http://dx.doi.org/10.5935/0103-5053.20140120

J. Braz. Chem. Soc., Vol. 25, No. 8, 1380-1390, 2014.
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0103 - 5053 $6.00+0.00

Article

Historical Evolution of Organic Matter Accumulation in a Coastal Bay in the SW Atlantic, Brazil: Use of Sterols and *n*-Alcohols as Molecular Markers

Eduardo C. Richard,*[*,a] Cláudia Hamacher,[a] Cássia O. Farias,[a] Marina P. Dore,[a] Natália C. M. Ribeiro,[a] Michelle A. Passos,[a] Plinio F. Martinho,[a] José M. Godoy[b] and Renato S. Carreira[b]

[a]*Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, 4º andar, 20550-900 Rio de Janeiro-RJ, Brazil*

[b]*Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente, 225, 7º andar, 22453-900 Rio de Janeiro-RJ, Brazil*

Os processos de deposição e preservação de matéria orgânica (OM) em sedimentos da Baía de Ilha Grande, sudoeste Atlântico, foram avaliados através de análise elementar (C e N) e de marcadores moleculares (esteróis e *n*-alcoois). Foram analisadas amostras de quatro testemunhos datados, representando diferentes níveis e tipos de influência antrópica. A concentração total de esteróis variou de 71 a 9.320 ng g^{-1}, com predomínio de 24-etil-colesta-5,22-dien-3β-ol, 24-etil-colest-5-en-3β-ol e 24-etil-5α-colest-3β-ol. Entre os *n*-alcoóis, com concentrações totais entre 130 a 15.407 ng g^{-1}, houve predomínio de compostos de cadeia longa (> C_{22}). A confirmação da origem terrestre ou marinha dos marcadores moleculares selecionados foi realizada por análise de componentes principais (PCA). A PCA revelou, ainda, tendência e eventos que influenciaram o acúmulo de OM nas últimas décadas, como o aumento na ocupação humana na região e a remoção de uma floresta de manguezal ocorrida entre 1940 e 1960. A ausência ou baixa contaminação fecal foi revelada através do esterol coprostanol e índices associados.

The deposition processes and preservation of organic matter (OM) in the sediments of Ilha Grande Bay, SW Atlantic, were evaluated based on elemental composition (C and N) and molecular markers (sterols and *n*-alcohols). Samples from four dated sediment cores, representing distinct levels and type of human influence, were analyzed. The concentration of total sterols ranged from 71 to 9,320 ng g^{-1}, with 24-ethyl-cholesta-5,22-dien-3β-ol, 24-ethyl-cholesta-5-en-3β-ol and 24-ethyl-5α-cholesta-3β-ol as the most abundant compounds. The *n*-alcohols, with a total concentration between 130 and 15,407 ng g^{-1}, were dominated by long-chain compounds (> C_{22}). Assignments of the selected markers to terrestrial or marine sources were evaluated using principal component analysis (PCA). The trends and events that influenced the OM accumulation in the last decades were also revealed by the PCA, as the increasing human settlement in the region and the removal of a mangrove forest occurred between 1940 and 1960. The absence or low level of sewage contamination was indicated by the sterol coprostanol and associated indexes.

Keywords: molecular markers, sediment cores, land-use, eutrophication

Introduction

Coastal ecosystems are essential components for understanding the global carbon cycle.[1] These regions have high rates of primary and secondary productions, and the transformations and deposition of organic matter (OM) occurring in these regions are notably more intense than those observed in the continental margin.[2] Because the majority of the global population lives within the coastal zone,[3] human perturbation of coastal ecosystems has being intensified in the recent decades. Eutrophication, increasing hypoxia, land use changes, industrial and domestic wastewaters, fossil fuels and organic and inorganic contaminants, among other biological and physical alterations, are threats to the environmental health of coastal ecosystems throughout the world.[4]

Land plants, seaweeds, phytoplankton, zooplankton and bacteria are important sources that contribute to the

*e-mail: eduardocrichard@gmail.com

total pool of OM in coastal sediments.[5] The wide range of OM sources, in addition to ecosystems dynamics and human perturbation, have all posed a challenge to the understanding of OM geochemistry in the coastal oceans.[1,6] The distinct reactivities of OM according to its source is another relevant factor in this context, whose effect on the preservation of OM in the sedimentary record has been addressed in detail in the last years.[7,8]

Molecular markers are organic compounds whose origin and/or chemical transformation may be related to a particular source of OM, both autochthonous and allochthonous.[9] Lipids are less abundant in OM than proteins and carbohydrates, but they are the most common molecular markers in organic geochemistry because of their source-specificity and better resistance to diagenesis than other organic groups.[10] Different lipid classes such as *n*-alkanes, fatty acids, sterols, *n*-alcohols and many others, have been extensively used as molecular markers to assess the inputs of natural and anthropogenic OM to coastal environments.[11,12] Sterols are hydrophobic molecules that has a tendency to associate with solid phases which, in conjunction with *n*-alcohols, are good tracers of source changes and OM preservation.[5]

Sedimentary profiles have the potential to be natural archives of environmental changes under historical and/or geological time frames.[13] This is possible in places where the sedimentary column structure was not destroyed by physical or biological action and post-depositional diagenesis are (or are considered to be) negligible.[14] There is a relative large literature addressing the history of organic contaminants accumulation in Brazilian coastal systems,[15,16] whereas similar information for OM characterization is comparatively more restricted.[17,18]

In the present work, four sediment cores were collected in the Ilha Grande Bay, SW Atlantic, Brazil (Figure 1) to evaluate the historical evolution, deposition and transformation of OM in this environment. Sedimentation rates, based on ^{210}Pb measures, were used to estimate the age of each sediment layer. Lipid biomarkers (sterols and *n*-alcohols) and elemental composition were used to assign the sources of OM to natural and anthropogenic inputs and to infer post-deposition OM transformation.

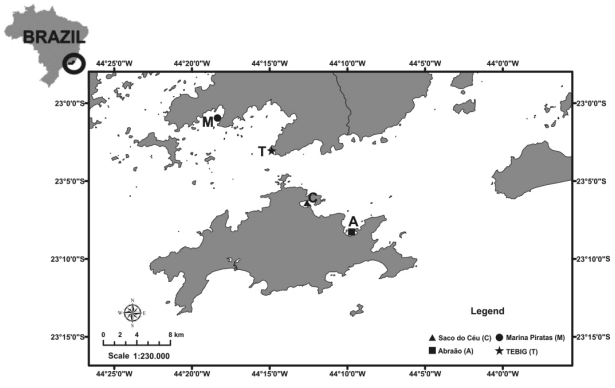

Figure 1. Study area showing station locations in Ilha Grande Bay, Rio de Janeiro, Brazil.

Experimental

Study area

Ilha Grande Bay (IGB) is located in the southern portion of Rio de Janeiro state, SE Brazil (22°50'–23°20' S and 44°00'–44°45' W). With 650 km² of area and 2,300 km² of a drainage basin, the IGB is unique because of its proximity to Serra do Mar, a long mountain ridge that reaches to over 2,000 m.[19] The region has great ecological importance and is composed of two water bodies separated by a large island (Ilha Grande, IG). Two of the most remarkable characteristics of IGB are its highly angled coastline and the presence of small rivers, which cause a sharp variability in freshwater discharge to the system when there are seasonal variations in rainfall. Several coastal ecosystems can be found in the surrounding areas, such as mangroves, coastal lagoons, tidal flats, sandy beaches, rocky shores, etc.

Three sectors were defined for IGB, namely the West Portion, Central Channel and East Portion.[20] In general, the bathymetry within the East and West portions is very smooth and the depth slowly increases seawards.[20] The tidal wave reaches the IGB simultaneously at the West and East portions and is divided by the IG, which might explain the enhanced contribution of tide-related frequencies to water circulation.[21] There is an apparent quasi-steady clockwise circulation around Ilha Grande,[21,22] thus, seawater from the Atlantic Ocean enters the bay from the west, is mixed inside with lower salinity waters inside the bay and its outflux leaves the eastern portion. There is also evidence for communication with Sepetiba Bay,[23] which raises the possibility of organic matter importation from this adjacent region.

Along with industry, tourism and economic growth, the two cities surrounding IGB, namely Angra dos Reis and Paraty, have experienced dramatic population increases during recent decades.[24] This increase is much higher than the one experienced in Rio de Janeiro state as a whole, and it was over two times higher for Paraty and almost four times higher for Angra dos Reis. The increase is even more impressive over a longer period of time; the population in the two cities has grown from 28,256 inhabitants in 1940 to 207,044 in 2010.[25] Notwithstanding the increasing human pressures, the IGB on the whole remains well-preserved.[19]

Sampling

Four sediment cores were collected from IGB using a 1 m long Kullemberg-type sediment profiler (Husky-Duck, Brazil). Sampling points were chosen according to their different types and levels of anthropogenic impact, as follows: core T is near a large oil terminal; core M is located close to Angra dos Reis city center; core A is located at Abraão, Ilha Grande's second largest village and main quay; and core C is in a very pristine location with minimal human occupation. The cores were sliced in 2-cm intervals until 20 cm, and from this depth on, 5-cm intervals were used until the end of each core, except for the cores taken for ^{210}Pb measures, which were sliced in 1-cm intervals.

The sediment cores were analyzed for elemental composition, grain size parameters, radionuclides and molecular markers (sterols and *n*-alcohols). The total organic carbon (OC) and total nitrogen (TN) were determined using a Carlos Erba 1110 Elemental Analyzer. Acetanilide ($C_6H_5NH(COCH_3)$) was used to plot the calibration curve, and precision was determined with certified sediment (PACS-2). Grain size, organic matter and $CaCO_3$ content were determined by weight difference after reacting with H_2O_2 and HCl according to usual methods.[26] Sediment chronology and sedimentation rates were obtained by ^{210}Pb dating method described by Godoy *et al.*.[27] The summarized procedure starts with the leaching of 3 g aliquots with 40 mL of 0.5 mol L^{-1} HBr for two hours at 80 °C. The resulting solution was centrifuged, and the residue was leached with 40 mL of 0.5 mol L^{-1} HBr and 1.0 g hydroxylamine hydrochloride for two hours at 80 °C. A lead carrier was added to the solution, and the mixture was transferred to an ion-exchange column containing Dowex 1X8, 50-100 mesh. This procedure was followed by a cleaning step with 0.5 mol L^{-1} HBr and 1.0 g hydroxylamine hydrochloride and further elution with 1 mol L^{-1} HNO_3. Lead was precipitated as chromate, and the chemical yield was obtained gravimetrically. A two week-period was awaited prior to the concentration of ^{210}Pb, which was determined based on its daughter decay product (^{210}Bi) by beta counting on a ten channel, low level proportional counter (Perkin-Elmer Prof Berthold LB-750). The minimum detectable activity for this technique is 3 Bq kg^{-1} (1 Bq^{-1} for 1 g sample) for 1000 min of counting time.

The ^{210}Pb sediment dating method is based on the measurement of excess or unsupported ^{210}Pb activity, which is incorporated rapidly into the sediment from atmospheric fallout and water column scavenging. Once incorporated into the sediment, unsupported ^{210}Pb decays with time according to its known half-life (22.3 years). The logarithm of the ^{210}Pb concentration *vs.* sediment depth were first plotted, and excess ^{210}Pb was then calculated by subtracting the constant ^{210}Pb value observed in the core bottom.

Reagents and chemicals

The standards of of 5α-androstan-3β-ol (98% purity), 5α-cholestane (99% purity), 5β-cholestan-3β-ol (> 98% minimum purity), 5β-cholestan-3α-ol (> 95% minimum purity), cholest-5-en-3β-ol (94% purity), 5α-cholestan-3β-ol (95% purity), 24-ethylcholest-5,22E-dien-3β-ol (95% purity), 24-methylcholest-5-en-3β-ol (ca. 65% purity) and 24-ethylcholest-5-en-3β-ol (98% purity) were purchased at Sigma Aldrich. Hexanes (95% purity) and dichloromethane (99.9% purity) were supplied by M. Chemicals, while methanol (99.96% purity) was purchased at J. Baker. Sodium sulfate anhydride (> 99% purity) and alumina (aluminum oxide activated and neutral, 150 mesh) were purchased at Sigma Aldrich and silica (silica gel 60, 0.063-0.200 mm) was supplied by Merck. N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) was purchased at Supelco.

Lipid analysis

Sediments were dried at 60 °C and 10 g subsamples were portioned out. Samples and eleven procedure blanks were spiked with the surrogate standard 5α-androstan-3β-ol. The samples were Soxhlet extracted for 24 h with 200 mL of dichloromethane and later concentrated to 1 mL with a rotary evaporator. Concentrated extracts were taken with hexanes prior to clean-up and fractionated by passing them through a glass chromatography column (with 7 g of deactivated aluminum oxide, 10 g of deactivated silica gel, 2 g of copper and 2 g of sodium sulfate). The sterols and *n*-alcohol fraction (F3) was isolated by elution with 50 mL of a mixture of dichloromethane-methanol (9:1, v:v), after isolation of aliphatic hydrocarbons (F1; 30 mL of hexanes) and aromatic hydrocarbons (F2, 75 mL of 1:1 mixture of n-hexane-dichloromethane). The F1 and F2 fractions were not considered in the present study. Prior to gas chromatography/mass spectrometry (GC/MS), the F3 extracts were derivatized into their trimethylsilyl (TMS) derivatives with BSTFA using acetonitrile (CH_3CN) as a catalyst. After that, an internal standard (5α-cholestane, 2500 ng) was added. The fractions were analyzed using a gas chromatography-mass spectrometer (GC/MS; Finnigan Focus DSQ GC/MS system), which was operated at full-scan (*m/z* 50-550), and a VF-5MS column (30 m × 0.25 mm × 0.25 μm film) was used. Quantification was performed using a calibration curve (six points between 100 ng mL^{-1} and 20,000 ng mL^{-1}) with commercial standards

(*n*-C_{18}OH, *n*-C_{19}OH, cholest-5-en-3β-ol, 5β-cholestan-3β-ol, 5α-cholestan-3-one, 5β-cholestan-3-one, 5α-cholestan-3β-ol, 24-methylcholest-5-en-3β-ol, 24-ethylcholest-5,22E-dien-3β-ol and 24-ethylcholest-5-en-3β-ol) and by considering the peak areas of key ions (*m/z* 129 or 215 for sterols and *m/z* 103 for *n*-alcohols) and response factors relative to the internal standard (5α-cholestane, m/z 217). Similar response factors for key ions were assumed for structurally related compounds for which standards were not commercially available. GC/MS component identification was based on a full spectrum scan obtained from the available standards or by comparing them with spectra in the literature from other compounds. The limit of detection (LOD), determined for each compound, was the standard deviation of at least seven replicate analysis of a standard solution with a concentration near the estimated LOD, multiplied by 3 and divided by the mean extracted sediment mass.[28] The limit of quantificationt (LOQ) was the lowest point of the calibration curve divided by the mean extracted sediment mass. The LOD and LOQ obtained for this study was, respectively, 3.00 ng g^{-1} and 10.00 ng g^{-1}.

Samples with recovery out of 50-120% range were disregarded and reextracted. Average recovery of surrogate standard in sediment samples was 84 ± 15%. The recovery of same standard for procedure blanks was 83 ± 14%.

Statistical analysis

Principal component analysis (PCA) was used to identify the dominant factor contributing to the variance in the molecular markers dataset. The following compounds were considered, including the symbols used to represent them in the corresponding PCA figures: the stenols cholest-5-en-3β-ol (27Δ^5), 24-methylcholest-5-en-3β-ol (28Δ^5), 5α-cholestan-3β-ol (27Δ^0), 24-methylcholest-5,22E-dien-3β-ol (28$\Delta^{5,22}$), 24-methylcholest-5,24(28)-dien-3β-ol (28$\Delta^{5,24(28)}$), 24-ethylcholest-5-en-3β-ol (29Δ^5), 24-ethylcholesta-5,22E-dien-3β-ol (29$\Delta^{5,22}$) and 4α,23,24-trimethylcholest-22-en-3β-ol (30Δ^{22}), the stanols 5α-cholestan-3β-ol (27Δ^0), 24-methyl-5α-cholestan-3β-ol (28Δ^0), 24-ethyl-5a-cholestan-3β-ol (29Δ^0) as a single group, phytol and the *n*-alcohols (*n*-C_{14}OH, *n*-C_{16}OH, *n*-C_{18}OH, *n*-C_{22}OH, *n*-C_{24}OH, *n*-C_{26}OH, *n*-C_{28}OH, *n*-C_{30}OH and *n*-C_{32}OH). Prior to PCA analysis, the dry weight concentrations (μg g^{-1}) were normalized by dividing each observation by the sum of all observation of that variable, followed by subtraction of this calculated values by the mean concentration and division by the standard deviation.[29] Varimax rotation was selected to represent the planar projection of the loadings (variables) and scores (samples) for the two principal components, using the Statistica® v7.0 package.

Results

Sedimentation rates and estimated age

The constant flux sedimentation (CF:CS) model was applied to find the sedimentation rates, and the ages were calculated according to the sediment depth and respective sedimentation rate. Profiles for total and excess of ^{210}Pb are available as Supplementary Information (Figure S1). Higher rates were found in M (0.55 ± 0.09 cm yr^{-1}); on the other hand, lower and very similar rates were found for A and T (0.33 ± 0.03 and 0.30 ± 0.04 cm yr^{-1}). The rate could not be found for core C because of surface layer mixing; nevertheless, because A has similar oceanographic features and is close to C, the sedimentation rate for A was also used on C. Thus, the last layer of core A was approximately 245 years old, core C was 260 years old, core T was 235 years old, and core M was only 109 years old. Difference in age of cores A and C is length related. Since dating methods based on ^{210}Pb model is limited to 150 years,[27] the age determination of samples older than that are not reliable. Nevertheless samples with calculated ages older than 150 years should be considered as natural condition for Ilha Grande Bay.

Bulk parameters

Detailed results for the bulk sediment parameters are presented in Table 1, and the complete dataset are presented as Supplementary Information (Tables S1-S3). OC and TN results were used to separate the four cores into group I (cores A and C) and group II (cores T and M). Group I cores are enriched in C and N when compared to group II cores. As shown in Figure 2, cores A and C have OC concentration usually as high as 28 mg g^{-1}, whereas cores T and M show, on average, approximately 10 mg g^{-1} of OC. The same grouping is observed for fine sediment contents, where again, A and C contribute more fine sediments and cores T and M have fewer of these sediments, although some sharp changes can be observed in the M profile.

Along the sedimentary record, cores A and C show very little variation in both fine sediments and OC (Figure 2), although some punctual changes might be observed in the OC profile of core A. In contrast, core T and most of core M had marked variations in both parameters, with fine sediments increasing from the base to the top of core M from approximately 15% to 90%. The contents of fine sediments increased in core T from 40% at core base to 70% at core top. OC also increases in both cores (T and M), while core T has a gradual increase, core M presents OC contents increasing rapidly in the two superficial samples

(from about 7 mg g^{-1} to approximately 20 mg g^{-1}). The total nitrogen levels in all four cores have similar variations to those observed in OC.

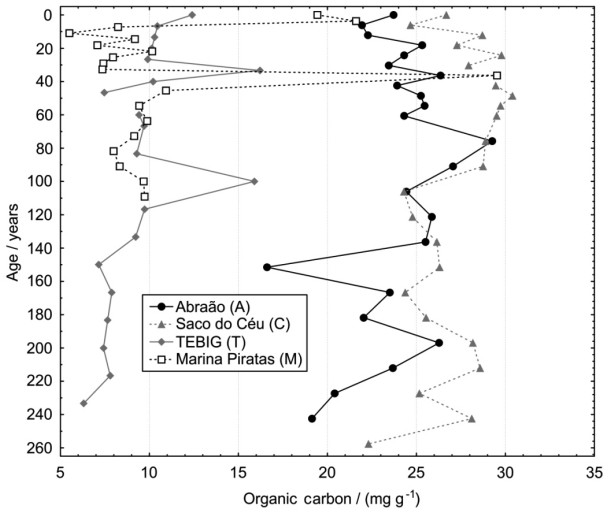

Figure 2. Organic carbon concentrations (mg g^{-1}) in the sediments cores: A: Abraão (black circle); C: Saco do Céu (gray triangle); T: TEBIG (gray diamond) e M: Marina Piratas (open black square).

Molecular markers

Total sterols (Σ sterols) show concentrations (in ng g^{-1}) of 1,441 ± 1,858 in core A, 930 ± 354 in core C, 516 ± 652 in core T and 834 ± 726 in core M. The highest Σ sterols (9,320 ng g^{-1}) were found in sample A02-04, and the lowest value (71 ng g^{-1}) was found in T30-35 (Table 1). The *n*-alcohols are much more abundant in core C than in any other sediment core, with the average total *n*-alcohols concentration reaching 4.39 ± 2.82 ng g^{-1}. In core A, the Σ *n*-alcohol was 2,615 ± 883, with M and T yielding lower concentrations (1,135 ± 370 and 968 ± 800 ng g^{-1}, respectively).

As a general rule, the higher molecular marker concentrations were found in the surface layers and lower concentrations were observed downcore. In fact, sterols were enriched several times in the surface layers, especially at core M, whereas the core C shows little or no enrichment. The *n*-alcohols, however, do not present an up-core enrichment pattern, and core C showed an opposite trend, with decreasing *n*-alcohols concentration towards recently deposited sediments (Table 1).

Sterols containing 29-carbon atom chains were predominant in almost all samples. 24-ethyl-cholesta-5,22-dien-3β-ol, 24-ethyl-cholest-5-en-3β-ol and 24-ethyl-5α-cholest-3β-ol ($29\Delta^{5,22}$, $29\Delta^{5}$ and $29\Delta^{0}$) accounted for at least 33.5 ± 8.8% on average (at core M) and a maximum of 52.9 ± 11.9% (at core C) of the total sterols. Cholest-5-en-3β-ol, 5α-cholestan-3β-ol and 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol ($27\Delta^{5}$, $27\Delta^{0}$ and $30\Delta^{22}$) were also relatively abundant in most samples (Figure 3).

The most abundant *n*-alcohols were the long-chain compounds (LCOH; > C23) with 22 to 30 carbon atoms (Table 1). The sum of *n*-C_{26}OH, *n*-C_{28}OH and *n*-C_{30}OH represented approximately 45.0 ± 11.5% of Σ *n*-alcohols in all the samples; in M30-35, these three alcohols accounted for up to 72% of the total *n*-alcohols. At T and M, where lower total concentrations were found, short-chain *n*-alcohols (SCOH; < C22) were more important compared to the long-chain *n*-alcohols. *n*-C_{20}OH appears to be the main contributor to SCOH. Phytol was found in very small concentrations in all four cores, and only 33 out of a total of 84 samples (39%) exhibited quantifiable amounts of this compound. Only the sample A14-16 had concentration higher than 100 ng g^{-1}. Cores A and T had a modest enrichment trend in *n*-C_{20}OH towards recently deposited sediments, although the trend could not be considered linear.

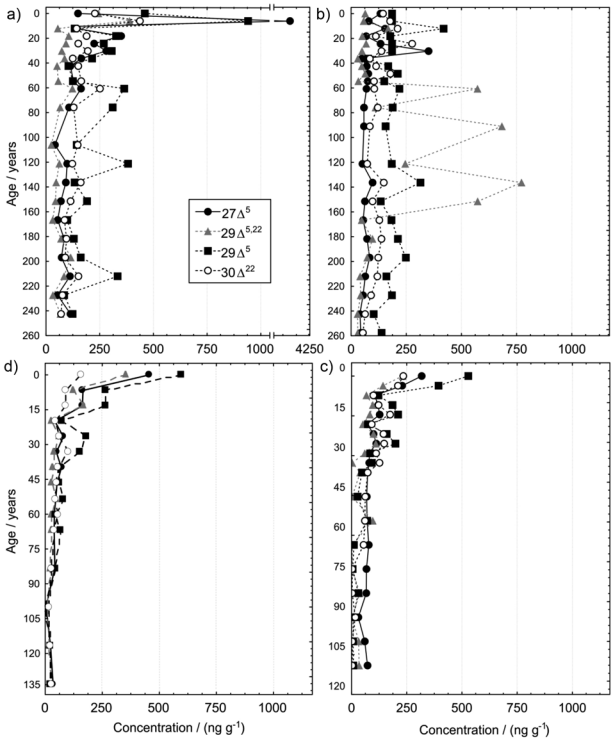

Figure 3. Four majors sterols in the sediment profiles: (a) Abraão; (b) Saco do Céu; (c) TEBIG and (d) Marina Piratas. $27\Delta^{5}$: black circles; $29\Delta^{5,22}$: gray triangle; $29\Delta^{5}$: black squares; $29\Delta^{0}$: open circles.

Discussion

Evolution of sediment bulk geochemistry

Concentrations of OC and TN were in the same range of other cores collected from water bodies along the Brazilian

Table 1. Organic carbon, total nitrogen, C/N ratio, grain size, lipid biomarkers (sterols and *n*-alcohols) concentrations in Ilha Grande Bay sediment cores

	Abraão				Saco do Céu				TEBIG				Marina Piratas			
	$\bar{x}$	σ	Min	Max	$\bar{x}$	σ	Min	Max	$\bar{x}$	σ	Min	Max	$\bar{x}$	σ	Min	Max
OC / (mg g^{-1})	23.7	2.7	16.6	29.3	27.3	2.2	2.2	3.3	9.7	2.6	6.3	16.2	11.0	6.0	5.5	29.5
TN / (mg g^{-1})	2.7	0.3	2.0	3.4	2.8	0.3	22.3	30.4	1.5	0.4	0.9	2.3	1.3	0.7	0.6	3.0
C/N	10.1	0.6	8.8	11.6	11.6	0.6	10.6	13.1	7.5	0.6	6.1	8.5	10.0	3.2	5.9	15.6
% Fine sediment	87.3	13.4	40.2	96.2	91.1	4.1	83.6	96.5	60.4	11.0	39.8	77.0	48.6	26.4	13.3	91.2
Sterols / (ng g^{-1})																
$27\Delta^{5}$	323	882	41	4,158	87	64	40	351	72	97	ND	456	102	66	31	318
$27\Delta^{0\beta}$	20	12	ND	44	12	7	8	37	10	14	ND	67	28	21	ND	87
$28\Delta^{5,22}$	72	146	ND	684	11	33	ND	153	16	43	ND	195	27	33	ND	123
$28\Delta^{5,24(28)}$	28	71	ND	333	19	7	ND	28	11	9	ND	29	5	8	ND	37
$28\Delta^{5}$	80	148	ND	714	36	13	18	75	27	33	ND	155	27	38	ND	130
$29\Delta^{5,22}$	92	83	25	389	172	232	29	772	53	80	10	356	63	59	ND	232
$29\Delta^{5}$	254	192	84	941	190	69	77	419	98	139	ND	597	119	140	< LOQ	529
$27\Delta^{0}$	91	61	30	294	54	23	31	135	51	63	ND	290	100	63	20	286
$28\Delta^{0}$	61	42	22	192	41	13	26	76	29	22	ND	95	51	50	ND	184
$29\Delta^{0}$	168	71	87	357	141	35	89	221	76	72	ND	319	126	105	23	370
$30\Delta^{22}$	156	80	71	437	125	50	54	277	47	36	15	156	92	71	ND	234
Total	1,441	1,858	575	9,320	930	354	448	1,730	516	652	71	3,024	834	726	148	2,800
n-Alcohols / (ng g^{-1})																
$C_{14}OH$	38	29	< LOQ	114	51	32	13	169	49	31	< LOQ	102	33	19	ND	73
$C_{16}OH$	55	72	< LOQ	274	99	174	< LOQ	789	47	57	< LOQ	179	48	56	< LOQ	194
$C_{18}OH$	40	54	< LOQ	210	74	114	< LOQ	508	38	42	< LOQ	115	13	18	< LOQ	70
Phytol	26	22	ND	64	6	13	ND	52	5	9	ND	43	28	28	ND	125
$C_{20}OH$	127	66	< LOQ	312	116	39	< LOQ	197	120	73	< LOQ	283	68	39	ND	140
$C_{22}OH$	160	59	42	264	167	34	119	235	78	34	ND	176	67	34	ND	145
$C_{24}OH$	214	84	15	397	320	115	110	574	90	27	11	137	63	28	31	137
$C_{26}OH$	335	116	55	543	547	233	138	933	143	55	20	316	97	53	20	214
$C_{28}OH$	466	187	168	870	954	605	119	3,014	144	54	< LOQ	275	141	167	24	782
$C_{30}OH$	492	238	172	984	1,257	1,147	87	5,996	157	63	10	272	188	398	10	1,802
$C_{32}OH$	381	248	104	1,040	888	614	45	2,938	133	60	10	246	76	110	< LOQ	510
Total	2,615	883	964	4,485	4,930	2,819	1,702	15,407	1,135	370	130	1,649	968	800	160	3,798

ND: not detected; LOQ: limit of quantification; LOQ = 10 ng g^{-1}.

coast,[16,17,30] although T and M may exhibit lower values. There was a clear separation between the four cores, A and C had higher OC contents and higher proportions of fine (silt and clay) sediments, whereas T and M were OC and fine sediments depleted. The differences are most likely related to different levels of organic matter input to sediments, grain-size and hydrodynamic patterns, which together control the OM input and preservation in the sedimentary record.[8] Fine sediments are usually enriched in OC; thus, there is a not surprisingly significant correlation between these two variables ($r = 0.70$, $p < 0.05$ when all cores are considered).

The C/N ratios between 5.9 and 15.6 indicate a mixture of allochthonous and autochthonous OM sources. Here again, cores A and C are set apart from cores T and M because the former presented higher C/N values. These data indicated that allochthonous OM seems to have greater importance in the areas represented by cores A and C. At core M, the C/N ratio profile was highly variable, with lower values found at the top of the core. Core T, however, showed no sign of changes in the OM source, and the same statement might be applied to core C. The presence of preserved Atlantic rain forest and mangrove areas, together with local depositional basin morphology, could

explain the higher contribution of land plant-derived OM for cores A and mostly C. Falling leaves and twigs are likely an important source of allochthonous OM; nevertheless, soil organic matter (SOM) is probably the main source of allochthonous material. Unlike leaves and twigs, SOM has lower C/N ratios.[31] The elevated precipitation combined with dense forest cover is capable of supporting sediments with soil-derived OM throughout the year.

For samples deposited during the 1960s, it is possible to observe an abrupt alteration in sediment input, with increasing proportions of fine sediments. Since that time, Angra dos Reis has faced a demographic boom related to economic growth. Areas that are now occupied with urban facilities were mangroves or other coastal environments in prior years.[24] The Σ *n*-alcohols in core M presents an isolated peak just a few years prior to the increase in fine sediments and a few years after that, the C/N ratio dropped from 12 to approximately 6. Thus, it is possible that the deforestation of mangroves is registered through the episodic increase in Σ *n*-alcohols [mostly long-chain alcohols (LCOH)], and fine sediments that were retained in mangrove systems were slowly released and reached areas that were inaccessible before. The reduction in mangrove-derived OM is suggested by the diminution of the C/N ratio that approaches phytoplankton-derived OM.[32]

Organic matter sources and degradation process

The sterol distribution in the sediment from the four analyzed cores was very similar, with higher concentrations of 29Δ^5 and 27Δ^5 compared to the other sterols. These two sterols are commonly considered markers of planktonically-derived OM,[5,10] and are therefore the input of autochthonous OM into the studied sediments. However, the dominance of LCOH is an indication of a relevant terrestrial OM addition because these compounds are mainly produced by higher land plants.[33,34]

Stigmasterol (29Δ^{5,22}) is known as a land plant-derived sterol,[35] although its specificity is sporadically questioned.[36] Diatoms and phytoflagellates[37] are also important sources for this sterol. Over the last two to three decades, the major sterol concentrations in T and M grew several times, from approximately 50 ng g^{-1} up to 500 ng g^{-1} (Figure 3), and because no pattern was observed in the 5α(H)stanol/Δ^{5}stenol ratio variation, we believe that this increase in concentration is caused by increasing OM input rather than degradation processes since OC concentrations also increased.[38] Enhanced OM delivery to sediments might be caused by eutrophication related to population growth and an inadequate sewage treatment system.

Although planktonic community assessment is limited for IGB, the dominance of diatoms over other microphytoplanktonic classes has been documented.[39] In addition, dinoflagellates are also quantitatively important and sometimes dominate the microphytoplanktonic community.[40] Such scenario has been observed in Angra dos Reis since the 1970s. Two diatoms commonly found in great abundance, namely *Pseudo-nitzschia seriata* and *Cylindrotheca closterium*, are 27Δ^5 producers and might be important sources of this sterol.[41] Another important diatom called *Skeletonema costatum* produces large amounts of 28Δ$^{5,24(28)}$, which are found only in minor amounts in IGB cores. Nevertheless, nanoplanktonic phytoflagellates are considered to be the most abundant phytoplanktonic group in the IGB,[40] and despite its small cell size,[42] the group is likely to be a very important source of OM in sediments. Two sterols, 29Δ^5 and 29Δ^{5,22}, are between the major sterols of a few phytoflagellates classes,[43] such as *Chlorophyceae* and mostly *Chrysophyceae* species. Therefore green algae classes contribution cannot be neglected because they are sources of C_{29} sterols, which were abundant in sediment samples.[44]

The very low concentration of most SCOH is consistent with the labile characteristics of these planktonic markers.[8] We believe that the planktonic contribution for *n*-alcohols is lessened by the efficient degradation occurring within the water column. The oxic conditions found all around the IGB,[19] favor the aerobic degradation process.

As mentioned earlier, soil organic matter leaching is most likely to be the main source of allochthonous OM in sediments. Thus, refractory terrestrial OM, which is composed primarily of Atlantic forest plant litter, reaches the IGB and resists degradation processes. On the other hand, autochthonous material seems to be affected by these degradation processes. The relative abundance of individual LCOH gives insights about the sources of the allochthonous OM to the studied area. It is noteworthy that in cores A and especially ate core C, the *n*-C_{30}OH is the major long-chain *n*-alcohol (Figure 4). This profile is associated with the presence of C3 land plants,[33] which is consistent with the location of these cores, i.e., in a region

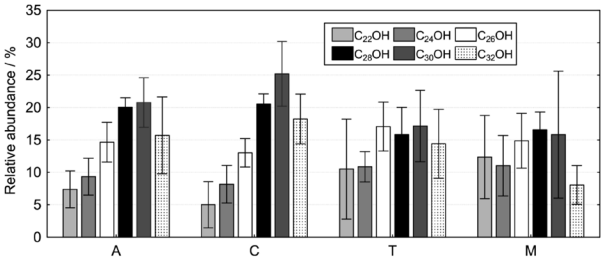

Figure 4. Relative abundance of *n*-alcohols, in % LCOH, for cores: A: Abraão; C: Saco do Céu; T: TEBIG and M: Marina Piratas. Bars represent average and error bars represent standard deviation.

surrounded by mangrove and Atlantic forest, with minimal human interference (in the case of core C).

The evidence provided by lipid biomarkers on the sources of OM is apparently conflicting, as discussed before. Therefore, to appraise the OM portion in the studied sediments, a principal component analysis (PCA) was performed to gain some insight into this subject.

Principal component analysis (PCA)

The PCA analysis resulted in two factors, which explain a total of 57.2% of the molecular markers data variance (Figure 5). Most sterols presented a positive correlation with factor 1 (44.2% of the total variance), with the highest loadings observed for $30\Delta^{22}$, $28\Delta^{5}$, stanols ($27\Delta^{0}$, $28\Delta^{0}$ and $29\Delta^{0}$) and $29\Delta^{5}$. On the other hand, negative loadings on factor 1 were observed for the LCOH, especially the *n*-C_{28}OH, *n*-C_{30}OH and *n*-C_{32}OH. Based on that, factor 1 was able to separate the inputs from terrestrial OM (with negative loadings) with the planktonic OM (with positive loadings). factor 2 (12.4% to total data variance) give additional resolution to separate sterols associated with ambiguous sources (i.e., $29\Delta^{5}$ and $29\Delta^{5,22}$) from sterols from known planktonic sources.[43]

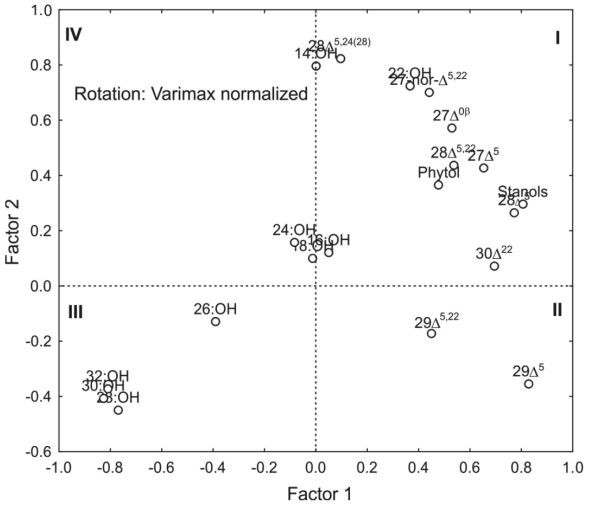

Figure 5. Projections (Varimax rotated) of the variable (lipids) loadings obtained in the PCA analysis considering the four sediment cores collected.

The results of the loadings (Figure 5) suggested four quadrants with respect to OM sources: quadrant I has a strong signal of planktonic-OM; quadrant III is dominated by allochthonous OM; quadrant II is a mixture between autochthonous and allochthonous OM; quadrant IV is non-specific.

The plot of the scores (i.e., samples) from the PCA analysis allowed the assignment of the main sources of OM to each studied core (Figure 6). Samples from core A were distributed roughly between quadrants II and III, although a few samples were found in quadrants I and IV. As a general trend, samples from the base of core A, the oldest ones, were distributed in quadrant III whereas the recently deposited samples from this core were found in quadrant II (Figure 6). This trend suggested a change from a predominance of allochthonous sources in the past to a mixture of sources in the present in the core A. The shift of samples from one quadrant to another matches the 1960s, a time of important population growth all around the IGB.[24] Even though that information about land-use in Ilha Grande is lacking, it is probable that these changes were caused by a reduction of land plant OM input caused by deforestation and an increasing planktonic-OM contribution promoted by continuous nutrient enrichment.[45]

Most samples from core C were placed at quadrant III, suggesting a predominance of allochthonous sources to the bulk of sedimentary OM over the entire period represented by this core (over 200 years). On the other hand, as few samples from core C are found in quadrant II, with maximum loadings in factor 1 observed for the surface samples of this core (Figure 6). This might suggest an increased contribution of planktonic inputs of OM in recent times, but may also be derived from a contribution of $29\Delta^{5,22}$ and $29\Delta^{5}$ from terrestrial sources only at the location of core C.[35]

Core T samples, which present low concentrations of most molecular markers, had a negative correlation with factor 1 for almost all samples collected prior to the 1930s, and after that, the positive correlation with factor 1 increases, with more recent layers located in quadrant II. Because there were low concentrations of most compounds (sterols and *n*-alcohols) in almost all samples at core T, it was concluded that the PCA must be evaluated with caution.

Because core M sample distribution was mostly based on quadrants I and II, this core is considered to be strongly dominated by autochthonous OM. Despite that designation, samples from 1940 to 1960 are scattered throughout quadrants III and IV, and within this period, Angra dos Reis faced several dramatic changes in land use such as the removal of mangrove forests.[24] In the areas around M, the mangrove destruction could have released a large amount of soil and land plant-OM, which would have affected the molecular marker fingerprint of sediments deposited at that time.

Sewage contamination

Coprostanol ($27\Delta^{0\beta}$) is a marker for domestic waste-derived organic matter.[12] It is an sterol produced in the

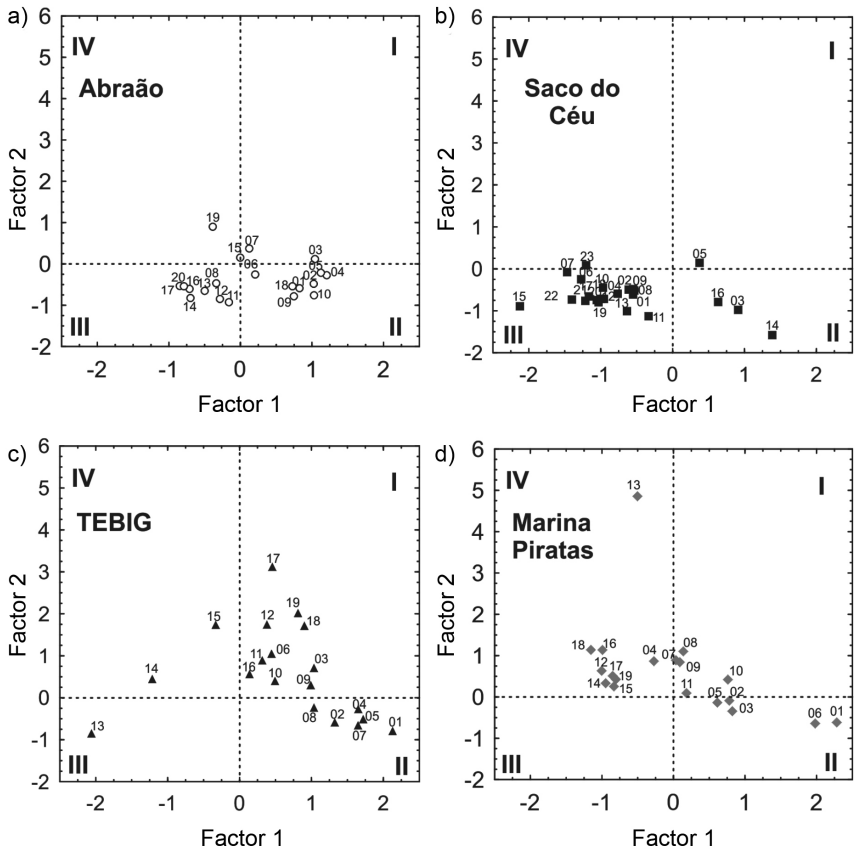

Figure 6. Projections (Varimax rotated) of the scores obtained in PCA analysis. All the samples in the cores A, C, T and M are represented respectively by open circles, black squares, gray diamond and black triangles. Samples of each core were numbered starting at 01 following the order of core depth from top to base layers, where lower numbers represent younger samples and higher represent older ones.

digestive tracts of humans and higher vertebrates by the degradation of cholesterol.[46] The concentration of coprostanol in IGB sediments is much lower than the concentration reported in previous studies of coastal environments along the Brazilian coast (Table 2). These include areas ranging from low (e.g., Ubatuba-SP and Paranaguá-PR bays) to extremely high (e.g., Guanabara and Espírito Santo bays) levels of sewage contamination. The coprostanol concentrations obtained in the present study may be considered, therefore, as typical of coastal bays in Brazil with no contamination by sewage. On the other hand, not all the IGB is free of sewage contamination, as higher concentrations of coprostanol were found near the city of Angra dos Reis (Oliveira, A.C., unpublished material; reference available under request from authors).

Table 2. Coprostanol concentrations in coastal areas from different Brazilian regions

Location	Sedimentary layer / cm	Deposition environment	Concentration range / (μg g^{-1})	Reference
Ilha Grande bay-RJ	0-90	Estuarine-coastal	< 0.01-0.09	Present study
Sepetiba bay-RJ	0-2	Estuarine	0.01-0.42	47
Guanabara bay-RJ	0-3	Estuarine	0.34-40.00	18
Ubatuba bay-SP	0-3	Coastal	0.01-0.27	48
Cubatão area-SP	0-3	Mangrove	4.21-8.32	49
Santos bay-SP	Surperficial	Coastal	< 0.01-8.51	50
Paranaguá bay-PR	0-2	Estuarine	< 0.10-2.22	51
Esp.Santo bay-ES	0-50	Coastal	< 0.01-2.72	52
Recife-PE	0-3	Fluvial-estuarine	0.52-7.31	53
Mundaú Manguaba-PB	0-47	Lagoonal-estuarine	0.15-5.65	54

Conclusions

The four sediment cores could be clearly differentiated into two groups, one with high organic carbon content, high proportions of fine sediments and higher concentrations of molecular markers, whereas the other group is poor in organic carbon, fine sediments and molecular markers. Forest litter is thought to be the major source of organic matter to sediments though planktonic contribution cannot be neglected.

The study has revealed important changes in organic matter delivery and preservation at sedimentary record during the last century. Eutrophication and deforestation evidences were found at cores analyzed demonstrating the relevance of analyzing sediment cores.

Despite the population growth experienced in the last century, fecal contamination is still a minor issue for IGB sediment pollution; nevertheless, all efforts should be made to avoid an increase in pollution levels.

Brazilian coastline occupation has experienced major growth over the last decades. Therefore, studies aiming the comprehension of its fingerprints at sedimentary record are of great value for further application elsewhere along Brazilian coast.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

We thank FAPERJ for financial support for the project. ECR was a MSc student granted by a scholarship from CNPq. The authors also thank the comments provided by three reviewers. RSC is a research fellow of CNPq (proc. No. 303399/2010-4).

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Submitted on: November 22, 2013
Published online: May 27, 2014