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Determination of water quality, toxicity and estrogenic activity in a nearshore marine environment in Rio de Janeiro, Southeastern Brazil

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ABSTRACT

Endocrine disrupting compounds (EDCs) can be found in domestic sewage, wastewater treatment plant effluents, natural water, rivers, lakes and in the marine environment. Jurujuba Sound, located in the state of Rio de Janeiro, Southeastern Brazil, receives untreated sewage into its waters, one the main sources of aquatic contamination in this area. In this context, the aim of the present study was to evaluate the estrogenic potential of water sampled from different depths and from areas with differential contamination levels throughout Jurujuba Sound. Water quality was evaluated and acute toxicity assays using *Allviibrio fischeri* were conducted, while estrogenic activity of the water samples was determined by a Yeast Estrogen Screening assay (YES). Water quality was mostly within the limits established for marine waters by the Brazilian legislation, with only DOC and ammoniacal nitrogen levels above the maximum permissible limits. No acute toxicity effects were observed in the *Allivibrio fisheri* assay. The YES assay detected moderate estrogenic activity in bottom water samples from 3 sampling stations, ranging from 0.5 to 3.2 ng L^{-1}, as well as in one surface water sample. Estrogenic activity was most frequently observed in samples from the bottom of the water column, indicating adsorption of estrogenic compounds to the sediment.

1. Introduction

Currently, marine contamination by potentially harmful compounds in the μg L^{-1} and ng L^{-1} range is a main environmental concern (Bila and Dezotti, 2007; Caldwell et al., 2012; Nascimento. et al., 2015). Contamination by endocrine disrupting compounds (EDCs) is noteworthy in this regard. EDCs include certain pharmaceuticals, personal care products, pesticides, antioxidants, plasticizers, among others (Ferguson et al., 2013; Locatelli et al., 2011; Sodré et al., 2010), and are capable of leading to biological effects similar to those induced by natural and synthetic hormones, such as 17α-ethinylestradiol, 17β-estradiol, estrone and estriol, among others (Rodil et al., 2016; Sodré et al., 2010). These compounds can cause alterations in the endocrine system of several organisms, including mussels, fishes, birds, reptiles, and mammals, and may lead to reproductive disturbances like imposex, hermaphroditism, masculinization and feminization, as well as disturbances in sexual relationships and population declines (Janex-Habibi et al., 2009; Verbinnen et al., 2010). Substances presenting estrogenic activity can have long lasting effects in human reproductive system, even if this exposure occurred *in utero* (Halem et al., 2014; OECD, 2012; Rocha et al., 2014).

Coastal areas such as estuaries have been increasingly compromised due to improper use of coastal areas and excessive industrial exploration of water resources (Gao et al., 2013; IIfelebuegui, 2011). The release of treated or/and untreated wastewater and the associated organic and inorganic contaminants in these environments affects water quality by decreasing dissolved oxygen levels, increasing turbidity and altering water pH, hence posing a risk to wildlife and humans (Baptista Neto et al., 2008). Contaminants that reach estuaries are, in many cases, retained in the sediment and ressuspended along the water column as part of a complex environmental mixture, which may adversely affect biota, due to endocrine disruption, or mutagenic,

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genotoxic and carcinogenic effects (Lapworth et al., 2012). Studies on estrogenic activity in estuaries are extremely valuable, since these environments show significant productivity and biodiversity, and are responsible for several processes crucial to the maintenance of the surrounding ecosystems (Longhurst et al., 1995).

Both *in vivo* and *in vitro* assays have been conducted in order to assess the effects of substances with estrogenic potential. *In vivo* assays assess parameters such as sexual organ weight, cell behavior, protein expression and enzyme activity (Baker, 2001). However, this type of analysis requires longer exposure periods and higher personnel effort and costs. Some *in vitro* assays may be less complex than *in vivo* tests, although this is not always the case. Concerns regarding ethical issues involving the use of animals in scientific research should also be taken into account (Soto et al., 1995). For environmental monitoring purposes, the assessment of chemical substances should be conducted by simple, inexpensive, sensitive and specific tests. Thus, *in vitro* assays may be preferred, and may aid in elucidating mechanisms of action of these compounds. In this context, the Yeast Estrogen Screening (YES) assay represents a quick, sensitive, effective and low cost tool to assess the estrogenic potential of all the compounds in mixture (Bila, 2005; Dias et al., 2015; Routledge and Sumpter, 1996). This technique has been applied to detect estrogenic activity in several water bodies worldwide, such as in sewage effluents from the river Rhine at Worms, Germany (Pawlowski et al., 2005) and surface waters of the Baltic Sea (Beck et al., 2006).

Jurujuba Sound, located in the state of Rio de Janeiro, Southeastern Brazil, is an estuary with several sources of pollution distributed throughout its surroundings and is, therefore, highly impacted. Only a small portion of the area's domestic sewage is treated (Baptista Neto et al., 2006), leading to the discharge of untreated sewage directly into nearby rivers and small streams along the coastline, the main sources of aquatic contamination in this area. As raw sewage is known as one of the main sources of EDCs. the aim of the present study was to evaluate the estrogenic activity and toxicity at Jurujuba Sound alongside water quality parameters.

2. Material and methods

2.1. Study area and water samplings

Guanabara Bay, one of the most polluted bays in the world (Silva et al., 2015), is surrounded by the biggest population center of the country, the metropolitan area of Rio de Janeiro, and presents serious pollution problems, with high rates of domestic and industrial wastewater discharges and accelerated sedimentation (Tucci, 2008). Jurujuba Sound is a restricted and shallow water body situated on the east bank of Guanabara Bay, between latitudes 22°54'S and 22°56'S and longitudes de 043°05′W and 043°07′30″W. It spans a 9 km^2 area, with depths ranging from 5 to 7 m at its entrance and from 3 to 4 m at the center. It is located in an area with both high-standard housing with access to goods and services and a large contingent of irregular occupations and *favelas* (slums) towards hillsides and subject to erosion by the less favored population, with little or no sanitation. Fig. 1 displays the map of the study area.

Therefore, this area is continuously significantly impacted due to untreated sewage (Baptista Neto et al., 2006). This has led to eutrofication processes of the Sound's water bodies. Surrounding the area are also a ferry station, a cemetery and several hospitals, as well as a mussel farm that markets these animals for human consumption, leading to further environmental and health risk concerns (Nascimento. et al., 2015).

Two sampling campaigns were conducted, the first in June 2011, in winter (dry period, low tide), at six water sampling locations throughout the area, and the second in May 2012, in autumn (dry period, low tide), at the same water sampling locations, with an extra sampling performed at the mouth of a sewer pipe. Samples were collected with the aid of a Van Dorn 2 L bottle (AFK 34) and transfered to amber glass bottles. Samples were taken from the water surface(S), the middle (M) and the bottom (deep, D) of the water column. Ten milliliters of methanol were added to each sample subsequently analyzed by YES (1% v/v), to avoid microbiological degradation of the compounds of interest. Samples were taken to the laboratory in styrofoam boxes on ice and maintained at 4 °C in the laboratory until analysis. Table 1 displays the water sampling sites characteristics.

2.2. Reagents

An 17β-estradiol (E2) solution (98% purity, Sigma-Aldrich) was prepared at 100 mgL^{-1} in acetone and stored at 4 °C. All medium constituents were obtained from Sigma-Aldrich. Purified water was obtained from a Milli-Q Biocell system (Millipore). Chlorophenolred-β-D-galactopyranoside (CPRG) was supplied by Merck. For sample extractions, hexane, methanol, ethanol and acetone (HPLC and spectrophotometric grade solvents) were supplied by Tedia Brazil. HCl was P.A. grade (Merck).

2.3. Water quality analyses

Water quality was evaluated based on the physico-chemical parameters established by Brazil's National Council for the Environment (CONAMA), Resolution 357 (Brasil, 2005). Samples were characterized in terms of dissolved organic carbon (DOC), pH, conductivity, turbidity, alkalinity, salinity, ammoniacal nitrogen ($N\text{-}NH_3$), and suspended solids, according to methodology described by the American Public Health Association APHA (2012).

2.4. Acute toxicity assays

Acute toxicity assays were conducted on an SDI Microtox500 analyzer wand with the MicrotoxOmni® software package using *Aliivibrio fischeri*, a luminescent, gram-negative and optional, anaerobic marine bacterium, according to Brazilian normative resolution NBR 15411-3 (ABNT, 2006). This toxicity assay requires small sample amounts and was deemed adequate to evaluate the toxicity of Jurujuba Sound waters, since *Aliivibrio fischeri* is a salt-water organism. Toxicity was determined by the decrease of bacteria luminescence comparing initial levels with values recorded after 0, 15 and 30 min of exposure to the water samples from the different locations throughout Jurujuba Sound. The bacteria were exposed to the water samples at a 81.9% dilution, at 4 serial dilutions using NaCl 2% as diluent.

2.5. Sample preparation for the estrogenic activity assays

The day after the sample collection, 1 L of each sample was filtered through a 0.45 μm cellulose membrane (Merck) using a vacuum pump. Solid phase extraction (SPE) was performed using a Strata X 500 mg cartridge; 3 mL (Phenomenex). The filtered samples were acidified to pH 3.0 with HCl, 3.0 mol L^{-1}, and stored at 4 °C until extraction. The Strata X cartridges were conditioned immediately before use with 3 × 2 mL of hexane, 1 mLof acetone, 3 × 2 mL of methanol and washed with 5 × 2 mL of purified water at pH 3.0 to eliminate interferents. The pre-filtered samples were forced under vacuum through the cartridge at a flow rate of approximately 10 mL min^{-1}.

The cartridges were kept under vacuum aspiration for 30 min after finishing the extraction. The elution of the extracts from the cartridges was performed using 4 mL of acetone. The solvent was evaporated under a gentle nitrogen stream, until dryness. Finally, the dry samples were reconstituted with 2 mL of ethanol and stored at 4 °C until analysis.

2.5.1. Yeast estrogen (YES) assay

The estrogenic potential of water samples can be measured through