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Baseline

Evaluation of anthropogenic contamination using sterol markers in a tropical estuarine system of northeast Brazil

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ABSTRACT

The São Francisco River estuarine system, located in the Northeast coast of Brazil, has great economic, tourist and social importance. Its waters are used for activities such as agriculture, aquaculture, navigation and fishery, which supplies the surrounding communities. In this study, sterols markers were determined in twenty-eight sediment samples from São Francisco River estuary by gas chromatography - mass spectrometry (GC–MS). Sterol analysis was useful to distinguish between anthropogenic and biogenic organic matter (OM) sources in the studied area. Six sterols were quantified, suggesting different sources. Concentrations of fecal sterol (coprostanol) were lower than 500 ng g^{-1}, suggesting no indicative of severe sewage contamination.However, two stations showed concentrations around 100 ng g^{-1} and the values for the coprostanol/(coprostanol + cholestanol) and coprostanol/cholesterol ratios indicates sewage contamination. The results in this study may be considered as baseline concentrations to be used as future reference for monitoring programs to prevent anthropogenic impacts.

Sewage pollution is a major cause of decreasing water quality in rivers and lakes throughout the world (Vane et al., 2010). Understanding what impact the anthropogenic effluent has on urbanized coastal waters and on environment is critical to sustainable marine management (Krepakevich and Pospelova, 2010). Therefore, the assessment of contamination in aquatic systems is of great interest both for the environment and human health, once it can improve water quality and reduce the risk of infectious diseases (Wang et al., 2010).

Sterols are ubiquitous compounds, therefore they might be used to determine the current and past ecosystem health, as well the relative contributions of natural versus anthropogenic input to the environment. Due to their source specificity and resistance to degradation, sterols are classically used to characterize the natural OM in estuarine systems and have been applied as molecular tracers to identify anthropogenic inputs (e.g. fecal contamination) in various environmental compartments, such as water and sediments (Peng et al., 2002; Abreu-Mota et al., 2014; Matic et al., 2014).

The São Francisco River, with a drainage basin of 639,219 km^2 and average flow of 2850 m^3 s^{-1}, is one of the most important Brazilian water resources and is considered the river of national integration, draining seven states. The estuary area, located at the boundary between the Northeast and East geographical regions of the tropical coast of Brazil, has great economic tourist and social importance. Its waters are used for activities such as agriculture, aquaculture, navigation and fishery, supplying communities which live on its banks. Several fish processing industries are distributed on this area, characterized mainly by artisanal fishery activities. The climate in this area is tropical semi-humid with a mean annual temperature of 25 °C, showing two outstanding seasons: one rainy, between April and August, and another dry, between September and March (Knoppers et al., 2006; Bernardes et al., 2012; Santos et al., 2014).

There is limited information regarding the current distribution of sterols in the sediments of the São Francisco River estuary. Carreira et al. (2015) evaluated the presence of fecal steroids to trace the input of sewage to the shelf along the coast of Sergipe and Alagoas states. However, to the best of our knowledge, no previous studies using sterols as markers to distinguish between anthropogenic and biogenic sources of the OM from São Francisco River estuary have been conducted. The aim of this study was to evaluate the distribution and sources of OM in sediments using sterols markers. Twenty-eight surface sediment samples were collected during two seasons: the rainy season (July 2013) and the dry season (December 2013). Sampling sites were chosen to give a wide spatial coverage of the system, as shown in Fig. 1.

Sediment samples were collected using a Van Veen dredge and stored at 4 °C during transportation to the laboratory. Prior to the laboratory work, all sediment samples were freeze-dried, pulverized with a mortar and sieved with a stainless steel sieve (<2 mm) and stored at 4 °C before analysis.

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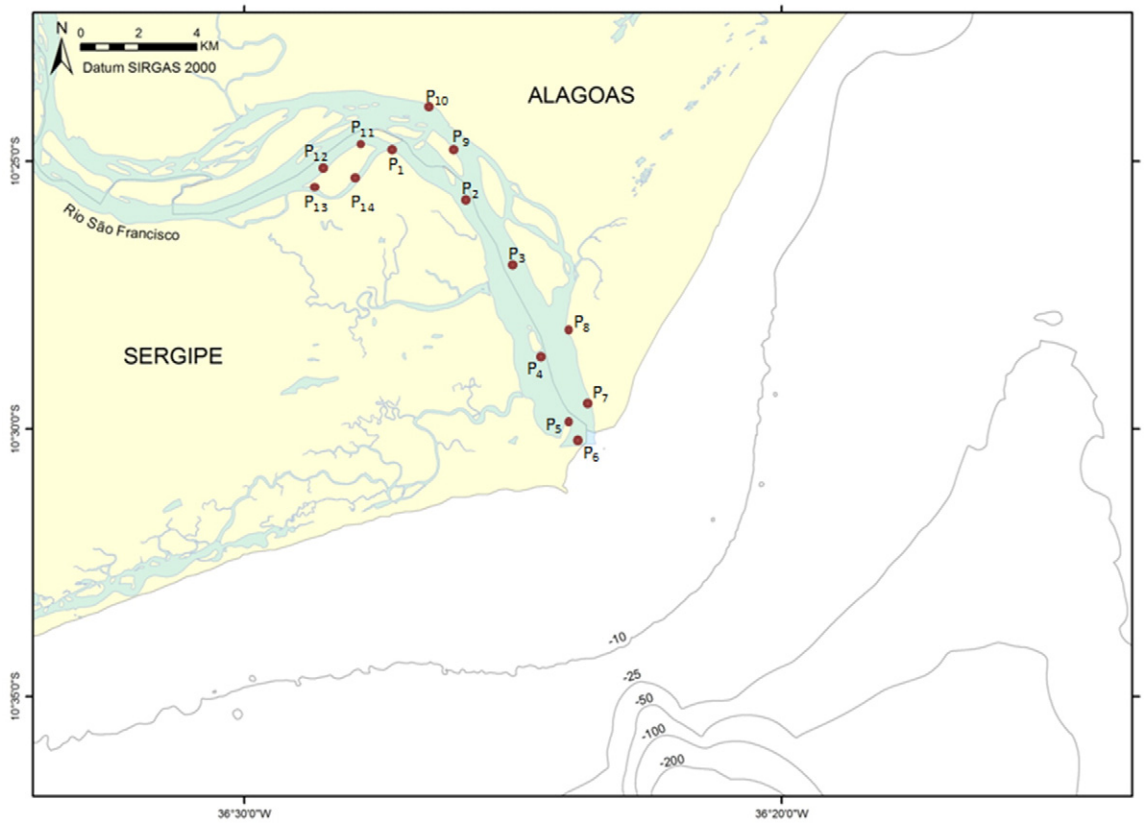

Fig. 1. Map of study area showing the sampling stations.

Cholesterol (3β-cholest-5-en-3-ol), coprostanol (3β,5β-cholestan-3-ol), stigmastanol (3β,5α-stigmastan-3-ol), cholestanol (3β,5α-cholestan-3-ol) and androstanol (5α-androstan-3β-ol) were obtained from Sigma Aldrich (St. Louis, USA) and stigmasterol (3β,22E)-stigmasta-5.22-dien-3-ol), β-sitosterol (3β-stigmast-5-en-3-ol) from Spectrum (Gardena, CA). 5α-cholestane was used as internal standard (IS) for quantification of all compounds. Stock solutions containing sterols (10 mg L^{-1}) were prepared in HPLC-grade dichloromethane (99.9% purity). Working standard solutions were prepared from these solutions and diluted with 95% hexane prior to analysis. HPLC-grade 95% hexane and dichloromethane were purchased from Tedia (RJ, Brazil). BSTFA/TMCS (99:1) was obtained from Supelco (Bellefonte, PA).

Sample extraction was performed using 5 g of lyophilized sediment (mesh < 2 mm), 10 mL of dichloromethane and 5 mL of methanol. Each sediment sample was spiked with the surrogate androstanol (5α-androstan-3β-ol) at a constant concentration of 500 ng mL^{-1}, according to previous studies reported in the literature (Cordeiro et al., 2008; Martins et al., 2010; Adnan et al., 2012; Puerari et al., 2012; Tolosa et al., 2014; Carreira et al., 2015; Frena et al., 2016). The samples were immersed during 30 min (three times) in an ultrasonic bath (model UltraCleaner 1400; Callmex, São Paulo, Brazil) operating at a frequency of 40 kHz and the extracts were combined and concentrated to approximately 2 mL by rotoevaporation. The extracts were then evaporated to dryness under nitrogen stream (99.996% purity) and sterols were derivatized into trimetylsilyl ethers form using 50 μL of BSTFA (bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane). The derivatization was performed at 60 °C for 60 min. Finally, the extract was reconstituted in 1 mL of hexane, the internal standard 5α-cholestane was added at a constant concentration of 500 ng mL^{-1} and 1 μL of the derivatizated sample was injected into the GC–MS.

Sterols analysis was conducted on a Shimadzu GC–MS QP2010 system (Kyoto, Japan), equipped with split/splitless injector. A Zebron ZB5-MS capillary column (30 m, 0.25 mm i.d., 0.25 μm d_f) supplied by J.W. Scientific (Santa Clara, CA, USA) was used under the following conditions: 100 °C (held for 3 min), increasing at 25 °C min^{-1} to 280 °C (held for 2 min), then increasing at 1 °C min^{-1} to 300 °C (held for 1 min). The injection was performed in splitless mode (1 min), at 280 °C, using an AOC-20i automatic injector.

The mass spectrometer ion source was operated in electron impact (EI) mode at 70 eV. The GC–MS interface and the ion source temperatures were set at 300 °C and 280 °C, respectively. Helium (99.995% purity) at a flow rate of 0.95 mL min^{-1} was used as the carrier gas. Analysis was performed in SIM (selective ion monitoring) mode (Table 1). The data was acquired by GC Solution software (Shimadzu, Kyoto, Japan).

Calibration curves for each sterol were obtained from standard solutions at different concentration levels. 5α-cholestane at a constant concentration of 500 ng mL^{-1} was used as internal standard. Cholesterol, coprostanol, stigmastanol, cholestanol, stigmasterol and β-sitosterol were identified on the basis of mass spectra and retention times obtained by using authentic standards. Quantification was based on response factors of authentic standards relative to 5α-cholestane.

Quality assurance/quality control was checked by the following: precision ranged from 0.5 to 7.0% for individual sterols based on three replicate analysis of a sediment sample; calibration was conducted daily; the first injection after instrument tuning was a calibration standard, followed by a blank, and sample analysis was performed only after correct instrument response. Procedural blanks were performed with each series of ten samples, and no peaks interfered with the analyses of the target compounds. The recovery of surrogate (androstanol) ranged from 76% to 99%, which is acceptable considering environmental samples (Ribani et al., 2004). The limit of quantification (LOQ) of each analyte was defined as the first point of the analytical curve divided by the mass of sediment and the limit of detection (LOD) as three times lower than the LOQ (Dias et al., 2013).