b}	–	–	–	–	0.05 ± 0.00^{c}
T*f*	130.0 ± 4.5^{d}	0.06 ± 0.00^{c}	0.15 ± 0.01^{c}	0.64 ± 0.05^{a}	0.17 ± 0.01^{d}	1.17 ± 0.85^{b}	112.4 ± 8.9^{e}	1.0 ± 0.1^{a}	0.48 ± 0.10^{a,b}	0.84 ± 0.56^{a}	4.54 ± 0.07^{c}	2.0 ± 0.3^{a}	–	0.51 ± 0.02^{c}
Summer														
IW	*NA	3738 ± 6^{a,b}	<LOD^{a}	101 ± 2^{b}	7309 ± 65^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	37 ± 12^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}
SE	<LOD^{a}	21492 ± 230^{a}	19.1 ± 0.3^{a}	53.6 ± 1.3^{b}	23810 ± 713^{c,d}	5.60 ± 0.10^{a}	3.79 ± 0.11^{a,b}	<LOD^{a}	3.96 ± 0.23^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	9.45 ± 0.12^{e}
RO	<LOQ^{a}	1189 ± 50^{a}	0.82 ± 0.05^{a}	19.1 ± 1.4^{a}	1319 ± 71^{a}	3.42 ± 0.22^{d}	<LOQ^{a}	11.2 ± 1.8^{b,c}	<LOQ^{a}	<LOQ^{a}	<LOD^{a}	<LOD^{a}	1.08 ± 0.07^{b}	0.55 ± 0.01^{c}
LE	19.79 ± 0.30^{b}	58.9 ± 0.4^{a}	0.09 ± 0.00^{a}	32.9 ± 0.4^{a}	104.3 ± 0.8^{a}	<LOQ^{a}	0.85 ± 0.01^{b}	11.74 ± 0.05^{b,c}	±0.03^{a,b}	<LOQ^{a}	0.01 ± 0.00^{c,d}	<LOQ^{a}	<LOQ^{a}	0.04 ± 0.00^{a}
BCF	3.00 ± 0.00^{a}	0.06 ± 0.00^{a}	0.04 ± 0.00^{a}	0.36 ± 0.02^{a}	0.06 ± 0.00^{a}	0.61 ± 0.03^{d}	–	224 ± 35^{b,c}	0.04 ± 0.00^{a}	–	–	–	22 ± 1^{b}	0.06 ± 0.00^{c}
T*f*	131.9 ± 2.0^{d}	0.05 ± 0.00^{b,c}	0.11 ± 0.01^{b}	1.73 ± 0.11^{c}	0.08 ± 0.00^{b,c}	0.01 ± 0.00^{a}	56.4 ± 0.8^{b}	1.1 ± 0.2^{a}	0.60 ± 0.35^{b}	–	2.39 ± 0.08^{a,b}	–	0.14 ± 0.01^{a}	0.08 ± 0.01^{a}
Serra														
Winter														
IW	*NA	5324 ± 356^{c}	9.7 ± 1.1^{b}	56 ± 4^{a}	7845 ± 483^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	9.5 ± 2.6^{b}
SE	14 ± 3^{b}	20496 ± 1478^{a}	84.0 ± 5.8^{d}	65.4 ± 4.8^{c}	17482 ± 1100^{a}	22.21 ± 1.59^{d}	6.41 ± 0.61^{c}	<LOD^{a}	7.65 ± 0.57^{c}	0.6 ± 0.2^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	8.76 ± 0.36^{d}
RO	<LOQ^{a}	5183 ± 197^{e}	4.59 ± 0.19^{e}	53.0 ± 2.2^{c}	4072 ± 190^{c}	0.76 ± 0.03^{c}	<LOQ^{a}	14.2 ± 1.5^{c}	1.03 ± 0.06^{b}	<LOQ^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	1.63 ± 0.06^{e}
LE	22.75 ± 0.41^{c}	94.1 ± 5.1^{a,b}	0.22 ± 0.01^{b,c}	74.9 ± 4.5^{b}	186.1 ± 7.4^{b}	0.02 ± 0.02^{a}	1.04 ± 0.05^{c}	22.89 ± 1.00^{e}	±0.01^{a,b}	0.03 ± 0.02^{a,b}	0.01 ± 0.00^{c}	0.01 ± 0.00^{b}	<LOQ^{a}	0.13 ± 0.00^{c}
BCF	0.01 ± 0.00^{a}	0.25 ± 0.02^{c}	0.05 ± 0.00^{a,b}	0.81 ± 0.08^{d}	0.23 ± 0.02^{c}	0.03 ± 0.00^{a}	–	283 ± 30^{c}	0.14 ± 0.02^{b}	0.18 ± 0.05^{a}	–	–	–	0.19 ± 0.00^{e}
T*f*	151.6 ± 2.7^{e}	0.02 ± 0.00^{a}	0.05 ± 0.00^{a}	1.41 ± 0.05^{b}	0.05 ± 0.00^{a}	0.03 ± 0.02^{a}	69.3 ± 3.5^{c}	1.6 ± 0.1^{a}	0.06 ± 0.01^{a}	0.28 ± 0.08^{a}	2.23 ± 0.05^{a,b}	2.6 ± 0.2^{a}	–	0.08 ± 0.00^{a}
Summer														
IW	*NA	3811 ± 360^{b}	<LOD^{a}	275 ± 17^{d}	7702 ± 467^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}
SE	<LOD^{a}	26572 ± 240^{d,e}	21.1 ± 0.4^{a}	52.7 ± 1.0^{a,b}	19940 ± 421^{b}	6.14 ± 0.17^{a,b}	4.45 ± 0.10^{b}	<LOD^{a}	5.67 ± 0.14^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	7.12 ± 0.03^{c}
RO	3.0 ± 2.0^{b}	2138 ± 341^{c}	2.07 ± 0.38^{c,d}	33.1 ± 5.6^{b}	1705 ± 301^{a}	0.59 ± 0.31^{b,c}	<LOQ^{a}	11.6 ± 3.9^{b,c}	0.36 ± 0.22^{a}	<LOQ^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	0.91 ± 0.05^{d}
LE	15.53 ± 0.08^{a}	67.3 ± 1.4^{a}	0.12 ± 0.01^{a}	38.4 ± 0.8^{a}	97.4 ± 1.6^{a}	<LOQ^{a}	0.78 ± 0.02^{b}	10.86 ± 0.20^{a,b}	<LOQ^{a}	<LOQ^{a}	0.01 ± 0.00^{b}	<LOQ^{a}	<LOQ^{a}	0.11 ± 0.00^{b,c}
BCF	59.49 ± 39.66^{b}	0.08 ± 0.01^{a}	0.10 ± 0.02^{d}	0.63 ± 0.09^{c}	0.09 ± 0.01^{a,b}	0.10 ± 0.05^{b,c}	–	233 ± 78^{b,c}	0.06 ± 0.04^{a,b}	–	–	–	–	0.13 ± 0.01^{d}
T*f*	8.3 ± 7.3^{a,b}	0.03 ± 0.00^{a,b}	0.06 ± 0.01^{a}	1.18 ± 0.17^{b}	0.06 ± 0.01^{a,b}	0.06 ± 0.02^{a}	52.2 ± 1.6^{b}	1.0 ± 0.3^{a}	0.50 ± 0.23^{a,b}	–	1.44 ± 0.12^{a,b}	–	–	0.12 ± 0.01^{a}
Santa Maria														
Winter														
IW	*NA	12034 ± 706^{e}	17.8 ± 1.1^{d}	323 ± 18^{d}	17783 ± 748^{d}	<LOD^{a}	3 ± 1^{b}	<LOD^{a}	<LOD^{a}	26 ± 23^{a,b}	18.25 ± 0.05^{b}	<LOD^{a}	<LOD^{a}	6.7 ± 0.3^{b}
SE	<LOD^{a}	23581 ± 312^{b}	23.6 ± 0.4^{a,b}	47.6 ± 0.9^{a,b}	26915 ± 224^{e}	6.32 ± 0.10^{a,b}	4.06 ± 0.10^{b}	<LOD^{a}	6.00 ± 0.15^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	14.34 ± 0.23^{h}
RO	<LOQ^{a}	1569 ± 38^{a,b}	1.12 ± 0.03^{a}	18.2 ± 0.5^{a}	1408 ± 32^{a}	<LOQ^{a}	<LOQ^{a}	0.5 ± 0.4^{a}	<LOQ^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	0.19 ± 0.03^{a}
LE	22.30 ± 0.48^{c}	491.4 ± 15.4^{d}	0.59 ± 0.02^{e}	66.2 ± 2.0^{b}	521.6 ± 17.5^{e}	0.02 ± 0.01^{a}	1.27 ± 0.05^{d}	20.53 ± 0.71^{e}	0.17 ± 0.01^{c}	0.06 ± 0.03^{b}	0.01 ± 0.00^{d}	0.01 ± 0.00^{b}	<LOQ^{a}	0.44 ± 0.01^{e}
BCF	3.00 ± 0.00^{a}	0.07 ± 0.00^{a}	0.05 ± 0.00^{a,b}	0.38 ± 0.02^{a}	0.05 ± 0.00^{a}	–	–	10 ± 8^{a}	0.03 ± 0.00^{a}	–	–	–	–	0.01 ± 0.00^{a}
T*f*	148.7 ± 3.2^{e}	0.31 ± 0.01^{g}	0.52 ± 0.01^{d}	3.63 ± 0.03^{f}	0.37 ± 0.01^{e}	0.63 ± 0.28^{a,b}	84.5 ± 3.5^{d}	61.8 ± 37.4^{b}	1.15 ± 0.08^{c}	1.23 ± 0.61^{a}	2.65 ± 0.26^{b}	2.8 ± 0.6^{a}	–	2.31 ± 0.26^{d}
Summer														
IW	*NA	5328 ± 381^{c}	<LOD^{a}	134 ± 6^{b}	10302 ± 615^{b}	<LOD^{a}	3 ± 1^{b}	<LOD^{a}	<LOD^{a}	39 ± 13^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}
SE	<LOD^{a}	23976 ± 1113^{b,c}	28.4 ± 1.6^{b,c}	51.1 ± 2.9^{a,b}	24792 ± 1096^{d}	7.38 ± 0.46^{b,c}	4.53 ± 0.37^{b}	<LOD^{a}	6.16 ± 0.41^{b}	<LOD^{a}	<LOD^{a}	<LOD^{a}	<LOD^{a}	10.71 ± 0.07^{f}
RO	<LOQ^{a}	1846 ± 161^{b,c}	1.34 ± 0.19^{a,b}	22.8 ± 1.9^{a}	1877 ± 196^{a,b}	0.15 ± 0.05^{a}	<LOQ^{a}	7.3 ± 1.7^{b}	<LOQ^{a}	<LOQ^{a}	<LOQ^{a}	<LOD^{a}	1.00 ± 0.19^{b}	0.29 ± 0.10^{a,b}
LE	14.46 ± 0.09^{a}	164.7 ± 7.0^{b,c}	0.14 ± 0.01^{a,b}	28.5 ± 1.3^{a}	174.3 ± 7.7^{b}	0.11 ± 0.02^{b}	1.04 ± 0.05^{c}	9.31 ± 0.50^{a}	0.07 ± 0.01^{a,b}	<LOQ^{a}	0.01 ± 0.00^{c,d}	<LOQ^{a}	<LOQ^{a}	0.12 ± 0.00^{b,c}
BCF	3.00 ± 0.00^{a}	0.08 ± 0.00^{a}	0.05 ± 0.00^{a,b}	0.45 ± 0.01^{a,b}	0.08 ± 0.00^{a}	0.02 ± 0.01^{a}	–	147 ± 34^{b}	0.02 ± 0.00^{a}	–	–	–	20 ± 4^{b}	0.03 ± 0.01^{b}
T*f*	96.4 ± 0.6^{c}	0.09 ± 0.01^{d}	0.11 ± 0.02^{b}	1.26 ± 0.15^{b}	0.09 ± 0.01^{c}	0.80 ± 0.38^{a,b}	69.5 ± 3.6^{c}	1.3 ± 0.3^{a}	0.45 ± 0.06^{a,b}	–	0.78 ± 0.03^{a}	–	0.15 ± 0.03^{a}	0.43 ± 0.15^{b,c}

Sediments correspond to the fraction < 63 μm < LOD (below detection limit); <LOQ (below quantification limit). LOQs: B, Fe, Zn, As, Se and Hg (0.15 mg L^{-1}); Al, Cr and Ni (0.03 mg L^{-1}); Mn, Cu, Ag, Cd and Pb (0.015 mg L^{-1}). Equal letter in the same line data do not differ significantly (Tukey test; $P < 0.05$).

Table 3
Anatomical data of the roots of *A. shaueriana* ($n = 5$ in each site) sampled in estuaries of Santa Cruz, Lameirão, Serra and Santa Maria. Values are expressed as mean ± SD. Equal letter in the same line data do not differ significantly (Tukey test; $P < 0.05$).

	Santa Cruz	Lameirão	Serra	Santa Maria
Pneumatophores				
Root diameter (mm)	6.20 ± 0.01^{b}	6.00 ± 0.02^{a}	6.50 ± 0.01^{c}	7.00 ± 0.01^{d}
Vascular cylinder diameter (mm)	2.10 ± 0.01^{c}	1.70 ± 0.01^{a}	2.00 ± 0.01^{b}	2.10 ± 0.01^{c}
Cortex thickness (mm)	3.50 ± 0.02^{a}	4.00 ± 0.08^{b}	4.20 ± 0.01^{c}	4.30 ± 0.04^{d}
Periderm thickness (mm)	0.50 ± 0.02^{b}	0.30 ± 0.08^{a}	0.30 ± 0.01^{a}	0.60 ± 0.04^{b}
Air gap area (mm^2)	0.0200 ± 0.0020^{c}	0.0100 ± 0.0004^{b}	$0.0100 \pm 0.0002^{b,c}$	0.0100 ± 0.0009^{a}
Cortex/Vascular cylinder ratio	1.700 ± 0.010^{a}	2.300 ± 0.050^{c}	2.100 ± 0.003^{b}	2.100 ± 0.020^{b}
Absorption roots				
Root diameter (mm)	1.20 ± 0.08^{a}	2.10 ± 0.22^{c}	1.50 ± 0.05^{a}	1.80 ± 0.01^{b}
Vascular cylinder diameter (mm)	0.40 ± 0.08^{b}	0.50 ± 0.02^{c}	0.20 ± 0.02^{a}	0.40 ± 0.04^{b}
Cortex thickness (mm)	0.50 ± 0.02^{a}	1.50 ± 0.23^{c}	1.10 ± 0.02^{b}	1.30 ± 0.04^{c}
Air gap area (mm^2)	0.0100 ± 0.0031^{a}	0.0300 ± 0.0039^{c}	0.0200 ± 0.0009^{b}	0.0200 ± 0.0028^{b}
Cortex/Vascular cylinder ratio	1.30 ± 0.30^{a}	2.90 ± 0.53^{b}	5.00 ± 0.51^{c}	3.80 ± 0.57^{b}

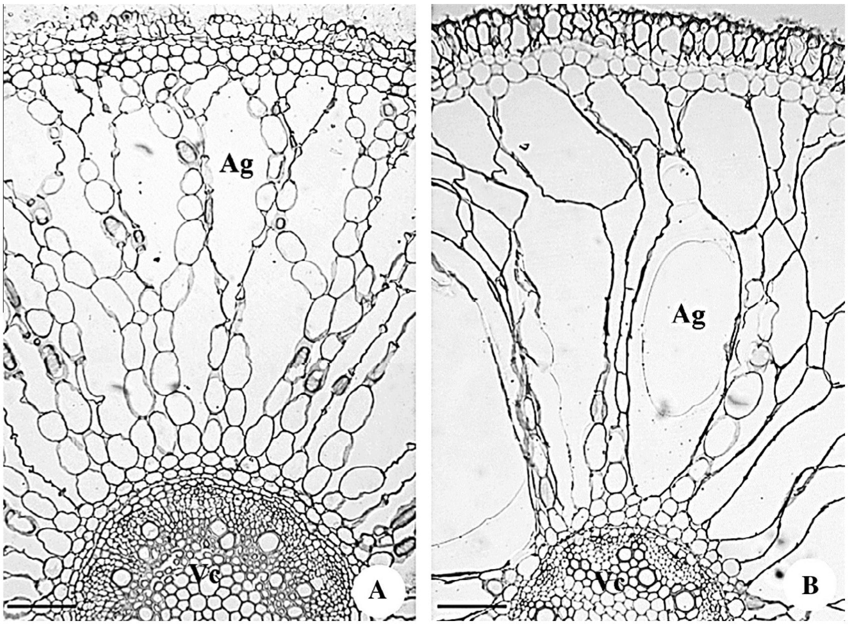

Fig. 2. Anatomical analysis showing absorption roots of *Avicennia schaueriana* in (A) Santa Cruz and (B) Serra. Bars = 70 μm (Ag = air gap; Vc = vascular cylinder).

manganese value in sediment. Manganese reflects the greater influence of continental water in this estuary as it is transported by watercourses adsorbed on suspended particulate matters (Pereira et al., 2007). Serra differs from others sites due to higher concentrations of metals–metalloids in sediment (B, Se, Cr, Ni and Cu). The differentiation between Santa Maria and Lameirão occurred due to metals in the interstitial water and the presence of iron and lead in sediment (Fig. 4A).

Physical, chemical and biological parameters were integrated by the GPA for each studied site (Fig. 4B). As there is no temporal difference in physical and chemical parameters, as shown by overlaps along the CP2 axis (data not shown), the evaluated parameters in both summer and winter seasons were combined in GPA. Significant differences between the studied sites were described primarily by the first axis (CP1), which explained 77% of the total variance, while the second axis (CP2), accounts for an additional 21% (Fig. 4). So far, results from GPA confirm spatial differences between studied areas.

Table 4
Classification functions corresponding to LDA of studied parameters, considering both spatial and temporal variations. C/V ratio: cortex/vascular cylinder ratio; AR: absorption roots; PN: pneumatophores; RT: roots; LV: leaves.

Sites	Santa Cruz	Lameirão	Serra	Santa Maria
	$P = .2500$	$P = .2500$	$P = .2500$	$P = .2500$
Classification functions LDA				
C/V ratio – AR	3582.7	4686.8	4363.2	4157.9
Cortex thickness – PN	−956.1	−944.9	−861.0	−819.8
Root diameter – PN	193.2	46.8	−24.2	17.1
Air gap area – AR	3230.4	−2042.5	−2119.6	−2027.7
Zinc – RT	2.9	5.8	5.9	4.2
Nickel – LV	−133.1	−268.0	−259.8	−220.6
Constant	−2907.4	−4833.2	−4097.8	−3774.9

Classification functions from stepwise LDA, considering the four mangrove areas evaluated in this study show that 6 out of 35 parameters pointed out by FA and all biological parameters distinguished between studied sites with 100% correct classification (classification matrix, data not shown) (Table 4). It is of note that LDA indicates four biological parameters (cortex/vascular cylinder ratio, air gap area in pneumatophores, cortex thickness and root thickness in absorption roots) and two chemical parameters (zinc in roots and nickel in leaves). Thus, the complex data matrix obtained by physical, chemical and biological studies at estuaries could be reduced by chemometrics to only 6 parameters, which were sufficient to spatially differentiate the four sites, point out the most important variables to enable such distinction. Anatomical parameters in roots were the most important for such differentiation. Also, it is of note that LDA selected only biological parameters.

So far, parameters indicated by LDA reinforce earlier conclusions, e.g. the thickness of the cortex of absorption roots was negatively correlated with most accumulated metals–metalloids in roots (r_s −0.79 for B, r_s −0.56 for Al, r_s −0.57 for Cr, r_s −0.52 for Mn, r_s −0.68 for Fe, r_s −0.84 for As and r_s −0.41 for Pb) (Fig. 3A and B). In *A. schaueriana*, the cortex is composed of aerenchyma, which plays an important role in radial oxygen loss and subsequent formation of iron plaque, so that a larger area of aerenchyma provides a higher oxygen diffusion outward from the root, allowing precipitation of potentially toxic metals before its uptake (Pi et al., 2009). Thus, current results could indicate an adaptive response of *A. schaueriana* to high metals–metalloids concentration in estuaries, so that a greater thickness of cortex provides less uptake and accumulation of metals–metalloids in

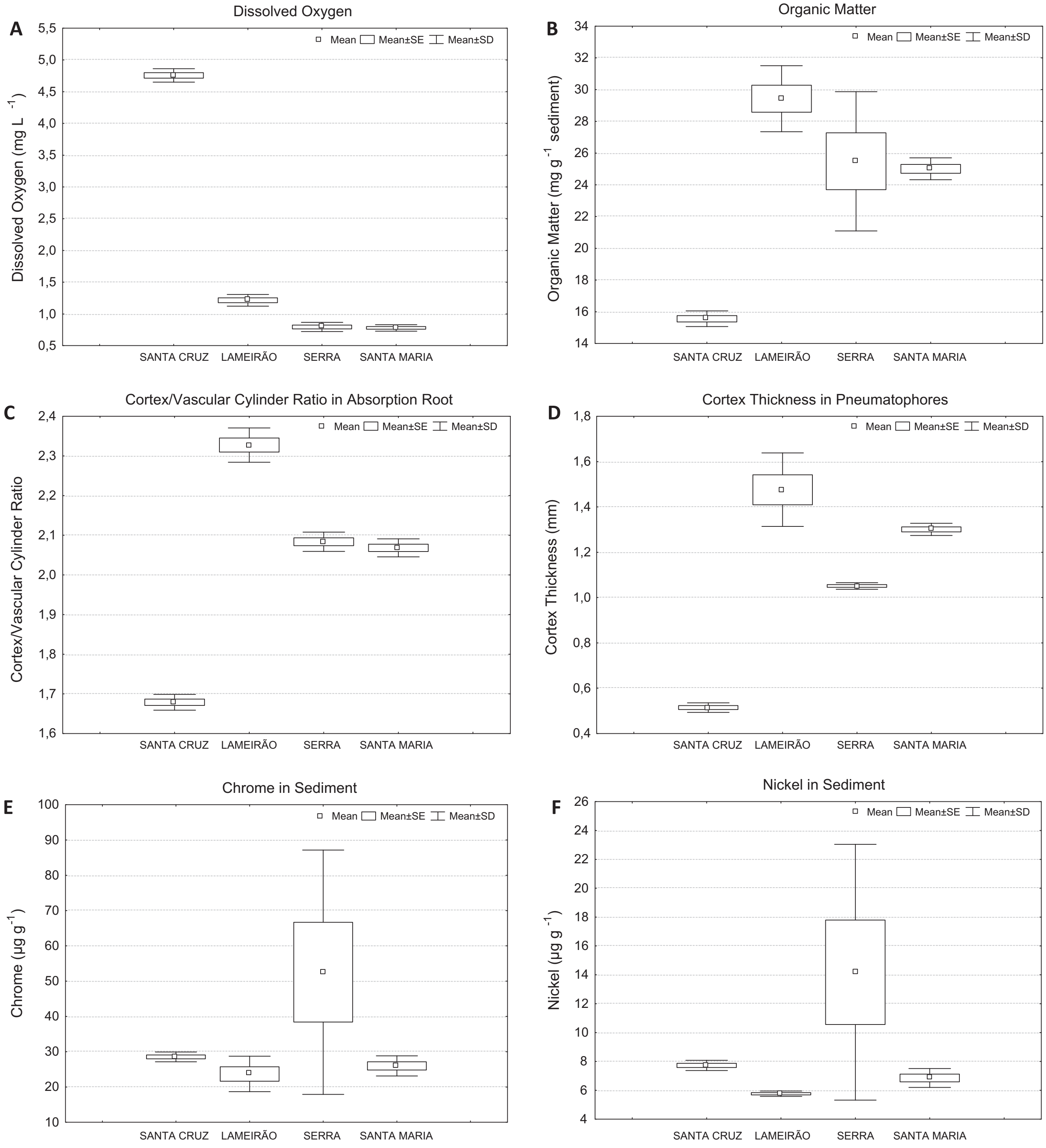

Fig. 3. Box and whisker plots from some selected parameters measured in sediment, root and leaves from *Avicennia schaueriana*. Values are reported as mean ± SD and SE.

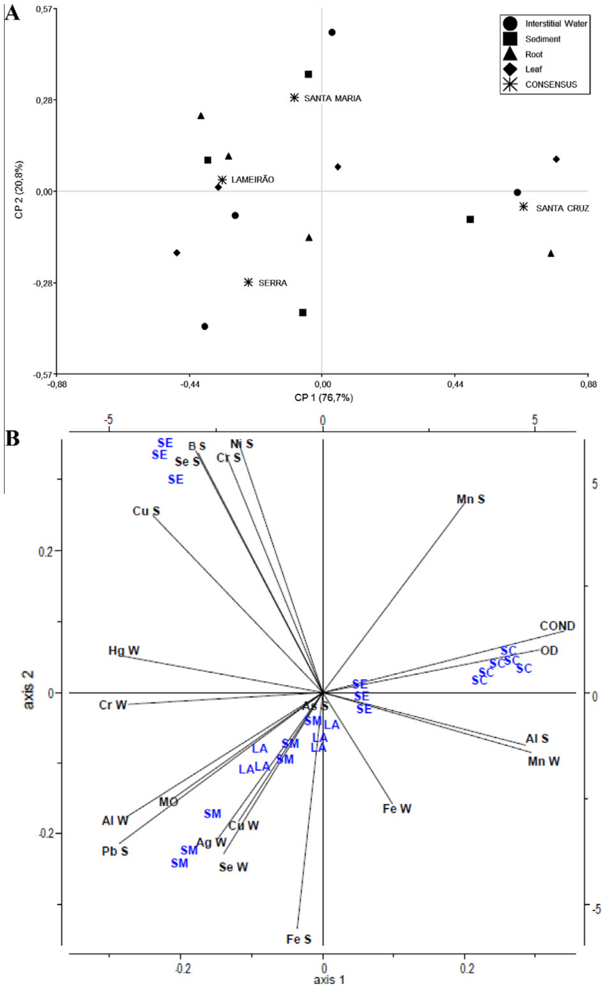

Fig. 4. Generalised procrustes analysis – GPA (A) and Principal component analysis – PCA (B) of studied parameters from each sampling site.

roots. This negative correlation may also be caused by metals–metalloids such as Pb, As, Cr and Al, impairing plant metabolism, which may cause less development of root (Weier, 1974; Singh et al., 2007; Yu et al., 2011).

The Zn values in roots were negatively correlated with root diameter (r_s −0.50) (Fig. 3C and D), thickness of vascular cylinder (r_s −0.68) and periderm (r_s −0.56) of pneumatophores, probably due to reductions in root growth caused by the increase of this element (MacFarlane and Burchett, 2002). However, there is still a lack of specific studies that show the effect of this metal in root tissues and how Zn uptake is affected by root anatomy.

The Ni values in leaves were positively correlated to Cr (r_s 0.60), Al (r_s 0.62), Fe (r_s 0.61), As (r_s 0.55) and Pb (r_s 0.44) (Fig. 3D and E). This may be because nickel comes from the same source as other metals–metalloids (ore or anthropogenic), or due to a synergistic effect of nickel with others chemicals, probable assisting in the simultaneous uptake of other metals (Luoma et al., 2008; Monferran et al., 2012).

Comparing with the others neotropical mangrove species, like *Laguncularia racemosa* (Souza et al., 2014a) and *Rhizophora mangle* (Souza et al., 2014b), *A. schaueriana* is the specie that accumulated higher levels of metals–metalloids in their tissues. In this study, anatomical parameters analysed in root corroborate chemical parameters found in soil and root, which was verified a greater thickness of both absorption and pneumatophore roots, combined with lower BCF values observed in roots from less polluted areas. Moreover, these results were associated with greater translocation values observed at highest polluted sites.

4. Conclusions

Our current results indicate that *A. schaueriana* presents adaptive changes, leading to reduce the amount of metals–metalloids in roots, by reducing the uptake and/or increasing the translocation, or both. However, is not fully understood the pathway that triggers these anatomical and physiological changes to face environmental contaminants, preventing excessive absorption of potentially toxic metals–metalloids. Future studies, evaluating gene expression and epigenetic factors could help to elucidate questions rose from current results.

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