

Leaf traits and mineral contents of *Rhizophora mangle* L. subject to environmental pollution

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Abstract

Rhizophora mangle L. (*Rhizophoraceae*) is a halophyte from two mangroves in the municipality of Rio de Janeiro in Rio de Janeiro state, Brazil. This work aimed to determine the presence of environmental contaminants in the leaf of *R. mangle* through morphological and chemical analyses. We collected samples in Coroa Grande mangrove (CG), near an industrial park, and Pedra de Guaratiba mangrove (PG), which is currently under threat of anthropization by domestic occupation. Energy Dispersive X-Ray Spectroscopy (EDS) coupled to Scanning Electron Microscopy (SEM) revealed the presence of chemicals and their relative quantities in leaves. Light microscopy and Scanning Electron Microscopy (SEM) were used to examine leaf tissue. Na, Ca and Cl were found in both CG and PG. Specific leaf area was significantly lower in PG. Leaves from CG also presented the three heavy metals Mn, Zn and Cu. *R. mangle* presents succulent, hypostomatic leaves with dorsiventral structure. Abundant mucilage and crystals in idioblasts were observed. Slight differences in anatomical qualitative traits between leaves from both sites were detected. Leaves from CG were slightly thicker than those examined from PG, especially on main vein vascular system, and a high quantity of stomata was observed in abaxial surface of leaves from PG. Collectively, these findings indicate that *R. mangle* is resilient and tolerant to the environmental pollutants and presents functional traits related with saline environments; therefore, the morphoanatomical variations are probably related to abiotic factors.

Highlights

- The main minerals detected by EDS were Na, Ca and Cl.
- Zn and Cu were the only heavy metals detected in *R. mangle* leaves in samples from CG.
- A large number of stomata in leaves were observed in plants from PG.
- Differences in spongy parenchyma and stomata were noted between leaves of PG and CG.
- No anatomical changes related to pollution were noted.

1. Introduction

Rhizophoraceae is a pantropical family with six species and three hybrids (Mantiquilla et al. 2021). *Rhizophora mangle* L., known as red mangrove, is a tree with an aerial structure system formed by unique branches with positive geotropism and rhizophores that firmly establish the tree in permanently swampy soils, forming a large number of roots (Pitot 1958; Menezes 2006; Costa et al. 2017). Rhizophores are also interpreted as stilt roots or aerial roots and are considered an adaptation in flooded areas (Tomlinson 1986; Raven et al. 1992; Evans et al. 2005). Three species are found in Brazil: *R. mangle*, *R. racemosa* and *R. harrisonii* (Menezes et al. 2008). *R. mangle* is a multipurpose tree, the only species that occurs in Rio de Janeiro (Costa et al. 2017). It can be used as a food in times of emergency. Its bark and leaves have medicinal properties and are used in folk medicine for treatment of diabetes, dysentery,

angina, elephantiasis, and diarrhea. They are also attributed with antifungal, antibacterial and antiulcer activity (Cruz et al. 2015). Bernini et al. (2010) suggest that *R. mangle* may play a role in mangroves as biogeochemical barriers against the transit of heavy metals. Located on the fringes of mangroves, *Rhizophora* spp. trap plastic waste that arrives in large quantities through multiple tidal cycles, posing a risk to this ecosystem (van Bijsterveldt et al. 2021; Videla and Araújo 2021).

Mangroves are resilient ecosystems where biodiversity is transitory. Some species have a permanent place in mangroves, such as *Rhizophora* spp. the most representative genus, but others, such as some migratory birds and fish, only make temporary visits to feed, reproduce and grow (Lo et al. 2014; Alongi and Mukhopadhyay 2015). Mangrove plants are restricted to the typical intertidal mangrove habitats where seawater salinity is often 17.0–36.4 (Wu et al. 2008). Brazil has the second largest area of mangrove forests worldwide. They are distributed in estuaries, lagoons and bays along a little more than 7.408 km of coastal areas. In Brazil, estuarial mangroves are areas of permanent conservation, and no type of degradation is allowed by Law 4,771/1965 (Brazilian Forest Code). Nonetheless, the health of this ecosystem is severely affected by anthropogenic activities (Nguyen et al. 2020).

West Zone of Rio de Janeiro city has historically been an industrial location. In the past, this area was a major textile center. Nowadays, large-scale enterprises are found with correspondingly large efflux of pollutants, mainly with considerable pollution potential, including metallurgical, petrochemical, and pyrometallurgical smelters (Fonseca et al. 2013; Silva and Victório 2021). These areas are around Sepetiba Bay and very close to Coroa Grande (CG) mangrove, but farther from Pedra de Guaratiba (PG) mangrove, the collection points of this study. The area is advantageous for industry because it is close to Itaguaí Harbor and the *Arco Rodoviário Metropolitano* (SEMA 1998; Wasserman et al. 2013; Silva and Victório 2021). Consequently, the West Zone is subjected to high pollution of substrate, water and air that can be detected in plant organs (Flores et al. 2018; Victório et al. 2020; Flores et al. 2021). The backfilling of mangroves to accommodate illegal housing construction has also led to the suppression of flora and fauna of mangroves around Sepetiba Bay, revealing socioenvironmental vulnerability. Domestic sewage and agricultural activities, textile, and beverage industries put mangroves at risk by the influx of chemical pollutants (Souza et al. 2018). Fruit and vegetable growers have transformed important fragments of the tropical rain forest on the Serra do Mar mountain slopes, resulting in the aggregation of phosphate fertilizers and herbicides that contribute to heavy metal pollution (Molisani et al. 2004).

Reports have shown that leaf organ from mangrove areas can adsorb, bioaccumulate or degrade metals and other pollutants using specific mechanisms (Moreira et al. 2011; Flores et al. 2018; Victório et al. 2020; Victório et al. 2021; Flores et al. 2021). This work aimed to determine the presence of environmental contaminants in the leaf of *R. mangle* through microchemical analyses and evaluate morphoanatomical features using microscopy.

2. Materials And Methods

2.1. Study areas and plant materials

Rhizophora mangle L. leaves were collected in two areas in the state of Rio de Janeiro (Figures 1, 2), around Sepetiba Bay, in June-July 2012: Coroa Grande (CG) (22°54'42.23"S and 43°52'48.88"W) and Pedra de Guaratiba (PG), (23° 0'27.98"S and 43°37'22.41"W). CG mangrove, Itaguaí City, is a plain with 10 meters of mud and then extending out for 20 meters of sand (Coimbra 2003). CG is located near the industrial park (Fig. 1). Mangroves in PG feature a landscape of well-integrated ocean, estuary, river and canals, mangrove forests, hypersaline plains and swamps. PG is located along the eastern portion of Sepetiba Bay (Estrada et al. 2013) next to the *Reserva Biológica de Guaratiba* (Fig. 1).

2.2. Analysis of Sepetiba Bay: physicochemical and heavy metals

Sepetiba Bay has brackish water according to resolution n. 357/2005 of CONAMA, Brazil (Environmental Brazilian Council), considering the parameters DO (7.0-8.3 mL/L), salinity (20-33%) and pH (6.5-8.1) (Ferreira et al. 2010; Silva 2017; Monte 2014). The presence of heavy metals has been noticed in several reports. The minerals and heavy metals of interest present in Sepetiba Bay are summarized in Table 1.

2.3 Leaf mass, area and SLA determination

From each sampling location, ten fully expanded and undamaged leaves were removed from the branches collected from five individuals for determination of the leaf area. Mature leaves were collected from the third to fifth node, from the apex of the branches, at a height of approximately 1.75 cm above substrate level. Leaves (Fig. 2C) were photographed individually on a known area for determination of leaf area using ImageJ, v. 1.42q (Rasband 2011), for subsequent calculation of extract yields. The experiment was conducted in a randomized design with three replicates for each locality. The means of leaf areas and their standard deviations were calculated. Weighing was done with a digital analytical balance with four decimal places, applying the parameter in grams (g). For fresh weight, ten leaves of five individuals were used. They were conditioned in paper at room temperature for drying and maintained in an oven at 50°C. After seven days, the leaves were unwrapped and weighed to calculate the dry weight. Later, they were weighed for confirmation of the values. Specific leaf area (SLA) was calculated using the ratio of the leaf area to the corresponding leaf dry weight. These procedures were repeated in the three areas, and the average dry and fresh weight for each species was calculated.

2.4 Analysis of minerals by Energy Dispersive X-Ray Spectroscopy coupled to Scanning Electron Microscope (EDS-SEM)

The ashes for metal presence analysis were obtained by burning ten leaves from each locality in a muffle furnace, as described by the Association of Official Analytical Chemists (AOAC 1995). Ten leaves randomly collected from five individuals from each study area were deposited in porcelain crucibles and previously dried at 60°C in an air circulation oven for one hour, followed by storage at room temperature. Afterwards, porcelain crucibles that contained leaves were put in an EDG 3P-S muffle furnace for 60 min at 800°C.

Ashes from the burned leaves were analyzed by Energy Dispersive X-Ray Spectroscopy (EDS or EDX, Thermo®, Noran System Six model, coupled to SEM, Jeol JSM-6380L), following the protocol of Resende (2013) with adaptations. The main electron beam of the Scanning Electron Microscope (SEM) focuses on the sample. Its atoms emit X-rays. Each element of the periodic table has specific and well-defined energy peaks; consequently, it is possible to identify elements present in the sample. The detector will capture the X-ray photons emitted by the sample and measure its energy in electron volts (Ev), counting how many photons are detected with certain energy content. From these results, a spectrum is produced indicating the number of photons for each X-ray-emitted value. Ev is the amount of energy gained by a single electron when accelerated by a power of one volt in vacuum. Data were obtained by means of three replicates from each set of ten burned leaves for each study area.

2.5 Microscopy analyses

For anatomical analyses and leaf measurements, three leaves from each site were fixed in FAA (3.7% formaldehyde, acetic acid and 70% ethanol (1:1:18), Johansen 1940), dehydrated in ethanol series and stored in ethanol 70%. Middle portions of leaves were cut in appropriate pieces and embedded in glycolmethacrylate (Historesin®), cross sectioned (8 µm thickness) in a rotary microtome (Leica RM 2155), and stained with Toluidine Blue 1% (O'Brien et al. 1965). Histochemical tests were performed on free-hand sections with Sudan IV to identify lipophilic substances (Pearse, 1972), Ruthenium Red for mucilage (Gregory and Bass 1989), and Toluidine blue 1% and ferric chloride for phenolic compounds (Johansen 1940). To observe the leaf epidermis, we prepared epidermal peels. Briefly, 0.5 cm² of leaves from three individuals were treated with glacial acetic acid and hydrogen peroxide for 12h, according to leaf consistency. Afterwards, the epidermal segments were stained with basic fuchsin (Luque et al. 1996), mounted in 20% glycerin, and sealed with nail polish. Photomicrographs of leaf sections were made using light microscopy (Leica) fitted with a digital camera. The measurements were taken from images obtained from leaf cross sections using a light microscope (LM, Nikon Eclipse Ci) equipped with a digital camera (Moticam Pro 252b).

For leaf surface observations, scanning electron microscopy (SEM) was performed. Segments of dry leaves, not previously treated, were mounted on stubs and coated with a thin layer of gold (Denton Vacuum Desk IV, LLC). The abaxial and adaxial surfaces of leaves were analyzed with a JEOL-JSM 6390 LV scanning electron microscopy (JEOL, Tokyo, Japan).

2.7 Statistical analyses

Morphoanatomical data from leaves and mineral content were subjected to a one-way analysis of variance (ANOVA), considering data for each mangrove (Coroa Grande and Pedra de Guaratiba), followed by a Tukey's multiple comparisons test. Data are expressed as mean±SD. A difference was considered to be statistically significant when $p < 0.05$. All statistical analyses were performed with GraphPad Prism software, version 8.0 for Windows.

3. Results

Fresh and dry leaf mass showed similar values between the two mangroves. Leaves from PG had a smaller mean area in comparison with CG. SLA was significantly lower in PG (Figure 3).

EDS spectra (Table 2) showed the elemental composition and relative distribution of nutrients in leaves from mangrove plants. All leaves contained micronutrients (Mn, Zn and Cu) that are also heavy metals, macronutrients (Na, Mg, Ca, K, P and S) and other elements, such as Si and Cl (Table 2).

Minerals in high amounts found in leaves of *R. mangle* collected in CG and PG were Na, Ca and Cl. These minerals are common in saline ecosystems that have input of seawater. The presence of heavy metals was found mainly in CG: Mn, Zn and Cu (Table 2). Only Mn (1.005 ATOM%) was found in leaves of *R. mangle* from PG. For this analysis, we quantified by the percentage of atoms of each element present in each leaf, or ATOM.

Leaf anatomy

Intercostal region - Epidermis

In frontal view, the leaf surface of *R. mangle* under scanning electron microscopy shows an epicuticular wax layer forming projections on the upper surface giving a reticulated appearance. On the underside, the wax appears to form granules (Fig. 4A, B). The cuticular layer that covers the adaxial surface of the leaves of individuals from Coroa Grande is slightly thicker than that of Pedra de Guaratiba leaves; however, on the abaxial surface, we did not observe any differences (Tab 3.). Stomata of the leaf blade are randomly distributed only on abaxial surface (Fig. 4B, F) and common epidermal cells with straight cell walls (Fig. 4E, G). We observed 25 stomata/mm² in plants from CG and 74 stomata/mm² in plants from PG, some of which seem to give rise to lenticels (Fig. 4D, F, H). In cross section, we observed that the epidermis is uniseriate (Fig. 5) The stomata are located below other common epidermal cells. Stomatal cells have well-developed ridges that project outwards (Fig. 4C, 6A). The subsidiary cells also contain mucilage, are very bulky compared to stomatal and other epidermal cells, and protrude on the inner side of the epidermis towards the stomatal pore (Fig. 5A). Quantitative differences observed in the epidermis by comparing leaves from individuals collected in the Coroa Grande and Pedra de Guaratiba mangroves are presented in Table 3

Mesophyll

In cross section, under the epidermis, we observed a thick layer of the hypodermis more developed on the adaxial side (Fig. 5A). The hypodermis is formed by cells with very thick walls, achlorophyllous, and containing phenolic compounds, especially in the strata close to the epidermis with no marked differences observed in these tissues between the two sampled populations. Since only anatomical aspects of the subepidermal layer were considered with no further ontogenetic studies, we identified it as an aquiferous hypodermis. Under the hypodermis, we noted palisade parenchyma formed by 1-3 layers

with narrow cells. Large mucilaginous idioblasts are evident in this tissue, as well as others containing phenolic compounds (Fig. 5B). The spongy parenchyma is formed by a varied number of cell layers (8-12) between which large intercellular spaces are observed (Fig. 5A). Along the abaxial face, however, in the regions between stomata, the parenchyma cells are organized in a more compact way, containing phenolic compounds in abundance. Idioblasts containing druse crystals can be seen throughout the mesophyll (Fig. 5A). The vascular system is formed by collateral vascular bundles with a parenchyma sheath (endoderm) surrounding the vascular bundles. No significant differences were observed regarding the organization of the mesophyll in leaves of either population evaluated.

Main vein

In cross section, the main vein is also covered by a uniseriate epidermal layer, absent of stomata, on the adaxial surface. Under the upper surface, the hypodermic layer is continuous, extending over the entire leaf blade, as well as the palisade parenchyma, which shows no break in continuity, at least in part of the region (Fig. 5C). Under the hypodermis, the cortical region is occupied by regular parenchyma, containing large intercellular spaces, and chlorophyll in the innermost portion. Idioblastic sclereids are dispersed in the cortical region, having thick and lignified walls. Mucilaginous and crystal-containing idioblasts are also seen (Fig. 5C). Internally, the vascular system is composed of a set of collateral vascular bundles organized in a flat-convex cylinder (Fig. 5D). The vascular system of the main vein was more robust in leaves from CG compared to those from PG. The vascular system was apparently the most developed region owing to greater width and height of vascular tissues (primary xylem and phloem, Table 3). Phenolic and crystalline idioblasts were seen in abundance in vascular tissues, especially phloem. Fiber caps may be associated with vascular bundles forming an outer layer.

4. Discussion

Mangrove is an ecosystem whose woody plants serve as a natural water and wastewater treatment system in addition to dampening various inappropriately discarded residues that arrive by the movement of oceanic tides and get stuck in the stems and roots (Ouyoung and Guo 2016; Biswas and Biswas 2019). The ability of mangrove plants to tolerate high concentrations of salts may be associated with tolerance to heavy metals since both have similar physiological mechanisms (Manousaki and Kalogerakis 2011). Sediment analyses of Sepetiba Bay has shown high contamination by heavy metals, mainly Zn and Cd (Wasserman 2001), and recent data showed elevated levels of Mn (Flores et al. 2021). Heavy metals are largely retained in mangrove roots, and because of this, the amount in aerial parts is lower than sediment (Liu et al. 2009). However, high amounts of heavy metals have been recorded in leaves by deposit of particulate air pollution or by root uptake from soil (Bayen 2012; Arrivabene et al. 2015). The accumulation of heavy metals in plant organs reflected their concentrations in the environment and their bioavailability (Thanh-Nho et al. 2019). Using EDS, three heavy metals were detected in leaves of *R. mangle*. Reports have shown that the *Rhizophora* genus can absorb, translocate and accumulate heavy metals, and for this reason, it is considered to be involved in the phytoremediation process (Pinheiro et al. 2012; Agbogidi 2011). For example, young mangrove trees of *R. apiculata*

bioextracted chromium (Richter et al. 2016). Meanwhile, *R. mucronata* is considered a bioindicator by its good accumulation and translocation rate of Pb (Ganeshkumar et al. 2019). *Rhizophora mangle* is also considered an important bioindicator of mangrove areas contaminated by various metals based on atomic absorption spectrophotometry analysis (Pinheiro et al. 2012). In comparison, EDS analysis did not detect Cd or Cr, considering the same leaf stage, only Cu and Mn. This technique is limited because the results are shown as relative values (%ATOM), rather than absolute numbers. Excepting Cu, Mn and Zn were present in leaves from CG, which is closer to the industrial center. The presence of Mn was detected in Sepetiba Bay and in leaves of *Avicennia shaueriana* in high quantity, using inductively coupled plasma optical emission spectrometry (ICP-OES) (Flores et al. 2021).

The availability of heavy metals in water and soil can lead to bioaccumulation along the food chain. As heavy metals are not degraded, they can be absorbed by plants and displaced at trophic levels. Herbivores, as primary consumers, consume plants and are, in turn, consumed at other trophic levels. Senescent leaves are decomposed by bacteria and fungi, and the metal can again return to the substrate (Mathivanan and Rajaram 2014). Consequently, pollutants are remobilized as heavy metals (Almahasheer et al. 2018). Thus, heavy metals are persistent pollutants that can act in the long term, affecting biotic and environmental health. The presence of heavy metals in mangroves points to the contamination of ecosystems and damage to ecological relationships. Considering the same minerals, we have compared the detection of heavy metals between the leaves of *R. mangle* and *A. shaueriana* from the same mangroves of Sepetiba Bay (Victório et al. 2020). We conclude that *A. shaueriana* is a better biomonitor and could contribute more effectively to phytoremediation in mangrove restoration initiatives since higher concentrations of metals were found in its leaves.

The EDS leaf spectra showed a similar chemical composition among leaf samples collected in mangroves, considering the major nutrients. According to EDS spectra, Na, Ca and Cl accounted for over 50% of the total plant mineral composition. The high concentration of Na, Ca and Cl is compatible with the brackish water that is constantly influenced by seawater. Na, Ca and Cl constitute about 99% of the total content of the earth's crust (Alloway 2013). NaCl is the most abundant salt in salinity-affected land. Sepetiba Bay suffers from the influence of several channels that flow into it, causing water to pile up and stagnate. However, the contribution of fresh water to the bay, mainly originating from the Guarda and Guandu Rivers, does dilute marine waters entering the bay, generating variation in salinity (Droppo and Ongley 1994). During winter, the tide has greater influence because of the rise in sea level influenced by cold fronts, and the entry of oceanic waters promotes the renewal of the waters of Sepetiba Bay (SisBaHiA 2018). This renewal of waters occurs both through the entry of oceanic waters through Barra de Guaratiba to the east and restinga of Marambaia to the west (SEMADS 2001).

We observed that leaves from PG had smaller SLA in comparison with that of leaves from CG. Silva et al. (2015) comment that sun-exposed and shade-grown *R. mangle* leaves are not easily distinguishable, but are very different when considering leaf area and average length, with sun-exposed leaves tending to be smaller, corroborating the data here found. The decrease of leaf size reduces the air layer adjacent to the

leaf, allowing greater heat loss to the environment by convection, thereby requiring less transpiration to cool the leaf (Leigh et al. 2017).

Leaves of *R. mangle* herein evaluated presented leaf anatomical features indicated in classical literature for the family and genus. The thickness of the cuticle+epidermis layer did not show significant difference between the two evaluated sites and can be considered as a constant attribute for adult leaves, as suggested by Farnsworth and Ellison (1996) in an ontogenetic study of *R. mangle* in different life forms (seedling, sapling and tree).

Rhizophora mangle presents a dorsiventral, hypostomatic leaf with uniseriate epidermis and hypodermis. We found no differences in tissue composition between the leaves of the compared areas, corroborating information previously reported (Metcalf and Chalk 1950; Silva et al. 2015; Pelozo et al. 2016). Stomata located only on abaxial surface is a common trait of many angiosperms exposed to sunlight. In the plants investigated here, we observed high values of stomata in plants from PG compared with those from CG (74 stomata/mm²), similar to sun-exposed *R. mangle* leaves (Silva et al. 2015).

In the leaf blade of *R. mangle* herein evaluated, we observed 2-3 lenticels/mm², a high number compared with results cited for plants from the shores of Indian Rocks Beach, Florida, coming in at 0.17 lenticels/mm² (Evans et al. 2005). In addition to occurring in the leaf blade and petiole, these structures can be seen in the stem and aerial roots and roots buried in mud in association with the transfer of O₂ from the leaves and aerial parts to the small roots growing in anoxic substrate (Evans et al. 2005). Leaf lenticels are additional sites of gas exchange and can originate from scars resulting from the fall of trichomes, stomata or over secretory structures (Vaz et al. 2018). Lenticels can form on submerged parts of stems and on roots of a wide variety of woody plants through which potentially toxic compounds associated with anaerobiosis, including the release of acetaldehyde, ethanol, and ethyl (Koslowiski 1997). Under the epidermis, on both sides of the leaf blade, we observed a continuous layer of cells containing phenolic compounds, a characteristic not mentioned for the same species occurring in other regions of Brazil. This layer under the epidermis can provide chemical protection to the mesophyll and underlying tissues, not only against high rates of radiation, but also against pathogens, given its remarkable antimicrobial activity (Castro and Demarco 2008). In leaflets of *Prosopis strombulifera* treated with Na₂SO₄, Reginato et al. (2014) observed a strong accumulation of phenolic compounds in the epidermis and subepidermal mesophyll layers that led the authors to propose a fundamental role of polyphenols in the protection of the photosynthetic apparatus under severe stress since epidermis and subjacent tissue are the main sites of flavonoid accumulation in plants.

On the leaves of plants living in areas of saline soils, succulence is often observed (Da Silva et al. 2020). In the mesophyll of *R. mangle* leaves, succulence and leaf thickness were related to the upper hypodermis. We did not identify significant differences in the thickness of the palisade parenchyma, which can be associated with the probable similarity of light conditions received by the plants in the two areas evaluated here. In a comparative study on the effects of sun and shade on leaves of *R. mangle*, Silva et al. (2015) found a significant difference in the thickness of the palisade parenchyma, but no

changes in the thickness of the spongy parenchyma, indicating the role of light on the development and length of cells in the palisade parenchyma (Silva et al. 2015). Idioblastic mucilage cells detected in palisade parenchyma can be interpreted as multifunctional secretory structures in view of their complex chemical composition, including water retention, owing to their hydrophilic property, or filtration for certain wavelengths of solar radiation (Rocha et al. 2011). Some anatomical characteristics observed, such as thick leaf, tissue for water reserve, mucilaginous idioblasts, abundant phenolic compounds, in addition to thick cuticle can be considered relevant functional traits for survival in saline environments and with water deficit.

Flooding is a big problem for the survival of plants in many parts of the world because when it happens, it restricts gas exchange, especially in the roots (Fukao et al. 2019). In the case of *R. mangle* leaves, we noticed the development of spongy parenchyma, characterized by wide intercellular spaces, similar to an aerenchyma, occurring in the region located along the lower side of the epidermis of the leaf blade and main vein. Intercellular spaces improve gas diffusion and reduce the metabolic requirements of the flooded organs. Considering that *R. mangle* belongs to the group of flood-tolerant mangrove species (Mantiquilla et al. 2021), we consider that the greater development of the spongy parenchyma, connected to the stomata on abaxial surface, contributes to the better diffusion of CO₂ and O₂ between the leaf and the outside, especially during periods of high tide and flooding of aerial roots. Given the parameters evaluated, no variation in the anatomical structure can be attributed to pollution.

Conclusion

EDS microanalysis allowed assessment of the mineral nutrition in leaves of *R. mangle* from different mangroves, highlighting the macronutrients Na, Ca and Cl and including such heavy metals as Zn. The heavy metals detected included Mn, Cu and Zn and were found in leaves of *R. mangle* from CG. Micromorphology has a high diagnostic value, allowing us to distinguish plant species and alterations in leaves under different conditions in the ecosystem. Leaf anatomy did not change when comparing the two study sites, but some quantitative differences were observed, and both origin and functions need further investigation.

Declarations

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Author contributions

All authors contributed to the study conception and design. Plant material was collected in mangrove by Cristiane P. Victório, Mayara S. dos Santos, Guilherme T. Alves and Marcelo da C. Souza. Material preparation, data collection and analysis were performed by all authors according to their expertise. The first draft of the manuscript was written by Cristiane P. Victório and Rosani do C. O. Arruda and all authors commented on previous versions of the manuscript.

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Tables

Table 1

Total concentration of minerals and metals in sediments of Sepetiba Bay, as analyzed by ICP-OES and meteorological data (temperature and pluviosity).

Minerals	Mangrove* (mg/L)
Na	21.3-27.93 ^a
Ca	3.97-6.87 ^a
Cl	27.64-33.32 ^a
Heavy metals	(mg/kg)
Cd	0.3 – 23 ^b
Cu	4 - 1.16 ^b
Mn	227 – 872 ^d 60.24 ^c
Zn	17 - 3.4 ^b
Meteorological data**	
T (°C)	20-22 ^e
P (mm)	53-67 ^e
Salinity (%)	20-33 ^e
*Sediment or water of Sepetiba Bay. **Approximate ranges of data obtained from two points in Sepetiba Bay. (-) not determined. Letters indicate references: ^a Comitê da Bacia Hidrográfica Guandu 2012, ^b Rocha et al. 2012, ^c Flores et al. 2021 (phase where metals are soluble), ^d Gomes et al. 2009, ^e Silva, 2017.	

Table 2

Content of minerals in leaves (%ATOM) of *Rhizophora mangle* L. (Rhizophoraceae) by Energy Dispersive X-Ray Spectroscopy (EDS) coupled to SEM (EDS-SEM). Mangroves around Sepetiba Bay, Rio de Janeiro: Coroa Grande (CG) and Pedra de Guaratiba (PG). Minerals were divided into micronutrients, macronutrients and other elements.

Minerals		(%ATOM)	
		Coroa Grande	Pedra de Guaratiba
Micronutrients	Mn*	0.1	1.005
	Zn*	0.2	nd
	Cu*	0.27	nd
Macronutrients	Na	35.04 ^a	23.81 ^b
	Mg	1.39	11.46
	Ca	17.9	24.69
	K	4.005	4.925
	P	0.685	2.96
	S	5.76	3.13
Other elements	Si	nd	0.305
	Cl	34.93	27.71

The data expressed mean of minerals for leaf samples. *Heavy metals. nd – not detected. Different letters indicate statistical differences for nutrients ($p < 0.05$).

Table 3

Leaf anatomical traits (μm) from plants of *Rhizophora mangle* L. (Rhizophoraceae) collected in two mangroves in Rio de Janeiro state (CG- Coroa Grande and PG – Pedra de Guaratiba).

Leaf anatomy	Parameters	CG	PG
Epidermis (adaxial surface)	Lenticels/ mm^2	0	0
Epidermis (abaxial surface)	Stomata/ mm^2	25 ^a	74
	Lenticels/ mm^2	3	2
Intercostal region (μm)	Adaxial cuticle	7.57 $\pm$ 1.40	6.16 $\pm$ 1.21
	Abaxial cuticle	3.53 $\pm$ 0.70	3.10 $\pm$ 0.86
	Adaxial epidermis height	13.64 $\pm$ 1.26	13.09 $\pm$ 1.79
	Adaxial hypodermis	112.22 $\pm$ 12.29	101.47 $\pm$ 22.79
	Abaxial epidermis height	11.40 $\pm$ 2.09	11.14 $\pm$ 1.96
	Mesophyll thickness	370.19 $\pm$ 9.13	377.35 $\pm$ 27.23
	Palisade parenchyma	123.89 $\pm$ 12.46	120.23 $\pm$ 11.23
	Spongy parenchyma	148.56 $\pm$ 9.83 ^b	165.91 $\pm$ 25.93
	Adaxial cuticle	11.21 $\pm$ 0.78	10.07 $\pm$ 1.08
	Abaxial cuticle	5.82 $\pm$ 0.68	5.96 $\pm$ 0.43
Midvein region (μm)	Adaxial epidermis + hypodermis height	14.28 $\pm$ 1.01	13.21 $\pm$ 0.71
	Abaxial epidermis height	12.30 $\pm$ 0.94	11.52 $\pm$ 0.82
	Midvein total height	1244.22 $\pm$ 7.69	1069.83 $\pm$ 20.77
	Vascular bundle height	606.30 $\pm$ 5.12	530.30 $\pm$ 9.08
	Vascular bundle width	745.67 $\pm$ 5.63	690.09 $\pm$ 4.94
	Hypodermis	112.22 $\pm$ 3.41	101.47 $\pm$ 6.32
Values represented the means of 12 replications per treatment $\pm$ SE. Letters indicate significant difference for each parameter, $p < 0.05$.			

Figures

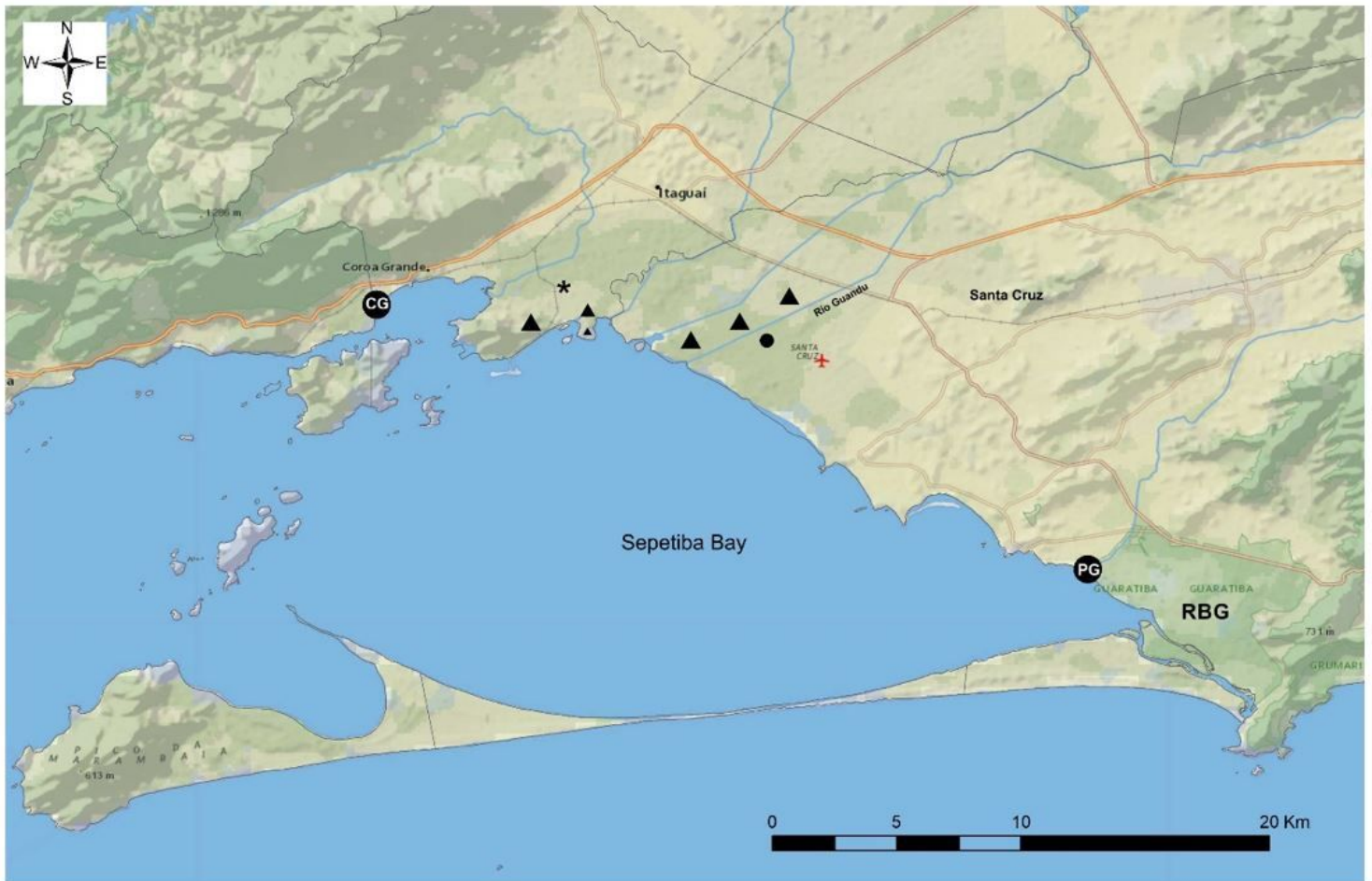


Figure 1

Mangrove collection areas of *Rhizophora mangle* L. (*Rhizophoraceae*) around Sepetiba Bay. CG: Coroa Grande (Itaguaí City) and PG: Pedra de Guaratiba (Rio de Janeiro City). ● Santa Cruz Industrial Park (Rio de Janeiro City, circle). ▲ Itaguaí Industrial Park (triangle), both in Rio de Janeiro state, Brazil. *Itaguaí Harbor, RBG: Conservation Unit *Reserva Biológica de Guaratiba*.

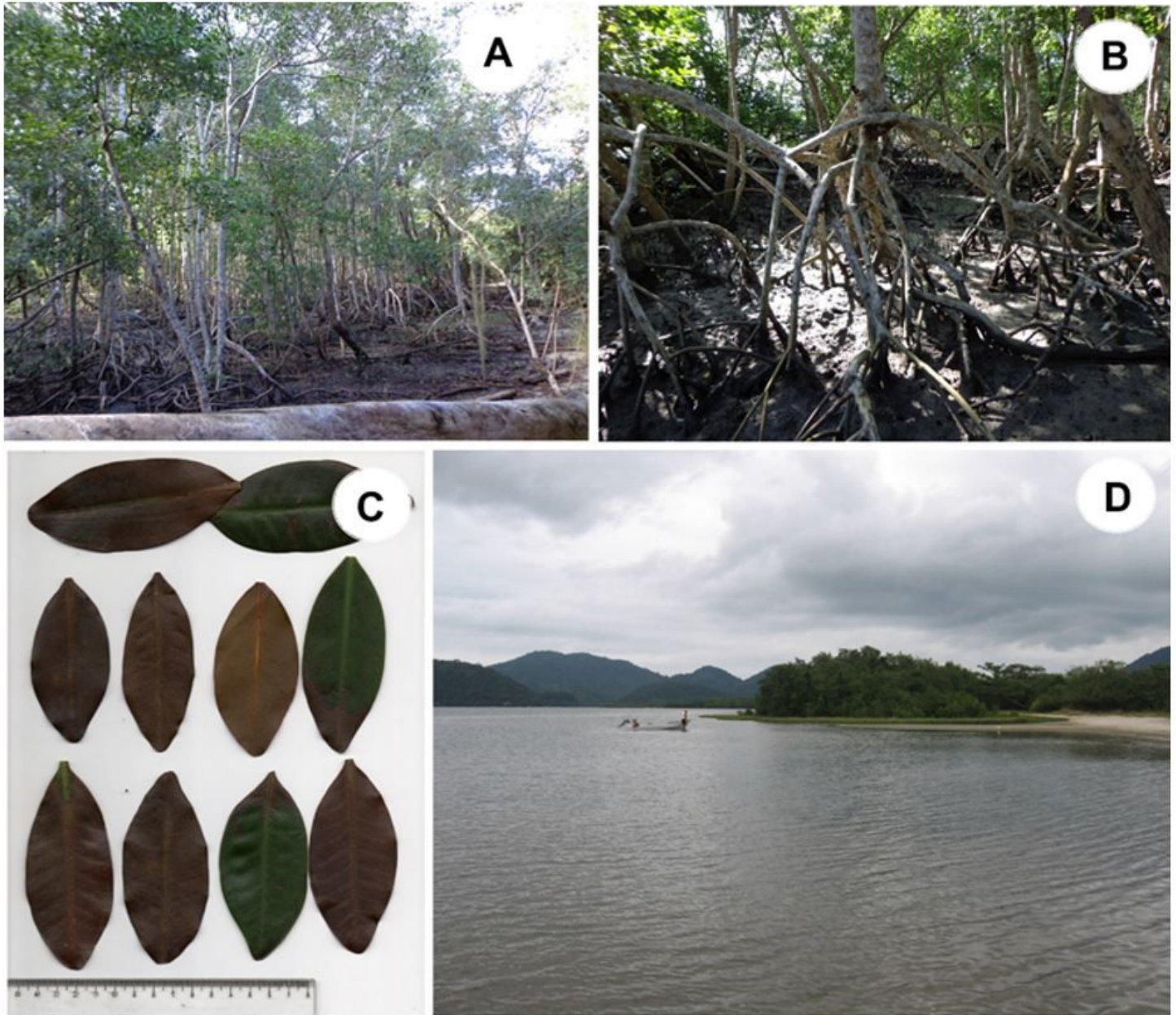


Figure 2

Plant habit and collection sites of *Rhizophora mangle* L. (*Rhizophoraceae*) leaves around Sepetiba Bay in Rio de Janeiro state, Brazil. A-B. Rhizospheres (detail B) from *R. mangle* from Pedra de Guaratiba mangrove. C. Leaves of *R. mangle* organized for microscope scanning and analysis of leaf area. D. Coroa Grande mangrove around Sepetiba Bay, Rio de Janeiro.

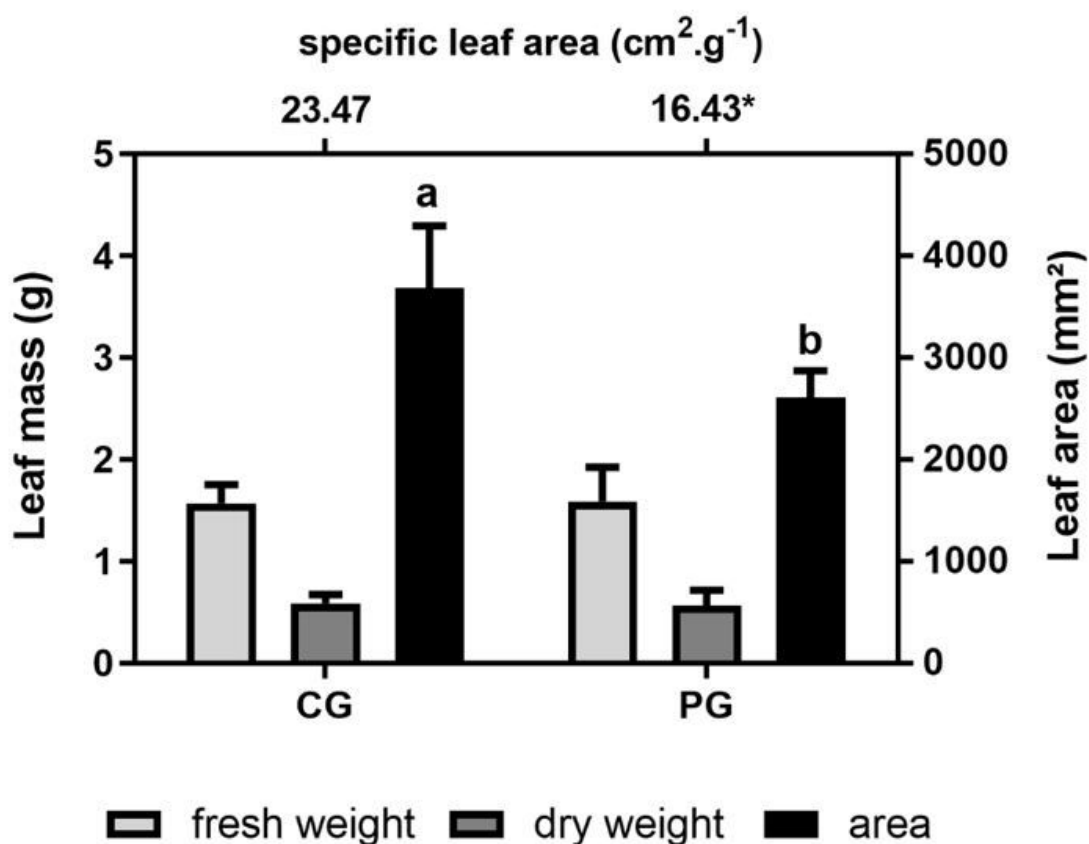


Figure 3

Leaf traits: fresh and dry mass, area and specific leaf area (SLA) of *Rhizophora mangle* L. (*Rhizophoraceae*) collected in mangroves of Coroa Grande (CG) and Pedra de Guaratiba (PG), around Sepetiba Bay, Rio de Janeiro state. Values represent the means of 10 replications per treatment $\pm$ SD. Different letters and (*) indicate statistical differences ($p < 0.05$) using the Tukey test.

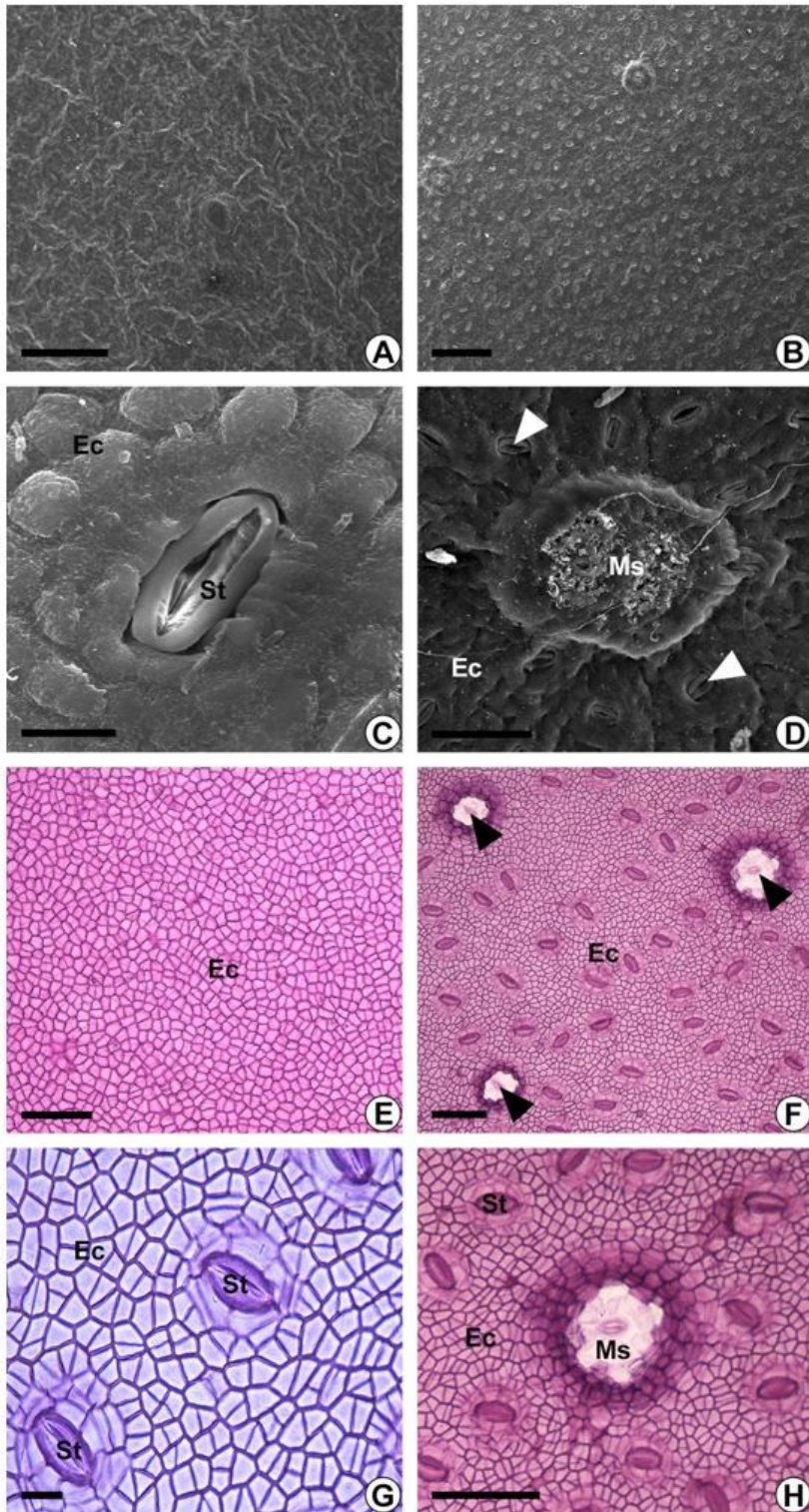


Figure 4

Rhizophora mangle L. (Rhizophoraceae): micromorphology of leaf surface (A, B, C, D - surface electronic microscopy, SEM) and details of epidermis (E, F, G, H, light microscopy, ML). A, D-H: Coroa Grande mangrove. B-C: Pedra de Guaratiba mangrove. EC: common epidermal cells; St: stomata; Ms: lenticels formed by modified stomata; white arrows: normal stomata seen in SEM; black arrows: lenticels formed by modified stomata seen in ML.

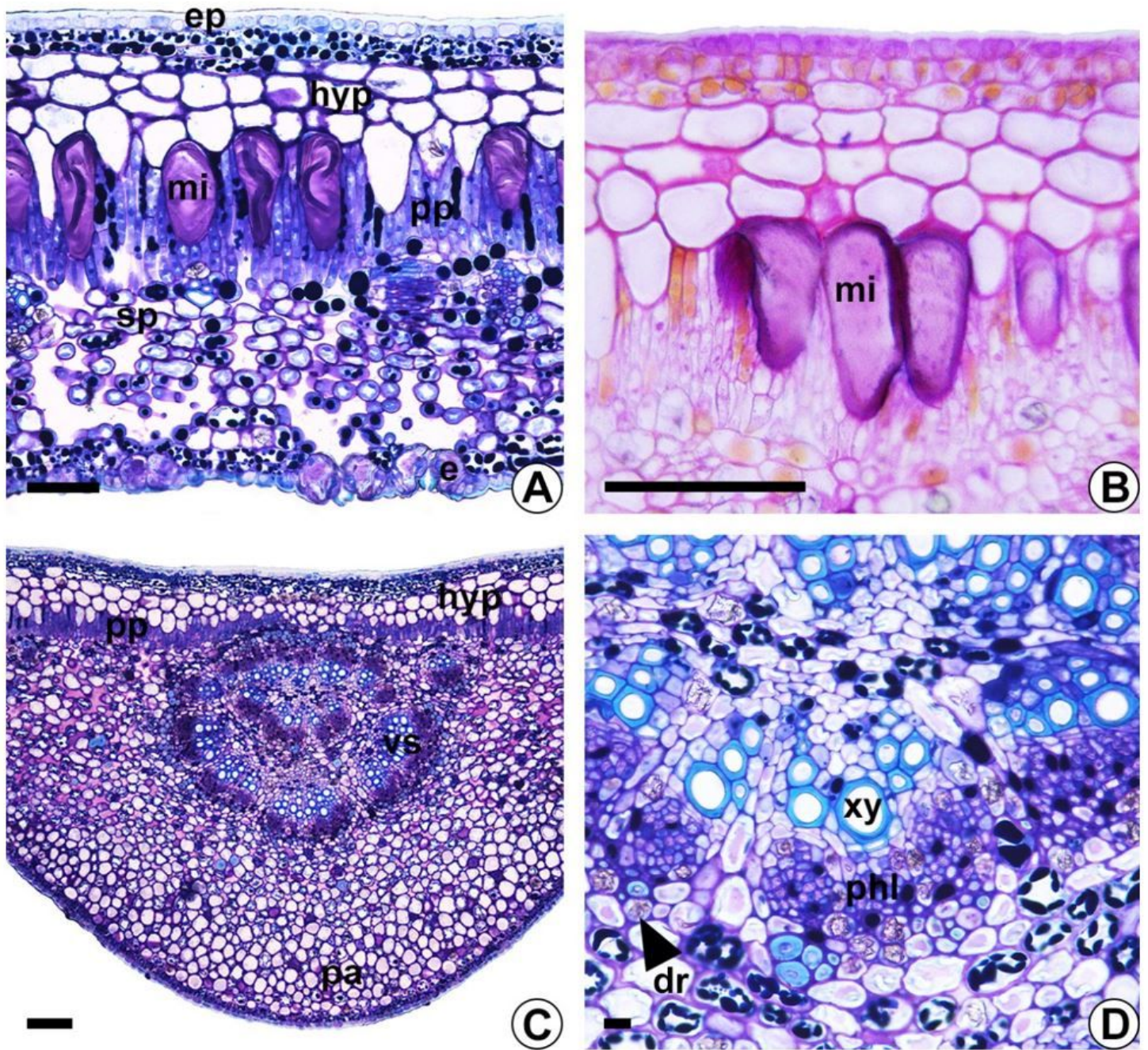


Figure 5

Rhizophora mangle L. (*Rhizophoraceae*). Leaf blade in cross section in light microscopy (ML): intercostal (A, B) and midvein region (C, D). ep: epidermis; e: stomata; hyp: hypodermis; pp: palisade parenchyma; sp: spongy parenchyma; mi: mucilage idioblast; pa: regular parenchyma; xy: xylem; ph: phloem; dr: druse idioblasts.