



Accumulation of six metals in the mangrove crab *Ucides cordatus* (Crustacea: Ucididae) and its food source, the red mangrove *Rhizophora mangle* (Angiosperma: Rhizophoraceae)

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ABSTRACT

The crab *Ucides cordatus* and the red mangrove *Rhizophora mangle* are endemic mangrove species and potential bio-accumulators of metals. This study quantified the accumulation of six metals (Cd, Cr, Cu, Hg, Mn and Pb) in different organs (claw muscle, hepatopancreas and gills) of *U. cordatus*, as well as in different maturation stages of the leaves (buds, green mature, and pre-abscission senescent) of *R. mangle*. Samples were collected from mangrove areas in Cubatão, state of São Paulo, a heavily polluted region in Brazil. Data for metal contents in leaves were evaluated by one-way ANOVA; while for crabs a factorial ANOVA was used to investigate the effect of different tissues, animal size and the interactions between them. Means were compared by Tukey test at five percent, and the association between the metal concentrations in each crab organ, depending on the size, was evaluated by Pearson's linear correlation coefficient (r). Concentrations of Pb and Hg were undetectable for the different leaf stages and crab tissues, while Cd concentrations were undetectable in the leaf stages. In general, the highest accumulation of metals in *R. mangle* leaves occurred in pre-abscission senescent and green mature leaves, except for Cu, which was found in the highest concentrations in buds and green mature leaves. For the crab, Cd, Cu, Cr and Mn were present in concentrations above the detection limit, with the highest accumulation in the hepatopancreas, followed by the gills. Cu was accumulated mostly in the gills. Patterns of bioaccumulation between the crab and the mangrove tree differed for each metal, probably due to the specific requirements of each organism for essential metals. However, there was a close and direct relationship between metal accumulation in the mangrove trees and in the crabs feeding on them. Tissues of *R. mangle* leaves and *U. cordatus* proved effective for monitoring metals, acting as important bioindicators of mangrove areas contaminated by various metals.

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1. Introduction

Anthropogenic metal contamination and bioaccumulation in ecosystems have become a worldwide concern (Kabata-Pendias, 2011). In coastal regions, particularly in mangrove areas, the hydrodynamics of the region, due to the typical vegetation, makes the problem worse (Struve and Falconer, 2001). These regions are also a natural and productive environment for many species of fishes and crustaceans (Rajendran and Kathiresan, 1999;

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Sheridan and Hays, 2003; Tse et al., 2008). The sediments in mangrove areas act as a chelating matrix for trace metals, reducing mobilization of these chemicals to the mangrove plant tissues (Zoumis et al., 2001) and, consequently, their availability to the local biota (Silva et al., 1990; Zheng et al., 1997; Spalding et al., 2010). The roots of mangrove plants have an important role in depurating the water and the sediment, retaining large quantities of organic material and trace metals brought by the tides (Schaeffer-Novelli, 1995; MacFarlane and Burchett, 1999).

Metallic contaminants usually accumulate in permanent tissues of the vegetation (Zheng et al., 1997), but can be transported to deciduous parts such as leaves and buds, where the metals can combine with macromolecules of the cell membrane, affecting important physiological processes. The subsequent use

of leaf litter by primary consumers allows these metals to enter the trophic web, and can consequently affect human health (Das et al., 1997; Onder and Dursun, 2006), due to their persistence in the various environmental compartments.

Animal contamination by metals can occur through the water, soil and trophic web (Rainbow, 1995, 1997; Ahearn et al., 2004). Environmental contamination by these chemicals is measured through their concentrations in animal and plant tissues. Since the 1990s, the vegetation from mangrove areas has been successfully used to quantify metal contamination (Aksoy and Öztürk, 1997), particularly species of the genus *Rhizophora* (Zheng et al., 1997; Fruehauf, 2005; Ramos and Geraldo, 2007). For the fauna, the species most studied are the benthic macroinvertebrates, particularly those with low mobility, which accumulate larger concentrations of metals compared to animals that live in open water (Chapman et al., 1998). Among the most-studied are the 'uçá'-crab *Ucides cordatus*, which has important characteristics that allow the study of bioaccumulation: (i) they feed mainly on litter of mangrove areas (Nordhaus et al., 2009), as well as the sediment itself (Christofolletti, 2005); (ii) they promote bioturbation and incorporate organic matter into the sediment during burrow-making (Nordhaus et al., 2006); (iii) they have a slow growth rate and long life cycle (Pinheiro et al., 2005); and (iv) they are abundant and easy to capture in the field (Pinheiro and Fiscarelli, 2001). Therefore, this mangrove species is especially appropriate for use in studies of environmental impact by metals, from an ecosystemic (Jesus et al., 2003; Nudi et al., 2007) or genotoxic (Toledo, 2007; Banci, 2008) point of view.

The study of metal accumulation in biotic and abiotic compartments of mangrove areas can contribute to discussions about the state of conservation of these coastal areas. The present study measured the accumulation of six metals (Cd, Cu, Pb, Cr, Mn and Hg) in two ecologically related mangrove species: (i) the 'uçá'-crab *Ucides cordatus*, and three tissues (claw muscle, hepatopancreas and gills), and their relationship to the body (cephalothorax) size; and (ii) the red mangrove tree *Rhizophora mangle*, for three foliar stages (bud, green mature and senescent).

2. Materials and methods

2.1. Area of study: history and location

Studies were conducted in Cubatão, at Santos–São Vicente estuary on the central coast of the state of São Paulo (68 km from São Paulo city), Brazil. This region is heavily impacted by the Port of Santos (the most important port in Brazil) and the Industrial Pole of Cubatão (comprising 23 industrial complexes, 111 factories, and more than 300 polluting sources), aggravated by unregulated and disorganized human occupancy (Luiz-Silva et al., 2006; Nascimento et al., 2006; Zündt, 2006). During the 1960s, Cubatão was known worldwide as one of the most polluted cities in the world (Viola, 1987; Pinheiro et al., 2008), with damage to its watershed (Ramos and Geraldo, 2007) and effects on the health of the human population (Rocha et al., 1988; Jasinski et al., 2011).

Samples were obtained on August 8 2010 in two mangrove areas with a predominance (> 80 percent) of red mangrove (*R. mangle*), near the Piaçaguera Channel in Cubatão Municipality (Fig. 1). A sample of *R. mangle* leaves was obtained at CUB-1 (23°53'2.4"S–46°21'55.6"W), 2 km from the Paulista Steel Company (named Usiminas-Cosipa). The mangrove crabs (*U. cordatus*) were caught at CUB-2 (23°54'2.4"S–46°22'56.9"W), 4 km from this same company. Therefore all region around are contaminated by metals.

2.2. Sampling and processing of *R. mangle* leaves

R. mangle specimens with a minimum height of 4 m were selected at CUB-1, where leaves were sampled according to three maturation stages ($n=50$ each)—buds, green mature, and pre-abscission senescent. Leaves were removed with pruning shears and placed in labeled plastic bags for transport to the laboratory. Leaves were immediately washed (1st washing in running water; water with 5 percent neutral detergent; 2nd washing in running water; solution of distilled water saturated with HCl; and a large volume of distilled water), to prevent atmospheric contamination of these tissues (Ramos and Geraldo, 2007). Shortly

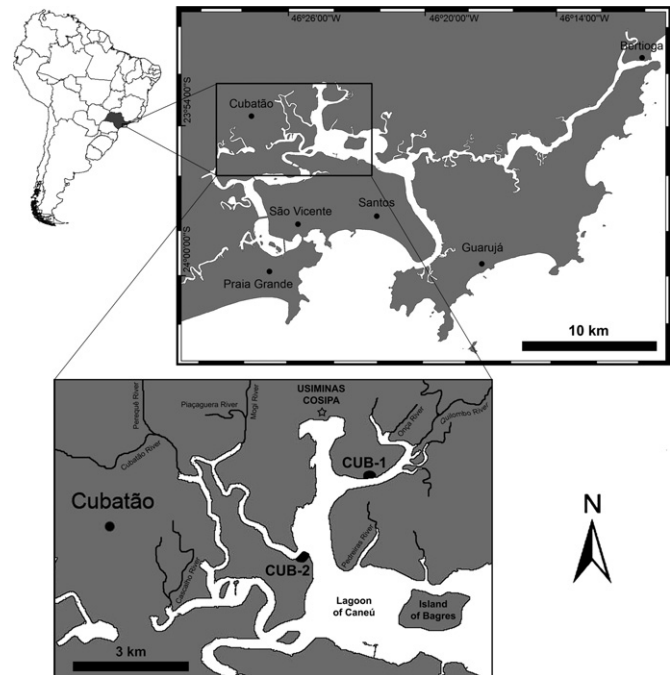


Fig. 1. Locations of two mangrove areas (CUB-1 and CUB-2) where crabs (*Ucides cordatus*) and leaves (*Rhizophora mangle*) were obtained, at Cubatão, state of São Paulo, Brazil.

after, the leaves were dried with cloth towels, dehydrated in a stove with forced-air ventilation (60 °C for 72 h) and milled in a knife mill. Samples of each leaf stage (20 g powder each) were placed in labeled plastic pots and transported to the CEATOX Laboratory at IB/UNESP Botucatu for analysis.

2.3. Sampling and processing of *U. cordatus* tissues

U. cordatus specimens were hand-caught inside their galleries by the 'braceamento' method (directly by inserting the crab-catcher's arm into the gallery) or with a 'Redinha' (an artisanal trap constructed by crab-catchers with nylon cord), according to Fiscarelli and Pinheiro (2002).

For purposes of standardization, metals in the crab organs were analyzed only for intermolt males (see Pinheiro and Fiscarelli, 2001), avoiding any effects of molting stage and sex, as previously reported for other decapod crustaceans by Jeckel et al. (1996), Sanders et al. (1998), Chou et al. (2000) and Chen et al. (2005).

Captured specimens ($n=11$) were placed in coolers with bags of ice and transported to the laboratory, where they were brushed to remove mud and measured with 0.05-mm precision calipers (CW, carapace width). The crabs were dissected with sterilized scissors and tweezers to remove samples of three tissues (claw muscle, hepatopancreas and gills), in standardized locations: (i) muscle of the chelar propodus, due to higher metal accumulation verified by Chen et al. (2005); (ii) middle lobe of the hepatopancreas, which has a particularly high metabolic rate (Mourete, 1996); and (iii) posterior gills, because of their osmoregulatory function (Mantel and Farmer, 1983).

Tissue samples ($n=33$) were placed in Eppendorf vials, kept frozen (−20 °C) and transported cold to the CEATOX Laboratory. Each sample was analyzed for six metals (Cd, Cu, Pb, Cr, Mn and Hg) by the mineralization method with HNO₃ at 65 percent, according to Basset et al. (1981). Analyses were optimized by hollow cathode lamps (LCO), according to the metallic element analyzed, and samples were read using a GBC-932 AA atomic absorption spectrophotometer (Athanasopoulos, 1993). The equipment was calibrated using metal stock solutions (1000 ppm). The metal concentration of each sample is expressed in micrograms of metal per gram of dry tissue (μg/g), with the minimum detected concentration represented as μg/g (Cd < 0.01; Cu and Mn < 0.02; Pb and Cr < 0.05) and ng/g (Hg < 0.001).

Data for metal concentrations obtained for each tissue sample were also used to evaluate a possible effect of crab size ($n=11$), testing four size classes of 10 mm each: 50–60 ($n=1$), 60–70 ($n=3$), 70–80 ($n=3$) and 80–90 mm ($n=4$).

2.4. Statistical analysis

Metal accumulation in relation to leaf stages of *R. mangle* was evaluated by one-way ANOVA. Metal concentrations in different crab tissues were analyzed by factorial ANOVA, represented by two independent variables (CW and tissues) and

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