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ARTICLE

Input of organic matter in a large south american tropical estuary (Paranaguá Estuarine System, Brazil) indicated by sedimentary sterols and multivariate statistical approach

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ABSTRACT

The Paranaguá Estuarine System is one of the most important environments of the South American coast. Fishing, urban and tourist activities, industries, sewage and the main shipping port for the export of grains in Brazil are sources of environmental impacts. The sources of sedimentary organic matter were evaluated by sterol concentrations which were obtained by gas chromatography with a mass spectrometer (GC-MS). The coprostanol levels were comparatively low, except at sites close to Paranaguá City, where high concentrations have shown sewage contamination. The principal component analysis showed the distinction between sterols from marine (cholesterol, cholestanol, stigmasterol and campesterol), fecal (coprostanol and epicoprostanol) and terrigenous inputs (β-sitosterol). The sterol composition of the sediments indicated that the estuary system is an embayment dominated by inputs of marine organic matter associated with phytoplankton. Terrestrial inputs were detected only at sites close to mangroves, uncontaminated rivers and semi-closed inlets. Fecal input is restricted around Paranaguá City.

Keywords: sterols, sediments, sewage, Paranaguá Bay, organic matter

RESUMO

O Estuário de Paranaguá é um dos ambientes mais importantes da costa Sul-Americana. A ocupação urbana, a pesca, o turismo, as indústrias, a introdução de esgoto e o principal porto de exportação de grãos do Brasil são fontes de impactos ambientais. As fontes de matéria orgânica sedimentar foram avaliadas através das concentrações de esteróis, obtidas por cromatografia gasosa acoplada à espectrometria de massas (GC-MS). Os níveis de coprostanol foram comparativamente baixos, exceto próximo da cidade de Paranaguá, onde as altas concentrações indicaram contaminação de esgotos. A análise de componentes principais mostrou a distinção entre os esteróis de origem marinha (colesterol, colestanol, estigmasterol e campesterol), fecal (coprostanol e epicoprostanol) e aportes terrígenos (β-sitosterol). A composição dos esteróis nos sedimentos indicou predomínio de matéria orgânica marinha associada ao fitoplâncton. Contribuições terrígenas foram verificadas próximas a manguezais, rios e pequenas baías não contaminadas. Contribuição de esgotos é restrita ao entorno da cidade de Paranaguá.

Introduction

The Paranaguá Estuarine System (25º30'S; 48º25'W) is the third most important estuarine environment of the Brazilian coast. Its economic importance is related to fishing activities, urban and tourist areas and industries, associated with fertilizer plants, fuel terminal and the main shipping port for the export of grains in South American, being the third most important in loading and unloading operations with 22,563,975 ton (in 2008).[1] The estuary is encompassed by an Environmental Protection Area (EPA) and has a major ecological function, grouping at least ten conservation units, with a great diversity of environments such as islands, mangroves, salt marshes, rivers, tidal creeks, rocky shores and sand beaches.[2]

Despite the ecological and economic importance of the Paranaguá System, it has been influenced by anthropogenic inputs from different sources, including domestic discharges and sewage from the port and industries. The population living on the margins of this estuary is 181,249, being Paranaguá (population: 126,076) and Antonina (population: 20,228) the most important cities in the region (Figure 1). These cities have very poor sewerage systems and most of the sewage from Paranaguá City is discharged into two rivers (Itiberê and Emboguaçú Rivers) (Figure 1) or directly into the estuary.[3]

One approach to investigate the geochemistry of organic matter in marine environment is based on information of specific organic compounds, known as molecular markers or biomarkers.[4,5] Sterols are one of the categories of molecular markers currently in use to elucidate the presence of marine, terrestrial and fecal input of organic matter in sediments. They are source-specific, relatively resistant to microbial degradation and may be quantified at low levels.[6,7]

Fecal sterols, such as coprostanol (5β(H)-cholestan-3β-ol) and epicoprostanol (5β(H)-cholestan-3α-ol) present in human feces and sewage effluents, have previously been used as tracers for human waste along several coastal areas of industrial and urban centers in several regions.[8,9] Coprostanol has been widely used as a marker of fecal contamination because it is produced in the digestive tracts of humans and higher vertebrates by microbial reduction of cholesterol (cholest-5en-3β-ol; $27\Delta^5$). On the other hand, epicoprostanol (a coprostanol isomer) can be used as an indication of the level of treatment or age of the fecal matter because it is formed during wastewater treatment and sewage sludge digestion.[10]

Other sterols, like cholesterol and cholestanol (5α(H)-cholestan-3β-ol; $27\Delta^0$), are indicators of marine organic matter input patterns in the sediments. In addition, sterols like β-sitosterol (24-ethylcholest-5-en-3β-ol; $29\Delta^5$), stigmasterol (24-ethylcholesta-5,22E-dien-3β-ol; $29\Delta^{5,22E}$) and campesterol (24-methylcholesta-5-en-3β-ol; $28\Delta^5$) help to detect the contribution to the organic pool by vascular plants or bacteria.[11]

The organic geochemistry on the Paranaguá Bay remains practically unknown. Only three Brazilian estuarine environments has been selected for diagnosis of organic geochemistry and environmental pollution: Guanabara Bay and Campos Basin (both at Rio de Janeiro State);[12] Santos Estuary (São Paulo State)[13] and, recently, the Mundaú-Manguaba Estuarine System (Alagoas State).[5,14]

Despite their ecological importance, the sources of the organic matter found in the Paranaguá System have barely been examined. No previous studies using sterols as biomarkers, to distinguish between sewage and marine/terrestrial sources of organic matter, have been conducted in this region. In this respect, several sterols have been analyzed in surface sediments at selected sites in the Paranaguá Estuary to assess the main sources of organic matter to this system.

Experimental

Study area

The Paranaguá Estuarine System is located at Paraná State on the southeastern Brazilian coast (Figure 1). It has a water volume of *ca.* 2×10^9 m^3, an area of 612 km^2 and is divided into two main sections: (*i*) western section formed by Paranaguá and Antonina Bays and (*ii*) northern section formed by Laranjeiras, Guaraqueçaba and Pinheiros Bays and also Benito and Itaqui Inlets.[3]

Surrounded by Serra do Mar Mountains and Atlantic Forest, the Paranaguá Estuarine System is connected to the ocean through three channels: (*i*) the Galheta Channel is the most important link between the estuary and the continental shelf, and it is periodically dredged to allow ships to enter Paranaguá Harbor, (*ii*) the Barra Norte Channel between Mel and Peças Islands and (*iii*) the Superagui Channel between Peças and Superagüi Islands.[2]

The hydrodynamics are driven by tidal forcing and river runoff. The bay has semi-diurnal tides characterized by diurnal inequalities with maximum amplitudes of *ca.* 2 m, a mean depth of 5.4 m and a residence time of 3.5 days.[2] The climate can be defined as transitional tropical, the temperature ranges from 16 ºC in the winter to 34 ºC in the summer and the total annual precipitation is *ca.* 2000 mm.

The tidal flats on Paranaguá Bay were divided into three main sectors according to physicochemical characteristics: (*i*) an inner mesohaline sector with muddy sediments and high organic content (up to 25%), low energy and average salinity (PSU) 0-15; (*ii*) a middle polyhaline sector with very fine sand bottoms and organic content ranging from 5 to 15% and; (*iii*) an outer euhaline sector with well sorted sand, with organic percentages around 1.5%, high energy and average salinity (PSU) 30.[15]

On the Paranaguá Bay, *Spartina alterniflora* marshes colonize tidal flats or creeks as narrow, monospecific, discontinuous belts in front of mangrove woodland (*Laguncularia racemosa* and *Rhizophora mangle*).[15]

Sampling

In order to evaluate steroid inputs in the Paranaguá Estuarine System, 39 sediment samples were collected between August 2003 and January 2005 (Figure 1; Table S1). The sites were divided into four different sectors: Antonina Bay (A), Paranaguá Bay (B), Laranjeiras Bay (C) and Pinheiros Bay (D) for better discussion of the results. Sediments were sampled utilizing a stainless-steel grab. Only the top (2 cm) of undisturbed surface sediment were placed into pre-cleaned aluminum foil, and then stored at -15 ºC until analysis. The sediments were oven dried (40 ºC) and then sieved through a stainless steel mesh (250 µm). Sub-samples were taken for total organic carbon (TOC) and total nitrogen (TN) determination and grain size analysis.

Bulk organic matter analysis

TOC and TN were determined in samples collected until February 2004 (Table S1) using a dry combustion method with a Perkin Elmer 2400 CHN (carbon, hydrogen and nitrogen) analyser (series II). Inorganic carbon was removed prior to the analysis by treatment with HCl. Replicates analysis were not performed. Quantification was performed using calibration curves and acetanilyde (71.1% C, 6.71% H and 10.36% N) as standard. A reference material MESS-2 (National Research Council of Canada) was used to verify accuracy and the results were within 90-115% of the certified values.

Reagents and chemicals

In this study, standards of 5α-androstan-3β-ol (98% purity), 5α-cholestane (99% purity), coprostanol (> 98% purity), epicoprostanol (> 95% purity), cholesterol (94% purity), cholestanol (95% purity), stigmasterol (> 95% purity), campesterol (*ca.* 65% purity) and β-sitosterol (> 95% purity) were purchased at Sigma Aldrich. Hexanes (95% of n-hexane), methanol (PA ACS) and dichloromethane (> 99.8% minimum purity) were supplied by J. Baker. Sodium sulfate anhydride (98% purity) was purchased at J. Baker while silica (silica gel 60, 0.063-0.200 mm) and alumina (aluminum oxide 90 active, 0.063-0.200 mm) were supplied by Merck. N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA + TCMS, 9:1) was purchased at Supelco.

Steroid analysis

Each sediment sample and blank (25 g) were spiked with surrogate, 5α-androstan-3β-ol, and then Soxhlet extracted for 12 h with 200 mL of hexanes/dichloromethane (1:1) following the USEPA 3540 method. The extracts were concentrated down to 1 mL using rotary evaporation and under gentle

nitrogen stream. Sulfur was removed with activated copper. Clean-up and fractionation were performed by passing the extract through a silica/alumina column (the silica and alumina were activated at 200 ºC for 4 h and then partially deactivated with 5% Milli-Q water), following the modified USEPA 3640 method. The chromatography column was prepared by slurry packing 8 g of silica, followed by 8 g of alumina and finally 1 g of sodium sulfate. Elution was performed using 20 mL of hexane to yield the first fraction (which contains the aliphatic hydrocarbons, not presented in this study), then 30 mL of hexane/dichloromethane (90:10), followed by 20 mL of hexane/dichloromethane (50:50) (a combination which contains the polycyclic aromatic hydrocarbons, not presented in this study). Steroids were then eluted with 50 mL of dichloromethane/methanol (90:10).

The fractions containing the steroids were evaporated to dryness and derivatized using 40 µL of BSTFA/TMCS (9:1) for 90 min at 65 ºC.[16] The sterane 5-cholestane was added in the end of the process as internal standard.

Table 1. Characteristic ions (*m/z*) of the analyzed steroids, *m/z* (1) is a significant fragment used in the quantitation while *m/z* (2) and (3) were used as confirmation ion

Compound	*m/z* (1)	*m/z* (2)	*m/z* (3)
Coprostanol	370	355	215
Epicoprostanol	370	355	215
Coprostanone	386	231	316
Cholesterol	329	458	368
Cholestanol	445	460	355
Campesterol	382	343	367
Stigmasterol	394	484	355
β-Sitosterol	396	357	486

Instrumental analyses and quality assurance procedures

Steroid analyses were performed with a Perkin Elmer Clarus 500 coupled to a Perkin Elmer Mass Spectrometer Detector (model 500MS) and an Elite 5MS capillary fused silica column coated with 5% diphenyl/dimethylsiloxane (30 m × 0.25 mm ID × 0.25 µm film thickness). Helium was used as carrier gas. The oven temperature was programmed from 60 to 250 ºC at 15 ºC min^{-1}, then to 280 ºC at 1.0 ºC min^{-1}, and finally to 300 ºC at 5 ºC min^{-1} (holding for 5 min). Data acquisition was done in simultaneous collection of full scan and selected ion recording mass spectral data. Compounds were identified by matching retention times and ion fragments (Table 2) with results from 7 sterols (coprostanol, epicoprostanol, cholesterol, cholestanol, stigmasterol, campesterol and β-sitosterol) (Figure 2) and 1 ketone (coprostanone) (Figure 3). Quantification was undertaken by comparing compounds from standard mixture and surrogate response factors in the total ion chromatogram. Calibration of the peak area to concentration was done using the steroid standards in the derivatized form within the range of 0.25 to 8.0 µg $µL^{-1}$ and the linear response was > 0.995 to all compounds.

Procedural blanks were performed for each series of 10 extractions and interfering peaks did not interfere with the analyses of target compounds. Blank samples corresponds a portion of 25 g of sodium sulfate anhydride, heated at 450 ºC, prior the extraction procedure. It can be used to the evaluation of contamination during laboratory procedures. Surrogate recoveries ranged from 70-120%. Sediment and blank samples were spiked with a mixture of analyzed steroid and the standard recoveries ranged from 70-95%. Limits of detection (LOD), defined as three times the standard deviation of the signal in the same retention time of steroids in the blanks, was 1 ng g^{-1} for all the analyzed compounds. Measured concentrations of selected sterols (coprostanol, epicoprostanol, cholesterol and cholestanol) in the IAEA-417 reference material were within 90-110% of the certified values from International Atomic Energy Agency (IAEA).

Multivariate statistical

Principal component analysis (PCA) and Cluster analysis were performed using the Statistical package for Windows (version 5.1, 1997) to identify similarities or distinctions among the different steroids in marine sediments and between stations. For treatment, samples were taken as cases and the analyzed steroid concentrations were the variables. The PCA datasheet consisted of original values from the steroid concentrations at each site, while for the Cluster the data were the percentage of fecal, marine and terrestrial sterols in the total sterols detected.

Results and Discussion

Bulk organic matter

The bulk organic matter is shown in Table 2. High values of TOC (> 2.50%) and TN (> 0.40%) content were found at stations A1, A2 and A4 (Antonina Bay) and B1 (MTZ - Maximum Turbidity Zone).

A scatterplot of TOC *vs.* TN content showed a discreet linear coefficient of determination obtained by linear regression (R^2 = 0.54) and indicated that these elements are derived from multiple sources, such as fecal material, terrestrial organic matter input and marine contributions.

Carbon-nitrogen (C/N) ratios varied between 1.0 and 10.5 suggesting different origins of the sedimentary organic matter, depending on each station. Around 1/3 of stations analyzed (B4, B6, B8, B11 and C1) presented values between 4.0 and 7.0, reflecting a marine input.[17,18] On the Santos Bay (Southeastern Brazilian coast), typical marine organic matter presents C/N ratio values ranging from 5 to 9; while terrestrial C/N values are around 24.[19] Values between 7.0 and 10.5 were found at sites A1, A4, B1, B9 and B10 suggesting some contribution of terrestrial organic matter.

The C/N ratio variability is common in semi-enclosed and shallow environments, like the studied areas, owing to the mixed nature of the organic matter.[20] The proximity to the estuarine channels explains the input of terrestrial material at the above mentioned sites.

Low C/N (< 4.0) was presented at sites B2, B3, B5, B12 and B13, where high concentration of coprostanol (< 0.48 µg g^{-1}) (Table 2) was found suggesting input of fecal material what also contribute with high concentration of nitrogen. Site B3 is

located close to Paranaguá Harbour and sewage sources. Although low coprostanol contents (0.04 μg g^{-1}) (Table 2) have been found in this site, high nitrogen from these sources may be considered.

The carbon and nitrogen percentages in the sediments indicated that the embayment may be classified as an organic-rich system, which is dominated by phytoplankton primary production. This may be favored by a great excess of nutrients associated with domestic effluents and terrestrial organic matter inputs. However, an extensive discussion about fecal and marine or/and terrestrial sources of organic matter using sterols is provided in the next section to corroborate the carbon and nitrogen data.

Sewage contribution indicated by fecal steroids

Concentrations of coprostanol, epicoprostanol and coprostanone (5β-cholestan-3-one) are shown in Table 2. These results are well discussed by Martins *et al.*[21] In general, the levels decreased with the distance from the main sources, as in the downtown area of Paranaguá City, and this is compatible with patterns associated with mixing/dilution processes acting in the estuary. Stations located close to Anhaia River (B2 and B11) may be considered greatly contaminated by sewage, while sites B12, B5 and B9 located close to Paranaguá and Pontal do Sul Cities, respectively, showed major influence of sewage effluent. Other stations located at the mouths of the Itiberê and Correias Rivers (B6, B14 and B13), Encantadas Beach (B10) and Corisco Island (A1) presented coprostanol levels between 0.10 and 0.50 μg g^{-1}, suggesting discreet sewage contribution.

Although the fact that large quantity of discharged fecal material into Itiberê River reaches a semi-enclosed environment and finds Cotinga Island acting as a barrier to sewage dispersion, the hydrodynamic conditions seem to be effective in preventing critical fecal contamination around Paranaguá City. In the other studied places, such as Antonina, Laranjeiras and Pinheiros Bays, the absence of sewage contamination can be associated with low input of fecal material and regular dispersion providing low or undetectable levels of coprostanol and coprostanone. Small communities established close to the Paranaguá system, and far from large cities such as Paranaguá and Antonina, are a source of sewage discharges, but not sufficient to significantly change the natural conditions of this environment.

Sterol contribution from fecal, marine and terrestrial sources

According to Tolosa *et al.*[22] and recently cited by Santos *et al*.,[23] the relative contribution of distinct sources of organic matter can be summarized and assessed by grouping specific sterols as follows: (*i*) zooplankton/phytoplankton: cholesterol and cholestanol; (*ii*) higher plants/algae: campesterol, stigmasterol and β-sitosterol and; (*iii*) sewage: coprostanol and epicoprostanol. However, some sterols, such as cholestanol, stigmasterol, campesterol and β-sitosterol, could be associated with multiple sources.[24] The concentrations of these sterols are shown in Table 2.

The application of multivariate statistical techniques in the steroidal composition has been helpful overcoming the limited specificity of some lipid markers.[25] One way to try to distinguish the main sources of sterols is to apply statistical approaches, as principal component analysis (PCA) for the further investigation of main sources of organic matter in the Paranaguá system.

This analysis was undertaken based on the coefficient of variation for each variable. The first two components (PC1, 60.7% and PC2, 19.6%) were responsible for

80.3% of total variance. The loading variables and score values are shown in Figure 4. The PC1 axis (PCA loadings) showed a negative correlation with all compounds, except β-sitosterol. The PC2 axis had a positive correlation with coprostanol, epicoprostanol and coprostanone (fecal steroids) and a negative one with campesterol and stigmasterol (biogenic sterols) and cholestanol (marine or microbial reduction sources).

The discrimination presented by PC1 showed positive correlations between β-sitosterol (terrestrial sources), while other compounds associated with negative correlations came from marine sources or sewage and they could be distinguished according to discrimination presented by PC2 axis.

Basically, the PCA scores distinguished three different groups of samples. The PC1 axis provided the separation between terrestrial (positive scores) and marine and microbial reduction sources (negative scores). The PC2 scores grouped stations with high fecal steroids, indicating sewage contamination (positive scores) and low sewage with biogenic contribution (negative scores).

Cholestanol has also been found in sediments influenced by the biosynthesis of plankton organisms.[26] However sediments contaminated by sewage may also produce this sterol through the diagenetic transformation of coprostanol into cholestanol and by the anaerobic microbial reduction and hydrogenation of cholesterol in an anoxic environment.[27,28]

Early diagenesis of sterols has thus been estimated by stanol/stenol ratios (*e.g.* cholestanol/cholesterol). The general increase of the stanol/stenol ratio may illustrate the progressive reduction of stenols to stanols.[26] The rates of sterol degradation in sediments are a group of several processes, which hydrogenation by bacteria appears to be relatively more important.[11,24]

Stanols can be formed by the bacterial reduction of sterols[27] and some authors have suggested that the reaction rate of the stenol-to-stanol transformation increases as the redox potential decreases.[29] The cholestanol/cholesterol ratio (ratio I) was lower than 1.0 for all the analyzed samples, indicating oxic conditions and microbial reduction[27] (Table 3). Values around 1.0 for this ratio were found at stations B1 (MTZ) and D1 (Fátima Village) indicating limited oxic conditions compared to the rest of the Paranaguá System.

These values indicated the absence of ideal conditions to produce cholestanol, either by diagenetic or hydrogenation processes, and the source of this sterol is marine organisms. The PCA axis showed that cholestanol comes from single source (marine organisms) due to the high correlation with cholesterol. It was confirmed by the results for ratio I.

Campesterol, stigmasterol and β-sitosterol have been commonly used as markers of terrigenous organic matter, although the origins remained largely uncertain. This happens because marine sources (algae and bacteria) were associated with major occurrences in environments where organic matter from the land seemed unlikely.[10]

Volkman[30] and Laureillard and Saliot[31] proposed to evaluate campesterol/stigmasterol/β-sitosterol ratio to assess terrigenous organic matter input. Ratios close to 1:1.6:6.6 indicate inputs of these compounds from terrestrial vascular plant sources. It is clear in the stations grouped close to β-sitosterol in PCA, such as B8, B19 and B16 (Cotinga Island), C3, C4 (Rasa Island), C7 (Itaqui

Inlet), C8 (Benito Inlet), C10 (Lanço Point), D1 and D2 (Pinheiros Bay), A5 (Yatch Club Antonina) (Table 3).

At the remaining stations, the clear correlation with β-sitosterol (by PCA) did not occur when the campesterol/stigmasterol/β-sitosterol ratio (II) was calculated. These compounds (mainly β-sitosterol) are also present in contaminated sediments from urban areas, being vegetable oils from discharging of domestic wastewater/sewage,[32] and suggesting multiple sources (sewage and higher plants) at sites with higher levels of fecal steroids.

In addition, the evidence of marine sources for campesterol and stigmasterol, verified by a higher correlation between these sterols and typical marine compounds, such as cholesterol and cholestanol in PCA, contributed to the lack of conclusive values for ratio II.

In general, the PCA analyses and the specific ratios among biogenic sterols helped to estimate the main sources of the studied sterols in the Paranaguá System, described as: (*i*) sewage: coprostanol and epicoprostanol; (*ii*) terrestrial sources: β-sitosterol; (*iii*) marine sources: campesterol and stigmasterol (algae), cholesterol and cholestanol (plankton). The ketones, coprostanone and cholestanone were not included here.

A cluster analysis using the percentage of each compound in the total sterols concentration and all sites is shown in Figure 5, and it splits the stations into two main groups at 60% of Euclidean distance. Group I contains the station where the proportion of sterols from marine sources is higher (> 85.5% for Ia and between 49.4-78.8% for Ib) and the fecal sterols present a significant proportion (29.0-44.3%) in addition to marine sterols (41.8-50.2%) (Ic). Group II contains the stations where the proportion of fecal sterols is extremely low (< 0.5%, except B14: 7.4%) and terrigenous sources are slightly predominant over marine sources (50.0-63.5%)(IIa). A higher β-sitosterol content showing a strong terrigenous contribution occurs at site D1 and was responsible for the splitting of group II.

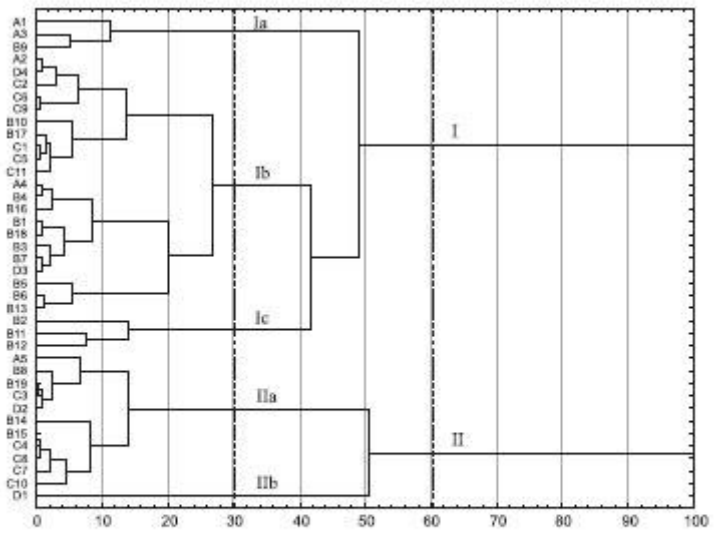

Figure 5. Cluster analyses according to general sources (fecal, terrestrial or marine sources) in the total sterols for the sediments in the Paranaguá Estuarine System. I and II represent subgroups explained in the text.

In general, the marine sources of organic matter seem to be the main source of sedimentary sterols in the Paranaguá System, as verified through the analysis of organic carbon and total nitrogen content. Fecal material is a major component of total sterols mainly close to Anhaia and Itiberê Rivers (B2, B11 and B12-Ic), while terrestrial contributions are limited to the stations close to uncontaminated rivers (*e.g.* Guaraguaçu River, B15), semi closed inlets such as Benito and Itaqui (C7 and C8), near the banks of estuarine channels (C3, C4, D1 and D2) and Cotinga Island (B8 and B19). The mangrove vegetation (mainly *Laguncularia racemosa*) is the source of plant sterols for these sites. The same tendency was verified by studies of Koch *et al.*[33] and Mater *et al.*[34] of the mangrove ecosystem in Bragança Peninsula (Northern of Brazil) and Santa Catarina Island (Southern of Brazil), respectively.

The majority of the stations on the Antonina Bay presented higher proportions of sterols from marine sources. The input of marine waters is more important than terrestrial input for several rivers that discharge at this site. A different situation occurs on the Laranjeiras and Pinheiros Bays, where the terrigenous input seems more effective to change the sterol composition of sediments.

Conclusion

The present work is the first study on the distribution and origin of sterols in sediments of the Paranaguá Estuarine System. The results showed that sedimentary sterols consisted in a mixture of compounds from marine, terrestrial and fecal sources.

The coprostanol concentration (> 1.00 μg g^{-1}) showed strong sewage contamination only at the sites close to Paranaguá City (Anhaia River).

The multivariate statistical approach allowed us to identify the origin of studied sterols and was successfully applied to recognize the main sources of sedimentary organic matter in different sectors of the Paranaguá System.

In general, the sterol composition of the sediments indicated that this estuary is an embayment dominated by predominant inputs of marine organic matter associated with phytoplankton primary production. Sterols from terrestrial inputs (β-sitosterol) were mainly detected at sites close to mangrove areas, uncontaminated rivers and semi-closed inlets while fecal sterols were restricted to areas close to Paranaguá City.

The tidal currents may explain the higher percentage of sterols from marine sources on the Antonina Bay and the gradient decrease of the fecal contamination from Itiberê River onwards.

The results of this work demonstrated that sewage pollution can be considered a problem for a small part of the Paranaguá basin ecosystem. The serious damage caused by organic enrichment, pollutant input and pathogenic microorganism is well known and it is enough to justify a strong environmental policy for this region to avoid, reduce and eventually eliminate the contamination of the studied area.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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References

1. http://www.antaq.gov.br acessed in January 2011. [Links]

2. Lana, P. C.; Marone, E.; Lopes, R. M.; Machado, E. C.; *The Subtropical Estuarine Complex Paranaguá Bay, Brazil*, Springer-Verlag: Berlin, 2001, p. 131. [Links]

3. Kolm, H. E.; Mazzuco, R.; Souza, P. S. A.; Schoenenberger, M. F.; Pimentone, M. R.; *Braz. Arch. Biol. Technol.* **2002**,*35*,27. [Links]

4. Xu, Y.; Jaffe, R.; *Mar. Environ. Res.* **2007**,*64*,666. [Links]

5. Costa, T. L. F.; Araujo, M. P.; Knoppers, B. A.; Carreira, R. S.; *Aquat. Geochem.* **2011**,*17*,1. [Links]

6. Saliot, A.; Laureillard, J.; Scribe, P.; Sicre, M. A.; *Mar. Chem.* **1991**,*36*,233; [Links] Wakeham S. G.; *Deep-Sea Res. Part I-Oceanog. Res. Pap.* **1995**,*42*,1749; [Links] Canuel, E. A.; *Org. Geochem.* **2001**,*32*,563. [Links]

7. Azevedo, D. A., *J. Braz. Chem. Soc.* **2003**,*14*,97. [Links]

8. Mudge, S. M.; Bebianno, M. J.; *Mar. Pollut. Bull.* **1997**,*34*,163; [Links] Maldonado, C.; Venkatesan, M. I.; Phillips, C. R.; Bayona, J. M.; *Mar. Pollut. Bull.* **2000**,*40*,680. [Links]

9. Carreira, R. S.; Ribeiro, P. V. R.; Silva, C. E. M.; Farias, C. O.; *Quim. Nova* **2009**,*32*,1805. [Links]

10. Mudge, S. M.; Lintern, D. G.; *Est. Coast. Shelf Sci.* **1999**,*48*,27. [Links]

11. Volkman, J. K.; *Org. Geochem.* **2005**,*36*,139; [Links] Hudson, E. D.; Parrish, C. C.; Helleur, R. J.; *Mar. Chem.* **2001**,*76*,253; [Links] Volkman, J. K.; Barret, S. M.; Blackburn, S. I.; Mansour, M. P.; Sikes, E. L.; Gelin, F.; *Org. Geochem.* **1998**,*29*,1163. [Links]

12. Carreira, R. S.; Wagener, A. L. R.; Readman, J. W.; *Est. Coast. Shelf Sci.* **2004**,*60*,587; [Links] Azevedo, L. A.; Brüning, I. M. R.; Moreira, I.; *Mar. Pollut. Bull.* **2004**,*49*,1120; [Links] Carreira, R. S.; Araújo, M. P.; Costa, T. L. F.; Ansari, N. R.; Pires, L. C. M.; *Org. Geochem.* **2010**,*41*,879. [Links]

13. Bícego, M. C.; Taniguchi, S.; Yogui, G. T.; Montone, R. C.; Silva, D. A. M.; Lourenço, R. A.; Martins, C. C.; Sassaki, S. T.; Pellizari, V. H.; Weber, R. R.; *Mar. Pollut. Bull.* **2006**,*52*,1784; [Links] Martins, C. C.; Mahiques, M. M.; Bícego, M. C.; Fukumoto, M. M.; Montone, R. C.; *Mar. Pollut. Bull.* **2007**,*54*,240; [Links] Martins, C. C.; Ferreira, J. A.; Taniguchi, S.; Mahiques, M. M.; Bícego, M. C.; Montone, R. C.; *Mar. Pollut. Bull.* **2008**,*56*,1359; [Links] Martins, C. C.; Gomes, F. B. A.; Ferreira, J. A.; Montone, R. C.; *Quim. Nova* **2008**,*31*,1008; [Links] Martins, C. C.; Bícego, M. C.; Mahiques, M. M.; Figueira, R. C. L.; Tessler, M. G.; Montone, R. C.; *Environ. Pollut.* **2010**,*158*,3355; [Links] Luiz-Silva, W.; Machado, W.; Matos, R. H. R.; *J. Braz. Chem. Soc.* **2008**,*19*,1490. [Links]

14. Costa, T. L. F., Araújo, M. P., Knoppers, B. A., Carreira, R.S.; *Quim. Nova* **2010**,*3*,1915; [Links] Araujo, M. P.; Costa, T. L. F.; Carreira, R. S.; *Quim. Nova* **2011**,*34*,64. [Links]

15. Netto, S. A.; Lana, P. C.; *Est. Coast. Shelf Sci.* **1997**,*44*,641; [Links] Netto, S. A.; Lana, P. C.; *Hydrobiologia*, **1999**,*400*,167. [Links]

16. Kawakami S. K.; Montone, R. C.; *J. Braz. Chem. Soc.* **2002**,*13*,226. [Links]

17. Stein, R.; *Accumulation of Organic Carbon in Marine Sediments. Lecture Notes in Earth Sciences*, Springer-Verlag: Berlin, 1991, p. 217. [Links]

18. Jaffé, R.; Mead, R.; Hernández, M. E.; Peralba, M. C.; DiGuida, O. A.; *Org. Geochem.* **2001**,*32*,507. [Links]

19. Fukumoto, M. M.; Mahiques, M. M.; Tessler, M. G.; *J. Coast. Res.* **2006**,*SI39,*1737. [Links]

20. Bordovskiy, O. K.; *Mar. Geol.* **1965**,*3*,33. [Links]

21. Martins, C. C.; Braun, J. A. F.; Seyffert, B. H.; Machado, E. C.; Fillmann, G.; *Mar. Pollut. Bull.* **2010**,*60*,2137. [Links]

22. Tolosa, I.; LeBlond, N.; Copin-Montégut, C.; Marty, J.-C.; Mora, S.; Prieur, L.; *Mar. Chem.* **2003**,*82*,161. [Links]

23. Santos, E. S.; Carreira, R. S.; Knoppers, B. A.; *Braz. J. Oceanogr.* **2008**,*56*,97. [Links]

24. Volkman, J. K.; *Org. Geochem.* **2005**,*36*,139. [Links]

25. Dachs, J.; Bayona, J. M.; Fillaux, J.; Saliot, A.; Albaiges, J.; *Mar Chem.* **1999**,*65*,195; [Links] Yunker, M. B.; MacDonald, R. W.; Veltkamp, D. J.; Cretney, W. J.; *Mar. Chem.* **1995**,*49*,1. [Links]

26. Fernandes, M. B.; Sicre, M.-A.; Cardoso, J. N.; Macedo, S. J.; *Sci. Total Environ.* **1999**,*231*,1. [Links]

27. Jeng, W. L.; Han, B. C.; *Mar. Pollut. Bull.* **1994**,*28*,494. [Links]

28. Martins, C. C.; Montone, R. C.; Fillmann, G.; *J. Braz. Chem. Soc.* **2007**,*18*,106. [Links]

29. Wakeham, S. G.; Canuel, E. A. In *Handbook of Environmental Chemistry: Reactions and Process*, vol.2 ; Volkman, J. K., ed.; Springer: Berlin, 2006. [Links]

30. Volkman, J. K.; *Org. Geochem.* **1986**,*9*,83. [Links]

31. Laureillard, J.; Saliot, A.; *Mar. Chem.* **1993**,*43*,247. [Links]

32. Quéméneur, M.; Marty, Y.; *Water Res.* **1994**,*28*,1217. [Links]

33. Koch, B. P.; Rullkötter. J.; Lara, R. J.; *Wet. Ecol. Manag.* **2003**,*11*,257. [Links]

34. Mater, L.; Alexandre, M. R.; Hansel, F. A.; Madureira, L. A. S.; *J. Braz. Chem. Soc.* **2004**,*15*,725. [Links]

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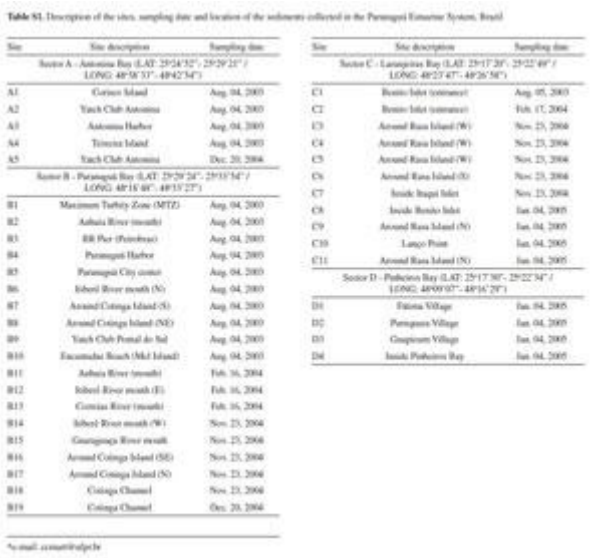
Supplementary Information



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