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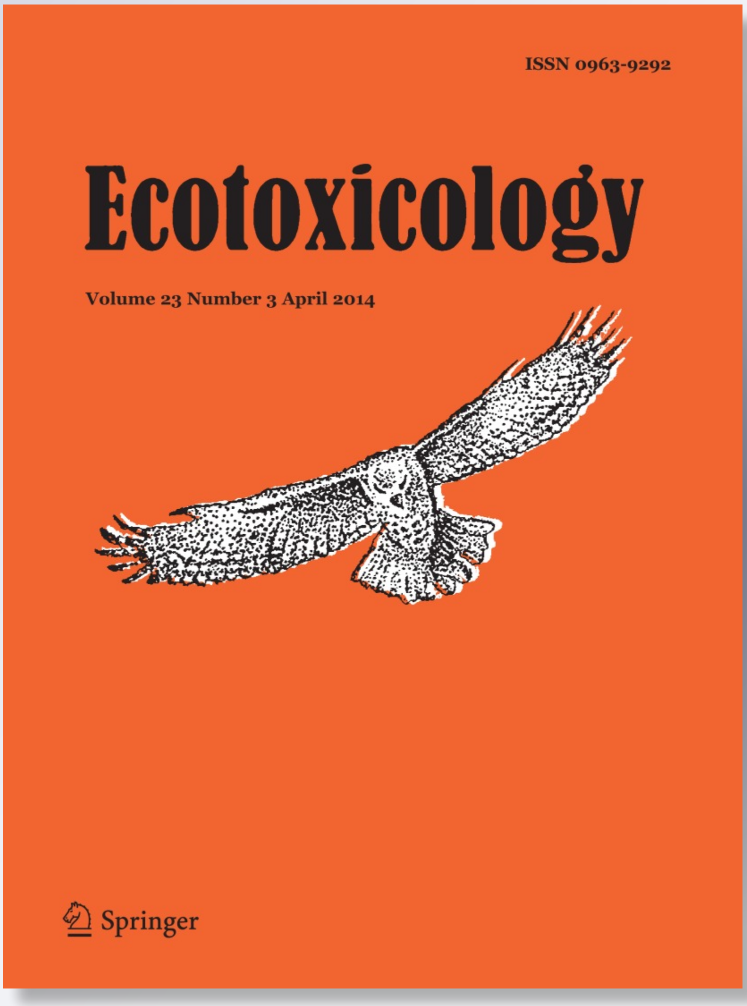

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Adaptive plasticity of *Laguncularia racemosa* in response to different environmental conditions: integrating chemical and biological data by chemometrics

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Abstract Mangroves are dynamic environments under constant influence of anthropic contaminants. The correlation between environmental contamination levels and possible changes in the morphology of plants, evaluated by multivariate statistics helps to highlight matching between these variables. This study aimed to evaluate the uptake and translocation of metals and metalloids in roots and leaves as well as the changes induced in both anatomy and histochemistry of roots of *Laguncularia racemosa* inhabiting two estuaries of Espírito Santo (Brazil) with different pollution degrees. The analysis of 14 elements in interstitial water, sediments and plants followed by multivariate statistics, allowed the differentiation of studied sites, showing good match between levels of elements in the environment with the corresponding in plants. *L. racemosa* showed variations in their root anatomy in different collection areas, with highest values of cortex/vascular cylinder ratio, periderm thickness and air gap area in Vitória Bay, the most polluted sampling area. These three parameters were also important to differentiate the mangrove areas by linear discriminant analysis. The development stage of aerenchyma in roots reflected the oxygen availability in the water, being found a negative correlation between these variables. The combined use of chemical and biological analyses responded quite well to different pollution scenarios, matching morphological responses to physical and chemical parameters, measured at different partitions within the estuary. Thus, *L. racemosa* can be confirmed as a reliable sentinel plant for biomonitoring of estuaries impacted by anthropic pollution.

Keywords Chemometrics · Estuary · Contaminants · Aerenchyma · White mangrove

Introduction

Mangrove ecosystems have received increased attention in the last years due to frequent records of toxicity and contamination by metals and the effects of potential accumulation of these contaminants in the team highlights parts, such as leaves, where they may affect the cellular physiological processes and the tissue anatomy as well (Liu et al. 2009; Pi et al. 2009; Pinheiro et al. 2012; Cheng et al. 2012).

Studies on metal distribution in the sediment and in plant compartments have great importance for biomonitoring, helping to assess changes in these ecosystems (Gutiérrez-Ginés et al. 2012). So, the concentration of chemicals determined in the biota have been often compared to those

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present in sediments to determine bioconcentration factors (Mejías et al. 2013). Translocation is a common issue in mangrove plants, where the presence of metals is usually greater in roots than in aerial tissues (Lewis et al. 2011). Despite all existing data concerning metal evaluation, its significance on plant physiology and survival remains poorly explored (Macfarlane et al. 2007).

Mangrove constitute one of the most productive ecosystems, operating in carbon sequestration and transforming nutrients in organic matter, functioning as an integrated unit between terrestrial and marine environments (MacFarlane et al. 2007; Bouillon et al. 2008). Its plants are capable to colonize areas with variable environmental conditions, including locals exposed to pollution (Machado et al. 2005). *Laguncularia racemosa* (L.) Gaertn is a typical mangrove specie that tolerates high salinity, characterized as salt-including (Parida and Jha 2010), and that has been used in several environmental studies (Lewis and Pryor 2013; Mejías et al. 2013; Sodré et al. 2013). The analysis of plant responses upon exposure to contaminants allows the utilization of resident species as environmental quality bioindicators (Ernst and Peterson 1994; Lewis et al. 2011).

In this context, the aim of this study was to evaluate the responses of *L. racemosa*, commonly known as white mangrove, growing in impacted Brazilian mangrove areas, looking for the correlation between environmental contamination levels and the changes in the root anatomy and histochemistry by use of multivariate statistics to help with result interpretation and also to evidence matching between the surrounding environment, namely sediment and interstitial water, and the plant. So far, this study looks to test an integrated approach, using chemistry, biology and multivariate statistics for the evaluation of changes in the environment and their consequences on the inhabiting biota.

Materials and methods

Studied area

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 ocean influence (Fig. 1). Vitória Bay is an estuarine complex formed by five rivers, that has been suffered several environmental impacts caused by harbors, steel mills and mining activities. Three sites were selected in this estuary: Santa Maria (S20°14′31.5″ and W40°19′84.7″), an area with major continental water influence, Serra (S20°14′19.6″ and W40°18′48.7″), an area under impact of industrial and sanitary effluents, and Lameirão (S20°14′60.6″ and W40°18′68.6″), a legally protected environmental area. The second estuary, Santa Cruz (S19°56′26.2″ and W40°12′87″) is formed by two rivers, has an extensive mangrove area (Souza et al. 2013). This is considered a well-preserved mangrove area (Souza et al. 2013) and was considered as the reference area in this study (Fig. 1). Both studied areas were geo-referenced during

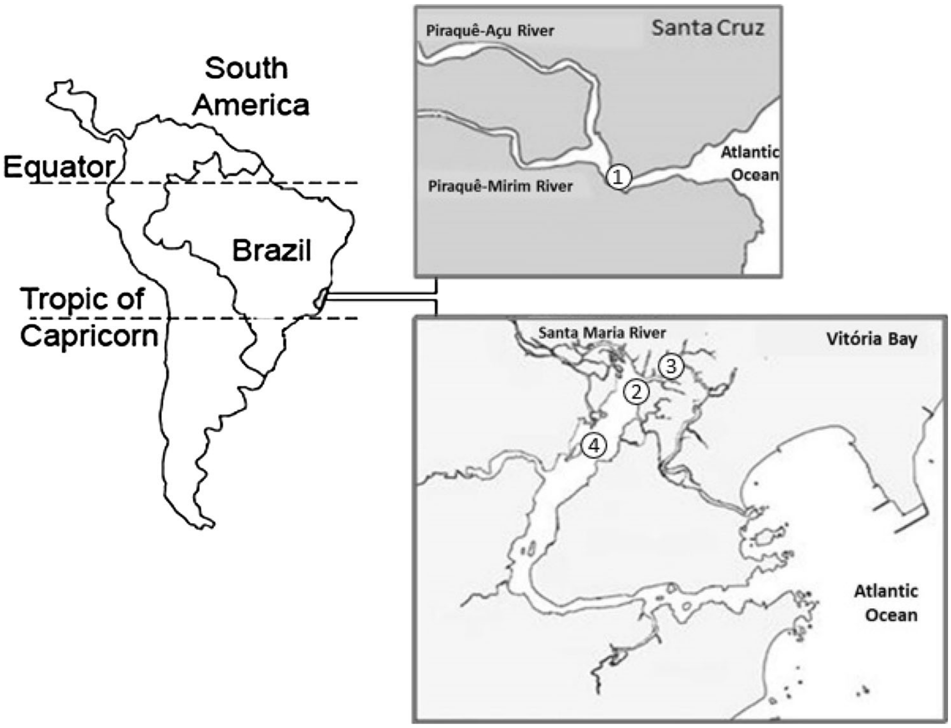


Fig. 1 Localization of the State of Espírito Santo (Brazil), showing sampling sites. *1* Santa Cruz; *2* Lameirão; *3* Serra; *4* Santa Maria

field sampling, using a portable GPS 368 (Garmin Vista, USA).

Water, sediment and plant sampling

Ultra-pure water (<5 µg L^{-1} TOC) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Multi-element standard solution Merck VI CertiPUR® was obtained from Merck Química Argentina (Buenos Aires, Argentina). Nitric acid (63.7 %) sub-boiling grade was prepared from analytical grade acid using a distiller (Figmay Sub-boiling distiller, Córdoba, Argentina). Purity of nitric acid was verified by Mass Spectrometer Inductively Coupled Plasma (ICP-MS). Filters (0.45 µm, HAWG04756) were obtained from Millipore (São Paulo, Brazil). All glassware and plastic bottles/containers were left within a sulfuric/nitric acids solution overnight and washed with ultra-pure water. ICP probes and pipes were of PTFE (Teflon) previously washed with nitric acid (2 % v/v). The pH was determined in the field using a multiparametric probe (YSI model 85, USA), operated 20 cm below the water surface.

Sediment and root samples were collected from all sites along two seasons (winter-2009 and summer-2010). Sediment samples were taken throughout the experiment simultaneously with plant sampling. Sample collection, containers, stabilization, and transportation to the laboratory as well as sample storage were done in accordance with previously described methods (Monferrán et al. 2011, 2012). Sediment samples (approximately 20 cm depth interval from the sediment–water interface), close to areas where *L. racemosa* rhizospheres, were collected using a plastic spoon and quickly transferred into clean plastic containers (1 L) without head space, for elemental analyses. Subsequently they were dried at room temperature and sieved through nylon meshes (63 µm) with an acrylic frame to avoid the transfer of metals from metallic meshes during sieving. In the laboratory, interstitial water was extracted from sediment samples by centrifugation (3 000 rpm; 40 min) and the supernatant was filtered using 0.45 µm nitrocellulose filters. Water dissolved oxygen (DO) and conductivity were determined in the interstitial water using a multiparametric probe (YSI model 85, USA) in the laboratory. Interstitial water samples were acidified with ultrapure HNO_3 (sub-boiling) and stored at 4 °C until analysis. Prior to measurement, the samples were filtered using 0.45 µm nitrocellulose.

Absorption roots, pneumatophores and leaves samples were collected from five individuals of *L. racemosa* from each site and were immediately washed with distilled water. Roots and leaves were also sampled, dried at 40 °C until constant weight, stored at 4 °C until analysis and homogenized with a mortar. Sediment and plant samples (0.5 g each) were digested with nitric and hydrochloric acids (ultra-pure, 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). The assay of organic matter in the sediment was performed according to Walkley and Black (1934) method.

Multielement analyses

The analysis of metals and metalloids (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb) in both abiotic and biotic digested samples was performed with a ICP-MS, Agilent 7500 cx, USA, equipped with an ASX-100 autosampler (CETAC Technologies, Omaha, NE). All samples were digested in triplicate. Concentrations of elements were determined in triplicate; the repeatability of ICP-MS measurements was generally $\geq$ 97 %. Quality assurance (QA) and quality control (QC) 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 elements measured in the samples. Spiked samples were also prepared. Variable amounts of mix standard solutions, containing all the elements analyzed 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 rest 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 measured to compare the levels of heavy metals accumulated in root tissue in respect with the original concentration in soils. The translocation factor (T*f*) was applied to evaluate the metal concentrations in leaves and its correlation to values found in roots. Both factors were considered only when values found were greater than one, as described in Ali et al. (2013).

Biological analyses

The thickness of cortex, air gap area and vascular cylinder diameter of pneumatophores and absorption roots were measured (30 measurements/root section) and the cortex/vascular cylinder ratio was calculated. In pneumatophores the thickness of the periderm was also measured. Root samples were dehydrated in 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). To determine the chemical nature of the

Table 1 Physical ($n = 9$), chemical ($n = 9$) and biological parameters ($n = 5$), measured in superficial and interstitial water, sediment and *Laguncularia racemosa* sampled in the estuaries Santa Cruz, Lameirão, Serra and Santa Maria in the summer and winter

Physical, chemical and biological parameters	Santa Cruz		Lameirão		Serra		Santa Maria	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Conductivity (mS)	70.4 ± 4.4^{a}	72.9 ± 4.4^{a}	$51.0 \pm 1.6^{a,b}$	52.0 ± 1.6^{b}	49.6 ± 3.6^{b}	51.6 ± 3.6^{b}	49.7 ± 4.6^{b}	52.4 ± 4.6^{b}
Total solids (g/L)	30.9 ± 10.4^{a}	36.9 ± 10.4^{a}	26.1 ± 4.7^{a}	28.9 ± 4.7^{a}	16.8 ± 8.8^{a}	21.9 ± 8.8^{a}	29.5 ± 7.8^{a}	25.0 ± 7.8^{a}
Organic matter (mg g^{-1} sediment)	14.3 ± 0.1^{b}	14.4 ± 0.1^{b}	26.0 ± 7.2^{a}	30.1 ± 7.2^{a}	22.4 ± 0.9^{a}	21.8 ± 0.9^{a}	22.5 ± 0.5^{a}	22.8 ± 0.5^{a}
Dissolved oxygen (mg L^{-1})	4.8 ± 0.1^{a}	4.8 ± 0.1^{a}	1.2 ± 0.0^{b}	1.2 ± 0.0^{b}	0.8 ± 0.1^{b}	0.8 ± 0.1^{b}	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}
pH	7.60 ± 0.32	7.49 ± 0.37	6.76 ± 0.33	6.83 ± 0.25	7.15 ± 0.18	7.15 ± 0.23	7.16 ± 0.22	7.00 ± 0.15
Absorption roots								
Root thickness (mm)	1.64 ± 0.11^{c}		2.23 ± 0.09^{a}		$1.79 \pm 0.02^{b,c}$		1.86 ± 0.23^{b}	
Vascular cylinder diameter (mm)	$0.48 \pm 0.02^{a,b}$		$0.43 \pm 0.09^{a,b}$		0.41 ± 0.01^{b}		0.52 ± 0.08^{a}	
Cortex thickness (mm)	0.97 ± 0.07^{b}		1.63 ± 0.03^{a}		1.15 ± 0.01^{b}		1.13 ± 0.28^{b}	
Air gap area (mm^2)	0.01 ± 0.00^{d}		0.05 ± 0.00^{b}		0.03 ± 0.00^{c}		0.11 ± 0.02^{a}	
Cortex/Vascular cylinder ratio	2.02 ± 0.09^{b}		3.92 ± 0.65^{a}		2.81 ± 0.04^{b}		2.31 ± 0.92^{b}	
Pneumatophores								
Root thickness (mm)	6.8 ± 0.0^{b}		5.2 ± 0.0^{c}		7.0 ± 0.0^{a}		7.0 ± 0.0^{a}	
Vascular cylinder thickness (mm)	2.2 ± 0.0^{b}		1.4 ± 0.0^{d}		1.7 ± 0.0^{c}		2.3 ± 0.0^{a}	
Cortex thickness (mm)	4.29 ± 0.02^{b}		$3,37 \pm 0.12^{c}$		4.93 ± 0.03^{a}		4.26 ± 0.02^{b}	
Periderm thickness (mm)	0.31 ± 0.02^{b}		0.43 ± 0.12^{a}		$0.37 \pm 0.03^{a,b}$		0.44 ± 0.02^{a}	
Air gap area (mm^2)	0.01 ± 0.00^{b}		0.05 ± 0.02^{a}		0.02 ± 0.00^{b}		0.01 ± 0.00^{b}	
Cortex/Vascular cylinder ratio	1.95 ± 0.01^{c}		2.41 ± 0.08^{b}		2.90 ± 0.02^{a}		1.85 ± 0.01^{d}	

Values are expressed as mean ± SD. Equal letter in the same line data do not differ significantly (Tukey test; $P < 0.05$)

Table 2 Chemical characterization of Santa Cruz, Lameirão, Serra and Santa Maria

	Metals						
	B	Al	Cr	Mn	Fe	Ni	Cu
Santa Cruz							
Winter							
IW	*NA	4,141 ± 63^{d}	<LOD^{c}	896 ± 11^{b}	10,288 ± 107^{b,c}	<LOD^{b}	<LOD^{b}
SE	<LOD^{b}	30,226 ± 1284^{a}	27.3 ± 0.9^{d}	99 ± 4^{a}	19,380 ± 505^{e,f}	7.8 ± 0.3^{d}	3.5 ± 0.1^{e,f}
RO	43 ± 2^{c,d}	5,244 ± 113^{a,b}	4.32 ± 0.08^{b}	51 ± 2^{b}	5,898 ± 88^{a,b}	1.38 ± 0.07^{b}	<LOQ
LE	9.6 ± 0.1 ^{g}	137 ± 15^{b}	0.13 ± 0.02^{c}	40 ± 4^{a}	204 ± 23^{b}	<LOQ^{b}	0.48 ± 0.08^{d,e}
BCF	285 ± 13^{c}	0.17 ± 0.01^{b,c}	0.16 ± 0.00^{b}	0.51 ± 0.02^{c}	0.30 ± 0.01^{b}	0.18 ± 0.01^{b}	–
T*f*	0.23 ± 0.01^{c}	0.03 ± 0.00^{b,c}	0.03 ± 0.00^{c}	0.8 ± 0.1^{c,d,e}	0.03 ± 0.00^{c}	–	32 ± 6^{d,e}
Summer							
IW	*NA	1,781 ± 329 ^{g}	<LOD^{c}	3,166 ± 381^{a}	15,867 ± 2040^{a}	<LOD^{b}	<LOD^{b}
SE	<LOD^{b}	29,617 ± 823^{a}	28 ± 1^{d}	89 ± 3^{b}	20,719 ± 729^{d,e}	7.3 ± 0.4^{d}	3.3 ± 0.2^{f}
RO	47 ± 1^{c}	3,498 ± 511^{c}	3.2 ± 0.5^{c}	43 ± 6^{b,c}	6,124 ± 824^{a,b}	0.2 ± 0.2^{d,e}	<LOQ
LE	14.2 ± 0.4^{d}	109 ± 11^{c}	0.06 ± 0.01^{d}	24 ± 2^{c,d}	180 ± 16^{b,c}	<LOQ^{b}	0.40 ± 0.06^{e}
BCF	314 ± 7^{b}	0.12 ± 0.02^{d}	0.12 ± 0.02^{c}	0.49 ± 0.08^{c}	0.30 ± 0.05^{b,c}	0.03 ± 0.03^{d}	–
T*f*	0.30 ± 0.01^{c}	0.03 ± 0.01^{b}	0.02 ± 0.01^{c}	0.6 ± 0.1^{d,e,f}	0.03 ± 0.01^{c}	–	27 ± 4^{e}
Lameirão							
Winter							
IW	*NA	3,991 ± 114^{d}	6 ± 3^{c}	148 ± 3^{c}	6,732 ± 88^{d}	<LOD^{b}	<LOD^{b}
SE	<LOD^{b}	27,456 ± 682^{b}	23.1 ± 0.6^{e}	46 ± 1^{d,e}	23,059 ± 525^{c}	6.2 ± 0.2^{e}	4.4 ± 0.2^{c}
RO	56 ± 2^{b}	6,331 ± 278^{a}	5.2 ± 0.3^{a,b}	92 ± 3^{a}	4,932 ± 248^{b,c}	2.1 ± 0.2^{a}	<LOQ
LE	11.5 ± 0.2^{f}	58.9 ± 0.4^{e}	0.12 ± 0.00^{c,d}	27.7 ± 0.4^{b,c}	154 ± 5^{c}	0.03 ± 0.01^{a,b}	0.65 ± 0.01^{d,e}
BCF	370 ± 13^{a}	0.23 ± 0.01^{a,b}	0.22 ± 0.02^{a}	2.01 ± 0.09^{a}	0.21 ± 0.01^{d}	0.34 ± 0.03^{a}	–
T*f*	0.19 ± 0.01^{c}	0.01 ± 0.00^{d}	0.02 ± 0.00^{c}	0.30 ± 0.01^{f}	0.03 ± 0.00^{c}	0.02 ± 0.01^{b}	43.5 ± 0.4^{d,e}
Summer							
IW	*NA	3,142 ± 165^{e}	<LOD^{c}	163 ± 15^{c}	5,764 ± 347^{d}	<LOD^{b}	<LOD^{b}
SE	<LOD^{b}	23,006 ± 593^{d}	20.9 ± 0.5^{e}	50.4 ± 0.9^{d}	22,349 ± 520^{c,d}	5.5 ± 0.1^{e}	4.2 ± 0.1^{c,d}
RO	<LOQ^{f}	3,003 ± 121^{c}	2.8 ± 0.1^{c}	39 ± 1^{c}	2,571 ± 102^{e}	0.88 ± 0.09^{c}	<LOQ
LE	19 ± 0.6^{c}	68 ± 3^{d,e}	0.10 ± 0.01^{c,d}	20 ± 1^{d}	107 ± 8^{d}	<LOQ^{b}	0.50 ± 0.04^{d,e}
BCF	–	0.13 ± 0.01^{c,d}	0.13 ± 0.01^{b,c}	0.78 ± 0.03^{b}	0.12 ± 0.01^{e}	0.16 ± 0.02^{b}	–
T*f*	126 ± 4^{b}	0.02 ± 0.00^{b,c,d}	0.03 ± 0.00^{b,c}	0.52 ± 0.01^{e,f}	0.04 ± 0.00^{b,c}	0.01 ± 0.02^{b}	33 ± 3^{d,e}
Serra							
Winter							
IW	*NA	8,730 ± 30^{a}	16.1 ± 0.3^{a}	44 ± 1^{c}	12,229 ± 38^{b}	<LOD^{b}	<LOD^{b}
SE	39 ± 2^{a}	25,566 ± 464^{b,c}	42.7 ± 0.4^{b}	41.4 ± 0.3^{e}	22,609 ± 326^{c,d}	10.8 ± 0.2^{b}	5.8 ± 0.2^{a}
RO	68 ± 3^{a}	5,799 ± 285^{a,b}	5.5 ± 0.2^{a}	21 ± 1^{d}	5,022 ± 239^{b,c}	0.9 ± 0.1^{c}	<LOQ
LE	13.4 ± 0.1^{d}	91 ± 15^{c,d}	0.31 ± 0.06^{b}	26 ± 4^{b,c,d}	217 ± 32^{b}	0.08 ± 0.05^{a}	1.9 ± 0.3^{b}
BCF	1.7 ± 0.2^{e}	0.23 ± 0.01^{a,b}	0.13 ± 0.00^{b,c}	0.51 ± 0.02^{c}	0.22 ± 0.01^{c,d}	0.08 ± 0.01^{c}	–
T*f*	0.20 ± 0.01^{c}	0.02 ± 0.00^{c,d}	0.06 ± 0.01^{b}	1.2 ± 0.2^{a}	0.04 ± 0.01^{b,c}	0.09 ± 0.07^{a,b}	128 ± 21^{b}
Summer							
IW	*NA	2,318 ± 50^{f}	<LOD^{c}	118 ± 2^{c}	5,290 ± 57^{d}	<LOD^{b}	<LOD^{b}
SE	<LOD^{b}	25,109 ± 231^{c}	39.3 ± 0.9^{c}	44.2 ± 0.7^{e}	26,722 ± 635^{b}	9.6 ± 0.2^{c}	4.30 ± 0.09^{c,d}
RO	26.2 ± 0.9^{e}	2,930 ± 70^{c}	3.00 ± 0.08^{c}	22.2 ± 0.6^{d}	3,995 ± 105^{c,d}	0.15 ± 0.05^{d,e}	<LOQ
LE	24 ± 0.3^{a}	92 ± 4^{c,d}	0.10 ± 0.01^{c,d}	26 ± 1^{b,c,d}	209 ± 9^{b}	<LOQ^{a,b}	2.7 ± 0.1^{a}
BCF	175 ± 6^{d}	0.12 ± 0.00^{d}	0.08 ± 0.00^{d}	0.50 ± 0.02^{c}	0.15 ± 0.01^{d,e}	0.02 ± 0.01^{d}	–
T*f*	0.91 ± 0.02^{c}	0.03 ± 0.01^{b}	0.03 ± 0.02^{b,c}	1.16 ± 0.01^{a,b}	0.05 ± 0.00^{a,b}	0.13 ± 0.08^{a,b}	180 ± 8^{a}

Table 2 continued

	Metals						
	B	Al	Cr	Mn	Fe	Ni	Cu
Santa Maria							
Winter							
IW	*NA	4,882 ± 10^{c}	<LOD^{c}	60.5 ± 0.8^{c}	9,616 ± 31^{c}	<LOD^{b}	<LOD^{b}
SE	<LOD^{b}	21,491 ± 222^{d}	80 ± 2^{a}	63 ± 1^{c}	18,630 ± 323^{f}	19.9 ± 0.5^{a}	5.30 ± 0.06^{b}
RO	39 ± 2^{d}	5,163 ± 902^{b}	4.5 ± 0.8^{a,b}	36 ± 5^{c}	7,169 ± 1093^{a}	0.5 ± 0.3^{c,d}	<LOQ
LE	12 ± 0.1^{e}	337 ± 9^{a}	0.42 ± 0.02^{a}	31.5 ± 0.8^{b}	427 ± 10^{a}	0.06 ± 0.02^{a,b}	0.80 ± 0.03^{c,d}
BCF	263 ± 12^{c}	0.24 ± 0.04^{a}	0.06 ± 0.01^{d}	0.56 ± 0.08^{c}	0.38 ± 0.06^{a}	0.02 ± 0.02^{d}	–
T*f*	0.31 ± 0.02^{c}	0.07 ± 0.01^{a}	0.09 ± 0.02^{a}	0.9 ± 0.1^{b,c}	0.06 ± 0.01^{a}	0.17 ± 0.10^{a}	53 ± 2^{c,d}
Summer							
IW	*NA	5,979 ± 11^{b}	8.2 ± 0.1^{b}	154 ± 1^{c}	12,147 ± 11^{b}	13.5 ± 0.4^{a}	14.04 ± 0.04^{a}
SE	<LOD^{b}	22,246 ± 955^{d}	20.9 ± 0.9^{e}	45 ± 2^{d,e}	31,309 ± 1404^{a}	5.9 ± 0.3^{e}	3.9 ± 0.3^{d,e}
RO	<LOQ^{f}	3,936 ± 186^{c}	2.9 ± 0.1^{c}	36.9 ± 0.9^{c}	3,197 ± 32^{d,e}	<LOQ^{e}	<LOQ
LE	20.2 ± 0.2^{b}	82 ± 4^{d,e}	0.08 ± 0.01^{c,d}	31 ± 1^{b}	196 ± 6^{b,c}	<LOQ^{b}	1.03 ± 0.04^{c}
BCF	–	0.18 ± 0.01^{b,c}	0.14 ± 0.01^{b,c}	0.82 ± 0.03^{b}	0.10 ± 0.00^{e}	–	–
T*f*	134 ± 1^{a}	0.02 ± 0.00^{b,c,d}	0.03 ± 0.00^{c}	0.85 ± 0.04^{b,c,d}	0.06 ± 0.00^{a}	–	69 ± 3^{c}
	Zn	As	Se	Ag	Cd	Hg	Pb
Santa Cruz							
Winter							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	7.7 ± 0.2^{b,c}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	6.53 ± 0.04^{f}
RO	7 ± 1^{d,e}	4.3 ± 0.1^{b}	<LOD	<LOD	<LOD	<LOD	1.01 ± 0.04^{e}
LE	9 ± 1^{d,e}	<LOQ^{a,b}	<LOD	<LOD^{d}	<LOQ^{b}	<LOQ^{b}	0.08 ± 0.00^{c}
BCF	146 ± 22^{d,e}	0.56 ± 0.01^{b}	–	–	–	–	0.15 ± 0.00^{d}
T*f*	1.3 ± 0.3^{b,c}	0.01 ± 0.01^{b}	–	–	–	–	0.08 ± 0.00^{b}
Summer							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	8 ± 0.3^{a,b}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	5.73 ± 0.03 ^{g}
RO	5 ± 2^{e}	6 ± 1^{a}	<LOD	<LOD	<LOD	<LOD	1.21 ± 0.05^{d}
LE	11 ± 1^{c,d}	<LOQ^{b}	<LOD	0.03 ± 0.00^{a}	<LOQ^{b}	<LOQ^{b}	0.02 ± 0.00 ^{g}
BCF	97 ± 34^{e}	0.8 ± 0.2^{a}	–	–	–	–	0.21 ± 0.01^{c}
T*f*	2 ± 1^{a,b}	–	–	6.1 ± 0.2^{a}	–	–	0.01 ± 0.00 ^{g}
Lameirão							
Winter							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	4.3 ± 0.2^{e}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	11.4 ± 0.1^{d}
RO	10 ± 1^{b,c,d}	1.2 ± 0.1^{c,d}	<LOQ	<LOD	<LOD	<LOD	1.65 ± 0.06^{c}
LE	14.7 ± 0.3^{a}	<LOQ^{b}	<LOQ	<LOQ^{d}	0.01 ± 0.00^{a}	<LOQ^{b}	0.11 ± 0.00^{b}
BCF	202 ± 20^{b,c,d}	0.28 ± 0.04^{c}	–	–	–	–	0.14 ± 0.01^{d}
T*f*	1.5 ± 0.2^{a,b,c}	–	–	–	2.4 ± 0.4^{a}	–	0.07 ± 0.00^{c}
Summer							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	4.98 ± 0.08^{e}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	8.65 ± 0.02^{e}
RO	11 ± 1^{b,c}	0.06 ± 0.05^{d}	<LOQ	<LOD	<LOD	<LOD	0.83 ± 0.04^{f}
LE	7.6 ± 0.6^{e}	<LOD^{b}	<LOD	<LOD^{d}	0.01 ± 0.00^{a,b}	<LOQ^{b}	0.04 ± 0.00^{f}
BCF	229 ± 25^{b,c}	0.01 ± 0.01^{e}	–	–	–	–	0.10 ± 0.01^{e}
T*f*	0.67 ± 0.03^{c}	–	–	–	1.9 ± 0.2^{a}	–	0.05 ± 0.00^{d}

Table 2 continued

	Zn	As	Se	Ag	Cd	Hg	Pb
Serra							
Winter							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	6 ± 1^{a}	<LOD
SE	<LOD	8.82 ± 0.06^{a}	0.5 ± 0.2^{a}	<LOQ^{a}	<LOD	<LOD	12.2 ± 0.2^{c}
RO	20 ± 1^{a}	1.5 ± 0.2^{c}	<LOD	<LOD	<LOD	<LOD	2.97 ± 0.08^{b}
LE	14 ± 2^{a,b}	<LOQ^{b}	<LOD	<LOQ^{d}	0.01 ± 0.00^{a,b}	0.09 ± 0.01^{a}	0.07 ± 0.00^{d}
BCF	401 ± 24^{a}	0.17 ± 0.03^{c,d,e}	–	–	–	–	0.24 ± 0.01^{b}
T*f*	0.7 ± 0.1^{c}	–	–	–	2 ± 0.4^{a}	1.8 ± 0.1^{a}	0.02 ± 0.00^{f}
Summer							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	6.9 ± 0.3^{c,d}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	17 ± 0.1^{a}
RO	7.5 ± 0.2^{c,d,e}	0.6 ± 0.1^{c,d}	<LOD	<LOD	<LOD	<LOD	1.20 ± 0.03^{d}
LE	12.4 ± 0.6^{a,b,c,d}	<LOQ^{b}	<LOD	0.01 ± 0.00^{c}	0.01 ± 0.00^{a,b}	<LOQ^{b}	0.08 ± 0.00^{c}
BCF	149 ± 4^{c,d,e}	0.08 ± 0.02^{d,e}	–	–	–	–	0.07 ± 0.00^{f}
T*f*	1.65 ± 0.04^{a,b,c}	–	–	1.6 ± 0.1^{c}	2.2 ± 0.2^{a}	–	0.07 ± 0.00^{c}
Santa Maria							
Winter							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	7.5 ± 0.2^{b,c,d}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	6.7 ± 0.1^{f}
RO	12 ± 3^{b}	1.7 ± 0.4^{c}	<LOQ	<LOD	<LOD	<LOD	3.24 ± 0.05^{a}
LE	11.1 ± 0.3^{b,c,d}	<LOQ^{a}	<LOQ	<LOQ^{d}	0.01 ± 0.00^{a,b}	<LOQ^{b}	0.28 ± 0.00^{a}
BCF	249 ± 56^{b}	0.23 ± 0.06^{c,d}	–	–	–	–	0.49 ± 0.01^{a}
T*f*	0.9 ± 0.2^{c}	0.04 ± 0.01^{a}	–	–	2.2 ± 0.1^{a}	–	0.09 ± 0.00^{a}
Summer							
IW	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD^{b}	<LOD
SE	<LOD	6.8 ± 0.6^{d}	<LOD^{b}	<LOD^{a}	<LOD	<LOD	16.3 ± 0.3^{b}
RO	5.3 ± 0.2^{e}	<LOQ^{d}	<LOD	<LOD	<LOD	<LOD	1.22 ± 0.04^{d}
LE	13.8 ± 0.7^{a,b,c}	<LOQ^{b}	<LOD	0.01 ± 0.00^{b}	0.01 ± 0.01^{a}	<LOQ^{b}	0.05 ± 0.00^{e}
BCF	105 ± 3^{e}	0.00 ± 0.01^{e}	–	–	–	–	0.07 ± 0.00^{f}
T*f*	2.6 ± 0.2^{a}	–	–	2.9 ± 0.3^{b}	3 ± 1^{a}	–	0.04 ± 0.00^{e}

Metal concentrations in interstitial water, sediment and in *Laguncularia racemosa* roots and leaves ($n = 9$ in each site). Values are expressed as mean ± SD

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 column data does not differ significantly (Bonferroni test; $P < 0.05$)

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, *Tf* translocation factor

cell wall and cell content, free-hand sections were stained with Sudan IV to detect lipid substances (Johansen 1940), where a positive reaction was indicated by an orange colour. For negative control, samples without staining were also analyzed. Sections were documented in photomicroscope Nikon Eclipse 50i (Tokyo, Japan).

Statistical analysis

Data are 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 normal distribution. One-way analysis of variance (ANOVA) was applied to compare data followed by Tukey's post-test with significance $P < 0.05$.

Multivariate statistical methods were applied to datasets: lineal discriminant analysis (LDA), generalized procrustes analysis (GPA) and Spearman's rank correlation coefficient. LDA was performed in the stepwise mode to verify statistical differences in global parameters measurement during each season (winter and summer) and at sites, and to evaluate interaction considering both seasonal and spatial responses. GPA was used to evidence both spatial and temporal segregation and to evaluate the matching of interstitial water elemental data to the corresponding data of sediment and plant (both elemental and anatomical). Sperman rank correlation was also applied for assessing the correlation between the data matrix (biological, chemical and physical) using a more formal mathematical approach (Di Paola-Naranjo et al. 2011).

Results and discussion

Environmental physical and chemical variables

Conductivity and dissolved oxygen in the interstitial water of Santa Cruz were higher than the corresponding to other sites. Organic matter in the sediment was higher in three studied sites of Vitória Bay (Santa Maria, Serra and Lameirão, with no differences between them) in comparison with Santa Cruz (Table 1). No seasonal differences were observed in the analyzed parameters (Table 1). The oxygen level in the environment determines the nature and velocity of chemical and biochemical reactions. The reduction and oxidation processes of metals and metalloids can elevate or decrease its toxicity, then a higher value of dissolved oxygen results in a higher metal bioavailability, due to an increase of the redox potential (Azevedo and Chasin 2003).

In this context, lowest levels of dissolved oxygen, associated with the highest concentration of organic matter in Vitoria bay, probably decreases the bioavailability of metals in this environment. In Santa Cruz, despite sediment have lower concentrations of the analyzed elements, they are more bioavailable when correlated with organic matter and dissolved oxygen.

Metals and metalloids

Table 2 shows the metal and metalloids concentration in the interstitial water, sediments and in roots and leaves of *L. racemosa*. In this work were detected Al, Cr, Cu, Fe, Hg, Mn and Ni in interstitial water, in which the values of Hg in Serra exceed eight times limits recommended by Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME 1995).

Sediment analysis showed higher concentrations of most contaminants (As, Cu, Pb) in Serra, although their concentration in Santa Maria were also high (Table 2). According to Index Sediment Quality Guidelines (ISQG) of Canada (CCME 1995), Santa Maria and Serra in winter, and also Santa Cruz during both winter and summer, showed higher values of arsenic (ratio $\text{marine}_{\text{As}}/\text{estuary}_{\text{As}} > 7.24$). High levels of As in the soil may affect the growth and development of plants, resulting in several biochemical and physiological disorders, which can lead to morphological changes (Singh et al. 2007; Forino et al. 2012).

Zn, Mn and B bioconcentration was observed in *L. racemosa*. For Zn, bioconcentration occurred in all sampled sites and seasons, with the highest value observed in Serra during the winter. For B and Mn, the highest values were found in Lameirão during the winter (Table 2). These results corroborate the data presented by Lewis et al. (2011), showing that the bioconcentration is usually low in mangrove plants. Higher values found for Zn and Mn may be explained by its high mobility (MacFarlane et al. 2007).

Translocation of Cu, Cd, Zn and Ag from roots to shoots of *L. racemosa* was detected throughout this study, with the exception of Cd in Santa Cruz and Ag in Lameirão. Cu was the metal showing the higher T*f*, becoming 179 times higher in leaves with relation to its content in roots. On the other hand, the translocation of Ag occurred only in the summer. Moreover, translocation of Mn and Hg was found only in Serra (Table 2). In general, essential elements have greater mobility than non-essential ones (MacFarlane et al. 2007), which probably may explain the pattern found in the present study.

Biological parameter

Absorption roots showed highest values of air gaps and cortex/vascular cylinder ratio at Santa Maria and Lameirão, respectively; while lower values of air gaps were found at Santa Cruz. For pneumatophores, highest values of air gaps and cortex/vascular cylinder ratio were found at Lameirão and Serra, respectively (Table 1, Fig. 2). It its worthy to remark that plants subject to lower oxygen levels tend to develop wider air gaps or thicker cortex with aerenchyma (Parlanti et al. 2011; Abiko et al. 2012). Furthermore, the aerenchyma promotes internal aeration and is typical on species that tolerate soil conditions with excess of water and hypoxia (Wegner 2010). The oxygen deficiency also stimulates the production of ethylene, inducing formation of aerenchyma through cell disintegration (Taiz and Zeiger 2006). In this work, aerenchyma development in roots had a negative correlation with the availability of dissolved oxygen in surface water (Spearman 0.78).

Pneumatophores periderm thickness was higher at Santa Maria and Lameirao and lower at Santa Cruz (Table 1, Fig. 2). The development of periderm is related not only to the plant age but also to environmental conditions, including pollution (Sawidis et al. 2011; Lux et al. 2011). Previous studies showed the role of periderm as a barrier to the input of metals, since plants subjected to high levels of metals in soil exhibited greater development of that tissue (Lux et al. 2011). Thus, the anatomical changes observed in *L. racemosa* in Vitória bay seem to be related to the highest values of contaminants found in this estuary.

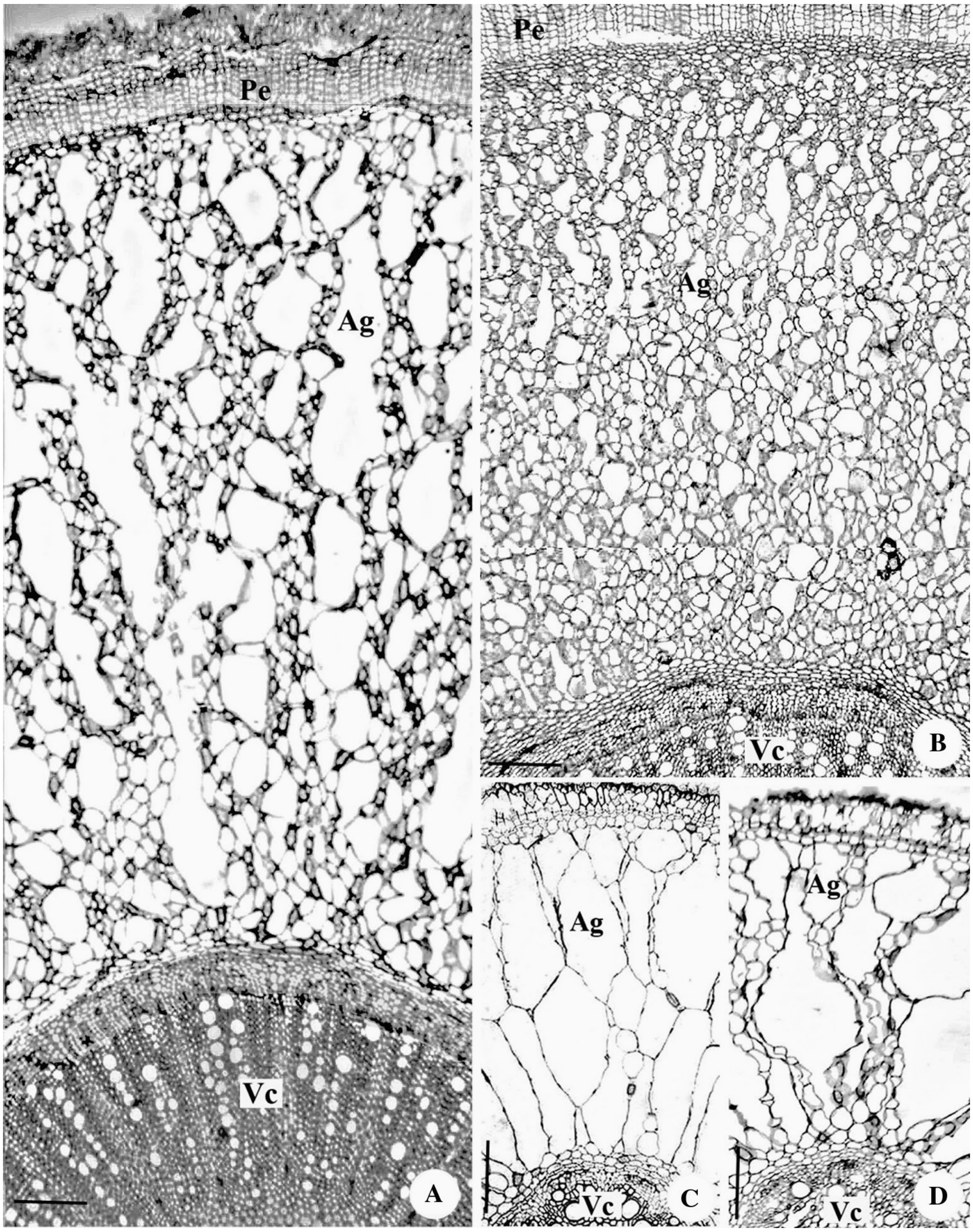


Fig. 2 Histological analysis in *Laguncularia racemosa* showing Pneumatophores: **a** plant from Serra, **b** plant from Santa Cruz; Absorption roots: **c** plant from Serra, **d** plant from Santa Cruz. *Bars* 0.4 mm (*Ag* air gap, *Pe* periderm, *Vc* vascular cylinder)

Interestingly, we observed oil droplets inside different tissues of absorption roots only in plants sampled at Serra in the summer season (Fig. 3). During that sampling, we also observed large amounts of oil in the sediment, probably caused by oil vessels, suggesting the permeability of the root to the input of this pollutant.

Overall discussion using multivariate statistics

Interpreting data from environmental biomonitoring is complex, particularly when multiple pollution sources are present and the multivariate analysis provided an integrated view of the overall situation, pointing out differences in the monitoring areas as well as associations between spatial and temporal variations and their effects on the biota (Wunderlin et al. 2001; Sinha et al. 2009; Monferrán et al. 2011).

LDA analysis was applied to verify the spatial and temporal differences between the studied areas, including the chemical parameters analyzed in interstitial water, sediment, root and leaves, and the anatomical root parameters. Table 3 presents the results of LDA analysis,

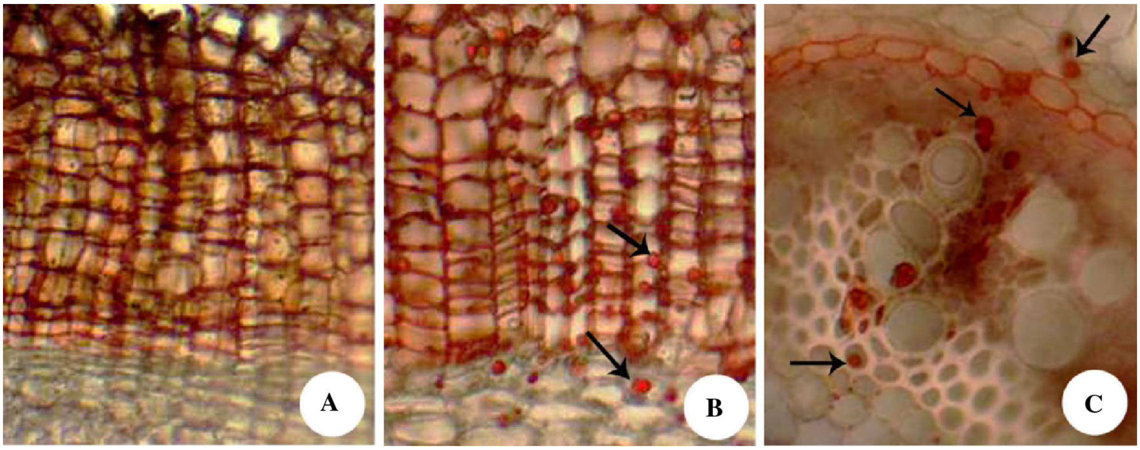


Fig. 3 Histochemical analysis in *Laguncularia racemosa*. Sections were stained with SUDAN IV. **a** Plant from Santa Cruz, **b**, **c** plants from Serra. *Arrows* indicate oil droplets

Table 3 Classification functions corresponding to LDA of studied parameters, considering both spatial and temporal variations

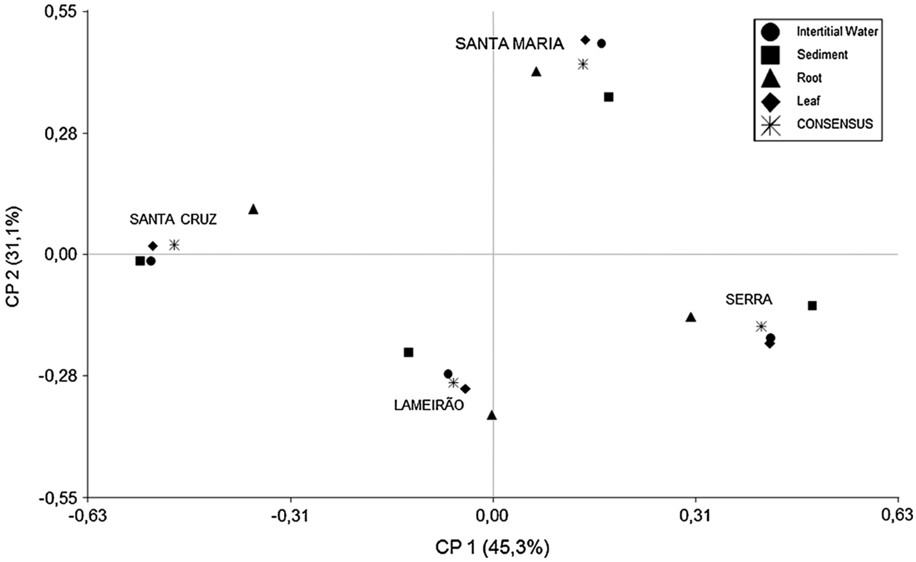
Sites	Santa Cruz $p = 0.2500$	Lameirão $p = 0.2500$	Serra $p = 0.2500$	Santa Maria $p = 0.2500$
Classification Functions LDA				
C/V ratio - PN	779890.9	953396.4	1126626.1	763286.0
Periderm - PN	543925.1	664851.3	784868.9	532043.1
Air gap area - AR	−114237.8	−128406.3	−136558.0	−90667.5
As - SE	1521.5	2318.6	3174.6	2279.2
Cu - LV	15406.2	19574.9	26815.3	17576.9
Cd - LV	10310.2	41818.4	55953.9	53158.4
Pb - LV	−142722.4	−163977.0	−200651.6	−129092.5
Mn - SE	761.9	835.2	929.3	611.1
Pb - RT	19819.4	23045.8	27844.9	18212.8
Ni - LV	−80639.8	−112076.8	−168267.7	−110291.2
Cr- LV	−43121.9	−43896.6	−38209.7	−26369.3
Fe - IW	−1.8	−2.2	−2.7	−1.7
As - LV	−38511.2	−49250.6	−65358.4	−43401.3
Root thickness - PN	−4517.4	−5133.3	−5607.9	−3657.6
Hg - LV	9738.6	16027.0	24924.8	17136.1
Constant	−878605.9	−1310549.2	1837840.3	−843468.3

C/V ratio cortex/Vascular cylinder ratio, *PN* pneumatophores, *AR* absorption roots, *SE* sediment, *LV* leaves, *RT* roots, *IW* interstitial water

showing classification functions for sampled sites. Fifteen (Fe in interstitial water, As and Mn in sediment, Pb in root, Cu, Cd, Pb, Ni, Cr, As and Hg in the leaves and four anatomical root parameters) out of 63 parameters (Table 3) were necessary to distinguish the areas with 100 % correct classification (classification matrix, data not shown). Thus, the complex data matrix obtained by performing chemical and biological monitoring at both estuaries could be reduced to only 15 parameters showing spatial but not temporal differentiation. No differences were detected between summer and winter.

Considering that 12 out of these 15 parameters belong to the plant (metals-metalloids and anatomical root parameters), it is clear that the plant condition can evidence differences between studied areas better than the single use of chemical measurements from sediment and interstitial water. Furthermore, LDA indicated that cortex/vascular cylinder ratio of pneumatophores, periderm of pneumatophores and air gap area of absorption roots were the parameters showing the maximal discriminating power (Table 3). This last point, reinforce the need of integrated studies on estuaries, including physical partitions (sediment,

Fig. 4 Generalized procrustes analysis (GPA) of studied parameters from each sampling site



water, etc.) and the inhabiting biota, considering the uptake of chemicals and the biological response of the biota to different levels of toxic elements.

In our current study, cortex/vascular cylinder ratio of pneumatophores, periderm of pneumatophores, air gap area of absorption roots and root thickness of absorption roots of *L. racemosa* were negatively correlated with the level of arsenic in the root (Spearman 0.61, 0.65, 0.62 and 0.62, respectively). Moreover, the level of arsenic in the root was positively correlated with arsenic in the sediment (Spearman 0.59). The strong affinity of As for aquatic particles, particularly iron and manganese oxides, results in its deposition in sediments in association with these materials. In sediments, the fate and persistence of As are intricately connected with that of iron oxides and are influenced by redox conditions, pH, and microbial activity in the sediments (CCME 1995).

A positive correlation between Cu concentration in leaves with Cu concentration in sediments was observed (Spearman 0.68), which show the uptake and translocation of this metal from sediment to aerial parts of the plant. Considering that Cu is a redox-active transition metal essential for plants, acting in photosynthetic and respiratory electron transport chains, cell wall metabolism and others processes (Peng et al. 2013; Yruela 2009), this translocation may occurs to provide the required copper level to the plant. Negative correlation was found between Cu levels in leaves of *L. racemosa* with Mn levels in interstitial water (−0.77), in sediment (−0.82), and roots (−0.74).

Thereafter, a GPA was performed, looking to demonstrate matching between interstitial water, sediment, root (metals-metalloids and anatomical parameters) and leaves, considering only spatial differences since temporal differences were discarded by LDA analysis. Figure 4 shows a graphical representation of the correspondence between the analyzed parameters. Significant differences between studied areas were described primarily by the first axis (CP1), which explained 45.3 % of the total variance, while the second function, described by the second axis (CP2), accounts for additional 31.1 %. Also from Fig. 4 it can be observed that the first function (CP1) contributes to the spatial differentiation between both studied areas (Santa Cruz and Vitória Bay–Lameirão, Serra and Santa Maria).

Box plots with patterns representing six out of 53 studied parameters are shown in Fig. 5. The levels of Pb in the root (Fig. 5a), leaves (Fig. 5b) and sediment (Fig. 5c) evidence the spatial difference between areas, showing that Santa Cruz estuary has the lowest values of Pb in the sediment, which clearly separate this area from Vitória Bay. Also the level of Cu in the sediment of Santa Cruz is the lowest in comparison to other three areas (Fig. 5d). Furthermore, different levels of these metals in the sediment are translated to the plant (Figs. 5a, b and e). The root periderm thickness of *L. racemosa* shows an adaptive response of this species, being thin in individuals from Santa Cruz and thicker as the level of toxic compounds rise in the sediment (Fig. 5f). Thus, by the parameters evaluated, *L. racemosa* showed to be a species able to adapt to different pollution scenarios. It is also worthy to remark the usefulness of LDA to extract valuable information from a complex data matrix (Table 1).

These results are in agreement with Souza et al. (2013), highlighting a higher pollution degree at Vitória Bay over the Santa Cruz estuary through metals-metalloids analysis in surface water, interstitial water, sediment and fish (*Centropomus parallelus*), confirming the results obtained by the GPA, which show the spatial differentiation of both estuaries.

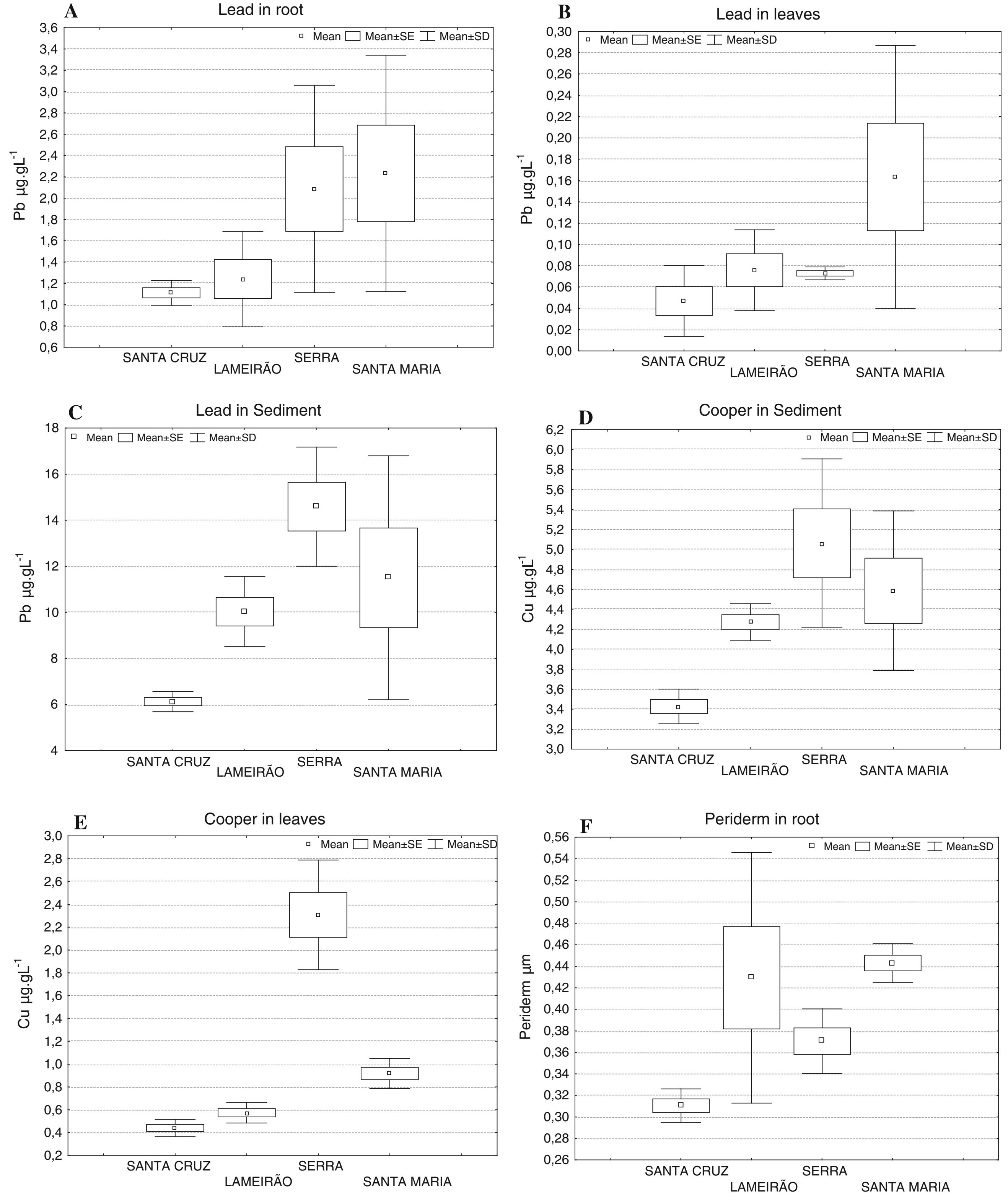


Fig. 5 Box & whisker plots from some selected parameters measured in sediment, root and leaves from *Laguncularia racemosa*. Values are reported as mean ± SD and SE

Multivariate statistical analyses indicated that plants from each station exhibited a distinctly response among studied areas, pointing out that the bioaccumulation of metal-metalloids contribute significantly to discriminate among studied areas, reinforcing the need of integrated monitoring, using both physical–chemical parameters and biota biomarkers to generate a complete scenario for the assessment of the water quality. This approach is especially important in neotropical estuaries, considering variations in salinity, DO and radiation, which may affect environmental parameters and their influence on inhabiting biota (Canário et al. 2007).

Conclusion

The root anatomy responded to different pollution scenarios, showing the adaptive plasticity of *L. racemosa* to different environments present within both estuaries. The use of multivariate statistics greatly contributes to extrapolate results from field and laboratory measurements, pointing out parameters that help to differentiate sites with different contamination sources and, consequently, different risks for the biota. Furthermore, statistical methods like LDA and GPA contribute to integrate the knowledge coming from different scientific disciplines, such as biology and chemistry, producing more complete results complementing both field and laboratory efforts.

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Conflict of interest The authors declare that they have no conflict of interest.

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