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Artigo

EVALUATION OF SEDIMENT CONTAMINATION BY URBAN SEWAGE IN A STRETCH OF CAPIBARIBE RIVER, PERNAMBUCO, BRAZIL

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Anthropic activities are responsible for the increase in environmental contamination, especially in aquatic systems, demanding studies to assess and monitor contamination levels in different water bodies. We have done a chemical characterization of eleven sediments in order to evaluate the level of contamination by domestic sewage from the Capibaribe River, between the cities of Santa Cruz do Capibaribe and Toritama in the state of Pernambuco, Brazil. The classical analyses were performed, as well as the determination of sterol biomarkers using liquid chromatography coupled to tandem mass spectrometry. Sterol ratios corroborated the presence of fecal-derived organic matter, mainly in the sediments collected in regions of higher population occupation. The multivariate statistical analysis clearly showed the regions from the most contaminated to the least contaminated, indicating the severe contamination by domestic sewage in the region. To the best of our knowledge, this is the first study to assess anthropic contamination of the Capibaribe River by using sterol biomarkers and classical analyses in an integrative data interpretation. This can be helpful when making decisions about preventive and corrective actions for human health concerns.

Keywords: environmental biomarkers; sediments; contamination; sterols; Capibaribe River.

INTRODUCTION

Contamination levels in aquatic environments are increasing due to anthropogenic activities, such as domestic and industrial waste discharges. Thus, it is necessary to assess these systems to determine the negative impact on the environment and human health.[1]

Chemical characterization of sedimentary organic matter possibility to collect information from the field of study makes it possible to evaluate the historical evolution of the aquatic and adjacent terrestrial ecosystems, enabling the collection of geological and geochemical information from the field of study.[2,3] A comprehensive proposal may include the integration of various chemical analyses, including the classic ones such as infrared spectroscopy, elemental composition, granulometry, and organic matter content. In addition, identification and quantification of environmental biomarkers may be carried out, example, by sterol compounds.[4,5] Sterols are a class of four-cyclic alcohols with a cyclopentaneperhydrophenanthrene core and a hydroxyl at position 3 of the A-ring. In addition, they have an aliphatic side chain at position 17 on the D-ring and methyl groups at positions 18 and 19.[6] Variations on aliphatic side chain long and double bonds result in a series of sterols, for instance, for example, cholesterol, brassicasterol, ergosterol, campesterol, β-sitosterol, and stigmasterol. These tend to associate with suspended particles and accumulate in sediments due to their hydrophobicity[7,8] and their source specificity, chemical stability, and resistance to degradation. Thus, sterols are used as biomarkers for events and processes in the environment,[9–11] being used to indicate fecal pollution and entry of domestic wastewater into systems around the world.[3,12,13] In these studies, coprostanol is used as the fecal marker since it represents around 60% of the sterol composition in feces.[14]

Usually, coprostanol concentration >100 ng g^{-1} is used as a parameter to indicate environmental contamination caused by domestic sewage.[14] The coprostanol concentration, however, cannot be used alone because of its possible formation from anaerobic hydrogenation or microbial reduction processes in the environment.[15] Furthermore, there is a conversion of some sterols into others, in this way diagnostic ratios of sterols are used as a useful approach to increasing the reliability of the contamination assessment. The main sterol ratios are: coprostanol/cholesterol,[16,17] coprostanol/(coprostanol + cholestanol),[12,18] coprostanol + epicoprostanol/(coprostanol + epicoprostanol + cholestanol),[19–21] coprostanol/cholestanol[22] and epicoprostanol/coprostanol.[19,21,23] The identification of these chemical markers aiming to assess anthropogenic contamination of aquatic systems, wich is frequently carried out by liquid chromatography coupled with mass spectrometry (LC-MS/MS), which has proven to be an appropriate technique due to its selectivity and sensibility.[22–24] There are studies in the literature aiming to identify and quantify sterol biomarkers for the assessment of environmental contamination in various aquatic systems.[17,21] Many potentially polluted rivers, however, have not yet been studied concerning this question, including regions in the northeast of Brazil. One of special interest is the Capibaribe River that passes through the cities of Santa Cruz do Capibaribe and Toritama in the state of Pernambuco where there are intense urban and industrial activities, a high population, and recent industrial growth.[25] The Capibaribe River basin crosses through 42 municipalities and extends for about 280 km, from the slopes of Serra do Jacarará (Poção city) down to the metropolitan region of Recife, located at the mouth.[26] The Capibaribe Estuary is important not only for its extension but mainly for its socio-economic status, relevant since the colonization of the city of Recife city (capital of the state). Furthermore, your basin influences 42 municipalities and guarantees water to around 2 million inhabitants of the state of Pernambuco.[27] According to the national database, The Brazilian Institute of Geography and Statistics,[28,29] the city of Santa Cruz do Capibaribe had a total of 87,582 inhabitants in the last census, while the city of Toritama had a population of 35,554 people, expected by 2020 to be about 109,897 and 46,164, respectively.

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The main objective of the present study is to assess the sediment contamination from domestic sewage of the Capibaribe River by applying an integrative approach using multiple chemical analyses. The classical analyses by infrared spectroscopy, granulometry, elemental composition, and organic matter content, as well as the determination of sterol biomarkers by LC-MS/MS were applied to characterize eleven samples of sediments. The results obtained from this comprehensive characterization were processed by multivariate statistical analysis in order to brings a rapid and clear definition of the level of contamination.

MATERIALS AND METHODS

Chemical standards

Standards of cholesterol, brassicasterol, ergosterol, epicoprostanol, coprostanol, cholestanol, campesterol, β-sitosterol, cholesterol-d_6 (internal standard-IS), stigmasterol, and stigmastanol were obtained from Sigma-Aldrich (Saint Louis, USA). HPLC-grade methanol and dichloromethane were purchased from Tedia (Rio de Janeiro, Brazil). Stock solutions containing the individual sterols at 1.0 mg mL^{-1} were prepared with dichloromethane. A standard working solution at 2.0 µg mL^{-1} containing all sterols was prepared by diluting the stock solutions in methanol. The 2.0 µg mL^{-1} solution was used to prepare the analytical curves in the concentration range of 10.0 to 1000.0 ng mL^{-1} (nine levels) of each analyte with 500.0 ng mL^{-1} of internal standard.[23] The LC-MS/MS method used in this study was previously developed and validated by Bataglion *et al.*[30] in which the instrumental and chromatographic conditions are summarized in Table 1S.

The curves were constructed by the internal calibration method and then applied to the quantification of sterols in sediments from the Capibaribe River.

Field of study and sampling

The study field comprises the aquatic system of the Capibaribe River, which crosses two cities: Santa Cruz do Capibaribe and Toritama. The sample collection was carried out in a 25 km transect. Eleven surface sediment samples (coded from ST1 to ST11) were collected at different sampling points, according to a previous analysis carried out during a field campaign (Figure 1). The regions were chosen to obtain a representative sampling, taking into account the characteristics of the collection points, including regions of low and high population occupation, visible discharge of sewage and garbage, and a high number of industrial activities. Sediments were collected outside and between the municipalities of Santa Cruz do Capibaribe and Toritama (Point ST1, ST4, ST5, ST6, ST7 and ST11) in regions of lower population, and also in points close to the centers of the municipalities (ST2, ST3, ST8, ST9 and ST10) that are corresponding for regions of high population occupancy and industrial activities, mainly textile.

The sediments were collected in its superficial portion (up to 10 cm) using a Van Veen dredger in September 2018, at the beginning of the dry season. Then, sediments were dried at 60 °C in an air circulation oven for 48 h, followed by grinding using a porcelain mortar/pestle and sieving with a 2 mm mesh.

Characterization by classical analysis (organic matter content, elemental analysis, granulometry, and infrared spectroscopy)

The organic matter content (OM) was determined by gravimetric analysis in triplicate, according to the method previously described.[31] For the elementary analyses, samples were previously decarbonated using the hydrochloric acid solution at 0.1 mol L^{-1}. Then, the elementary analyses were performed using 50 mg of sediment wrapped in aluminum foil, which was inserted in a CHN628

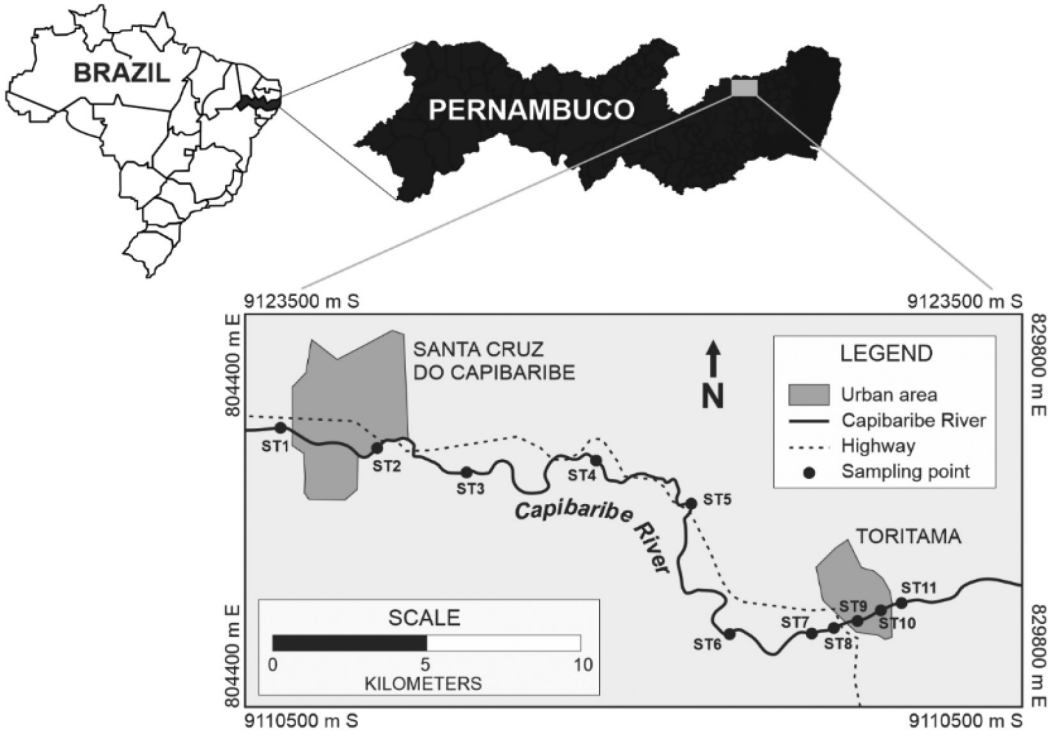

Figure 1. *Study field comprises the aquatic system of the Capibaribe River in its stretch crossing Santa Cruz do Capibaribe and Toritama cities, state of Pernambuco, Brazil. Sampling sites: ST1 (7°57'18.07"; 36°13'43.98"), ST2 (7°57'39.32"; 36°12'2.40"), ST3 (7°58'3.38"; 36°10'26.18"), ST4 (7°57'49.53"; 36° 8'5.11"), ST5 (7°58'36.45"; 36° 6'24.09"), ST6 (8° 0'54.71"; 36° 5'41.24"), ST7 (8° 0'54.28"; 36° 4'9.01"), ST8 (8° 0'50.12"; 36° 3'51.32"), ST9 (8° 0'44.32"; 36° 3'28.05"), ST10 (8° 0'31.12"; 36° 3'0.75"), ST11 (8° 0'23.18"; 36° 2'43.59")*

instrument from LECO with Software CHN628 version 1.30. The instrument was calibrated with the EDTA standard (41.0% C, 5.5% H, and 9.5% N) using a mass range between 10-200 mg.

Infrared spectroscopy (IV) analyses were performed in the spectral range of 400-4000 cm^{-1} using the IR TRACER-100 with Fourier Transform (Shimadzu Co., Japan) and resolution of 4 cm^{-1}. The data processing involved a normalization according to the highest transmittance obtained for each analysis, followed by the generation of the final spectra.

The granulometric analysis was performed using a sieve of 0.063 mm to separate the fine fraction (silt + clay) from the sandy fraction. The separation of the silt and clay fractions occurred by the pipetting method using the Stokes principle, following an adaptation of the conventional method described by Suguio.[32]

Extraction and fractionation of sterols

The extraction of organic compounds and the fractionation of crude organic extract were developed according to Rau and contributors.[2] Briefly, the extraction was done using 5 g of dry sediment added to the activated copper and cholesterol-d6 (IS) at a concentration of 2 μg g^{-1}. Then, 15 mL of a mixture of dichloromethane and methanol (2:1, v/v) was added and subjected to a vortex and ultrasonic bath for 30 min. The process was carried out three times and the organic extracts were combined. The final extract was subjected to rotary evaporation at 45 ± 5 °C, until complete the elimination of the solvent.

The extract was subjected to fractionation performed by open column chromatography using 5 g of silica gel and 1 g of alumina, both previously dried at 200 °C and disabled with 5% water. In total, four fractions of the organic extract were obtained: fraction one referring to aliphatic hydrocarbons, fraction two referring to aromatic hydrocarbons, fraction three referring to alcohols and sterols, and fraction four regarding the fatty acids. The fraction of sterols was re-dissolved in dichloromethane, diluted in methanol, and transferred to vial-type flasks for analysis by LC-MS/MS.

Determination of sterols by LC-MS/MS

The LC-MS/MS analyses were performed using an Agilent 1200 Series liquid chromatograph (Santa Clara, USA) to an API 4000 QTrap mass spectrometer from Applied Biosystems (Darmstadt, Germany). The analyses were carried out according to the Bataglion and contributors.[30] The atmospheric pressure chemical ionization (APCI) source was operated in the positive mode, with corona current at 4.0 μA, the temperature of 450 °C, curtain gas at 10 (arb), and ionization gas at 1-30 (arb). Detection was performed in Select Reaction Monitoring (SRM) mode, monitoring the *m/z* ratios referring to the products of water loss from protonated molecules as precursor ions and two fragments as product ions for each analyte.[33] A reverse phase column, Shimpack XR-ODS III octadecyl-C18 (15 cm long, 2.0 mm internal diameter, and 2.2 μm particle size (Shimadzu, Kyoto, Japan)) was used. The chromatographic separation was performed using deionized water as mobile phase A and methanol as mobile phase B, with the following gradient: 0-2 min (90% B), 2-8 min (100% B), 8-9 min (90% B), 9-10 min (90% B) at a flow rate of 0.6 mL min^{-1}. The temperature of the injector and the chromatographic oven were 10 and 30 °C, respectively, and the injection volume was 10 μL. The analytical curves were constructed by the internal calibration method from data acquired in triplicate in the concentration range of 10.0 to 1000.0 ng mL^{-1} with 500.0 ng mL^{-1} of internal standard.

Multivariate statistical analysis

Multivariate analyses were applied to all data obtained from the classical analysis (Organic matter content (%OM); Total carbon content (%TOC), hydrogen percentage (%H); Nitrogen content (%TN); Total carbon content/Nitrogen content (TOC/TN); Hydrogen/Carbon (H/C), % sand, % total silt, % clay and % silt + clay) and quantification of sterols (concentration of coprostanol, cholesterol, epicoprostanol, cholestanol, and their respective diagnostic ratios). Principal component analysis (PCA) and hierarchical component analysis (HCA) were obtained from those data. For the same data set, an assessment based on the construction of a heatmap was created.

RESULTS AND DISCUSSION

Classical analysis

Table 1 summarizes the results of the organic matter content (OM%), elementary analysis, and granulometry for the eleven surface sediments from the Capibaribe River. The granulometric distribution shows that most sediments were composed of sandy particles, with values between 19.9 and 93.1%, which denotes a characteristic of regions with strong hydrodynamic conditions.[2,13]

About OM content, a range was obtained from 1.32% to 21.37%, with the highest value being obtained for ST1 (10.64%), ST4 (8.89%), and ST10 (21.37%). The highest TOC values were

Table 1. Characterization of surface sediments from the Capibaribe River (PE) regarding organic matter content (OM%), elementary composition (TOC, %H, TN, TOC/TN, H/C), and granulometric distribution (sand, silt, clay, and silt+clay)

SAMPLES	OM%	TOC	%H	TN	TOC/TN	H/C	SAND (%)	CLAY (%)	SILT (%)	SILT+ CLAY (%)
ST1	10.64 ± 0.93	0.09	0.07	0.01	9.00	9.33	19.9	47.50	32.20	79.70
ST2	1.87 ± 0.15	0.61	0.08	0.07	8.71	1.57	93.1	2.70	1.80	4.50
ST3	1.62 ± 0.10	0.34	0.04	0.04	8.50	1.41	86.6	3.00	5.10	8.10
ST4	8.89 ±0.57	2.5	0.48	0.3	8.33	2.30	58.3	9.60	22.80	32.40
ST5	2.22 ± 0.13	0.4	0.08	0.06	6.66	2.40	88.5	3.10	5.10	8.20
ST6	1.97 ± 0.28	0.34	0.02	0.04	8.50	0.70	90.6	2.60	4.20	6.80
ST7	1.32 ± 0.01	0.29	0	0.05	5.80	0	90.6	4.70	1.60	6.30
ST8	2.13 ± 0.10	0.74	0.15	0.09	8.22	2.43	14.7	41.90	41.90	83.80
ST9	4.67 ± 0.27	0.77	0.27	0.11	7.00	4.20	81.2	7.40	8.60	160
ST10	21.37 ± 1.22	5.47	1.47	0.52	10.51	3.22	83.1	5.90	8.30	14.20
ST11	3.75 ± 0.31	0.53	0.03	0.06	8.83	0.68	90.6	4.70	0.00	4.70

found for the ST4 and ST10 sediments (2.50 and 5.47, respectively), which correlated with the highest OM content (8.89% and 21.37%, respectively) of these samples. Thus, the TOC concentration, which is influenced by the initial biomass production and subsequent degree of degradation,[1,34] represents the fraction of organic matter that escaped remineralization during sedimentation. Therefore, according to Otero,[35] the variation of TOC in sediments can be caused by structural differences in local vegetation, which implies a greater addition of biomass to the soil or more roots in the study area.

Sherwin e contributors[36] also reported that high levels of TOC may be related to an entry of fecal material, which in our study was confirmed by the sterol data that is described in the next section.

Table 1 also shows that the nitrogen content (TN) was less than 1 for all samples, which can be attributed to the more intense reducing conditions of the system associated with the denitrification process.[37,38] Furthermore, Meyers and Ishiwatari,[39] reported that environments influenced by MO of non-vascular aquatic plants tend to have low TOC/TN ratios (between 4 and 10), whereas those influenced by OM of terrestrial vascular plants have higher TOC/TN ratios (20 or higher). Based on this, our study showed a predominance of OM from non-vascular aquatic plants in the environment of all analyzed sediments. Furthermore, according to Grilo,[40] high TOC/TN values may be associated with the presence of sewage carbon. In the present study, the highest TOC/TN ratios were obtained for ST2 and ST10 (8.71 and 10.52, respectively), both samples collected in points close to the more urbanized areas of the cities of Santa Cruz do Capibaribe (ST2) and Toritama (ST10). The anthropogenic influence at these points was also confirmed by the higher concentrations of coprostanol (Table 2), with concentrations of 19,362 ng g^{-1} and 28,803 ng g^{-1}, respectively.

The H/C ratio infers the degree of aromaticity of OM, with ST7 and ST11 (0.00 and 0.68, respectively), the sediments with the highest aromatic content, whereas ST1 and ST9 (9.33 and 4.20, respectively) were those with less aromaticity. Considering that organic matter is based on chains of carbon molecules, forming a heterogeneous mixture of microorganisms, plant and animal residues, in which humus is the last and most stable part of them,[41] a decrease in this ratio can be caused by OM formed by the decomposition of terrestrial plants by microorganisms, which are responsible for the decomposition of lignin, carbohydrates, and aromatic proteins, leading to the formation of compounds that will result in a lower H/C ratio. On the other hand, in natural organic matter, formed by aquatic plants, the main source of OM is the decomposition of plankton, which uses lipids as an energy reserve. As lipids present an absence of aromaticity in their composition, an increase in the H/C ratio tends to be perceived.[42,43] Thus, according to Carreira,[44] the OM is classified according to the origin of organic carbon, in which contributions of aquatic origin are called autochthones (bacteria, fungi, phytoplankton, zooplankton, and aquatic plants) and terrestrial origin called allochthones (erosion coastline, rivers, and specific vegetation).

Figure 1S shows the IR spectra for superficial sediments of the Capibaribe River and Table 5S lists the wavenumbers (cm^{-1}) and their possible specific functional groups[45–50] of the main bands obtained for the samples of this study.

It is possible to make interesting correlations between the OM% (described in Table 1) and IR data. The highest OM% for ST1 and ST10 samples (10.64 and 21.37, respectively) corroborate the highest bands, corresponding to the OH elongation from kaolinite at ≈3697.26 cm^{-1},[45,47,49] C=O bands in ≈1648.81 cm^{-1} of carboxylate groups, ketones, and aldehydes,[38,46,51] and C-O stretch and aromatic C-H deformation in ≈1015.35 and 908 cm^{-1}.[38,48] This behavior is associated with the high content of humic substances into the sedimentary organic matter, since the humic substances contain a high number of carbonyl and phenolic groups and also aliphatic chains, aromatic rings, and mineral impurities.[52,53]

Analysis of sterols by LC-MS/MS

Diverse types of sterols (cholesterol, epicoprostanol, coprostanol, cholestanol, epibrassicasterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, and sitostanol) were identified in sediments from the Capibaribe River (Table 2), which suggests the presence of distinct sources of OM in the study area.

The phytosterols, campesterol, and stigmasterol showed concentration ranges of 118-6265 ng g^{-1} and 91-3910 ng g^{-1}, respectively, while β-sitosterol varied from 121 to 6953 ng g^{-1}. These sterols are widely considered to be biomarkers of OM derived from plants, being the main constituents of higher plants although other origins are possible. The sources of individual sterols can be confirmed by building linear correlations between their concentrations. In this study, strong correlation coefficients were found between campesterol and β-sitosterol (r = 0.922), campesterol versus stigmasterol (r = 0.971), and β-sitosterol versus stigmasterol (r = 0.965), showing that these three phytosterols had the same origin in the studied environment. Moreover, β-sitosterol, due to its presence in the composition of vegetable oils used in cooking, is likely to be present in domestic sewage discharges.[54,55] Regarding Brassicasterol, it was quantified in a concentration range of 7-609 ng g^{-1}, since

Table 2. The concentrations in ng g^{-1} of sterols obtained by LC-MS/MS of the eleven sediments from Capibaribe River

#	Chol	Epi	Cop	Choln	Epibra	Brass	Camp	Stig	β Sito	Sito	Total
ST1	4030	437	673	1506	87	609	4617	2881	5370	848	21059
ST2	3697	3362	19362	5951	71	113	1401	1096	3137	1644	39834
ST3	737	377	2169	735	27	54	762	430	860	268	6420
ST4	2652	1295	4665	4961	178	477	6265	3910	6953	5522	36879
ST5	627	222	675	636	12	55	1078	770	1954	373	6401
ST6	209	23	19	35	< LOQ	16	373	387	516	< LOQ	1579
ST7	9971	16	27	25	< LOQ	7	118	91	121	18	10394
ST8	1973	1050	6563	2120	119	192	1980	1128	2406	1466	18997
ST9	3142	833	4360	1977	121	216	3689	2732	4548	941	22558
ST10	4107	3768	28803	5119	86	160	3294	2859	6732	2222	57150
ST11	1067	168	482	4741	14	84	1268	1444	2193	649	12110

Chol = Cholesterol; Epi = Epicoprostanol; Cop = Coprostanol; Choln = Cholestanol; Epibra = Epibrassicasterol; Brass = Brassicasterol; Camp = Campesterol; Stig = Stigmasterol; β-Sito = β-Sitosterol; Sito = Sitostanol; LOQ = Limit of quantification.

cholesterol was determined in much higher concentrations, from 209 to 9971 ng g^{-1}.

In addition to biogenic sterols, anthropogenic ones were also detected in sediments of the Capibaribe River. The main fecal sterol (coprostanol) was found in concentrations varying from 19 to 28803 ng g^{-1}. Among the eleven samples, nine presented a coprostanol concentration greater than 100 ng g^{-1}, which indicates sewage contamination.[1,23,56] It is important to note that 82% of the samples showed concentrations greater than 500 ng g^{-1} of coprostanol, indicating expressive contamination by domestic sewage.[23,57] The coprostanol presence may be due to the direct or indirect input of domestic effluents, especially in the region of the ST2 and ST10 samples, where the highest values (19362 ng g^{-1} and 28803 ng g^{-1}, respectively) were observed. These samples were collected in the towns of Santa Cruz do Capibaribe and Toritama in regions of the cities where most of the population is concentrated, and consequently, show a greater anthropogenic impact. In contrast, ST6 and ST7 showed coprostanol concentrations lower than 100 ng g^{-1} (19 ng g^{-1} and 27 ng g^{-1}, respectively), which can be explained by their location outside of urban areas.

Diagnostic ratios of sterols

To support the assessment of anthropogenic contamination by sterols in sediments, absolute concentration should be accompanied by diagnostic ratios, as described in Table 2S, which is also shown in Figure 2S by graphs. The relative percentage of coprostanol/total sterols in sediments is a way to assess contamination by domestic sewage.[23,58,59] The samples in this study had percentages of coprostanol ranging from 0 to 50% (Figure 2S), with the highest values for ST2 and ST10 (49% and 50%, respectively) and the lowest for ST1, ST6, and ST7 (3%, 1%, and 0%, respectively). Some studies have considered 2-3% as a threshold for indicating moderate contamination by sewage and the threshold of 5-6% would be indicative of strong contamination.[23,58,59] In our results, it is clear that most of the sediments samples demonstrated strong contamination by sewage, in particular ST2 and ST10.

Given the total number of sterols considered in the analysis, however, care must be taken in using the ratio coprostanol/total sterols as the only means of contamination assessment.[23] Thus, it is necessary to increase the reliability of the results in terms of interpretation regarding sewage and/or fecal contamination using other diagnostic relationships, such as coprostanol/(coprostanol+cholestanol), coprostanol + epicoprostanol/(coprostanol + epicoprostanol + cholestanol), coprostanol/cholesterol, coprostanol/cholestanol and epicoprostanol/Coprostanol.[17,30,55] Figure 2 was created from the data of Table 2S, to present the results as a graphic, aiming to facilitate the visualization of the samples that are within the criteria used to define the level of anthropogenic contamination.

The coprostanol/(coprostanol+cholestanol) ratio is a parameter that indicates sewage contamination when values are between 0.5 and 1.0.[13,18] The highest values were found for ST2, ST8, and ST10 (0.76, 0.76, and 0.85, respectively), which is justified by the fact that these sediments were collected from points near urban centers with a higher population occupation and sewage disposal. In contrast, the lowest coprostanol/(coprostanol + cholestanol) ratios were found for ST1 and ST11 (0.31 and 0.09, respectively), which are located before the urban area of Santa Cruz do Capibaribe and after the urban area of Toritama, respectively.

The coprostanol + epicoprostanol/(coprostanol + epicoprostanol + cholestanol) ratio of the sediments, is used to compensate for

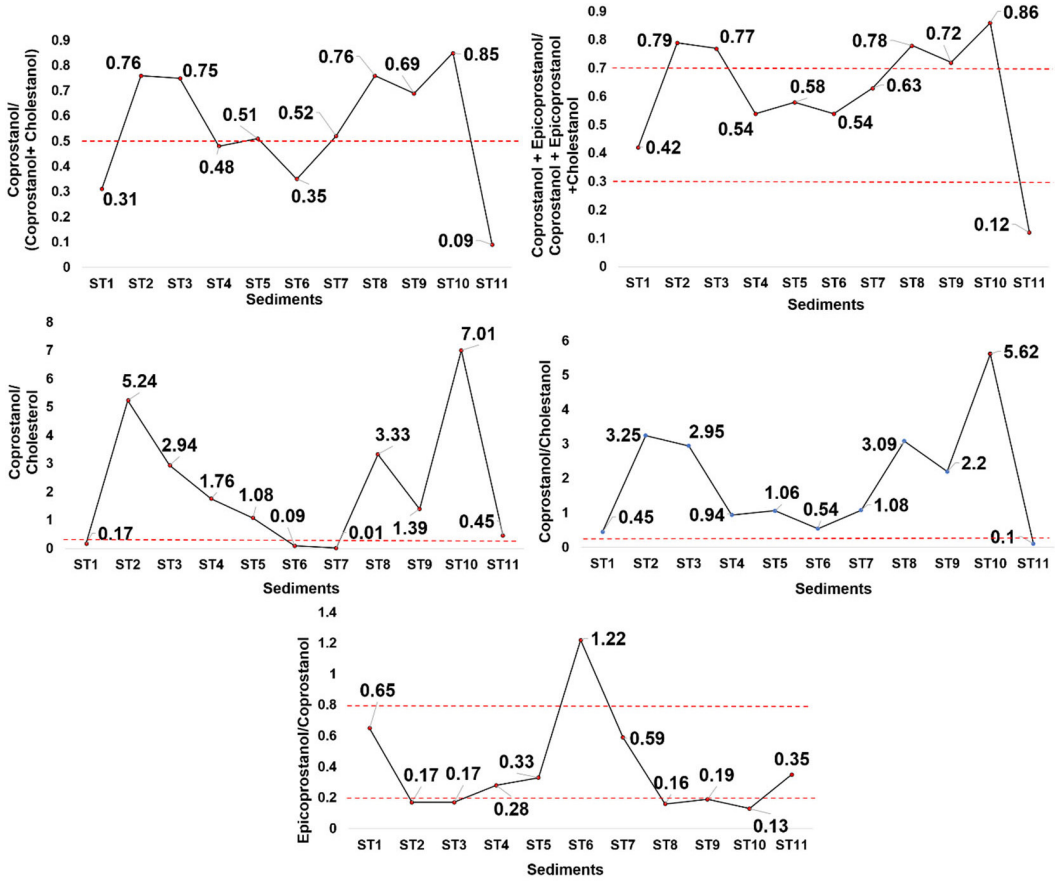

Figure 2. *Diagnostic ratios for assessing domestic sewage contamination in surface sediments from the Capibaribe River (PE). The dashed red line defines the value limits for each diagnostic ratio*

the microbial conversion of coprostanol into its epimer, thus, the concentration of epicoprostanol was incorporated into this ratio [19,20]. The sediments ST2, ST3, ST8, ST9, and ST10, presented values greater than 0.7, which indicates the contamination by sewage.[19,20] More specifically, the ST2 and ST10 sediments were again the most contaminated (0.79 and 0.86, respectively) and ST1 and ST11 had the lowest values (0.42 and 0.12, respectively), which indicates less contamination. This data agrees with that obtained for the coprostanol/coprostanol + cholestanol ratio presented above.

The coprostanol/cholesterol ratio indicates contamination by human feces for values greater than 0.2, while values less than 0.2 are related to biogenic sources.[16,30] Only the ST1, ST6, and ST7 sediments showed values below 0.2 (0.17, 0.09, and 0.01), whereas the highest values for this ratio were found in the ST2, ST8, and ST10 sediments (5.24, 3.33, and 7.01, respectively). Thus, the greater contamination from human feces is confirmed, mainly in sediments collected from the more populous regions around the Capibaribe River.

There is also the coprostanol/cholestanol ratio, by which it is possible to observe that ten of the eleven sediment samples presented values above 0.3, suggesting contamination by domestic sewage,[30] the only exception was the ST11 sediment, which presented 0.1. The highest values obtained were again found in sediments ST2 and ST10 (3.25 and 5.62, respectively), indicating samples with a high level of contamination.

The interpretation of the epicoprostanol/coprostanol ratio is described as follows: 1) values greater than 0.80 indicate partial sewage treatment; 2) values between 0.20-0.80 do not allow conclusive interpretation and 3) values lower than 0.20 indicate the presence of untreated sewage effluents.[5,20,54] Only ST6 showed a value greater than 0,80, which confirms the low concentration of coprostanol found in this sample (19 ng g^{-1}). On the other hand, several samples, including ST2 and ST10, showed values lower than 0.20, which indicates the presence of untreated sewage effluents. These results reinforce those mentioned previously, where ST2 and ST10 sediments presented a high level of anthropogenic contamination.

Although the highest concentrations of coprostanol, as well as the values of the diagnostic ratios between sterols, were found in the ST2 and ST10 sediments, high concentrations were also found in the ST3, ST4, ST8, and ST9 sediments, pointing to the high presence of anthropogenic environmental contamination at various points in the field of study.

Comparison with other studies

The results obtained were compared with those reported in other studies with the same objective of assessing anthropic contamination by determining sterols in sediments. First, we compare the values of the concentrations of fecal sterol coprostanol (Table 3S). Our study presented the highest concentrations of coprostanol in eight of the ten studies used in the comparison. Thus, this preliminary assessment indicates a greater amount of fecal sterol in the sediments than in other sediments analyzed that used the same diagnostic ratios, in other regions of Brazil and the world.

In addition, a comparison of the sterol ratios is shown in Figure 3S, with the percentages of the samples from each study that fit the characteristics of the values of each ratio, to provide a more effective comparison of contamination by fecal sewage. It is possible to observe for coprostanol/(coprostanol + cholestanol) that 63.63% of the samples in our study were contaminated by sewage; this value is only lower than the sediment studies of the Danube River, Serbia,[21] streams in Manaus, Brazil,[23] from the Sergipe-Poxim estuary, Brazil[11] and the Sergipe River, Brazil,[60] which presented 72.72%, 76.92%, 81.25% and 90.32% of the contaminated sediment samples, respectively.

In terms of contamination by human feces, for the coprostanol/cholesterol ratio, our study showed that 72.72% of the samples contained contaminated sediments and only 27.28% of sediments associated with biogenic sources. Therefore, our study presented less contamination than sediment studies of the Piauí-Real River estuary, Brazil,[13] Itajaú-Açu River estuary, Brazil,[17] Sergipe River, Brazil,[60] Danube, Serbia[21] and Sergipe-Poxim estuary, Brazil,[11] that presented 73.33%, 75%, 83.87%, 90.90%, and 93.75%, respectively. Finally, in the evaluation of the epicoprostanol/coprostanol ratio, our study showed a total of 45.45% of the sediment samples with no indication of sewage treatment in the effluent to which they were subjected, while only 9.10% of the sediments were characterized by possible sewage treatment. This result is inferior to the study with sediments from the streams in Manaus, Brazil[23] and Danube River, Serbia,[21] which presented values of 57.69% and 63.63%, respectively. Thus, this comparative investigation shows that the sediments of the Capibaribe River present several points of contamination by anthropic activities, in many cases, even greater than in other locations in Brazil and the world.

Correlation between the classical analyses and sterol data

In Table 4S it is possible to observe correlations between classical analyzes and sterol data. Regression analysis was used first to investigate the relationship between TOC and fine particles, and showed that there is no significant correlation between the data (R^2 = 0.0009). The weak correlation occurs due to additional sources of TOC that are not associated with the flow of the river, or with clay from landfills that are deposited in sediments. This suggests that sources (internal or external) were more important than hydrodynamic factors in the distribution of organic matter in the system studied.[1] The content of TN showed a strong linear correlation with TOC (r= 0.99). The regression line almost passed through the origin, suggesting that most of the nitrogen measured was related to sedimentary organic carbon and, therefore, probably in organic form.[4] According to Speranza and contributors,[61] it is also possible to perform a regression analysis to investigate the relationship between the total sterol concentration and the proportion of coprostanol. Our study obtained a satisfactory correlation (R^2 = 0.7814), which means that an important part of the total sterols came from coprostanol.

The correlation of stigmasterol and total sterols was calculated, indicating no good correlation (R^2 = 0.4504) in the data, as well as between campesterol and total sterols (R^2 = 0.3408). Thus, it can be seen that the proportions of stigmasterol and campesterol did not show a significant correlation to the proportion of total sterols, only with coprostanol, probably due to the high anthropogenic discharges found in most of the sediments.

It is possible to observe that some sediments (for example ST2, ST4, ST9, and ST10) presented high TOC values (0.61, 2.50, 0.77, and 5.47, respectively), as shown in Table 1, and high concentrations of total sterols (39834 ng g^{-1}, 36879 ng g^{-1}, 22558 ng g^{-1}, and 57150 ng g^{-1}, respectively as shown in Table 2). A reasonable explanation is that sterols are enriched in sediments with a high TOC content.[59,61,62] Mudge and contributors,[63] suggest a statistical correlation between TOC and individual sterols or total sterols. In our study, a correlation was obtained between the total sterol content and the TOC with an r = 0.6945. However, the correlation between TOC and cholesterol was very low (R^2 = 0.0158), as well as the correlation of total sterol content with campesterol (R^2 = 0.2613). This is probably because there was a small contribution of these sterols to the total organic content of sediments. In contrast, our study obtained a better correlation between TOC and coprostanol (R^2 = 0.588), which may be associated with a greater contribution of the coprostanol to the total organic content of these sediments.

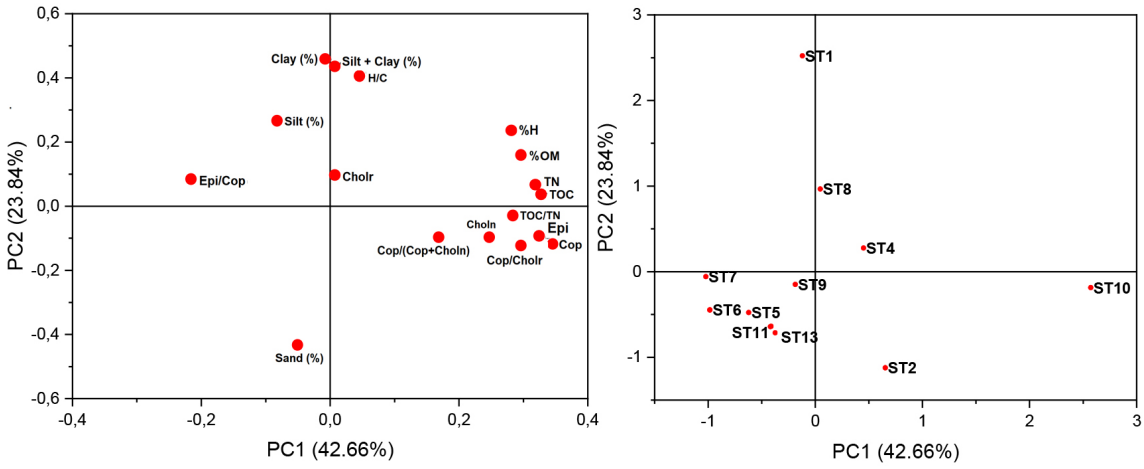

Figure 3. *Weight graphs and scores for main components 1 and 2 for all sterol and classical analysis data obtained for surface sediments from the Capibaribe River*

Multivariate statistical analysis

Principal component analysis – PCA

PCA was used for an integrative approach to the sterol and classical analysis data aiming to reveal relationships among different sediments. Figure 3 shows the graphs of weight and scores of main components 1 and 2 (PC1 and PC2), which indicate the association between the variables. PC1 and PC2 were responsible for 42.66% and 23.84% of the variance, respectively, with a total cumulative variance of 66.5%.

In PC1, it is possible to notice the formation of two groups, one formed by ST2 and ST10 samples associated with positive weight due to their highest values of organic matter, TOC, coprostanol/(coprostanol+cholestanol), coprostanol/cholesterol, coprostanol, and cholestanol and another grouping of samples formed by ST3, ST5, ST6, ST7 and ST11 with negative weight due to their lowest values of epicoprostanol/coprostanol. PC1 also shows that there is no influence of the granulometric fractions on the parameters evaluated.

In PC2, it is possible to note a grouping for ST1 and ST8 sediments, which have the highest values of clay, silt, and H/C with positive weights, which may be associated with the composition of clay and silt that present a greater amount of hydrogen, which increases the H/C ratio. Another grouping was obtained mainly due to the ST2 sample, which has a high sand content, with a negative weight in PC2.

The PCA indicates that the ST2 and ST10 samples behave differently from the other samples due to their greater anthropogenic contamination by domestic sewage due to their proximity to the urban areas of Santa Cruz do Capibaribe and Toritama. This difference confirms the assessment made individually by the absolute concentration and diagnostic ratios of coprostanol. The PCA graphs in Figure 3 also show that there is a contamination trend, which increases from left to right about PC1, indicating greater contamination by human activities in the ST2 and ST10 sediments. This reinforces the importance of providing more adequate sanitary conditions to the municipalities of the Capibaribe River.

HCA/Heatmap

A heatmap is a graphical representation that uses colors, from "hot" (red) to "cold" (blue), to identify areas of greater or lesser intensity. The HCA/Heatmap of Figure 4 was made using the same data that was used previously for PCA. It is possible to observe that there is a convergence between the results of the analyses, for example, the variables H/C, clay, and silt are in red for the ST1 sample, which also corroborates the results obtained in the PCA, which for this sample presented grouping of these variables.

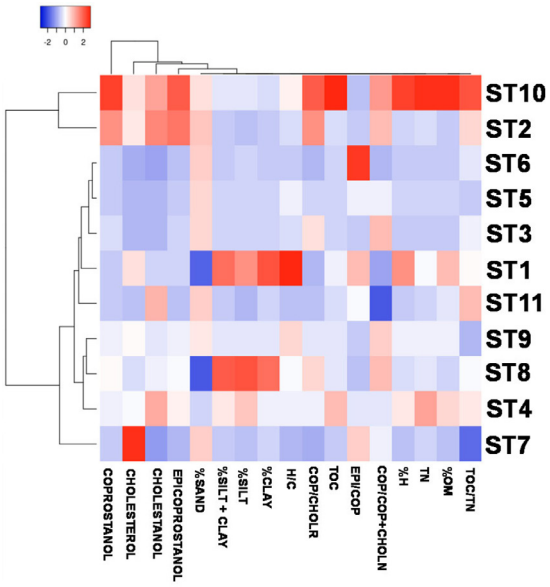

Figure 4. *HCA/Heatmap generated of data obtained from classical and sterol analyses for surface sediments from the Capibaribe River. H= Hydrogen; C= Carbon; Cop= Coprostanol; Cholr= Cholesterol; Epi= Epicoprostanol; Choln= Cholestanol; TN= Nitrogen content; TOC= Total carbon content; OM= Organic matter*

Note that the main variables associated with the highest weights (red color) are similar in the ST2 and ST10 samples, indicated as the ones with the highest contamination, showing an agreement with the PCA data discussed above. This finding is due to factors with positive displacement, mainly for sterol data and their proportions, such as coprostanol, cholesterol, cholestanol, epicoprostanol, coprostanol/(coprostanol + cholestanol), and coprostanol/cholesterol values. On the other hand, other samples are grouped due to variables associated with classical analyses.

CONCLUSIONS

This study presented a comprehensive chemical characterization of sediments from the Capibaribe River, Pernambuco, Brazil, based on classical analyses and quantification of sterols by LC-MS/MS. An integrative evaluation of data was able to show a clear indication

of contamination by domestic sewage. Individual concentrations of sterols, as well as diagnostic ratios, were key parameters in indicating the most contaminated areas. From these analyses, severe anthropogenic contamination was detected in sediments of the Capibaribe River near the urban centers of Santa Cruz do Capibaribe and Toritama, in particular for the samples from stations ST2 and ST10, which are located in the most populated areas of the cities. To the best of our knowledge, this is the first study that evaluates and indicates the level of contamination of the Capibaribe River by fecal OM, expecting to aid decisions regarding actions of prevention and remediation, given concern for human health and the environment.

SUPPLEMENTARY MATERIAL

The supplementary material, available free of charge at https://quimicanova.sbq.org.br, contains the following:

- Infrared spectra in the range of 400-4000 cm^{-1} for surface sediments from the Capibaribe River, Pernambuco, Brazil (Figure 1S);
- Percentages of coprostanol/total sterols of surface sediments from the Capibaribe River, Pernambuco, Brazil (Figure 2S);
- The percentages of samples that fit the characteristics of the values for the diagnostic ratios coprostanol/(coprostanol + cholestanol), coprostanol/cholesterol, and epicoprostanol/coprostanol indicate fecal contamination, in study versus studies reported in the literature (Figure 3S)
- Analytical parameters for detection and quantification of sterols in sediment samples by LC-MS/MS described by Bataglion et al.[30] (Table 1S)
- Diagnostic ratios for assessing domestic sewage contamination in surface sediments from the Capibaribe River (PE) (Table 2S);
- Comparison between the concentrations of fecal sterol coprostanol obtained in the sediments of our study with other sediments collected in other regions of Brazil and the world (Table 3S);
- Correlations among classical analyses and sterol data and the respective values of R^2 or r (Table 4S);
- Wavenumber (cm^{-1}) and its specific functional groups of the main bands were obtained for the samples of this study, using infrared analysis (Table 5S).

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