Microanalysis of minerals in *Rhizophora mangle* L. leaves from Marambaia mangrove

Microanálise de minerais em folhas de *Rhizophora mangle* L. do manguezal da Marambaia

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ABSTRACT

Marambaia mangrove, area bathed by the Sepetiba Bay, is located in an area protected by the Brazilian Army and has a remarkable level of environmental protection among ecosystems around the bay. Leaf content of minerals in a typical species of mangrove of *Rhizophora mangle* L. were analyzed from samples collected in Marambaia. Microanalyses of minerals were done by Energy Dispersive X-Ray Spectroscopy coupled to Scanning Electron Microscope (EDX-SEM) that showed the elemental composition and its relative quantity in *R. mangle* leaves. The major mineral deteced were Na, Ca and Cl, and low concentration of Zn. Leaves of *R. mangle* from Marambaia revealed low presence of potentially toxic elements.

Keywords: Atlantic Forest, EDS, heavy metal.

RESUMO

O manguezal da Marambaia, banhado pela Baía de Sepetiba, está localizado em área protegida pelo Exército Brasileiro e possui notável nível de proteção ambiental entre os ecossistemas do entorno da Baía. O conteúdo de minerais em folhas de uma espécie típica de manguezal - *Rhizophora mangle* L. foi analisado a partir de amostras coletadas na Marambaia. As microanálises dos minerais foram feitas por espectroscopia de energia dispersiva de raios-X acoplada ao microscópio eletrônico de varredura (EDX-MEV) que mostrou a composição elementar e sua quantidade relativa nas folhas de *R. mangle*. Os principais minerais detectados foram Na, Ca e Cl, e baixa concentração de Zn. Folhas de *R. mangle* da Marambaia revelaram baixa presença de elementos potencialmente tóxicos.

Palavras-chave: Mata Atlântica, EDS, metais.

INTRODUCTION

Mangrove is an important ecological ecosystem of Atlantic Forest biome geographic domain. The calm waters of the mangrove, combined with the root protection of arboreal plants and a high nutritional reserve, attract many animals that come to reproduce and remain in the initial stages of development. This ecosystem control erosive process and reduce environmental contamination of coastal (Filho and Silva, 2017). Acting as biogeochemical barriers to minerals transport and, in general, present relatively high concentrations in their sediments both by earth crust origins and by high anthropogenic pollution associated with economical developmental practices (Schaeffer-Novelli 2001; Alongi and Mukhopadhyay 2015; Almahasheer et al. 2018).

Brazil has a wide area of mangrove forests that suffers a high impact of anthropologic activities that harms the atmosphere, soil, water places and their biotic factors (Victório et al. 2020; Victório et al. 2021; Videla and Araujo, 2021; Flores et al. 2022). Marambaia in the Rio de Janeiro State comprises restinga and mangrove ecosystems, it has an area of 81 km², with an elongated shape in the West-East direction (Figure 1). Marambaia mangrove forest form a natural barrier island and is in an area under military jurisdiction part of the Piraquê Microbasin, with a series of restrictions on the movement of people and objects and the use of the island's natural resources, being therefore a preserved area although the dynamics of the tide entering Sepetiba Bay move waste and pollutants (Santos et al. 2019).

Woody plants as *Avicennia shaueriana*, *Rhizophora mangle*, *Laguncularia racemosa* are found in Marambaia mangrove. The genus *Rhizophora* (*Rhizophoraceae*) has a striking morphological characteristic- the stems form arcs towards the ground (rhizophores) that help in sustaining the plants in the face to tidal movement. The rhizophores are rich in lenticels whose are also seen in the other plant organs and contribute to the oxygenation (Menezes, 2006). *Rhizophora mangle* (Fig. 1), popularly known of red mangrove, is spread along Atlantic and Pacific coasts and in Brazil is found on the coast from South to North (Record et al., 2013).

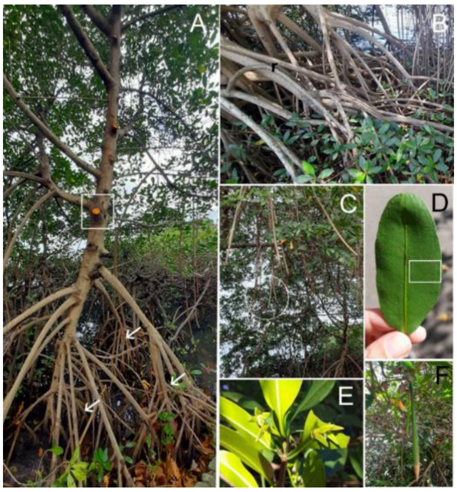

Fig. 1. *Rhizophora mangle* (*Rhizophoraceae*) in Marambaia mangrove (Rio de Janeiro State, Brazil): plant habit and morphological details. A. Arboreal habit showing reddish wood after cut (white square). B. Rhizophore (r) with lenticels. C. Adventitious roots (white circle). D. Leaves with lenticels in adaxial surface (white square). E. Plant with flowers. F. Seed germination inside fruit still attached to the mother plant.

The leaves are organs that reveal physiological and morphoanatomical responses associated with the evolution of plants in ecosystems, but also with eco-adaptive responses to periodic environmental conditions. Therefore, they are able to indicate nutritional and physical changes such as solar intensity and rainfall and environmental pollution of ecosystem (Victório et al. 2020; Flores et al. 2022) which turn leaf evaluation a key step in science. In this study we investigated the content of minerals in leaves of *R. mangle* collected in Marambaia mangrove, a protected remnant mangrove using method of microanalyses by EDX-SEM.

MATERIAL AND METHODS

Study areas and plant materials

The collection point is a plain adjacent to the narrow channel (Bacalhau Channel) of Barra de Guaratiba, through which the waters of the Atlantic Ocean enter into Sepetiba Bay (Fig. 2). Near to Marambaia collect point there are many houses and the main pollution is domestic sewage, plasticizers and agricultural activities (Souza et al. 2018; Silva and Victório, 2021; Victório et al., 2021). Industrial and metallurgical centers are located about 60 km away. Occupation by irregular housing is advancing in the surrounding mangrove areas around Marambaia that are landfilled, and there is real estate speculation in the area that has high scenic beauty. The mangroves in the Guaratiba region are a source of income for the local community due to the fishes, crabs and oysters, ancient activities since *Tupi-Guarani* and attested by the shell mounds (*sambaquis*) present in the region (Filho and Silva, 2017).

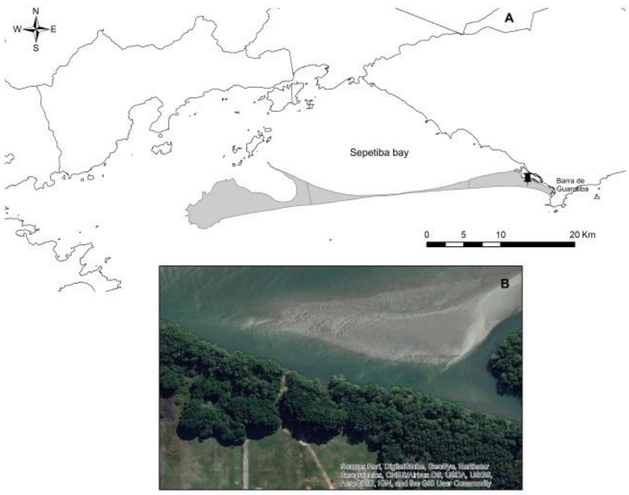

Fig. 2. A. Marambaia mangrove (strip of sand in gray), Rio de Janeiro (Brazil), and the collect point (black arrow) where leaves of *Rhizophora mangle* was collected (side Bacalhau Channel). B. Satellite photo of Marambaia mangrove: collecting point.

Anthropogenic pressure is a threat to mangrove biodiversity and also to adjacent areas such as restingas. Month average of precipitation was 43.9 mm in the dry season. The annual average temperature is 23.5ºC, with an amplitude of 5.7ºC (Chaves, 2001; Estrada et al. 2008). The access to the mangrove for the collection was made by Barra de Guaratiba, guardhouse of the Army of Brazil, with prior permission, since it is a militarily protected area with very restricted access.

Plant material and morphotraits

Leaves were collected from 5 individuals found in the study area. Representative samples of the studied plants were collected and processed for inclusion in the herbarium RBR (Universidade Federal Rural do Rio de Janeiro, UFRRJ).

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 the substrate level. The leaves were photographed individually on a known area for determination of leaf area using ImageJ. The experiment was conducted in a randomized design with three replicates. Average of leaf areas 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 50ºC. After seven days, the leaves were unwrapped and weighed to calculate the dry mass. Later, they were weighed again for confirmation of the values. These procedures were repeated, and the average dry and fresh masses was calculated and obtained.

Analysis of minerals by EDX-SEM

The ashes for mineral and metal presence analysis were obtained by burning ten leaves for each locality in a muffle furnace. Ten leaves randomly collected from five individuals were deposited in porcelain crucibles and previously dried at 60ºC in an air circulation oven for one hour, followed by storage at room temperature. Then, porcelain crucibles that contained leaves were put in an EDG 3P-S muffle furnace for 60 min at 800ºC.

Ashes from the burned leaves were analyzed by Energy Dispersive X-Ray Spectroscopy (EDX, also EDS or XEDS, Thermo®, Noran System Six model, coupled to Scanning Electron Microscope-SEM, Jeol JSM-6380L) for qualitative elementary analyzes, following the protocol of Resende (2013), with adaptations. It works such that the main electron beam of the 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 was produced indicating the amount 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.

RESULTS AND DISCUSSION

Morphometrical features revealed 0.55 g to dry leaf mass and leaf area 3580.3 mm^2 (Fig. 3).

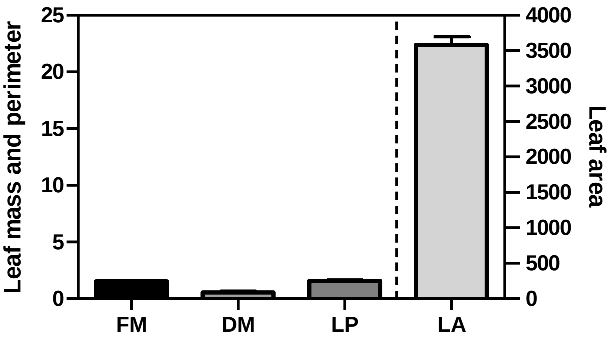

Fig. 3. Leaf traits: fresh and dry mass (g), leaf perimeter (LP, mm) and leaf area (LA, mm2) of *Rhizophora mangle* collected in Marambaia mangroves, Rio de Janeiro, Brazil. Values represent the means of 10 replications per treatment ± SE. FM: fresh mass; DM: dry mass.

EDX analyses enabled a chemical characterization and relative quantification of minerals in leaves from *R. mangle*. The concentration of each element present is expressed in ATOM%. It was detected the micronutrients Zn, macronutrients (Na, Mg, Ca, K, P and S) and other elements– Si and Cl (Fig. 4).

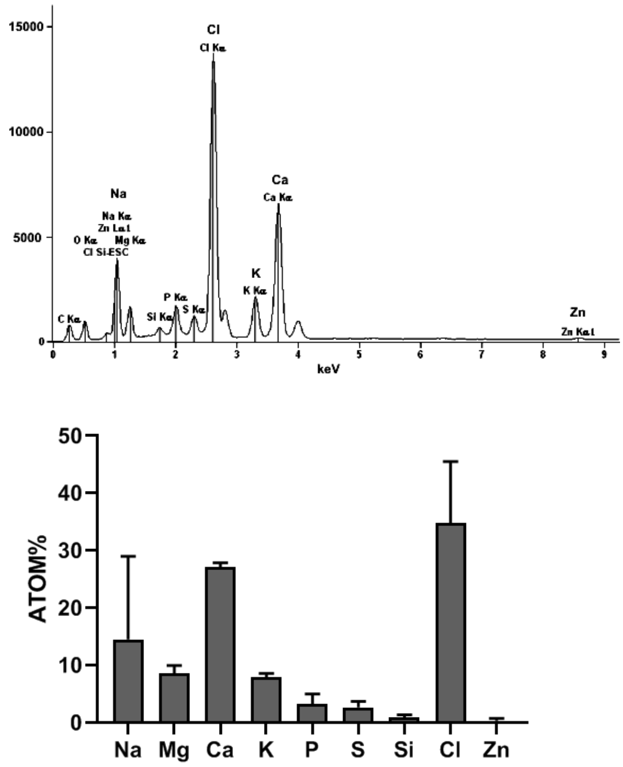

Fig. 4. EDX spectrum and relative content of minerals in leaves (%ATOM) of *Rhizophora mangle* by EDX-SEM collected in Marambaia mangrove, Rio de Janeiro (Brazil). This graphic shows the macronutrients, other elements as Si and Cl and micronutrient/metal Zn.

The major minerals in high amount found in leaves of *R. mangle* collected in Marambaia were Na, Ca and Cl. The unique metal- Zn (0.6 ATOM%) was detected in leaves of *R. mangle*.

The salinity, low oxygenation, flooding, processes of reduction and oxidation are pressures subject mangrove ecosystems that resulted in adaptation of the plants along the evolutionary dynamics (Madi et al., 2015; Nizam et al. 2022). According microanalyses by EDX spectra using SEM as visual resource, Na, Mg and Ca accounted for over 50% of the total plant mineral composition. The high concentration of

Na, Ca and Cl in leaves indicate salinity of mangroves that are constantly influenced by Atlantic Sea water. *R. mangle* samples were collected in physiographic fringe forest, found along protected margins and because of this it is washed daily by the tides. As a result of washing, salinity is low compared to other physiographic mangrove types, with no high physical-chemical gradients. NaCl is the most abundant salt in salinity-affected land. The contributions of fresh water to the bay originating mainly of the rivers of Guarda and Guandu, dilute the marine waters entering the bay generating variation in salinity (Droppo and Ongley, 1994).

Mangrove plants have different strategies to regulate the entry and accumulation and use of minerals (Madi et al., 2015), considering high salt mangrove concentration there are species that contain leaf glands that excrete salt as *A. shaueriana* and *L. racemosa* and not present in *R. mangle*; the fall of leaves in short period reported for *R. mangle* and other resources as lenticels suggested by ongoing studies (data not shown) may be involved. By these same pathways *R. mangle* can eliminate potentially toxic elements.

EDX-SEM has advantages of localize minerals in plant tissues fresh due to the opportunity to observe the sample under natural conditions right after collection (Pataca et al. 2005). Although it is not necessary to submit the plant material to any preparation before analyzing by EDX, in this study, leaves were burned to evaluate minerals. The ashes from the leaves are the result of the complete combustion of organic matter producing carbon dioxide and water, the volatile inorganic materials are also degraded, leaving only the target minerals for analysis, remaining inorganic materials (Pataca et al., 2005). EDX technique disadvantage is not reaching detection limits comparable to those of atomic spectrometry techniques as ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) commonly used in detection of minerals.

Leaf analysis is important to investigate process of entry of potentially toxic elements and other substances, since substances can rise via the root up to leaves or be deposited or absorbed by leaf surface and pass through the cuticle of leaf and epidermal structures (Terekhina and Ufimtseva, 2020). Fernandez and Eichert (2009) proposed that particles could penetrate inside the leaf tissue through pores present on the leaf cuticle and inside stomata what may be a pathway to metal entry.

Among the metals proven in the composition of the dynamics of the Sepetiba bay, only Zn was detected in the ashes of the leaves through the EDX method. In low concentrations, Zn is considered essential micronutrients to plants in general. There are reported that Zn is one of the main pollutants in Sepetiba bay mainly due to environmental liability of the metallurgical company Ingá Mercantil in mangrove areas around Sepetiba Bay that extracted Zn from calamine. The company went bankrupt in the late 20s resulting in a high level of exposure of Sepetiba Bay to Zn (Wasserman, 2013).

Data about metals in leaves of *Avicennia shaueriana* also by EDX methods in the same mangrove, and considering the same period and minerals, indicate that *A. shaueriana* accumulated more Zn than *R. mangle* (Victório et al., 2020).

Species of *Rhizophora* are known to have capacity to absorb and accumulate potentially toxic elements as Cr and Pb, polycyclic aromatic hydrocarbons (PAHs), plasticizer in epicuticular waxes, and may be a biotool in phytoremediation process or as bioindicator of environmental health (Pinheiro et al., 2012; Richter et al., 2016; Ganeshkumar et al., 2019; Verâne et al. 2020; Victório et al. 2021). The adsorption was also a manner to remediation verified in studies with R. mucronate where stem-bark showed be effective for dyes removal from industrial effluents (Oloo et al., 2020).

Mangrove species are constantly under anthropic pressure from water and air pollution. Because of this, many studies investigate the capacity of flora of this ecosystem to bioindicate and remediate different chemical compounds.

CONCLUSION

Chemical elements were detected by quick EDX-SEM technique with spotlight to macronutrients Na, Ca and Cl and it was verified Zn. The EDX technique combined with the observation of the leaves of trees can be very useful to signal environmental pollution and promote ecosystem conservation. Considering that Marambaia comprises an area far from the industrial and metallurgical center around the Sepetiba Bay the data are in agreement both under the qualitative and quantitative aspects of detected potentially toxic elements.

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