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Baseline

Evaluation of polycyclic aromatic hydrocarbons bioavailability on Santos Bay (Brazil) through levels of biliary metabolites

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ABSTRACT

This study evaluated the PAH bioavailability from Santos Bay (Brazil) in 4 species of fish, using PAH biliary metabolites. The collection was done monthly, between July and December, in three different regions of Santos Bay. The metabolites were analyzed through a high performance liquid chromatograph with fluorescence detectors. Total metabolites concentrations ranged from 65.5 to 589 μg g^{-1} of bile, evidencing PAH bioavailability on Santos Bay. Levels of phenanthrene and benzo[*a*]pyrene metabolites were in the classification range of areas moderate contaminated. Those concentrations were lower in *Nebris microps* and higher in *Sphoeroides testudineus* ($p < 0.05$). Naphthalene metabolites concentrations did not differ significantly among fish species and were in the classification range of low contaminated areas. There were no significant spatial and temporal differences in levels among sampled areas. These results are environmentally important given the high levels of urbanization and the absence of biomonitoring data in this area.

Urban development in coastal areas is intense in most part of the world. Consequently, marine environments located near urban areas are subjected to contaminant inputs (Sacchi et al., 2013). Polycyclic aromatic hydrocarbons (PAH) are a group of hydrophobic organic compounds that are widespread in the marine environment, mainly as result of anthropogenic activities (Beyer et al., 2010). This class of compounds affects the marine ecosystems and poses risks due its mutagenic and carcinogenic properties (Johnson et al., 2002).

PAH exposure in marine organisms is often assessed by measuring these compounds in their tissues (e.g. Ruiz et al., 2011; Devier et al., 2013; León et al., 2013). However, due to the presence of the mixed-function oxygenase enzymes (MFO enzymes) that efficiently convert aromatic compounds into more hydrophilic metabolites (Pikkarainen, 2006; Van der Oost et al., 2003), fishes from highly polluted areas often show only trace levels in their tissues, not reflecting the actual exposure levels they are potentially exposed (Beyer et al., 2010).

The PAH metabolites produced by the MFO enzymes in liver are secreted and stored in the gall bladder before being eliminated (Richardson et al., 2001). Determination of PAH metabolites in bile has become an integrative and useful method to characterize the recent PAH exposure (Brinkmann et al., 2013). Laboratory studies have shown that the presence of PAH metabolites in bile of fishes is well correlated with their exposure via contaminated food (Meador et al., 2008). Significant increases in the levels of biliary PAH metabolites were observed in the bile of fishes from polluted environments (Van der Oost et al., 2003). Blahova et al. (2013) and Meier et al. (2013) have found a good correlation between the biliary PAH metabolites levels and the sediments PAH concentrations. The assessment of PAH exposure through the measurement of PAH metabolites in fish bile has been successfully used in different marine environment studies (Richardson et al., 2001; Da Silva et al., 2006; Devier et al., 2013; Kammann et al., 2014; Nagel et al., 2012; Couderc et al., 2015).

Santos Bay is in the southeastern Brazil, in the central area of the Sao Paulo State coast (Fig. 1). This area is economically important to

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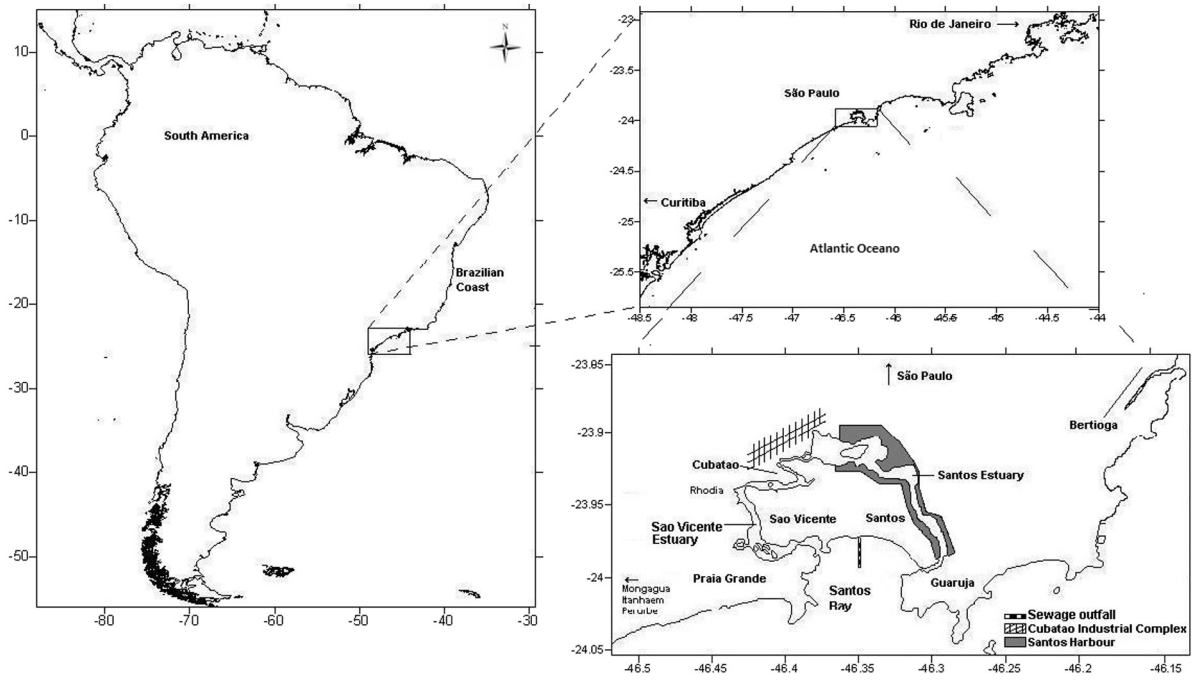

Fig. 1. Geographical location of the Santos Bay.

the country and it is also one of the most impacted zones of São Paulo State (CETESB, 1999). Domestic sewage is a significant source of contamination in Santos Bay (Rachid, 2002; Campos et al., 2012), once its population is around 740 thousand inhabitants (IBGE, 2010). Another source of contamination is the adjacent estuary complex that includes the largest harbor of South America (Santos Harbor, Fig. 1) (Martins et al., 2011) and one of the largest industrial complex of Brazil (Pole of Cubatão, Fig. 1). This industrial complex has 23 industries including steel mill, refinery, fertilizer, cement and chemical/petrochemical plants that sum up to 260 pollutant emission sources (CETESB 1999). In addition, due to the presence of the harbor, tons of sediment must be dredged every year from the Santos Estuary (Martins et al., 2007), which resuspend the contaminated material that has already been deposited. Studies regarding contamination in the Santos-São Vicente Estuarine Complex have shown concentrations of PAH in sediments ranging from 79,6 to 15.389 ng g^{-1} of dry weight sediment (Medeiros and Bicego, 2006). This contamination can be discharged into the Santos Bay (Taniguchi, 1995).

The aim of the present study was to determine the bioavailability of PAH in the Santos Bay through analyses of PAH metabolites in fish bile and to verify the specific, temporal and spatial influences of these levels. There are few studies in Brazil using this method (Da Silva et al., 2006; Azevedo et al., 2012; Albergaria-Barbosa et al., 2017).

The collection was done monthly, between July and December of 2005. Six sites in the bay (Fig. 2) were sampled by otter-trawl (towing speed: 2 knots; towing time: 10 min), using the research vessel *Velliger II* of the Oceanographic Institute – University of São Paulo - Brazil. Because there were limited numbers of specimens collected along each of these six areas, the areas were pooled into the following three groups: West (W); Central (C) and East (E) (Fig. 2).

Based on spatial and temporal occurrence, with emphasis on demersal fishes that feed on benthic invertebrates, four species of fishes were selected for this study: *Stellifer rastrifer* (Jordan, 1889), *Micropogonias furnieri* (Desmarest, 1823), *Nebris microps* (Cuvier, 1830) (Sciaenidae, Perciformes) and *Sphoeroides testudineus* (Linnaeus, 1758) (Tetraodontidae, Tetraodontiformes). The fish gall bladder was dissected, placed inside cryogenic vials and pierced using a blade. After the bile was drained inside the vial, the empty gall bladder was removed and the vial was placed in dry ice at − 20 °C until they could be transferred to a − 80 °C freezer. Due to the small volume of bile (< 10 μL in some cases) collected from each fish, it was not possible to replicate the samples and it was necessary to group bile of fishes with different gender, maturity stage and size.

The use of high-performance liquid chromatography coupled to fluorescence detectors (HPLC/F) has proven to be a powerful tool for estimating exposure of fish and other marine vertebrates to PAH, including nitrogen and sulfur homologs. This method is semi-quantitative and has been used in environmental monitoring studies (e.g., Krahn et al., 1986; Escartín and Porte, 1999; Da Silva et al., 2006; Scholz et al., 2011; Tairova et al., 2012; Tomy et al., 2014; Albergaria-Barbosa et al., 2017). Bile samples were analyzed by HPLC/F (Agilent Technologies 1200 series), according Scholz et al. (2011). The reverse-phase analytical column used was a 4.6 × 150 mm, C18, Synergi 4 μm Hydro-RP 80 Å (Phenomenex) with a 2 μm, 0.84 μL (internal volume) stainless-steel precolumn filter (Upchurch Scientific) and a 2 × 20 mm, C18 dry packed guard-column (Upchurch Scientific). The column temperature was 50 °C. Acetic acid/water (5 μL/L) (solvent A) and methanol (solvent B) were used in a linear gradient as follows: with a 0.8 mL min^{-1} flow from 90% solvent A to 100% solvent B in 5 min; 7 min at 100% solvent B, increase the flow to 1.5 mL min^{-1} in 1 min still with 100% solvent B, 2 min at 1.5 mL min^{-1} flow of 100% solvent B; 1 min to decrease the flow to 1 mL min^{-1} and to return to 90% solvent A, 3 min at 1 mL min^{-1} of 90% solvent A. The HPLC system was coupled with three fluorescence detectors connected in series. A standard solution containing naphthalene (NAP) (1.0 ng·μL^{-1}), phenanthrene (PHE) (0.5 ng·μL^{-1}), and benzo[*a*]pyrene (BaP) (1.0 ng·μL^{-1}) was used as an external standard. The chromatograms were recorded simultaneously and throughout the entire run, at excitation/emission wavelength pairs for each detector as follows: 290/335 nm for NAP; 249/364 nm for PHE; 380/430 nm for BaP. Peak areas eluting after 2 min were integrated, summed and quantified as NAP, PHE, or BaP equivalents. These peaks represent all compounds presented in bile that fluoresce at each wavelength pair. This current investigation reports the existence of fluorescent PAH in non-hydrolyzed bile samples at the given wavelengths and the possible presence of interfering compounds that also fluoresce at these wavelengths cannot be disregarded.

Bile from Atlantic salmon (*Salmo salar*) exposed to Monterey Bay crude oil was utilized as a control material (ASMBC) for quality