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Use of *Tisbe biminiensis* nauplii in ecotoxicological tests and geochemical analyses to assess the sediment quality of a tropical urban estuary in northeastern Brazil

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ABSTRACT

An approach pooling geochemical analyses and ecotoxicological tests has been applied to assess the sediment quality of the Capibaribe River Estuary, Brazil. Toxicity tests were performed to compare a well-established, labor-intensive protocol using ovigerous females to a new, easier and faster protocol using nauplii of the epibenthic marine copepod *Tisbe biminiensis*. The endpoints of the nauplii toxicity test were comparable to those of the female test. Nauplii proved to be more sensitive than females as a biological model for indicating sediment toxicity. All sediments collected had at least one contaminant above the threshold effects level (TEL) proposed in the literature. Furthermore, more than one-third of samples exhibited contaminants above the probable effects level (PEL). The PCA revealed that nauplii mortality was associated with metals in October 2014, which was confirmed by the Spearman correlation factor. In contrast, no strong association among contaminants and toxicological endpoints in May 2015 was found.

1. Introduction

Estuarine and riverine systems are among the environments most impacted by anthropogenic activities, frequently receiving pollutant inputs from diffuse and point sources, such as urban, industrial and agricultural wastewaters. Estuaries are especially vulnerable to these inputs since they are semi-enclosed systems that naturally act as sediment traps (Cindric et al., 2015; Schettini et al., 2017; Wolanski et al., 2006).

Sediment has been often used for assessing environmental quality because it is the fate of numerous chemicals from the water column involved in adsorption, precipitation and biological processes. Several toxic chemicals accumulate in sediments, being transferred to the food web and eventually having negative effects on living organisms (Cruz et al., 2014; NRC, 2003; Power and Chapman, 1995). Chemicals of concern include polycyclic aromatic hydrocarbons (PAHs), metals and persistent organic pollutants (POPs) (Abessa et al., 2017; Hauser-Davis et al., 2016; Lavradas et al., 2016; Maciel et al., 2015a; Pazi et al., 2011).

Sediment environmental quality is better assessed with integrated approaches that pool, for instance, chemical analyses and ecotoxicological assays (Buruaem et al., 2013; Choeuri et al., 2009; Krull et al., 2014; Perina et al., 2018). Although the use of standardized toxicity tests is important, protocols that make use of endogenous species are more relevant for extrapolating results to the local ecosystem (Nipper, 2000). The search for new species and protocols has been encouraged to attain results of ecological relevance and to allow faster management decisions (Forbes and Forbes, 1994; Jager et al., 2006; Lavorante et al., 2013).

The epibenthic marine copepod *Tisbe biminiensis* has been proposed as a test organism due to its easy laboratory culture (Ribeiro and Souza-Santos, 2011), short life cycle and sensitivity in toxicity tests (Araújo-

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Castro et al., 2009; Costa et al., 2014; Lavorante et al., 2013; Souza-Santos et al., 2015). The use of ovigerous females of *T. biminiensis* in sediment ecotoxicology tests is well developed and has presented good outcomes (Maciel et al., 2015b; D. Oliveira et al., 2014). However, this methodology is labor-intensive, especially for ecotoxicological studies involving a large number of samples. It requires certain ability, extensive training and many hours of work since ovigerous females must be individually added to the test containers.

The Capibaribe River estuary is a tropical estuary in the northeast of Brazil, the pollution of which has been the subject of only a few studies, and most of them have focused on its southern portion, known as the Pina Sound (Fernandes et al., 1999; Macedo et al., 2007; Maciel et al., 2015a; Maciel et al., 2015b; D. Oliveira et al., 2014). It is an important estuary that flows through one of the most densely populated areas along the eastern coast of South America, and despite its social, economic and ecological importance, the estuary receives a high input of urban wastewaters (Maciel et al., 2015b; Schettini et al., 2016a).

This study assessed sediment quality along the banks of the Capibaribe River Estuary main stem. An integrated approach pooling chemical data and ecotoxicological tests was employed for the first time at the area. The toxicity assays followed two protocols to verify the higher sensitivity of an alternative test using nauplii of *T. biminiensis*, which is faster and less laborious than the other using females of the same species. Multivariate statistics were used for investigating associations among sediment parameters, organic and inorganic chemical contamination, and lethal and sublethal toxicity endpoints.

2. Materials and methods

2.1. Study area

The Capibaribe River is 240 km long, and it is located on the northeastern coast of Brazil (Fig. 1). There are 42 municipalities in the watershed of the Capibaribe River. Recife - the largest one, with 1.6 million inhabitants (IBGE, 2016) - is located in the estuarine portion of the Capibaribe River, where the stream flows through narrows channels with depths ranging from 3 to 12 m. The Capibaribe Estuary has been characterized as a flat, shallow and partially mixed estuary, with its deepest portion closer to the mouth, where the Port of Recife is located (Araújo and Pires, 1998; Monteiro et al., 2011). Water circulation in the estuary is controlled by semidiurnal mesotides, with a tidal range up to 3 m in spring tides (Maciel et al., 2015a). The average discharge is 11 $m^3 s^{-1}$, although most of time, it is approximately 2 $m^3 s^{-1}$ (T. Oliveira et al., 2014; Schettini et al., 2016a). The rainfall in the Capibaribe River watershed is highly variable, as the stream flows from semiarid regions in the upper basin (annual mean: 702 mm of rain) to maritime tropical forest regions in the lower basin (annual mean: 2267 mm of rain) (SRH-PE, 2010).

Closer to its mouth, the Capibaribe River Estuary is connected to Pina Sound, which is formed by the confluence of several creeks (Tejipió, Jiquiá, Jordão and Pina). The waters of the sound carry a considerable amount of untreated industrial and domestic wastewaters (Fig. 1) (Santos et al., 2009). The amount of untreated sewage discharged into the estuary is unknown. However, it is known that only 32% of wastewaters from the city of Recife are collected for treatment prior to being discharged in estuarine waters (COMPESA, 2017). Fifty-one industrial plants are located within a distance of 3 km from the river banks, and 21 of them are considered to have high pollution potential (CPRH, 2007). There is also sugarcane agribusiness upstream the estuary. Pernambuco State is ranked as the second-largest sugarcane producer in Brazil (SINDAÇUCAR, 2017).

2.2. Sediment sampling

Surface sediment samples (from the top 2 cm) were collected from river banks at 7 stations in October 2014 and May 2015: S1 and S2 are located in the upper estuary, S3, S4 and S5 are located in a densely populated area, and S6 and S7 are located in the lower estuary closer to the Port of Recife (Fig. 1).

Three sediment samples (field replicate) per station were collected with a stainless-steel spoon from river banks during ebb tide and were stored according to standardized procedures (ABNT, 2006). Part of each sample was stored in an individual decontaminated glass recipient for toxicity tests, transported and tested immediately after the sampling. The remains of the samples were pooled and homogenized for geochemical analyses. Samples for metal analysis were stored in plastic bags, whereas samples for organic contaminants and grain size analysis were stored in aluminum containers (previously combusted at 450 °C for 4 h). Additionally, sediment samples were also collected from the Maracaípe estuary (8° 32′ S, 35° 0′ W), which has been considered as an unpolluted environment (Araújo-Castro et al., 2009) and used as a negative control for toxicity tests.

2.3. Toxicity tests

Prior to the tests, all sediment samples were sieved through a 64-μm sieve to remove sand, plant debris and benthic fauna. Subsequently, tests were performed with the sediment mud fraction (i.e., < 64 μm). Toxicity tests were performed with three laboratory replicates for each field sample, resulting in nine recipients per station. Each replicate consisted of a 40-mL flat-bottom glass container (4.5 cm in diameter and 5 cm in height) containing 2 g of sediment and 20 mL of an algal suspension of *Chaetoceros muelleri* at a concentration of 2.5×10^5 cells mL^{-1} in natural seawater at 34 ± 2 of salinity. The water used was collected from a preserved marine area and chlorinated as soon as it was brought to the laboratory. After 24 h, the water was dechlorinated by aeration for some days and stored for almost one year. One day before the bioassays, the seawater was filtered through a 0.45-μm filter. The test organisms were inoculated in these containers after at least 12 h. Toxicity tests were simultaneously performed following two different protocols (see the descriptions below).

2.3.1. *T. biminiensis* ovigerous females toxicity test

The protocol performed with *T. biminiensis* ovigerous females is described elsewhere (e.g., Araújo-Castro et al., 2009). Briefly, ten *T. biminiensis* ovigerous females were manually added to each test container, which was incubated at 28 °C with a 12-hours light/dark photoperiod for seven days. Every other day, 1 mL of concentrated *C. muelleri* was added to each test container. At the end of incubation, the contents of each container were fixed with 4% formalin and stained with rose bengal (Giere, 2009). Forty-eight hours after staining, the contents of the container were sieved through a 64-μm filter, and the animals were observed under a stereoscope microscope (20×). Stained animals represent living individuals at the end of the test and are easily distinguished from dead animal due to the fast degradation rate in sediment. The endpoints evaluated were the percentage of adult female survival, female fecundity rate (expressed as total offspring produced over 7 days), and percentage of offspring development (calculated as the ratio of copepodites to total offspring).

2.3.2. *T. biminiensis* nauplii toxicity test

The second protocol was performed with newly hatched (< 24 h) nauplii of *T. biminiensis* as test organisms, following a protocol proposed by Lavorante et al. (2013) for water samples but adapted for sediment samples in this study. A suspension with approximately 200 newly hatched nauplii was added to each test container with an automatic pipette. Recipients were incubated at 28 °C subject to a 12-h light/dark photoperiod for 3 days. At the end of tests, the samples were fixed and stained as described above. The endpoints evaluated were the percentage of copepod survival (calculated as the ratio of living animals at the end of the test to the initial mean number of nauplii added to the test container) and percentage of nauplii development (calculated as the