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Differential bioaccumulation and translocation patterns in three mangrove plants experimentally exposed to iron. Consequences for environmental sensing[☆]

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ABSTRACT

Avicennia schaueriana, Laguncularia racemosa and *Rhizophora mangle* were experimentally exposed to increasing levels of iron (0, 10, 20 and 100 mg L^{-1} added Fe(II) in Hoagland's nutritive medium). The uptake and translocation of iron from roots to stems and leaves, Fe-secretion through salt glands (*Avicennia schaueriana* and *Laguncularia racemosa*) as well as anatomical and histochemical changes in plant tissues were evaluated. The main goal of this work was to assess the diverse capacity of these plants to detect mangroves at risk in an area affected by iron pollution (Vitoria, Espírito Santo, Brazil). Results show that plants have differential patterns with respect to bioaccumulation, translocation and secretion of iron through salt glands. *L. racemosa* showed the best environmental sensing capacity since the bioaccumulation of iron in both Fe-plaque and roots was higher and increased as the amount of added-iron rose. Fewer changes in translocation factors throughout increasing added-iron were observed in this species. Furthermore, the amount of iron secreted through salt glands of *L. racemosa* was strongly inhibited when exposed to added-iron. Among three studied species, *A. schaueriana* showed the highest levels of iron in stems and leaves. On the other hand, *Rhizophora mangle* presented low values of iron in these compartments. Even so, there was a significant drop in the translocation factor between aerial parts with respect to roots, since the bioaccumulation in plaque and roots of *R. mangle* increased as iron concentration rose. Moreover, rhizophores of *R. mangle* did not show changes in bioaccumulation throughout the studied concentrations. So far, we propose *L. racemosa* as the best species for monitoring iron pollution in affected mangroves areas. To our knowledge, this is the first detailed report on the response of these plants to increasing iron concentration under controlled conditions, complementing existing data on the behavior of the same plants under field exposure.

1. Introduction

Mangroves are part of the ecosystem continuum from terrestrial to fully marine environments that occur at the boundary between land and sea, and are flooded regularly by the tide (Clough, 2013). Coastal wetlands have been recognized for their ability to stabilize shorelines and to protect coastal communities. Coastal wetland

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vegetation is an effective shoreline buffer, which acts as a natural barrier to stabilize fine sediment, preventing coastal erosion. Moreover, they reduce effects of storms and flooding, maintain water quality and biodiversity, and support a wide range of wildlife. Mangroves may have an indirect value in the protection of coastal property and economic activities such as fishery (Barbier et al., 2011; Vo et al., 2012). However, despite all the goods and services provided around the world, estimated at $1.7 to $2.8 billion per year (Brander et al., 2012; Costanza et al., 1997), mangroves have the fastest rates of loss of ecosystems worldwide, and are increasingly impacted by pollution (Valiela et al., 2001). Among the contaminants, pollution by metals is a concern, since these elements can be uptaken, transferred and involved in metal biomagnification along the food chain, generating toxicity for the biota (Cardwell et al., 2013; Nica et al., 2012).

Metal accumulation in mangrove sediment is favored by its high capacity of absorption of organic matter and small particles (Zhou et al., 2010). The anaerobic environment, in addition to high levels of organic matter and iron sulfide, in mangrove sediments enhance iron settling, and the accumulation of metals. Therefore, iron is responsible for modulating the bioavailability and redox characteristics of metals in sediments (Morse and Rickard, 2004).

Vascular plants mangroves are crucial to the dynamics of the estuarine ecosystem, strongly influencing the processes of metals retention, with unique biological mechanisms. Some mechanisms involved in the resistance of mangrove plants to metals, namely the accumulation of metals in roots in comparison with the aerial parts of the plants, have been reported (Nath et al., 2014). These mechanisms involve the formation of an iron (Fe) plaque on the root surface (Cheng et al., 2014; Du et al., 2013), and metal retention in the root (epidermis and endodermis) (Lu et al., 2014; MacFarlane and Burchett, 2000). Evidence suggests that the extent of Fe-plaque formed on mangrove root is species-specific (Pi et al., 2011) and that the amount of Fe-plaque can be related to adaptive changes in the root anatomy in response to pollution (Pi et al., 2010). An additional adaptive response in some mangrove plants is the secretion of metals through salt glands (MacFarlane and Burchett, 1999; 2000; Naidoo et al., 2014).

Avicennia schaueriana Stapf & Leechm. ex Moldenke, *Laguncularia racemosa* (L.) C.F. Gaertn. and *Rhizophora mangle* L. are mangrove species, commonly found in Brazil (Giri et al., 2011; Tomlinson, 1994). These plants are well adapted to saline wetlands, presenting aerenchymatose roots with air gaps that are usually bigger in *Avicennia schaueriana* and *Laguncularia racemosa* in comparison with *Rhizophora mangle*, which additionally shows warming root cells (Menezes, 2006; Youssef and Saenger, 1996). These three species show different ways to cope with the saline sediment. *A. schaueriana* and *L. racemosa* are species with salt glands in leaves; conversely, *R. mangle* have glabrous leaves (Tomlinson, 1994). Mangrove plants secreting salt through salt glands are capable of absorbing more salt through the root (salt-including species), which has been proposed as a mechanism that facilitates the absorption of metals, resulting in a higher bioaccumulation in these plants (Bernini et al., 2006; Cuzzuol and Campos, 2001; Sarangi et al., 2002).

The presence of iron mines close to mangrove areas can result in an additional load of metals in the sediment; particularly iron (Veerasingam et al., 2015). The state of Espírito Santo (Brazil) has the largest iron production of the country, being the biggest mining harbor in Brazil (IBEF, 2011). Levels of iron in sediments of this area reach 23 mg g^{-1} (Arrivabene et al., 2015), which are above levels found in other polluted mangrove areas (Defew et al., 2005; Lacerda et al., 1989; Sadiq and Zaidi, 1994; Sarangi et al., 2002; Sherman et al., 1998; Silva et al., 1990).

Some field studies have been carried out to evaluate the bioaccumulation of iron in mangrove plants (Souza et al., 2014a, 2014b, 2015). However, to our knowledge, there are few studies on the bioaccumulation of iron under controlled conditions (Cheng et al., 2012), looking to isolate the behavior of this metal from the complex matrix present in mangrove sediments, like the presence of different amounts of organic matter, dissolved oxygen, granulometry, etc. (Arrivabene et al., 2015). Furthermore, previous studies did not report the iron distribution in all plant compartments. It is worth to mention that, in addition to being a micronutrient, iron can reach concentrations toxic to the plant if its amount in sediments as well as its bioavailability are high enough (Kobayashi and Nishizawa, 2012).

Considering the above-described evidence, the main goal of this work was to evaluate the diverse capacity of these three plants to absorb, translocate and bioaccumulate iron in different plant compartments, as well as to evaluate changes in the plant anatomy. Thus, we look to verify if these plants can be used as pollution sentinels in an area with mangroves at risk because of iron pollution (Vitoria, Espírito Santo, Brazil).

2. Materials and methods

2.1. Experimental set up

Propagules of *A. schaueriana*, *L. racemosa* and *R. mangle* were collected at the ecological reserve of the Lameirão county (Estação Ecológica Municipal Ilha do Lameirão), State of Espírito Santo, Brazil, during the spring 2013 (*A. schaueriana*), or the summer 2014 (*L. racemosa* and *R. mangle*). Propagules were transported to a greenhouse at the Federal University of Espírito Santo, where they were cultivated in pre-cleaned PVC pots (2.8 L each) containing washed sand. Sand pots were stored in receptacles containing a nutritive medium (Hoagland and Arnon, 1950), with 0.25 ionic strength and a salt content of 7 g L^{-1}. The level of the nutritive medium was *ca.* 3 cm during plant grow, and *ca.* 7 cm during exposures, simulating mangrove conditions without the presence of organic matter. The nutritive medium was covered with a black PVC film to prevent photo-oxidation.

Propagules were developed during eight months, afterward plants were used for exposure. Exposures were performed by adding 0 (control), 10, 20 and 100 mg L^{-1} Fe(II)SO_4 (to simulate the bioavailable form of iron), disodium EDTA and MES buffer (1 mM, pH 6) to the nutritive medium (which already contained 0.53 mg L^{-1} Fe as $FeCl_3$). Iron concentrations of 10 and 20 mg L^{-1} were selected as they are close to values found in the interstitial water during field studies in the mangrove area of Espírito Santo (Arrivabene et al., 2015). The highest concentration (100 mg L^{-1}) was selected to simulate a more toxic condition, with iron levels exceeding current environmental levels, similar to the levels found during an incident like the recent mine dam break in the neighbor State of Minas Gerais (Brazil). The nutritive medium was renewed weekly.

Sets of five independent plants ($n = 5$) from each species (randomly selected) were exposed to different iron concentrations during eight weeks. After exposure, plants were harvested and analyzed as described below.

2.2. Chemical analysis of iron

Glassware and plasticware used for sampling preparation and analysis were previously washed using a neutral detergent Extran MA 02 (5% v/v), 15–50% v/v nitric acid (63.7%) sub-boiling grade and ultrapure water (<5 μg L^{-1} TOC). Ultra-pure water was obtained from a purification system Arium 61316-RO, plus Arium 611 UV (Sartorius, Germany). Nitric acid sub-boiling grade was