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Sedimentary sterol levels to track river contamination by sewage in one of the largest Amazonian cities (Belém – Pará), northern Brazil

Níveis de esteróis sedimentares para rastrear a contaminação por esgoto de um rio em uma das maiores cidades da Amazônia (Belém – Pará), norte do Brasil

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Abstract: Aim: The Aurá River, located in the second-largest Brazilian Amazon city, has been experiencing the effects of human activities from riverine communities and the Aurá landfill for many years. In this study, we assess the occurrence, sources, and distribution of selected sterol markers in surface sediments of Aurá River in order to evaluate the organic matter inputs in this water body. **Methods:** Gas chromatography-tandem mass spectrometry (GC/MS/MS) was used to identify and quantify sterol compounds. Pearson correlation, principal component analysis (PCA) and sterol ratios were used to assess sewage pollution. **Results:** The sterol markers identified, the related diagnostic ratios, and statistical analysis showed that Aurá River sediments presented two primary sterol sources: anthropogenic (domestic sewage and inputs from Aurá landfill) and biogenic sources (terrestrial higher plants). Station 1 (the closest site to the Aurá landfill) presented the highest level of coprostanol (219.8 ng g-1). This maximum level of coprostanol and the sterol ratios indicate moderate human fecal contamination in the upper reach of the Aurá River. Coprostanol levels were similar to the lower to midrange concentrations reported for surficial river sediments around the world. **Conclusions:** This study demonstrated that domestic sewage pollution from riverine communities and organic matter inputs from Aurá landfill might be assumed as potential threats to environmental and human health.

Keywords: surface sediments; organic matter; domestic riverine sewage; ecological and human health risk; amazonic aquatic systems.

Resumo: Objetivo: O rio Aurá, no nordeste da Amazônia brasileira, vem sofrendo influência antrópica de comunidades ribeirinhas e do aterro sanitário Aurá há muitos anos. Neste trabalho, avaliamos a ocorrência, fontes e distribuição de seis marcadores de esteróis em sedimentos superficiais do Rio Aurá para avaliar aportes orgânicos neste corpo d'água. **Métodos:** A cromatografia gasosa-acoplada a espectrometria de massas (GC/MS) foi empregada para determinar os esteróis. A análise de correlação de Pearson, análise de componentes principais (PCA) e razões de esteróis foram utilizadas para avaliar a poluição por esgoto. **Resultados:** Os analitos de interesse identificados e as razões diagnósticas indicaram que os sedimentos do rio estudado apresentam compostos orgânicos provenientes de fontes tanto antropogênicas (esgotos domésticos e MO do aterro sanitário) quanto biogênicas autóctones (plantas superiores terrestres). A Análise de Componentes Principais (PCA) corrobora com esse resultado e possibilitou o agrupamento dos pontos de amostragem segundo essas fontes. A estação 1 (ponto mais próximo do aterro Aurá) apresentou o maior nível de contaminação observado e o coprostanol foi detectado em maior concentração 219,8 ng g-1 nesse local, o que indica contaminação fecal humana moderada. **Conclusões:** Este trabalho demonstrou que a poluição por esgoto doméstico e insumos de MO do aterro do Aurá podem ser ameaças potenciais ao ecossistema e à saúde humana da região estudada.

Palavras-chave: sedimentos superficiais; matéria orgânica; esgoto doméstico em rios; risco ecológico e para a saúde humana; sistemas aquáticos amazônicos.

1. Introduction

Amazonia is the world's largest tropical forest, containing 15 to 20% of the world's freshwater supply (Melo et al., 2019). It is estimated that approximately 12-14% of global surface water drains through the twelve hydrographic regions in Brazilian territory. Although the importance of this region, there has been an increased deterioration of water quality due to the input of high amounts of pollutants related to the increase of human activities (Edokpayi et al., 2017; Duarte & Val, 2020) which includes the discharge of untreated domestic sewage, irregular landfills, and open dumps (Bacha et al., 2021; Melo et al., 2019; Siqueira et al., 2016). In addition, contaminants leached from landfills are subject to infiltration into groundwater (Amano et al., 2021). The organic pollution of surface waters affects the supply of clean water and threatens the ecological services provided by water bodies, especially near urban centers where there is poor management of multiple impacts (Hadlich et al., 2018; Häder et al., 2020).

Sterols are some of the most used chemical markers in studies that include sewage and biogenic organic matter inputs because of their specificity with the human fecal material, resistance to microbial degradation, and relative ease to track and quantify (Frena et al., 2016b; Cabral et al., 2020; Souza et al., 2020). Furthermore, sterols have been used to identify fecal pollution from landfill leachate (Zhang et al., 2008). These compounds have hydrophobic properties and are easily adsorbed to particles, allowing them to accumulate in sediments (Wen et al., 2020). Sterol markers provide much more consistent evidence of the source and the severity of sewage pollution compared to traditional methods that use microorganisms (e.g., Escherichia coli) (Cabral et al., 2019; Thomes et al., 2019; Wen et al., 2020). Particularly in tropical locations and low-density residential areas, microbiological indicators of household sewage, mainly fecal indicator bacteria of the coliform group, are thought to be non-specific and easily influenced by environmental conditions (Melo et al., 2023)

Coprostanol is a sterol synthesized in the digestive tracts of humans and higher vertebrates through the hydrogenation of cholesterol. This sterol is the most abundant in human feces, accounting for 60% of the total sterols (Leeming et al., 1996; Murtaugh & Bunch 1967). Thus, it has been used to trace anthropogenic fecal inputs in aquatic ecosystems (e.g., Araújo et al., 2021; de Oliveira et al., 2022). Investigations of sewage contamination in sediments comparing fecal sterols and coliform counts showed that coprostanol may be considered the best indicator of fecal contamination (Costa & Carreira 2005). Other studies in which pathogens, such as E. coli, have been destroyed by chlorination or heat have used coprostanol to indicate fecal contamination in the environment (e.g., Reeves & Patton 2005; Reichwaldt et al., 2017). However, sterol markers diagnostic ratios have been proposed to improve the reliability of the pollution assessment caused by domestic sewage, allowing confirmation of fecal pollution and distinguishing between human and animal sources (e.g., Bujagić et al., 2016; He et al., 2018).

Cholesterol and cholestanol support research on anthropogenic organic matter input patterns in sediments (Bull et al., 2002; Martins et al., 2014). Cholesterol is the main zoosterol. However, it can be attributed to other organisms, including algae, diatom, macrophytes, and a wide variety of phytoplankton (Volkman 1986; Sojinu et al., 2012, He et al., 2018;). Moreover, cholesterol can also enter into riverine ecosystems through sewage runoff and agricultural inputs (Thomes et al., 2019). Cholestanol, the epimer of cholesterol, is found in situ as a cholesterol bacterial reduction product and can be considered as a sewage sterol (Grimalt et al., 1990; Frena et al., 2016). It can also be produced by marine and terrestrial plants, zooplankton, and phytoplankton (Volkman 2005, 2006). Plant sterols such as campesterol, β-sitosterol, and stigmasterol are used to estimate plant-derived OM input to aquatic systems (Volkman 2005; Bataglion et al., 2016).

The Aurá River is relevant for Belém city (the second-largest city in Brazilian Amazon) because it directly influences the catchment springs of water to urban supply (Siqueira & Aprile 2013). There are two main sources of anthropogenic organic matter (OM) affecting the Aurá River basin. First, this river drains the surroundings of the Aurá landfill, actually deactivated but which did not treat correctly the garbage disposed on it for many decades. Consequently, this water body has constantly received organic anthropogenic contamination from soil runoff (Siqueira et al., 2016). And finally, the study river is under constant contamination as a consequence of the inadequate sewage system in the local riverside community (de Oliveira et al., 2013; Siqueira & Aprile 2013). To date, some research has been carried out in the Aurá river basin. In 2013, Siqueira & Aprile assessed the environmental risks due to contamination by metals. More recently, Carneiro et al. (2016) evaluated the spatial variation and sources of polycyclic aromatic hydrocarbons (PAH) in the surface sediments of this river. The results of these studies showed that the studied area is contaminated by metals (Al and Fe) and PAH, mainly close to the Aurá landfill. However, no research has been found that surveyed sewage pollution in this aquatic system so an approach based on sterols biomarkers is of special interest.

In this context, this study aimed to determine sterol markers concentrations in surficial sediments along the Aurá River, to evaluate: (i) organic contamination levels related to sewage input, and (ii) sterols distribution and sources to monitor the domestic effluents input and the influence of the Aurá sanitary landfill into the river. This research contributes to the literature concerning organic pollution in Amazonian aquatic bodies. In addition, the data can support the implementation of pollution control programs and sustainable decision-making in the region.

2. Materials and Methods

2.1. Study area

The Aurá River basin is located southeast of Belém city, Pará, Northern Brazil. This river is the third-largest in extension inside Belém Metropolitan Region (BMR) (Siqueira et al., 2016). The river mouth is 200 m from the water collection site of Companhia de Saneamento do Pará (COSANPA) on the Guamá River. The water captured is conducted to Bolonha and Água Preta lakes and supplies 75% of the BMR (Siqueira et al., 2016). A landfill, created in 1990 and closed in 2015, is located approximately 1400 m north of the study river; it operated uncontrolled and irregularly for about 24 years (Carneiro et al., 2016). According to previous studies, the water quality from Aura River is negatively impacted by organic and inorganic pollutants such as heavy metals and PAHs from runoff of the landfill soil (Siqueira & Aprile 2013; Siqueira et al., 2016; Carneiro et al., 2016).

Sampling was carried out in March/2020 along the Aurá River, and a total of 9 surficial sediment samples (depth 0-10 cm) were collected (Figure 1)

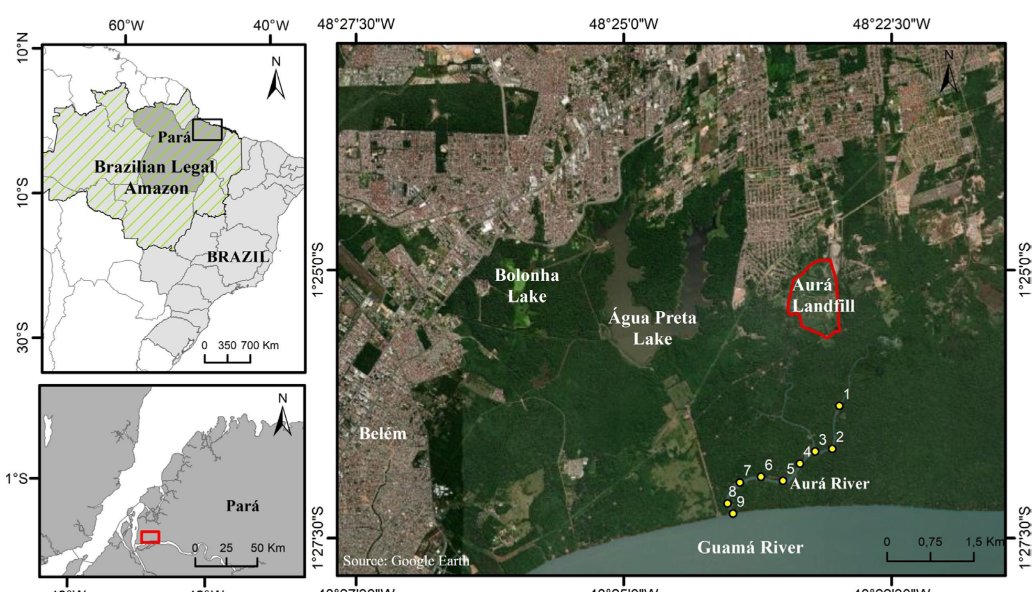

Figure 1. Study area and sampling site's location. The Legal Amazon is the territory under the supervision of the Amazon Development Superintendence – SUDAM, it covers a large part of Brazil's biodiversity (Garcia Junior et al., 2022; IBGE, 2020).

using a handheld Van Veen grab. Sediment samples were placed into pre-cleaned aluminum recipients and stored under refrigeration during transportation to the laboratory. Before initial chemical treatments, all sediment samples were freeze-dried, pulverized in a mortar, and stored at 4°C before further analysis.

2.2. Chemicals and reagents

Cholesterol (cholest-5-en-3β-ol), Coprostanol (5β-cholestan-3β-ol), Stigmastanol (5αcholestan-22E-en-3β-ol), Cholestanol (5α-cholestan-3β-ol), β-sitosterol (24-ethylcholest5-en-3β-ol), androstanol (5α-androstan-3β-ol) and 5α-cholestane (internal standard) were obtained from Sigma-Aldrich (St. Louis, USA). Stigmasterol (24-ethylcholest5, 22E-dien-3β-ol and BSTFA (bis(trimethylsilyl) trifluoroacetamide)/TMCS (trimethylchlorosilane) (99:1) from Spectrum (Gardena, CA). Stock solutions containing individual sterols were prepared in HPLC-grade dichloromethane (DCM). Working standard solutions were prepared from these solutions and diluted with 95% hexane before analysis. HPLC-grade 95% *n*-hexane, methanol, and DCM were purchased from Tedia (RJ, Brazil).

2.3. Sterols analytical procedures

Sterols extraction and analysis methods applied in this study were described by Frena et al. (2016b). An amount of 5 g of freeze-dried sediment from each sampling site was extracted in an ultrasonic bath (model UltraCleaner 1400; Callmex, São Paulo, Brazil). Sediment samples were immersed in a mixture of 10 mL of DCM and 5 mL of methanol (2:1, v/v) for 30 min (three times) at a 40 kHz frequency. Extracts were reduced to 2 mL by rotoevaporation and posteriorly evaporated to dryness under a nitrogen stream (99.996% purity). Sterols in the extracts were derivatized into the form of trimethylsilyl ethers using 50 µL of BSTFA with 1% TMCS, this process was carried out for 60 min at 60°C. The extracts obtained after derivatization were reconstituted in 1 mL of *n*-hexane. The internal standard 5α-cholestane (500 ng mL^{-1}) was added after extraction and dilution. Finally, an aliquot of 10 µL of the reconstituted and the derivatized extract was injected into the GC-MS/MS in splitless mode (1 min), at 280°C, for sterols markers identification and quantification.

The analysis was conducted on a Shimadzu GC-MS-MS QP2010 system (Kyoto, Japan), the extracts carried by helium (99.995% purity) at a 1 mL min^{-1} flow rate, and a Zebron ZB5-MS capillary column (30 m, 0.25 mm i.d., 0.25 µm thickness film) 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 rising at 1°C min^{-1} to 300 °C (held for 1 min). The mass spectrometer ion source was operated in electron impact (EI) mode at 70 eV, GC-MS interface temperature was set at 300°C, and the ion source at 280°C. Analysis was performed in selective ion monitoring (SIM) mode. Data were obtained by GC Solution software (Shimadzu, Kyoto, Japan).

Calibration curves for each sterol were obtained from standard solutions of coprostanol, cholesterol, stigmastanol, cholestanol, stigmasterol, and β-sitosterol at different concentration levels. The evaluated sterols were identified based on mass spectra and retention times obtained for standards and quantified based on response factors of standards relative to 5α-cholestane (internal standard). Procedural blanks were performed, and no peaks interfered with the analyses of the target compounds. An amount of 50 uL of the surrogate (androstanol 500 ng mL^{-1} solution) was added to samples before extraction to evaluate method recovery, which ranged from 76 to 99%, an acceptable rate considering environmental samples (Ribani et al., 2004). Each analyte's limit of quantification (LOQ) was defined as the first point of the analytical curve (10 ng mL^{-1}) divided by the sediment mass and the limit of detection (LOD) as three times lower than the LOQ.

2.4. Sterols origin

The diagnostic ratios of sterols origin (Table 1) were used to distinguish between human and animal fecal origins and assess the degree of pollution caused by residential sewage (He et al., 2018).

2.5. Bulk parameters

For grain size analyses, 4 g of dried sediment were treated with H_2O_2 10%, then centrifuged, and finally washed with distilled water to eliminate the organic matter (OM). The grain size was analyzed with a laser granulometer (SALD 2101 Shimadzu) (Suguio, 1973). The OM content in samples was determined by calcinating 5 g of dried sediment at 500°C for 4 h (Ranney, 1969).

2.6. Statistical analysis

Statistical and multivariate statistical analyses, such as Pearson correlation and principal component analysis (PCA), respectively, are generally used to assess sewage pollution in aquatic systems (Martins et al., 2008; Frena et al., 2016; Cabral et al., 2019). Statistical analysis was performed using Rstudio Statistical Software (Foundation for Statistical Computing, Vienna, Austria), version 2021.09.0.

It was applied principal component analysis (PCA) to identify relations among the sterol markers and bulk parameters (grain size and OM) and to distinguish sampling stations according to sterols sources. Pearson correlation was determined to better understand the relationship between the sterols and bulk parameters.

3. Results

The concentrations of sterols determined in nine samples of the superficial sediments are presented in (Table 2). The total sterols concentrations (Σsterols) ranged from 364.9 ng g-1 at site 7 up to 1319.6 ng g-1 at site 4, with a mean of 45.68 ng g-1. Coprostanol was identified in six of the nine samples, ranging from the below quantification limit at stations 6-9 up to 219.8 ng g-1 at site 1. This sterol represented 5.2% of the Σsterols in the study area and was found to be highest at site 1, which is located closest to the Aura landfill. The predominant sterol in all examined samples along the Aura River basin was β-sitosterol.

In order to evaluate the sewage contamination sources in the Aurá River sediments, various diagnostic ratios were considered (Table 1). The values for (R1) (coprostanol/(coprostanol + cholestanol) ranged from 0.2 to 0.7, while the ratio coprostanol/cholesterol (R2) ranged from 3.9 to 0.4 across the nine sites. Another ratio assessing fecal contamination in sediments of rivers is the coprostanol/(cholestanol + cholesterol) ratio (R3).

Table 1. Diagnostic ratios applied in this study.

Name	Diagnostic ratio	Value	Indicative	Reference
R1	$\frac{Coprostanol}{Coprostanol + Cholestanol}$	< 0.3	No Fecal contamination	Grimalt et al. (1990)
		0.3 – 0.7	Moderate Fecal contamination	
		> 0.7	Human Fecal contamination	
R2	$\frac{Coprostanol}{Cholesterol}$	≤ 0.5	Biogenic sources	Grimalt et al. (1990)
		> 0.5 and ≤ 1.0	Human fecal contamination	Leeming et al. (1996)
		> 1.0	Intense sewage contamination	Takada et al. (1994)
				Fattore et al. (1996)
R3	$\frac{Coprostanol}{Cholestanol + Cholesterol}$	> 0.06	Human fecal contamination	Writer et al. (1995)
R4	$\frac{Cholestanol}{Cholesterol}$	< 0.5	Fresh organic input	Chalaux et al. (1995)
		> 0.5	*In situ* reduction of cholesterol	

Table 2. Total (Σsterols) and individual sterol concentration (ng g^{-1} DW), diagnostic ratios values, and sedimentological characteristics for the 9 sediment samples from Aurá River (Northern Brazil).

Variables	Sampling Sites								
	1	2	3	4	5	6	7	8	9
Sterols									
Coprostanol	219.8	28.3	61.5	76.7	24.9	ND	ND	ND	ND
Cholesterol	55.7	138.2	146.1	158.6	66.7	21.8	56.3	16.5	34.4
Cholestanol	112.6	126.3	182.3	235.9	65.0	50.1	20.6	30.5	25.7
Stigmasterol	187.8	230.4	253.7	312.8	101.1	52.7	28.6	45.0	81.1
β-Sitosterol	335.7	220.2	102.3	320.7	317.4	319.1	222.7	340.4	348.4
Stigmastanol	355.4	243.4	354.4	215.0	333.7	330.1	36.7	106.0	219.5
Σsterols	1267.0	986.8	1100.3	1319.6	908.7	773.9	364.9	538.4	709.1
Ratios									
R1	0.7	0.2	0.3	0.2	0.3	ND	ND	ND	ND
R2	3.9	0.2	0.4	0.5	0.4	ND	ND	ND	ND
R3	1.3	0.1	0.2	0.2	0.2	ND	ND	ND	ND
R4	2.0	0.9	1.2	1.5	1.0	2.3	0.4	1.8	0.7
Bulk parameters									
%OM	10.8	8.8	16.8	6.3	5.7	9.8	7.5	7.2	11.8
%MUD	27.7	42.3	27.7	24.0	27.7	38.3	27.7	30.7	27.7

ND means non-detected; R1 = coprostanol/(coprostanol + cholestanol); R2 = coprostanol/cholesterol; R3 = coprostanol/(cholesterol + cholestanol); R4 = cholesterol/ cholestenol; %OM = organic matter content; %MUD = fine grain size content.

In the present study, all samples yielded values above 0.06 for this ratio. In addition, R4 was used to evaluate the level of diagenetic transformation in the sediments. In this study, values for R4 ranged from 0.4 up to 2.3.

Statistical and multivariate statistical analyses, such as Pearson correlation and principal component analysis (PCA), respectively, are generally used to assess sewage pollution in aquatic systems (Martins et al., 2008; Frena et al., 2016; Cabral et al., 2019). The first component (PC1) and the second component (PC2) explained 45.9 and 23.7% of the total variance, respectively. Stigmasterol, cholesterol, cholestanol, and coprostanol were the significant variables for PC1, while the dominant sterols for PC2 were stigmastanol and β-sitosterol. PC1 showed a positive correlation among the sterols (except β-sitosterol) and allowed to associate the studied stations in two groups according to the contamination sources.

Pearson's correlation analysis (r; $p < 0.05$) involving sterols compounds, %OM, and %MUD revealed a high positive correlation between two groups of sterols (Figure 2). The first group, (coprostanol and cholesterol) showed a low positive correlation coefficient (0.23). The second group of sterols, comprising cholestanol, cholesterol, and stigmasterol, demonstrated high positive correlation coefficients (>0.9). Furthermore, β-sitosterol demonstrated a positive correlation with coprostanol.

4. Disscussion

Coprostanol is the most abundant sterol found in human feces and constitutes a biomarker of human fecal contamination (Leeming et al., 1998; Bull et al., 2002). The threshold coprostanol values proposed by de Melo et al. (2019) of 10 ng g^{-1} for uncontaminated sediments, 100 ng g^{-1} for contaminated sediments, and 500 ng g^{-1} for severely polluted sediments were employed in this study. The coprostanol values found in the Aura River suggest a similar range of fecal material input to other aquatic systems in Brazil and worldwide (Table 3). Coprostanol levels were comparable to those in Tokyo Bay, Japan (243 ng g^{-1}) (Chalaux et al., 1995), Ubatuba Bay, Brazil (max. 270 ng g^{-1}) (Muniz et al., 2006), and slightly higher than sediments of the inner shelf adjacent to Sergipe River (184.1 ng g^{-1}) (Carreira et al., 2015) and those from Tarumã-Açu Stream in the Brazilian Amazon (142 ng g^{-1}) (Melo et al., 2019).

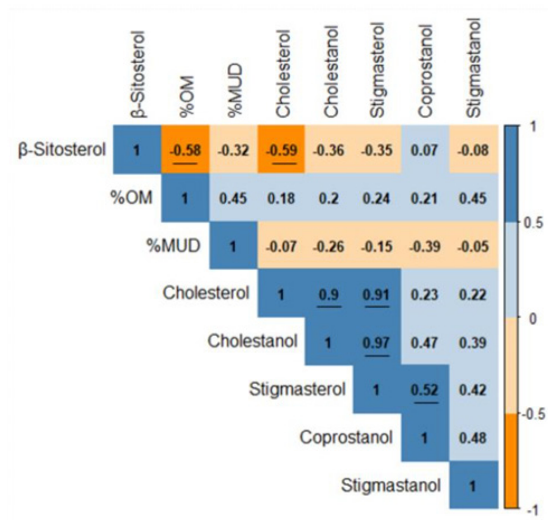

Figure 2. Pearson correlation matrix of individual sterol concentrations and bulk parameters from surficial sediments of Aurá River. The scale bar on the right shows p values and highlighted data indicates a significant correlation.

Table 3. Maximum coprostanol concentrations (ng g^{-1}) in different aquatic systems around the world.

Location	Maximum Coprostanol Concentration (ng g^{-1})	References
Ubatuba Bay, Brazil	270	Muniz et al. (2006)
Tay Estuary, Scotland	1.53	Reeves & Patton. (2005)
Inner shelf NE coast, Brazil	184	Carreira et al. (2015)
Tubarão River, Brazil	32,200	Cabral et al. (2020)
Sepetiba Bay, Brazil	430	Carreira et al. (2009)
Xiaoqing River, China	63,262	He et al. (2018)
Laizhou Bay, China	4,553	He et al. (2018)
Antonina Bay, Brazil	190	Cabral et al. (2019)
Paranaguá Bay, Brazil	70	Cabral et al. (2019)
Guaratuba bay, Brazil	6	Cabral et al. (2019)
The Sergipe River estuary, Brazil	1,072	Frena et al. (2019)
Mindu Stream, Brazilian Amazon	6,113	Melo et al. (2019)
Quarenta Stream, Brazilian Amazon	12,830	Melo et al. (2019)
Tarumã-Açu Stream, Brazilian Amazon	142	Melo et al. (2019)

The analysis of the sterol composition of the samples collected along the Aura River showed that the predominant sterol present was β-sitosterol. This finding suggests that a major source of organic matter in the Aura River basin is terrestrial vegetation, such as trees and plants.

Data determined for Station 1 indicated input of long-term discharge from the Aura landfill, which ceased activity in 2015, and may be the main source of coprostanol to the river. Coprostanol was not detected at Stations 6-9, suggesting that domestic sewage likely does not affect these sites. The absence of coprostanol at these sites implies that the hydrodynamics of the Aura River are effective for contaminant dispersion. A similar distribution pattern was observed for polycyclic aromatic hydrocarbons (PAHs) in the Aura River sediments by Rodrigues et al. (2018). Moreover, the study area is characterized by intense precipitation and fluvial-dominated hydrodynamics, which have effects on the depuration and dilution of sewage effluents.

The prevalence and high levels of β-sitosterol in all the samples suggest a significant input of terrigenous material to the studied area. However, the presence of β-sitosterol may also be attributed to domestic sewage discharges due to its typical presence in vegetable oils used for cooking (Froehner et al., 2009; Frena et al., 2016b). Other sterols that were prevalent along the Aura River basin included stigmastanol (at sites 1-3 and 5-6) and stigmasterol (at sites 1-4), which are indicative of herbivore feces and vascular plants metabolism, respectively (Frena et al., 2016b). High levels of phytosterols and sewage sterols at station 1 may also be related to eutrophication processes that favor the production of cholesterol, cholestanol, and phytosterols in addition to sewage markers (Melo et al., 2019).

According to Grimalt et al. (1990), the diagnostic ratio coprostanol/(coprostanol + cholestanol) R1 with values higher than 0.7 suggests sewage pollution, and values lower than 0.3 indicate the absence of sewage contamination. Station 1 exhibits moderate sewage pollution, as indicated by the relatively high levels of coprostanol (> 100 ng g^{-1}) and values close to 0.7 for R1 (Frena et al., 2016b). In contrast, the other studied sites had R1 values lower than 0.30, indicating a low influence of sewage. The coprostanol/cholesterol ratio R2 can be used to distinguish between biogenic and anthropogenic organic matter inputs. Values of R2 greater than 0.5 indicate sewage contamination, while values less than 0.5 are attributed to biogenic sources (Grimalt et al., 1990; Takada et al., 1994; Leeming et al., 1996,). This ratio showed that station 1 is the most affected by sewage pollution. The cross-plot representations of R1 and R2 versus coprostanol concentrations showed that station 1 is affected by sewage pollution, while the other sites are influenced by biogenic organic matter inputs. The cross plots of coprostanol levels versus coprostanol/(coprostanol+cholestanol) and coprostanol/cholestanol (shown in Figure 3) also indicate that only station 1 is contaminated by sewage.

Moreover, the ratio coprostanol/(cholestanol + cholesterol) (R3) has been used to indicate fecal contamination in sediments of rivers (Writer et al., 1995; Dsikowitzky et al., 2017). In the present study, R3 values above suggest that the presence of coprostanol in these points may be related to human fecal origin. The cholestanol/cholesterol (R4) ratio has been used to evaluate contamination by sludge (Froehner et al., 2009; Machado et al., 2014; Thomes et al., 2019). Values close to and above 0.5 for R4 were detected in all stations, indicating high rates of biohydrogenation processes (Nishimura & Koyama 1976; Souza et al., 2020).

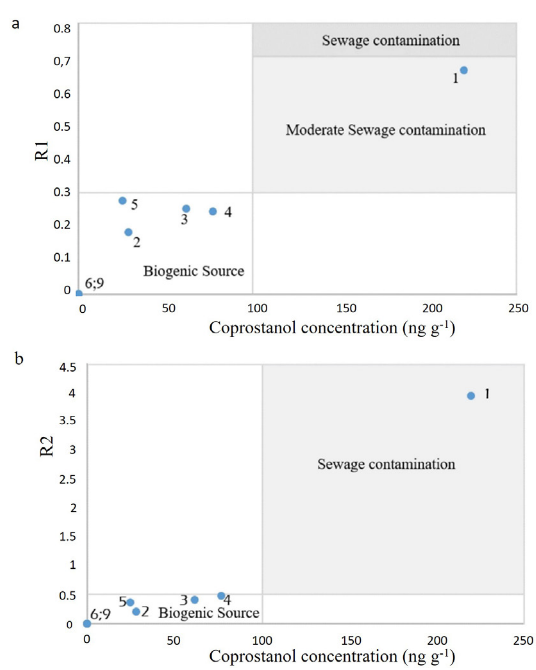

Figure 3. Cross plots of coprostanol/(coprostanol + cholestanol) (R1) and coprostanol/cholesterol (R2) *vs*. coprostanol concentrations for sampling sites from Aurá River. *Biogenic source* means a main input of natural sterols to the sampling site, and the *sewage contamination* (moderate or not) implies considerable urban/anthropogenic input of sterols.

These high rates may be related to an increase in terrestrial organic matter input, nutrients, and bacteria, which can affect primary productivity and redox conditions at the water/sediment interface (Ali & Mudge, 2005; Machado et al., 2014).

According to PCA (Figure 4), stations grouped in red exhibit both anthropogenic and biogenic sources, as indicated by coprostanol levels greater than 24.9 ng g-1. Stations grouped in blue are associated only with biogenic sources. This may be because plant sterols such as stigmasterol are derived from both municipal sewage and terrestrial sources (Bujagić et al., 2016; Melo et al., 2019; Wen et al., 2020).

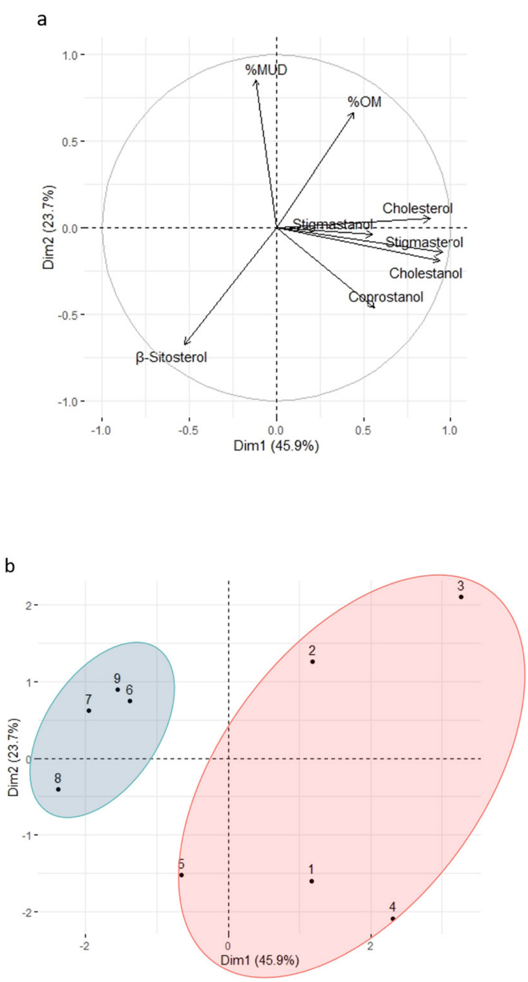

Figure 4. PCA analysis (Dim1 (45.9%): Principal Component 1; Dim2 (23.7%): Principal Component 2) of sterol markers and bulk parameters. **a** Variables (loadings): organic matter content (%OM), fine grain size content (%MUD), cholesterol (cholest-5-en-3β-ol), coprostanol (5β-cholestan-3β-ol), stigmastanol (5αcholestan-22E-en-3β-ol), cholestanol (5α-cholestan-3β-ol), β-sitosterol (24-ethylcholest5-en-3β-ol). **b** Sediment samples collected from the Aurá River were clustered according to their sterol source.

The correlation between coprostanol and stigmasterol evidenced through Pearson's correlation analysis may be attributed to the presence of plant sterols, such as stigmasterol, which could be derived from both municipal sewage and terrestrial sources (Bujagić et al., 2016; Melo et al., 2019; Wen et al., 2020). Previous studies have also reported a strong correlation between fecal and plant sterols (Yao et al., 2013; Melo et al., 2019; Wen et al., 2020). High positive correlation among cholestanol, cholesterol, and stigmasterol, could be due to the fact that the river in question is influenced by both anthropogenic and biogenic sources of OM. Pearson correlation confirms the observations made through PCA, as it also shows that the surficial sediments of the Aurá River are influenced by both anthropogenic and higher plants inputs.

The coprostanol and cholesterol positive correlation coefficient of 0.23 indicates the presence of human fecal contamination in the Aurá River because cholesterol, besides being found in zooplankton, a wide variety of phytoplankton, and several forms of marine animals, is also a byproduct of higher animals' feces, such as humans (Martins et al., 2007; Bataglion et al., 2016; Frena et al., 2016b). This finding is further corroborated by Pearson correlation, which also highlights the coexistence of anthropogenic and biogenic sources in the surface sediments of the Aurá River.

The results of this study suggest that sterols composition and levels in the sediments of Aurá River are mainly influenced by organic matter inputs from anthropogenic and higher plant sources. This is evidenced by the positive correlation between β-sitosterol and coprostanol, which is considered a fecal sterol. Moreover, sedimentological characteristics did not appear to influence sterols composition or levels, except for coprostanol.

5. Conclusions

For the first time, fecal pollution was determined in surficial sediments from Aurá River using sterol biomarkers, diagnostic ratios, and statistical analysis. Sterol ratios and coprostanol levels permitted the identification of anthropogenic OM from the Aurá Landfill and domestic sewage relied by riverine communities. Statistical analysis (PCA) corroborates this result. In addition, PCA allowed grouping the studied sites according to the main source of organic matter (anthropogenic and higher plants inputs). Although the Aura landfill is currently out of service, there is evidence of organic contamination reaching the Aurá River.

The absence of efficient sewage treatment for the riverine communities and the metropolitan region of Belém (PA) represents an ecological threat to Aura River. Hence, this study provides a basis for future management of the studied area and its surroundings which is an important source of water supply.

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Data availability

The authors have stored all the necessary databases for anyone who might be interested in making a query.

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