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Short-term cadmium exposure induces gas exchanges, morphological and ultrastructural disturbances in mangrove *Avicennia schaueriana* young plants

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ABSTRACT

Mangroves have been subject to more metal contamination, including cadmium (Cd). This study evaluated if a relatively short Cd exposure may induce metabolic, morphological and ultrastructural cell disturbance in *Avicennia schaueriana*. Cd induced evident constraints to seedlings since there was reduction in leaf gas exchanges and the plants did not survive for more than 10 days at a higher Cd exposure in controlled conditions. The highest Cd accumulation was observed in roots and gradually less in stem and leaves. Cadmium induced lignin deposition was observed in xylem cells of all vegetative organs. Intense sclerification in xylem cells, endoderm and change in the hypoderm organization were also detected. Cadmium clearly induced chloroplast deformities with ruptures of its membranes, thylakoids and core and provoked cytoplasm disorganization. These metal constraints under natural conditions for long term can lead to the accumulation of cellular and metabolic damages and jeopardize seedlings establishment and local biodiversity.

1. Introduction

Mangroves are marine costal ecosystems from tropical and subtropical regions with a vital role in biodiversity maintenance in estuarine environment, besides having an ecological, social and economic function for the surrounding communities (Feller et al., 2010). In the current scenario, the increase of toxic metals resulting from indiscriminate use of agricultural intake, mining, incorrect discharge of domestic and industrial waste has significantly contributed to the pollution of these ecosystems (Kathiresan and Bingham, 2001; Sandilyan and Kathiresan, 2012; Li et al., 2015).

Cadmium is a metallic element which does not have any known physiological function in animals and plants. It is highly toxic and therefore, in small quantities, may cause damage to plants and, consequently, to human health due to its accumulation in the food chain (Duffus, 2002). Cadmium can be found in nature through different ways and it contributes to environmental contamination: naturally in soil formation; excessive soil fertilization; incorrect battery disposal; steelmaking and petrol by-products (CONAMA, 257/99 and 263/99; Schianetz, 1999; Gao et al., 2011).

When Cd is absorbed by plants, it presents high mobility and affects plant growth and development (Benavides et al., 2005; Reef et al., 2010; Yan et al., 2017), besides basic metabolic processes such as photosynthesis (Gonzalez-Mendoza et al., 2007b). At cellular level, it affects the chloroplast structures and other cell organelles, induces changes in tissue and organs, and alters the compound production of the primary and secondary metabolism, stomatal movement, genes expression and enzymes functioning (Gonzalez-Mendoza et al., 2007a; Zhang et al., 2007; Liu et al., 2010; Xie et al., 2013). It affects water relations, leaf transpiration, cations efflux and it leads to the formation of amino acid and protein complexes (Das et al., 1997; Sandilyan and Kathiresan, 2014; Daud et al., 2015).

Avicennia schaueriana Stapf & Leechm. ex Moldenke (Acanthaceae) is a halophyte arboreal species, which grows under the influence of intermittent tides in saline and anoxic soils, which are frequent characteristics of mangroves ecosystems (Kathiresan and Bingham, 2001), and they occur in the coastal areas between the South coast of the East side of the Antilles and South America, from the Guyana to the South of Brazil (Schaeffer-Novelli et al., 1990). The *Avicennia* genus is able to absorb toxic metals which are translocated to the shoot, and when leaves fall metals return to the environment due to the process of decomposition of organic matter and may be ingested by animals resulting in trophic magnification (MacFarlane, 2002; Gonzalez-Mendoza et al., 2007b; Mahdavi et al., 2012; Nath et al., 2014). Over the years, a

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decline in mangrove area has been observed along the Brazilian coast. In 2000, it was estimated that the mangrove area in Brazil was 7721 km^2 while in 2014 it was reduced to 7663 km^2 (Hamilton and Casey, 2016). That was due to deforestation, urbanization and progressive waste discharge as being the main causes of disturbances in this ecosystem (Oliveira et al., 2014; Souza et al., 2014). These processes altogether significantly compromise the establishment and recruiting of seedlings in such environment. Due to the vulnerability and relevance of mangrove conservation, this study investigated the metabolic, morphological and ultrastructural disturbances induced by Cd. The *Avicennia schaueriana* seedlings were grown in different Cd concentrations in nutrient solutions, simulating the progressive Cd contamination in the mangroves.

2. Methods

2.1. Plant growth conditions

A. schaueriana seedlings were collected from Praia do Pontal, in the site of Itacaré (40° 45′ 36″ N and 73° 59´ 2.4″ W), Brazil. They were acclimated in a greenhouse in the State University of Santa Cruz (BA), in temperature and air relative humidity varying between 25 and 35 °C and 60 to 90%, respectively. Seedlings were cultivated in pots of 3 L of Hoagland and Arnon (1950) nutrient solution with 1/10 of ionic strength containing NaCl 10%$_0$, pH = 5–6, and kept under intermittent aeration, 8 h a day (4 h during the day and 4 h during the night), for 60 days.

The experiment was conducted in pots with nutrient Hoagland solution with ¼ of ionic strength plus 10%$_0$ of NaCl and $CdCl_2.5/2\ H_2O$ (Sigma-Aldrich, USA) in the following concentrations: 0; 5; 15; 30 and 45 mg L^{-1}. These solutions presented the following traits for the 3 L of diluted solutions: conductivity of 17.2 ± 0.02 mS cm^{-1}, salinity of 10.2, temperature of 24.5 ± 0.2 °C and pH varying from 5 to 6. During of experiment period of 10 days the solution volume and pH were kept constant and conductivity and salinity were monitored.

2.2. Leaf gas exchange evaluation

During plant growth period in increased Cd exposure, gas exchanges measurements were performed between 9:00 am and 11:30 am in young leaves of the second or third node which were intact and healthy. A portable system for photosynthesis measurement was used (LI-6400XT, LI-COR, USA) with temperature and relative humidity adjusted at 28 °C and 70% respectively. The photons density flow was 1000 μmol m^{-2} s^{-1} and environmental CO_2 concentration of ± 390 ppm. Leaves were adapted inside the photosynthetic chamber for 10 min before measurements. Leaf gas exchange data was obtained in 1, 5 and 10 days after the plants were subjected to Cd exposure.

2.3. Nutrients and Cd content in plants

After the growth period, plants were collected, separated in various parts viz., root, stem and leaf. The plant parts were placed to dry in a forced-air oven at 75 °C until reaching constant mass. Afterwards they were ground in a micro mill (Wiley TE-648, Tecnal, Brazil) and placed in plastic bags. The plant portions were weight to obtained samples of 0.2000 g for acid oxidant digestion ($HNO_3 + H_2O_2$) in a digestion block (TE-007MP, Tecnal, Brazil) at 50 and 80 °C for 30 min, 130 °C for 1.5 h. Samples were re-suspended at 25 mL with ultrapure water, and nutrients and Cd content in plants were analyzed using an optical emission spectrometry with inductively coupled plasma (ICP-OES) (Optima 5300 DV, PerkinElmer, USA) in the Fuel Test Laboratory in the Federal University of Minas Gerais (UFMG), Belo Horizonte-MG, Brazil.

2.4. Morphological analysis

Samples of the vegetative organs were collected at the end of a 10 day – treatment. Leaves from the second or third node were removed in order to obtained middle sections next to central vein; stems of the second internode and adventitious and apex root were also collected. All samples were fixed in neutral buffered formalin (NBF) (Conn's, 1948). Free hand cuttings were made to evaluate auto fluorescence under ultraviolet light (UV). Transversal sections were also submitted to phloroglucinol (Johansen, 1940) to detect lignin - a molecule which fluorescences under UV range of 340 to 380 nm. The micrographs were obtained through an imaging capturing system (DFC295, Leica Microsystem, Germany) coupled with the photonic microscope (DMI 300B, Leica).

2.5. Ultrastructural analysis

Leaf samples with approximately 3 mm were obtained in triples. Cuts were fixed in glutaraldehyde 2.5% in sodium cacodylate buffer 0.1 M and pH = 7.2. For the ultrastructural analysis samples were processed according to De Souza (2011). Then, fine sections at 70 μm thickness were cuts in a Leica UC6 and deposited in copper 300-mesh grids, contrasted with uranyl acetate for 25 min and lead acetate for 8 min. The sections were observed with a transmission electron microscope at kV (Morgagni 268D, FEI Company, EUA) at the Electron Microscopy Center (CME) of the State University of Santa Cruz (UESC), Brazil.

2.6. Statistics

The experimental design used was entirely at random with 5 treatments and 5 pots per treatment with 3 plants in each, being 15 plants per treatment kept in a greenhouse for 10 days. Data was subjected to normality test and variance homogeneity. Except the parameters of leaf gas exchange which were treated through measurements analysis repeated in time, data was subjected to a one-way variance analysis (ANOVA) to compare treatments in each evaluated period, and a two-way analysis to evaluate treatment and period with Tukey post-test at 5% probability.

3. Results

3.1. Symptoms of Cd toxicity in plants

Plants subjected to 45 mg L^{-1} of Cd presented leaf turgor pressure loss after 3 days of exposure to the metal, and the other plants did not present the same changes. In 7 days, plants growing at 30 mg L^{-1} of Cd also showed turgor loss, while the ones subjected to 45 mg L^{-1} presented advanced signs of leaf necrosis and chlorosis and necrosis in most of the root apex. These changes were not observed in plants growing at 5 and 15 mg L^{-1} which remained similar to the plants without Cd exposure. Only plants subjected to 15 mg L^{-1} started turgor loss at 10 days. Contrastingly, plants growing at 30 mg L^{-1} showed leaf apex necrosis and their roots were severely damaged, and the ones subjected to the highest Cd concentration were dry and presented black roots.

3.2. Leaf gas exchanges

The different Cd concentrations used in nutrient solutions, induced a significant effect on the all parameters of leaf gas exchanges (Fig. 1). Even after a quick 24 h of Cd exposure, plants presented a significant decrease in the CO_2 assimilation rates (*A*) when subjected to the highest Cd concentration (45 mg L^{-1}) (Fig. 1a). The *A* values were reduced from 12.8 (± 0.94) in plants without Cd to 7.4 (± 0.84) μmol CO_2 m^{-2} s^{-1} in 45 mg L^{-1} plants. Also, after 5 days of Cd-induced stress,