Ecotoxicology and Environmental Safety 145 (2017) 367–376

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Metal toxicity assessment by sentinel species of mangroves: *In situ* case study integrating chemical and biomarkers analyses

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ARTICLE INFO

Keywords:
Crab
Cytotoxicity
Genotoxicity
Mangrove
Metals
Ucides cordatus

ABSTRACT

Globally, there is a lack of knowledge about tropical ecotoxicology dealing with the potential impact of metal contamination in mangrove ecosystem. This habitat is considered a nursery for several animal species, among them the “uçá”-crab (*Ucides cordatus*), known as a key species due to its biological and economical importance. This study evaluated the association involving metal contamination (Cd, Cu, Pb, Cr, Mn and Hg) in water, sediment, red-mangrove vegetation (*Rhizophora mangle*) and tissues of uçá crab, together with its geno-cytotoxic responses, based on micronucleated hemocytes frequency and the retention time of neutral red in lysosomes. We assessed six mangrove areas with distinct pollution levels in São Paulo State, Brazil, where the water and sediment contamination by metals were associated with accumulation of these pollutants in biotic compartments (mangrove leaves and crab). In *U. cordatus,* metal accumulation was best explained by metal concentration found in leaves of *R. mangle* than in the water or sediment, indicating that feeding drives metal exposure in this organism. Mercury (Hg) concentration in sediment, copper (Cu) concentration in hepatopancreas of *U. cordatus* and lead (Pb) in water and green leaves of *R. mangle* showed a significant correlation with genotoxic impact in *U. cordatus*. However, copper concentration (in green/senescent leaves and hepatopancreas) and lead (in sediment), were the major metals affecting lysosomal membrane integrity. Therefore, representatives of all compartments were associated with cyto and genotoxicity in this species, thus requiring a holistic approach to issues related to sublethal damage. Probability estimates of cytogenetic impacts related to metal concentration in abiotic compartments (significantly correlated with known biomarkers: Hg in sediment; and Pb in water and sediment) are also presented. Our results highlight the need for environmental restoration of mangroves areas contaminated with metals, responsible for cytogenetic injuries and revealing a pre-pathological condition in this sentinel species, in addition to ecological disturbances.

1. Introduction

Tropical ecosystems account for most of the world's biodiversity (Barnes et al., 2014), even though the focus of ecotoxicological studies are, historically, almost exclusively on temperate environments (Lacher and Goldstein, 1997; Sueitt et al., 2015). This lack of knowledge comes from the increasing and constant threat of toxic substances released into the environment, and water bodies, which causes negative impact for tropical aquatic organisms (Peters et al., 1997; Harford et al., 2015).

Metals are among the known pollutants with the highest degree of toxicity and persistence (Rainbow, 1997, 2007; Ahearn et al., 2004; Rainbow and Black, 2005; Luoma and Rainbow, 2008), attracting interest at a global level, due to their large impact and potential risk to the biota. Metals are difficult to break down, accumulate in animal and vegetal tissues, and are magnified in trophic chains (Ahearn et al., 2004; Rainbow, 2007; Vilhena et al., 2012). There are metals considered essential (such as Cu, Cr and Mn), important for animal metabolism, although they become toxic if concentrations are high. Non-essential metals (such as Hg, Pb and Cd), on the other hand, cause toxicity even at small concentrations (Eisler, 2010). In all instances, and

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http://dx.doi.org/10.1016/j.ecoenv.2017.07.051
Received 14 March 2017; Received in revised form 14 June 2017; Accepted 22 July 2017
Available online 28 July 2017
0147-6513/

depending on the dosage, the presence of metals is of concern because of their mobility in water, their persistence in sediments (Luoma and Rainbow, 2008; Vilhena et al., 2012) and their bioavailability and toxicity in biota (Ahearn et al., 2004; Rainbow, 2007). Even if accumulation of these elements serves as an important parameter for quantifying the level of contamination on natural environments (Rainbow, 2007; Luoma and Rainbow, 2008), recent criteria on sublethal biological disturbances have been considered as the best way to monitor natural environments (Pereira et al., 2011, 2014; Duarte et al., 2016).

There are few "*in situ*" studies in the literature, and it is well known that pollution by metals can generate biological disturbances in organisms, which can be identified and quantified by specific biological assays known as biomarkers (Monserrat et al., 2007; Amiard-Triquet, 2011; Amiard-Triquet et al., 2013b; Pereira et al., 2014). Biomarkers can detect early sublethal disturbances that could potentially impact the entire population or communities (Amiard-Triquet et al., 2013b; Duarte et al., 2016; Pinheiro et al., in preparation). Genetic and physiological disturbations are one of the first sublethal modifications observed in organisms exposed to metals (Otomo and Reinecke, 2010; Amiard-Triquet et al., 2013b; Pinheiro and Toledo, 2010; Pinheiro et al., in preparation). Thus, to estimate these alterations, many authors have used the technique of micronucleated cells, expressed in MN‰ (Scarpato et al., 1990; Brunetti et al., 1988; Al-Sabti and Metcalfe, 1995; Burgeot et al., 1995; Hoshina et al., 2008; Collier et al., 2013; Pinheiro et al., 2013), as well as the neutral red technique, quantified through the retention time of the neutral red (NRRT) in lysosomes (Lowe et al., 1995; Svendsen et al., 2004; Daguano et al., 2007). These techniques have large ecological importance for populations of affected areas (Bonassi et al., 2000; Neri et al., 2003; Duarte et al., 2016), having been applied in freshwater (Falfushynska et al., 2014; Taylor et al., 2017), estuarine (Pereira et al., 2014; Duarte et al., 2016) and marine environments (Catharino et al., 2008; Buratti et al., 2012; Wyatt et al., 2014).

The use of biological models (bioindicators) may reveal "sentinel" species, named so because of early metal toxicity detection in natural environments, some of them having an important ecosystem function (Beltrame et al., 2010, 2011; Pereira et al., 2014). The land crab *Ucides cordatus* is semi-terrestrial and endemic of mangroves areas and is an example of a sentinel species (Fiscarelli and Pinheiro, 2002; Nordhaus et al., 2009), standing out as a testimony species of environmental quality (Pinheiro et al., 2012, 2013; Duarte et al., 2016; Ortega et al., 2016). *Ucides cordatus* is consumed by fish, birds and mammals, including man (Fiscarelli and Pinheiro, 2002), and is responsible for metal biomagnification in the mangrove trophic chain. In addition, they have an intimate relationship with the components of the environment they inhabit, namely: 1) water, through contact and ingestion, participating in various physiological and metabolic processes, among them breathing (Pinheiro et al., 2012); 2) sediment, through contact and ingestion, digging galleries and ingesting part of the sediment during feeding (Nordhaus et al., 2009); and 3) mangrove leaves, used as food by this species, mainly the red-mangrove *Rhizophora mangle* (Pinheiro et al., 2013; Christofoletti et al., 2013). Moreover, the species has a relatively long life cycle, reaching its maximum size at 10 years of age (Pinheiro et al., 2005). The uçá-crab is also a food source, particularly by coastal populations, and has a wide distribution in the mangroves of the Western Atlantic (Melo, 1996), many of which are subject to anthropic pressure, mainly in southeastern Brazil (Abessa and Ambrozevicius, 2008; Pinheiro et al., 2013, 2017; Duarte et al., 2016).

Duarte et al. (2016) demonstrated the effectiveness of uçá-crab as an indicator species for determining the conservation status of mangrove areas employing biological responses and their linkage to contamination. The authors addressed a multi-level biological approach (biomarkers, condition factors and crab density) and their strong association with the local contamination (determined *via* information reported in scientific and technical literature about metals, organochlorine pesticides, polychlorinated biphenyl and polycyclic aromatic hydrocarbon) and solid waste volume. This previous study also described that density of crabs was correlated with subcellular biomarkers, provided the normal baseline values for the biomarkers (frequency of micronucleated cells, MN‰ < 3; neutral red retention time > 120 min) and categorized three levels of human impacts in mangrove areas (PNI, probable null impact; PLI, probable low impact; and PHI, probable high impact). However, the group(s) of contaminants responsible for cyto-genotoxicity effects observed by Duarte et al. (2016) are still unknown. According to Eisynk et al. (1988), CETESB (2001) and Pinheiro et al. (2012, 2013, 2017), metals are the most historically threatening contaminants in state of São Paulo mangroves of state of São Paulo. Therefore, the present study aims to test the hypothesis that there is a significant relationship between cyto-genotoxicity effects and levels of metal contamination in abiotic (water and sediment) and biotic compartments (green/senescent leaves of *R. mangle* and tissues of *U. cordatus*). For this purpose, first, we assessed whether abiotic contamination by metals is linked to bioaccumulation and whether the association involving the presence of metals (Cd, Cu, Pb, Cr, Mn and Hg) in all compartiments is linked to sublethal responses recorded in a sentinel species of Western Atlantic mangroves.

2. Materials and methods

2.1. Study area

In Brazil, the coastal region of São Paulo State represents a contrasting scenario due to the presence of preserved areas, together with areas subjected to significant environmental impact (Pinheiro et al., 2008). In order to study the relationship between metals and sublethal disturbances, six mangrove areas of the State of São Paulo (Bertioga, BET; Cubatão, CUB; São Vicente, SAV; Iguape, IGU; Juréia, JUR; and Cananéia, CAN) were studied, based on their anthropic historical pressure, particularly with respect to contamination by metals (Eisynk et al., 1988; CETESB, 2001; Pinheiro et al., 2013). Therefore, the present study was developed in the same areas previously studied by Duarte et al. (2016) and Pinheiro et al. (in preparation), which represent the vast majority of the state's mangrove area (99%). Six mangrove areas have been established (Fig. 1, with local icons characterizing each mangrove area), three of them located on the central coast (BET, CUB and SAV) and extra three established on the southern coast (IGU, CAN and JUR - the latter an ecological station). Each mangrove area includes three subareas (replicates) that are similar in tree composition ($>$50% *Rhizophora mangle*), vegetation structure (tree height $>$5 m; and diameter at breast height $>$10 cm), flood tide height ($>$30 cm) and productivity (leaves available on the sediment $>$2 g m^{-2}).

Located on the central coast of São Paulo, the **Bertioga** Estuary (BET) receives domestic untreated sewage and effluents from a public garbage dump as its main source of pollution. Even so, the estuary is used for ecotourism and sport fishing (Eichler et al., 2006). **Cubatão** (CUB) is considered one of the most polluted regions in the world; in recent years, it has become the most important Brazilian industrial hub, formed by 23 industrial complexes, 111 factories, and more than 300 pollution sources. Home to the largest port in Latin America, it also experiences transit of large vessels carrying several types of chemicals (Martins et al., 2011; Pinheiro et al., 2012, 2013). Located within the same estuarine complex there is **São Vicente** (SAV), showing substantial human activity, illegal slums, a lack of wastewater treatment and solid waste collection (CETESB, 2007; Kirschbaum et al., 2009; Cordeiro and Costa, 2010; Mello et al., 2013), and intensified by 11 industrial sources of pollution. These areas, therefore, have a history of substantial human impact, with records of contamination by various xenobiotics, including metals (Azevedo et al., 2009; Pinheiro et al., 2012, 2013).

The mangrove areas on the southern coast of São Paulo are