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Article

Natural and Anthropogenic Sterols Inputs in Surface Sediments of Patos Lagoon, Brazil

César de Castro Martins,[*,a,b] *Gilberto Fillmann*[c] *and Rosalinda Carmela Montone*[b]

[a]*Centro de Estudos do Mar da Universidade Federal do Paraná, CP 50002, 83255-000 Pontal do Sul, Pontal do Paraná - PR, Brazil*

[b]*Laboratório de Química Orgânica Marinha, Instituto Oceanográfico da Universidade de São Paulo, Praça do Oceanográfico, 191, Cidade Universitária, 05508-900 São Paulo - SP, Brazil*

[c]*Fundação Universidade Federal do Rio Grande, Departamento de Oceanografia, Av. Itália, km 8, 96201-900 Rio Grande - RS, Brazil*

A Lagoa dos Patos (RS), considerada a maior lagoa costeira do mundo, tem sido afetada por diversas atividades antrópicas. Um risco de contaminação por esgoto ocorre devido ao estabelecimento de mais de 2 milhões de habitantes ao longo da bacia de drenagem desta região. Amostras de sedimentos foram coletadas e analisadas por CG-EM. Dez diferentes esteróis, incluindo coprostanol e epicoprostanol (fecais), e cetonas foram identificados e quantificados. A maior concentração de coprostanol foi encontrada próximo à cidade de Porto Alegre (1.423 ng g^{-1}). Nos demais pontos, a concentração variou desde o limite de detecção até 91,78 ng g^{-1}. As maiores concentrações foram encontradas próximo à descarga de esgotos e os níveis diminuíram com o aumento da distância desde as fontes. As razões isoméricas 5β / (5β+5α) indicaram sedimentos contaminados próximo a Porto Alegre. A razão epicoprostanol/coprostanol mostrou valores característicos do aporte de esgoto sem tratamento ao longo da Lagoa dos Patos.

The Patos Lagoon, the largest coastal lagoon in the world, has long been receiving considerable anthropogenic input resulting from urban, rural and industrial activities. Thus, sewage contamination possibly originating from the more than 2 million inhabitants living within its drainage basin is examined in this study. Sediment samples collected from various points along the Lagoon were extracted, purified and analysed by GC-MS. Ten different sterols and ketones, including coprostanol and epicoprostanol, were identified and quantified. The highest coprostanol concentration was found near Porto Alegre City (1,423 ng g^{-1} dry wt.), whilst most sediment levels ranged between < DOL and 91.78 ng g^{-1}. The highest concentrations were found at those three sites located closest to domestic outfalls though levels tended to decrease with distance from the sources. The 5β/(5β+5α) isomeric ratios indicated sewage-contaminated sediments near Porto Alegre City, while the epicoprostanol/coprostanol ratio showed values characteristic of untreated sewage input.

Keywords: sterols, coprostanol, sediment, Patos Lagoon

Introduction

Sterols have been used as molecular tracers to identify and distinguish between sources of contamination in various environmental compartments such as water and sediments because of their specificity as regards origin.[1] They are persistent in anoxic sediments, easily associate with particulate material and sediments, and present notable resistance to anaerobic degradation.[2,3]

Faecal sterols, such as coprostanol and epicoprostanol, and the ketone 5β-coprostanone present in human faeces have previously been used as tracers for human waste in coastal areas of industrial and urban centers in temperate regions[4-7] and also in the Brazilian tropical region.[8-10]

Coprostanol has been widely used as a marker of faecal contamination because it is produced in the digestive tracts of humans and higher vertebrates by the microbial reduction of cholesterol.[11] It accounts for 40-60% of the total faecal sterols excreted in human wastes.[12] As the main

*e-mail: ccmart@ufpr.br

excretion product of humans, this sterol may be associated with anthropogenic sewage.[7]

Epicoprostanol, a coprostanol isomer, can be used as an indication of the level of treatment or age of the faecal matter because it is formed during treatment of wastewaters and sewage sludge digestion.[13] Human faeces contain trace amounts of this isomer. During extensive anaerobic sewage treatment, however, epicoprostanol may be produced from cholesterol and the relative proportions of those compounds may be used to indicate the degree of sewage treatment, suggesting that this could be used as an indicator of the presence of untreated sewage in sediments.[11] In combination with cholesterol and cholestanol, faecal sterols are a reliable marker of human sewage contamination.[2,14]

5β-coprostanone is present in significant amounts in human faeces, sewage sludge and sediments collected at sewage outfalls. It can also be useful biomarker to evaluate faecal contamination since the presence of this compound implies that at least part of the conversion of cholesterol into coprostanol in the intestine of humans occurs *via* the formation of an intermediary compound.[15]

Marine organisms, including cyanobacteria, microalgae, phytoplankton (diatoms, coccolithophorids, dinoflagellates) and zooplankton, widely distributed in biological systems, produce no coprostanol, epicoprostanol or 5β-coprostanone. So sterol profiles and the ratios of faecal sterols to other sterols may be useful in the discrimination of faecal and biogenic sources.

The Patos Lagoon is the largest coastal lagoon in the world,[16] with a surface area of 10,360 km^2. The entire system is connected to the Atlantic Ocean through a narrow channel, 750 m wide. The lower lagoon, also called the estuarine area, occupies approximately 10% of the total area[17] (Figure 1). The system dynamics are essentially dependent on the wind and on the freshwater discharge.[18,19] The estuarine region is mostly shallow (generally less than 2 m deep), except for the navigation channel (in which depths can reach more than 14 m). Normally much weaker in shallow regions, currents at the entrance channel can flow at up to 1.5 m s^{-1} during periods of high river discharge (ebb flow) or during meteorological events coming from the southern quadrant (flood flow).[19]

This system suffers great anthropogenic pressure from urban, rural and industrial activities.[17] Porto Alegre (>1.5 million inhabitants), Pelotas (~350,000 inhabitants) and Rio Grande (~200,000 inhabitants) are the main metropolitan areas, which still dispose of a significant part of their untreated sewage in the lagoon. In addition to several diffuse urban inputs into the shallow marginal bays,[20] the activities related to one of the biggest harbors

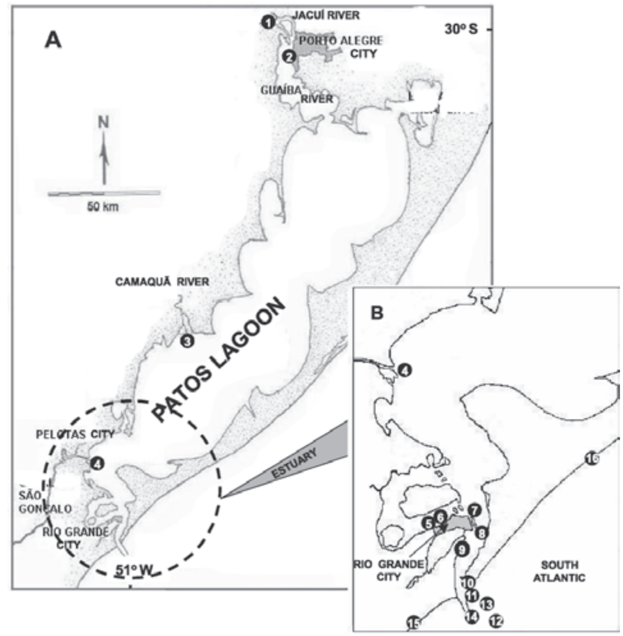


Figure 1. Sediment sampling sites in the Patos Lagoon, Rio Grande do Sul (RS), Brazil (main body (A) and estuary (B)).

of Brazil and numerous factories (oil refinery and terminal, fertilizer-producing plants, fishery industries) have an impact on the estuary.[21] Table 1 summarizes the main sources of organic contamination at each site studied.

Despite its ecological importance, the sources of the organic matter found in the Patos Lagoon have barely been examined.[22,23] No previous studies using sterols as biomarkers, to distinguish between sewage and biogenic sources of the organic matter in the Patos Lagoon have previously been conducted. In this respect, coprostanol and other sterols have been analysed, in the study, in surface sediments at selected sites in the Patos Lagoon.

Experimental

Sampling and analytical procedures

In order to evaluate sterols inputs from natural and anthropogenic sources in the Patos Lagoon, 17 sediment samples were collected at the sites shown in Figure 1, in 1997. Sediments were sampled utilizing a stainless-steel grab. Only the top 2 cm of undisturbed surface sediment was placed into pre-cleaned aluminum foils, and then stored at -15 ºC until laboratory analysis.

The sediments were freeze-dried, dry/wet ratios determined and then sieved through a stainless steel mesh (250 μm). Each sediment sample (10 – 20 g) was spiked with an internal standard, 5α-androstan-3β-ol (Sigma).

The samples were Soxhlet extracted for 12h into 200 mL of hexane/dichloromethane (1:1). The extracts were concentrated down to a few milliliters using rotary evaporation followed by gentle nitrogen "blow down". Sulphur was removed by shaking the extracts with activated copper.

Clean-up and fractionation was performed by passing the extract through a silica/alumina column (the silica and alumina were activated at 200 °C for 4 h and then partially deactivated with 5% Milli-Q water). The chromatography column was prepared by slurry packing 8 g of silica, followed by 8 g of alumina and finally 1 g of sodium sulphate. Elution was performed using 20 mL of hexane to yield the first fraction (which contains the aliphatic hydrocarbons), 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). Sterols were then eluted with 50 mL of dichloromethane/methanol (90:10). All solvents were pesticide grade.

The fractions containing the sterols were evaporated to dryness and derivatized to form trimethylsilyl ethers using BSTFA (bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) (Supelco) for 90 min at 65 °C.[24]

The sterols analyses were performed with an Agilent GC model 6890 coupled to a Agilent Mass Spectrometer Detector (model 5973) and an Ultra-2 capillary fused silica column coated with 5% diphenyl / dimethylsiloxane (50 m × 0.32 mm ID × 0.17 µm film thickness). Helium was used as carrier gas. The oven temperature was programmed from 40 to 240 °C at 20 °C min^{-1}, then to 255 °C at 0.25 °C min^{-1} (holding for 10 min), and finally to 300 °C at 20 °C min^{-1} (holding for 5 min). The data acquisition was done in scan mode. Compounds were identified by matching retention times with results from standard mixtures of 8 sterols and 2 ketones. The HP Enhanced Chemstation G1701 CA was used to perform the quantification and this was undertaken by the comparison of GC/MS compounds and internal standard response factors in the total ion chromatogram. Calibration of the peak area to concentration was done using the sterols and ketones standards (Sigma) in the derivatized form within the range of 0.25 to 20.0 µg L^{-1} and the linear response was >0.995. Procedural blanks were performed for each series of 10 extractions and interfering peaks did not interfere with the analyses of target compounds. Internal standard recoveries ranged from 70-120%. Detection limits (DOL), defined as three times the standard deviation of the signal in the same retention time of sterols in the blanks, was 1 ng g^{-1} for all compounds analysed.

Principal component analysis

Principal component analysis (PCA) was performed using the *Statistica* package for Windows (Version 5.1, 1997) to identify similarities or distinctions among the different sterols in marine sediments. For treatment, samples were taken as cases and the sterols were the variables. The PCA datasheet consisted of the original values of sterols concentrations at each site.

Results and Discussion

Sedimentary concentrations (ng g^{-1} dry weight) of the following faecal sterols and ketones are reported in Table 2: coprostanol (5β-cholestan-3β-ol), epicoprostanol (5β-cholestan-3α-ol), 5β-coprostanone (5β-cholestan-3-one), cholesterol (cholest-5-en-3β-ol), cholestanol (5α-cholestan-3β-ol) and 5α-cholestanone (5α-cholestan-3-one). In addition, campesterol (24-methylcholest-5-en-3β-ol), stigmasterol (24-ethylcholest-5,22(E)-dien-3β-ol), β-sitosterol (24-ethylcholest-5-en-3β-ol) and β-sitostanol (24-ethyl-5α-cholestan-3β-ol) were also quantified (Table 2).

Sewage markers

The highest coprostanol concentration was found at site #2 (Porto Alegre City; 1,423 ng g^{-1} dry wt.), while most sediment levels ranged between <DOL and 91.78 ng g^{-1}. The stations within the area of influence of Rio Grande city (#5-#9) presented a mean (± SD) concentration of 41.4 (± 31.4) ng g^{-1}, while the highest coprostanol levels in this area were found at sites # 6 (Yacht Club; 91.8 ng g^{-1}) and # 5 (North Channel; 64.6 ng g^{-1}). The highest concentrations were found at those three sites located close to domestic outfalls. In general, levels decrease with distance from the sources and this is compatible with patterns associated with mixing/dilution processes. However, some of the stations located in front of the Patos Lagoon entrance (#12, Ocean 1 and #13, Ocean 2) presented coprostanol concentrations at the same levels (76.0 and 54.0 ng g^{-1}, respectively) as those sites near Rio Grande city. Although these stations are located far from the main sources of sewage contamination, the coprostanol levels indicate the accumulation of coprostanol in these sediments. Since sterols may be found associated with suspended particulate matter[26,27] and the deposition of fine sediments carried out from the Patos Lagoon onto the inner shelf is well known,[17] it is expected that sterols originating from many diffuse sources, mainly adsorbed to fine particles, should be transported to the coast, where the sedimentation processes take place.

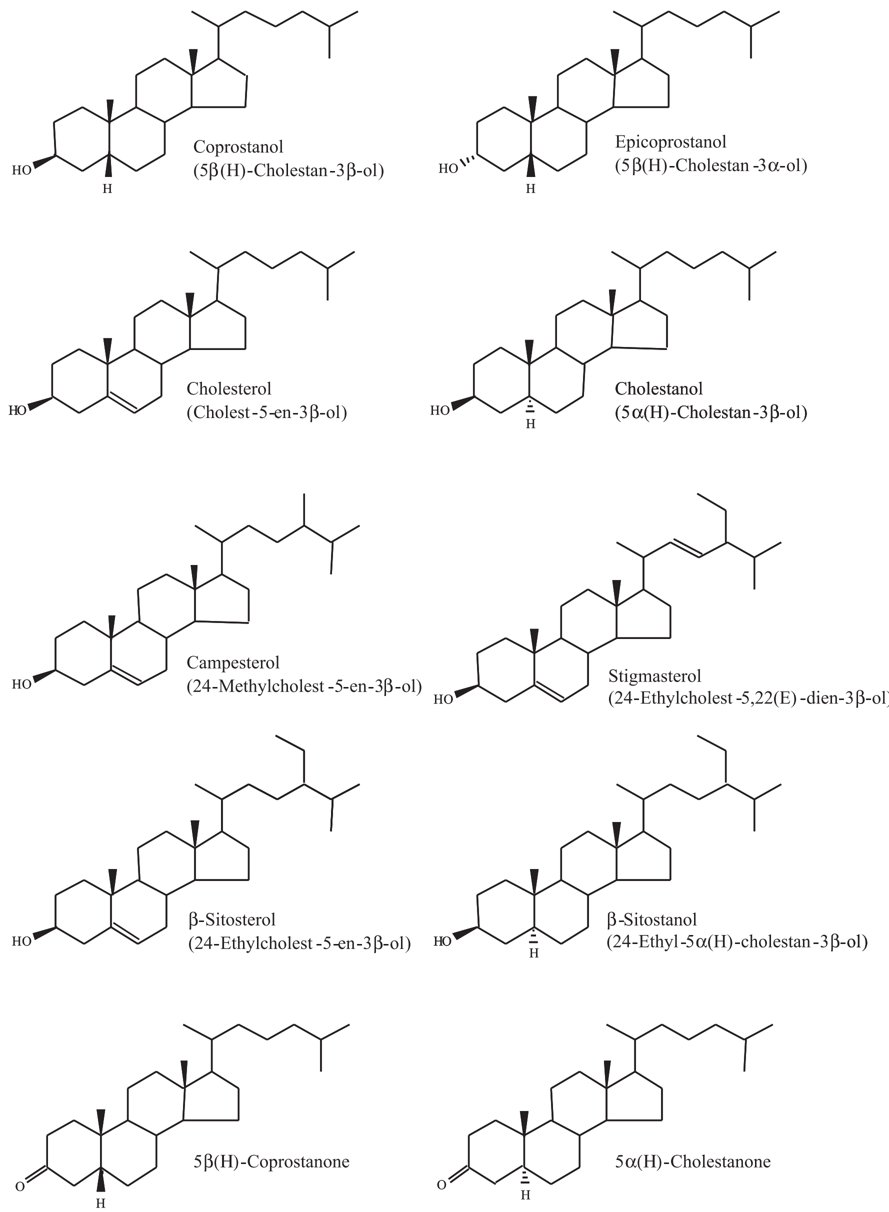
Table 1. Sampling sites and the most probable source of contamination for the sediment collected from each site in the Patos Lagoon, RS, Brazil

station	Site	possible pollution sources	additional information
1	Jacuí river	industrial and urban effluents	50 m from the margin, near petrochemical industrial complex
2	Porto Alegre City	industrial and urban effluents from Porto Alegre city.	sampled in the navigable channel
3	Camaquã River	diffuse sources from Camaquã drainage basin	NE margin of the Camaquã mouth
4	São Gonçalo output	diffuse sources from Mirim Lagoon drainage basin	500 m off São Gonçalo mouth
5	North channel	industrial and urban effluents from Rio Grande city.	100 m off Rio Grande secondary sewage outfall
6	Yacht Club	oil fuel, heavy metals, ship paint residues, urban effluents	50 m off Yacht club entrance
7	Shipyard	oil fuel, heavy metals, ship paint residues and urban effluents	100 m off shipyard entrance
8	Channel junction	harbour activities and multiple sources	sampled in the navigable channel
9	Oil terminal	petroleum input and industrial effluents	Oil terminal of petroleum discharge
10	East bar	diffuse sources from Patos Lagoon drainage basin	landward side of the jetty
11	East jetty	diffuse sources from Patos Lagoon drainage basin	ocean side of the jetty
12	South Atlantic Ocean 1	diffuse sources from Patos Lagoon drainage basin	10 km (SE) landwards from the Patos Lagoon estuary mouth
13	South Atlantic Ocean 2	diffuse sources from Patos Lagoon drainage basin	10 km (NE) landwards from the Patos Lagoon estuary mouth
14	South Atlantico Ocean 3	diffuse sources from Patos Lagoon drainage basin	5 km (SE) landwards from the Patos Lagoon estuary mouth
15	Altair shipwreck	diffuse sources from Patos Lagoon drainage basin	21 km (SW) from the Patos Lagoon estuary mouth
16 (a) & (b)	Conceição lighthouse (control)	far from anthropogenic sources	65 km (NE) from the Patos Lagoon estuary mouth. (a): 1997 (b): 1998

Table 2. Concentrations of individual sterols and ketones (ng g^{-1} dry weight), and selected ratios in surface sediments from the Patos Lagoon

sterols and stanones analysed	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16 a**	**16 b**
Coprostanol	6.5	1,423	11.6	22.1	64.6	91.8	11.0	18.7	20.8	< DOL	< DOL	76.4	54.0	35.7	< DOL	< DOL	< DOL
Epicoprostanol	< DOL	132.0	< DOL	3.7	7.3	11.7	5.5	2.7	2.4	< DOL	< DOL	9.4	6.9	3.0	< DOL	< DOL	< DOL
Cholesterol	12.3	73.5	185.6	7.0	151.8	56.6	16.3	95.3	88.5	4.4	6.7	474.1	92.0	333.1	14.4	369.2	131.7
Cholestanol	< DOL	998.3	50.4	174.2	585.3	166.0	107.6	183.5	72.1	20.7	< DOL	865.4	654.0	164.0	< DOL	< DOL	11.4
Campesterol	< DOL	< DOL	86.6	< DOL	122.4	< DOL	< DOL	52.7	34.2	< DOL	< DOL	510.7	55.3	68.0	< DOL	21.7	9.1
stigmasterol	< DOL	< DOL	88.0	< DOL	146.5	< DOL	< DOL	80.0	32.6	< DOL	< DOL	321.4	171.7	135.0	17.8	< DOL	< DOL
β-sitosterol	16.1	204.3	113.4	< DOL	219.5	< DOL	< DOL	102.3	38.8	< DOL	< DOL	758.7	95.6	63.4	84.3	21.6	8.8
β-sitostanol	61.5	1,336	< DOL	223.0	570.3	77.9	27.9	153.1	79.4	< DOL	< DOL	877.3	612.3	77.3	< DOL	< DOL	< DOL
5β-coprostanone	3.9	420.6	8.13	11.2	36.9	55.0	4.2	8.4	11.6	< DOL	< DOL	37.6	35.3	16.5	< DOL	< DOL	< DOL
5α-colestanone	6.9	141.4	12.7	44.7	167.3	109.5	14.9	36.1	30.9	< DOL	< DOL	172.2	116.1	68.6	< DOL	< DOL	< DOL
Σ-OLs	96.5	4,167	535.5	430.1	1,867	403.9	168.3	688.3	368.7	25.2	6.7	3,893	1742	879.4	116.5	412.4	161.0
ratios[ref.]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16 a**	**16 b**
e-cop/cop[13]	n.c	0.09	n.c	0.17	0.11	0.13	0.51	0.15	0.11	n.c	n.c	0.12	0.13	0.08	n.c	n.c	n.c
% (cop+e-cop)/Σ-OLs[4]	n.c	37.3	n.c	6.01	3.85	25.6	9.79	3.12	6.29	n.c	n.c	2.20	3.49	4.40	n.c	n.c	n.c
5α/(5β+5α) stanols[5]	n.c	0.59	0.19	0.11	0.10	0.36	0.09	0.09	0.22	n.c	n.c	0.08	0.08	0.18	n.c	n.c	n.c
5α/(5β+5α) ketones[5]	0.36	0.75	0.39	0.20	0.18	0.33	0.22	0.19	0.27	n.c	n.c	0.18	0.23	0.19	n.c	n.c	n.c
cholestanol/cholesterol[2]	n.c	13.6	0.27	24.9	3.86	2.93	6.60	1.92	0.81	4.66	n.c	1.83	7.11	0.49	n.c	n.c	0.09
β-sitostanol/β-sitosterol[13]	3.82	6.54	n.c	n.c	2.60	n.c	n.c	1.50	2.05	n.c	n.c	1.16	6.41	1.22	n.c	n.c	n.c
campesterol/stigmasterol/ β-sitosterol[33]	n.c	n.c	1.0:1.0: 1.3	n.c	1.0:1.2: 1.8	n.c	n.c	1.0:1.5: 1.9	1.0:1.0: 1.1	n.c	n.c	1.0:0.6: 1.5	1.0:3.1: 1.7	1.0:2.0: 0.9	n.c	n.c	n.c

< DOL: below limit of detection (< 1.0 ng g^{-1} dry weight);% (cop+e-cop)/Σ-OLs: percentage of coprostanol + epicoprostanol in the sum of sterols; Σ-OLs: sum of sterols; n.c: not calculated (one or more compounds in the ratio below the limit of detection); e-cop/cop: epicoprostanol/coprostanol.



Scheme 1. Chemical structures of sterols and ketones analysed.

Even though Gonzalez-Oreja and Saiz-Salinas[28] have suggested that coprostanol concentrations greater than 500 ng g^{-1} may be used to indicate sewage contamination, there is no consensus as to the sedimentary level of coprostanol that is indicative of environmental contamination by sewage. Writer *et al.*[37] have proposed that coprostanol levels above 100 ng g^{-1} should be associated positively with sewage input. Nichols *et al.*[29] found values of coprostanol greater than 500 ng g^{-1} when analyzing sediments near a sewage outfall, although much higher levels could be found in heavily contaminated sediments (eg. > 9,000 ng g^{-1}).[30]

Although there is controversy as regards the value of coprostanol indicative sewage contamination, it may be defined as the presence of levels of coprostanol above 1,000 ng g^{-1} and a potential source is enough preliminarily to confirm sewage contamination.

Thus, only the sediments from the navigable channel near Porto Alegre city (#2), may be considered as greatly contaminated by sewage. The untreated sewage and industrial effluents of Porto Alegre that are still being partially discharged into the Lagoon might explain the high values of coprostanol found there.

The coprostanol levels in the remaining sediments (100 ng g^{-1}) were quite low as compared to those of sites near densely populated areas, such as the Venice lagoon Italy (> 5,000 ng g^{-1}),[30] the Tan Shui estuary, Taiwan (33,300 ng g^{-1}, 2 km from outfall),[2] the San Pedro Shelf, USA (>

1,000 ng g^{-1} at stations near outfall),[7] Sochi, Black Sea, the Russian Federation (5,400 ng g^{-1}).[32]

As compared with other Brazilian marine systems, the concentration of coprostanol in the Patos Lagoon was lower than that found in Guanabara Bay, Rio de Janeiro (40.0 µg g^{-1} – site near the Iguaçú River),[9] and that in the Capibaribe River, Recife, Pernambuco (mean value = 3,026 ± 2,322 ng g^{-1}).[10] It is possible that these results might be associated with an input flux of sewage at each place, higher in Recife and Rio de Janeiro cities.

As an indication of the level of treatment or age of the faecal material,[13] the concentration of epicoprostanol at site #2 (132.0 ng g^{-1}) indicated limited sewage treatment at Porto Alegre. The much lower levels of epicoprostanol in the remaining sediments (< DOL to 11.7 ng g^{-1}) are consistent with the levels of coprostanol and the very limited sewage treatment in the other urban areas along the Lagoon. Rio Grande, for instance, had less than 10% of its sewage treated before 2004.

Concentrations of 5β-coprostanone ranged from < DOL to 55.0 ng g^{-1}, except at site # 2 (420.6 ng g^{-1}). This ketone, which has been used for sewage monitoring,[5] showed the highest values at the same sites at which coprostanol presented its maximum concentrations.

The regions considered as affected by sewage (site # 2 and, in lesser intensity, sites # 6 and # 5), were clearly associated, on the basis of on faecal sterols and 5β-coprostanone concentrations, with direct urban and industrial outfall discharges. Although no measurements were made of faecal sterols and microorganism indicators (e.g. faecal coliforms) in the water column, it is clear that sewage from Rio Grande[20] and Porto Alegre cities is affecting the quality of these waters. The estuarine dynamics around Rio Grande, however, might not allow sterol sedimentation, and the resulting exportation of these biomarkers to the inner shelf (as detected in sediments from sites #12 and #13) is, therefore, keeping sedimentary levels low.

Sterols from natural sources

Cholesterol is the major sterol of most marine plankton. High concentrations of this sterol are, therefore, generally attributed to zooplankton, a wide diversity of phytoplankton, and other marine fauna.[33] It may also be associated with sewage since this compound is a by-product of the excretion of higher mammals, such as humans.[12]

Concentrations of cholesterol, the only sterol present in all the samples, varied from 4.45 to 474.1 ng g^{-1}. The highest values of this sterol were found in stations close to the coast, indicating a considerable important marine organic matter contribution at sites # 12 (Ocean 1), # 16 (Control) and # 14 (Ocean 3) confirmed by the low values of campesterol/stigmasterol/β-sitosterol (discussed below). The high primary productivity due to nutrient fluxes from the Patos Lagoon sediment to the ocean from permeable sediments along the Rio Grande do Sul coast[34] and the autochthonous origin of phytosterols (e.g. cholestanol) are the most probable reasons for the higher cholesterol concentrations at these sites.

Cholesterol levels at those sites with no significant sewage contribution (e.g. Sites #3 – Camaquã river; # 8 - Channel junction; #9 – Oil terminal; #13 – Ocean 2) may also be attributed to biogenic sources, because the faecal contribution may be negligible (low levels of faecal sterols).

Cholestanol has also been found in sediments influenced by the biosynthesis of plankton organisms.[10] Sewage contaminated sediments, however, may also form this sterol through the diagenetic transformation of coprostanol to cholestanol (5β→5α) and the hydrogenation of cholesterol in anoxic environments.[2]

The highest values of cholestanol occurred at sites close to the coast (e.g. # 12 - Ocean 1 and # 13 - Ocean 2), and near the sewage outfalls of Porto Alegre (# 2, 998.3 ng g^{-1}) and Rio Grande (# 5, 585.3 ng g^{-1}).

Campesterol, stigmasterol and β-sitosterol have been commonly used as markers of terrigenous organic matter, although the origins remained largely uncertain since marine sources were associated with major occurrence in environments where organic matter from the land seemed unlikely.[33] Other authors have already addressed the problems of the source specificity of these sterols.[10] Volkman[33] proposed evaluating the ratio of campesterol/stigmasterol/β-sitosterol to overcome this limitation. Ratios close to 1:1.6:6.6 indicate inputs of these compounds from terrestrial vascular plant sources, while lower ratios suggest algae sources for these compounds. These compounds, mainly β-sitosterol, are also present in contaminated sediments from urban centers, originating in domestic vegetable oils discharged in wastewater and sewage.[36]

The highest values of stigmasterol (321.4 ng g^{-1}), campesterol (510.7 ng g^{-1}) and β-sitosterol (758.7 ng g^{-1}) were present at site # 12 (Ocean 1), which also received considerable marine contributions judging from the levels of cholesterol and cholestanol and the low value of the campesterol/stigmasterol/β-sitosterol ratio (1:0.6:1.5) at this station. The sites located near the sewage outputs of Rio Grande (#5) and Porto Alegre (#2) showed high concentrations of β-sitosterol (219.5 and

204.3 ng g^{-1}, respectively), which probably originated in the urban effluents.

β-sitostanol is found in marine sediments as a product of the hydrogenation of β-sitosterol.[13] The highest values of β-sitostanol occurred in sediments collected off the coast (# 12 and #13) and near urban wastewater discharges (#2 and #5), which was the same trend as that presented for cholesterol, cholestanol and β-sitosterol.

The ratio between reduced and oxidized sterols has been used to indicate microbial reduction in anaerobic environments.[35] The cholestanol / cholesterol and β-sitostanol / β-sitosterol ratios were higher than 1.0 for most of the samples analysed, indicating anoxic conditions and microbial reduction (Table 2). This might have provided the conditions necessary for the generation of cholestanol and β-sitostanol, either by diagenetic or hydrogenation processes.

The predominance of cholestanol over cholesterol and β-sitostanol over β-sitosterol for those sites, where high coprostanol levels were found (e.g. # 2 – Porto Alegre, # 5 - North channel and # 6 – Yacht Club), may be explained by the anoxic conditions (and microbial reduction processes) in the surface layer of these sediments as a result of the input of untreated sewage discharges.

At stations # 4 (São Gonçalo output), # 7 (Shipyard), # 10 (East bar) and # 13 (Ocean 2), the low concentration (# 13) and the absence of β-sitosterol over β-sitostanol suggest the occurrence of microbial reduction processes. This may be confirmed by the higher values of the cholestanol / cholesterol ratio at those sites. However, this may not be conclusive because the algae input of cholestanol may be attributed to the high concentration of this sterol.[38]

β-sitosterol has been considered to come from terrestrial inputs,[33] although this is not evident from the concentration values of this sterol and the low values of campesterol/stigmasterol/β-sitosterol found at the study sites. However, the strongly anoxic characteristic of the sediments studied (showed by reduced / oxidized sterol ratios) and the absence of potential sources of β-sitostanol in the marine/terrestrial environment studied allowed us to take β-sitostanol as a secondary biomaker of origin of β-sitosterol[13] and, in the area studied may be attributed to marine sources.

Although only a reduced number of sterols have been analysed, we were able to carry out a preliminary study of autochthonous and allochthonous sources of organic matter. This initial evaluation showed that the main contribution of organic matter to the Patos Lagoon comes from the marine system and will be useful for a better characterization of this region in future studies.

Qualitative evaluation of faecal contamination

The use of ratios between selected sterols has been proposed to enhance the reliability of contamination assessments based on sterol markers. Some commonly used ratios in sediments are: (*i*) percentage of coprostanols/total sterols;[4,39] (*ii*) epicoprostanol/coprostanol;[13] (*iii*) coprostanol/(coprostanol+cholestanol);[5] (*iv*) 5β/(5β+5α) isomeric ratios of cholestan-3-ol and cholestan-3-one.[5]

The percentage of coprostanols in total sterols (% (cop+e-cop)/Σ-OLs) has previously been considered to infer the degree of sewage contamination in marine sediments. The sewage effluents and strongly contaminated sediments generally show ratios of% (cop+e-cop)/Σ-OLs in the range of 50-80%. The majority of the Patos Lagoon sediments showed no predominance of coprostanols (ratios <10%). Higher relative contributions of coprostanols, indicating sewage contribution, were found at sites #2 (Porto Alegre, 37.3%) and #6 (Yacht Club, 25.6%) (Table 2).

Mudge and Seguel[13] proposed the ratio between epicoprostanol and coprostanol (e-cop/cop) for the assessment of the degree of sewage treatment. Typically, sediments that receive untreated sewage effluents show lower ratios (<0.2), while those higher than 0.8 are related to treated (primary or secondary) sewage. All sites (except those where coprostanols were not detected) have presented ratios characteristic of sewage inputs without treatment (Table 2). Even though there is no sewage treatment station in the vicinity, site #7 (Shipyard) presented ratio higher than 0.2.

Sedimentary reduction processes, influenced by microbial communities, especially in recent sediments, form 5α-stanol and ketone, which are thermodynamically more stable than their 5β epimers. In contrast, 5β stanols are dominant in sewage sludge. Thus, the combination of coprostanol levels and 5β/(5β+5α) stanol (coprostanol/(coprostanol+cholestanol)) and 5β/(5β+5α) ketone (5β-coprostanone/(5β-coprostanone+5α-cholestanone)) ratios can help better to elucidate sewage contributions.[5] 5β/(5β+5α) stanol and ketone ratios higher than 0.7 have been reported to indicate sewage contamination, while values lower than 0.3 are indicative of uncontaminated sites.[5] Intermediate values (0.3-0.7) make it difficult to confirm faecal contamination since it may be considered a biogenic input of cholestanol, a diagenetic production of 5α epimers or simply a mixture of different sources of sterols.

The 5β/(5β+5α) isomeric ratios are high at site #2 (Porto Alegre) (Figure 2), indicating, together with

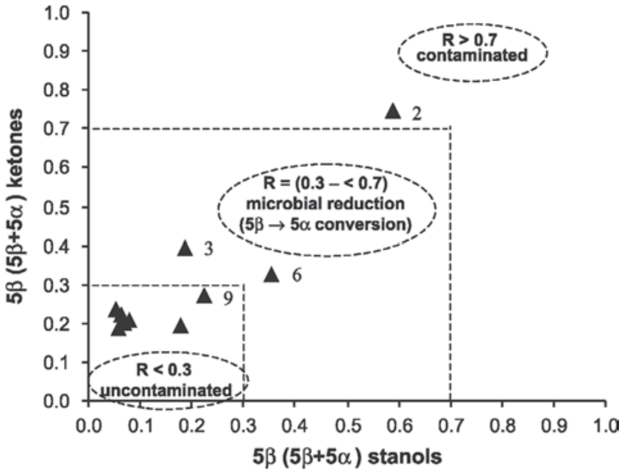


Figure 2. Comparison of the 5β/(5α+5β) stanols and 5β/(5α+5β) ketones ratios for sediment samples from the Patos Lagoon.

coprostanol levels (1,423 ng g^{-1}), that this location is contaminated by sewage. Other sediment samples from the Patos Lagoon do not appear to be contaminated, having very low coprostanol concentrations (<100 ng g^{-1}) and 5β/(5β+5α) isomeric ratios (Figure 2). The dominance of the 5α isomer indicates a predominance of natural reduction processes and/or biogenic inputs over sewage contamination. In addition, the estuarine dynamics around Rio Grande might not allow sterol sedimentation and export these biomarkers to the inner shelf (as detected in sediments from sites #12 and #13), thus keeping sedimentary levels low.

Principal Component Analyses (PCA)

The literature on biomarkers has highlighted the usefulness of multivariate statistical techniques to extract the maximum information from complex mixtures of lipid compounds.[40,41] While individual compounds and ratios using two or more sterols can provide some information regarding the origin of the organic matter in the sediments, multivariate statistical methods are able to extract more information from the data in a single analysis. This more holistic approach can be very useful but care needs to be exercised in explaining the results and some knowledge of the likely origin of the compounds is needed.[42]

The sterol data were subjected to principal component analysis (PCA) for the further investigation of the differences between the sources of the organic matter found in the Patos Lagoon sediments. This analysis was undertaken on the basis of the calculation of the coefficient of variation for each variable. The first two components (PC1, 56.9% and PC2, 32.9%) accounted for 89.8% of the total variance.

The loadings for the variables and the scores of the sites are shown in Figures 3a and 3b. The PC1 axis (PCA loadings) showed a negative correlation with all compounds, especially with 5α-cholestanone, cholestanol and β-sitostanol. The PC2 axis showed a positive correlation with coprostanol, epicoprostanol and 5β-coprostanone (faecal sterols and stanone) and a negative one with cholesterol, campesterol, stigmasterol and β-sitosterol (biogenic sterols) (Figure 3a).

The main discrimination was presented by PC2, which shows positive correlations between faecal sterols (sewage inputs), while the compounds with negative correlations are associated with natural sources (marine or terrestrial organic matter).

The PCA scores distinguished basically between two different groups of samples (Figure 3b). The PC1 scores grouped stations with high (>850 ng g^{-1}) and low (<700 ng g^{-1}) total sterols concentration (Σ-OLs). It possibly showed that the sites with the higher concentrations of Σ-OLs were grouped by negative PC1 scores 1 while the majority of stations had positive scores. On the basis of the PC2, only sites #2 (Porto Alegre) and # 12 (Ocean 1) were distinguishes from the other stations. Site #2 was separated because it is clearly contaminated by sewage

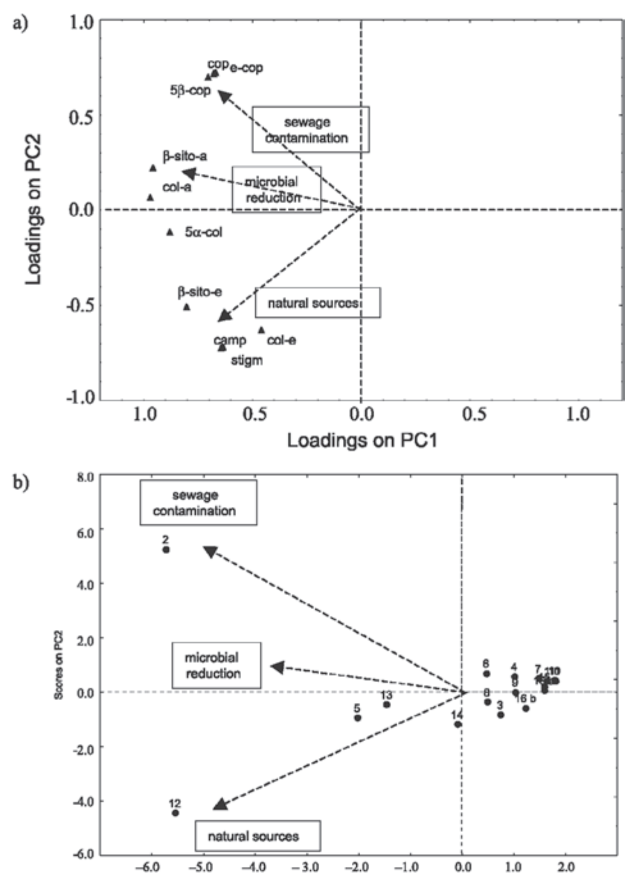


Figure 3. Factor loadings (a) and factor scores (b) of the PCA analysis of the sterols and ketones in surface sediments from the Patos Lagoon.

presenting with high values of faecal sterols and 5β-coprostanone while the higher values of biogenic sterols detected at site #12 explain this separation.

Conclusions

The present work is the first study ever made on the distribution and origin of sterols in the sediments of the Patos Lagoon. The results showed that the sedimentary sterols consisted of a mixture of compounds from both natural and anthropogenic sources.

Coprostanol levels were comparable with the lower to mid-range concentrations reported for coastal sediments worldwide. In the majority of samples, levels were comparatively low (<100 ng g^{-1}), indicating lesser sewage contamination. However, the coprostanol concentration (>1,000 ng g^{-1}) and ratios between selected sterols confirmed sewage contamination in the sediment collected off Porto Alegre.

The e-coprostanol/coprostanol ratio and low epicoprostanol concentration at sites where faecal sterols were detected shows that the wastewater discharge into the Patos Lagoon receives a poorly and inadequate sewage treatment.

Anaerobic microbial reduction at most of the sites is confirmed by the cholestanol / cholesterol and β-sitostanol / β-sitosterol ratios. The PCA analysis shows a strong correlation with 5α-compounds (cholestanol, β-sitostanol and 5α-cholestanone group), suggesting coincident sources and, most probably, the bacterial hydrogenation of 5β-isomers.

Although the sterols and ketones data are related to the sample collection date (1997) and the distribution of these compounds may differ from the present time, it is the author's expectation that this study may contribute to the knowledge of these organic biomarkers in the area studied.

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