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### Geomicrobiology and Biochemical Composition of Two Sediment Cores from Jurujuba Sound - Guanabara Bay – SE Brazil

Geomicrobiologia e Composição Bioquímica de Dois Testemunhos da Enseada de  
Jurujuba - Baía de Guanabara – SE Brasil

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#### Resumo

O objetivo deste trabalho foi quantificar os biopolímeros associados as enzimas esterase e identificar a atividade respiratória das bactérias em dois testemunhos amostrados na Enseada de Jurujuba, Baía de Guanabara, Rio de Janeiro. A concentração dos biopolímeros foram dez vezes menos do que o relatado na literatura, tornando necessária a criação e estabelecimento de novos índices dos níveis de eutrofização, compatível com os sistemas costeiros brasileiros. A relação representante bioquímicas dos testemunhos foi equivalente aos dados disponíveis para ambientes costeiros marinhos. A quantidade de enzimas esterases no sedimento mostrou que a mineralização de biopolímeros está em processo contínuo, mesmo com a preferência do metabolismo anaeróbico. Apesar do fato, dos estudos em geomicrobiologia serem incipientes, os resultados deste estudo indicam a possível aplicação da microbiologia para proporcionar uma melhor compreensão dos processos geoquímicos em ambiente tropical.

**Palavras chaves:** análises bioquímicas; análises enzimáticas; análises microbiológicas; geomicrobiologia

#### Abstract

The aim of this work was to quantify the biopolymers associated to esterase enzymes and identify the bacterial respiratory activity in two cores collected in Jurujuba Sound, Guanabara Bay, Rio de Janeiro State. Biopolymer concentrations were ten times less than reported in the literature, rendering necessary the creation and establishment of new eutrophication levels indices more compatible with Brazilian coastal systems. The biochemical representative relationship in the cores was equivalent to the available data for coastal marine environments. The amount of esterase enzymes in the sediment proved that the mineralization of biopolymers is in continuous process, even with preferentially anaerobic metabolism. Despite the fact that geomicrobiology studies are incipient, this study's results indicate the possible application of microbiology for providing a better understanding of the geochemical processes in tropical environment.

**Keywords:** biochemical analysis; enzymatic activity; microbiological analysis; geomicrobiology

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### 1 Introduction

Estuaries are among the world's most productive systems, and therefore autochthonous biological production is a major source of organic materials to sediments. However, estuarine sediments are also preferential sites of accumulation of particles of marine and terrestrial origin (Mayer *et al.*, 1988; Cifuentes, 1991). In addition, in anthropised estuaries, waste inputs may strongly influence the amount and quality of materials arriving at sediments. Therefore, the abundance and composition of organic materials in estuarine sediments depend on a complex combination of factors, which involves sources and physicochemical processes occurring in overlying water layers and in the sediment itself.

Formation of sediments in the marine environment which contain substantial amounts of organic matter consists in varied and complex processes involving multiple-source parameters from terrestrial and marine ecosystems. Initially this organic matter consists of all the major classes of naturally occurring organic compounds such as sugars, amino acids, pigments, phenolic substances, lipids, polypeptides, polysaccharides, and other constituents of living organisms. During the sedimentation process, only a small portion of the initial organic matter reaches the bottom (Premuzic *et al.*, 1982). The survival of organic compounds during sedimentation depends on a number of parameters including their chemical stability, biochemical usefulness, oxygen concentration and their interaction with clay minerals. After sedimentation, organic particles are equally subjected to a continuous degradation and mixing process, at the same time that the deposition of other materials continues (Colombo *et al.*, 1996; Cowie & Hedges, 1992; Danovaro *et al.*, 1999; Fiordelmondo & Pusceddu, 2004). Organic compounds and clays processing through and subsequently settling in a low-oxygen environment will accumulate and form sediment-enriched unoxidised organic matter (Demaison & Moore, 1980). Finally, although a part of the settled organic matter may return to the water column, a fraction will remain as a sedimentary record (Tselepides *et al.*, 2000).

Marine sediments are also intensively colonized by microorganisms (bacteria, cyanobacteria, fungi, algae; size  $\ll 150 \mu\text{m}$ ). Most are organized in biofilms, complex associations of microbes immobilized on surfaces and embedded

in an extracellular organic matrix, consisting of extracellular polymeric substances (EPS) secreted by the cells. Through their organization in biofilms, organisms create their own microhabitats with pronounced gradients of biological and chemical parameters. Along these gradients they can use substrates and energy effectively (Meyer-Reil, 1994). Microorganisms are present in sediments in high numbers (about  $10^{10}$  cells  $\text{g}^{-1}$  d.w.). Their biomass is greater than the biomass of all other benthic organisms. The cell surface of microbes by far exceeds that of all other organisms. Microbes possess a high surface-to-volume ratio, indicating their high metabolic activity rates. Dissolved inorganic and organic substrates can be metabolized with high substrate affinity and specificity. Particulate organic matter can be decomposed in close contact with the substrate by hydrolytic enzymes. Besides oxygen, microbes may use alternative electron acceptors (nitrate, manganese, iron, sulfate, and carbon dioxide) for the oxidation of organic material. Combined with their logarithmic growth and short generation times microbes possess a high metabolic potential (Demaison & Moore, 1980; Relexans *et al.*, 1992; Meyer-Reil & Koster, 2000).

The aim of this work was to characterise organic matter, total organic carbon, sulfur, biopolymers and grain size of two sediment cores from Jurujuba Sound, establishing the relationship of these parameters to the metabolism and enzymatic bacterial activity.

### 2 Environmental Setting

Guanabara Bay is located in Rio de Janeiro State – Southeast Brazil, between  $22^{\circ}40'$  and  $23^{\circ}00'$  S latitude and  $043^{\circ}00'$ – $043^{\circ}18'$  W longitude. It is one of the largest bays on the Brazilian coastline and has an area of approximately  $384 \text{ km}^2$ , including its islands (Kjerfve *et al.*, 1997). The Bay has a complex bathymetry with a relatively flat central channel, which is 400 m wide, stretches more than 5 km into the bay, and is defined by the 30-m isobath. The bay's deepest point (58 m) is located within this channel (Kjerfve *et al.*, 1997). According to the same authors, north of the Rio de Janeiro - Niterói Bridge the channel loses its characteristic features, as the bay rapidly becomes shallower, with an average depth of 5.7 m, due to the high rates of sedimentation, accelerated in the past century by anthropogenic activities in the catchment area.

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The drainage basin of Guanabara Bay has an area of 4,080 km<sup>2</sup> and consists of 32 separate sub-watersheds (Kjerfve *et al.*, 1997). However, only six of the rivers are responsible for 85% (JICA, 1994) of the 100 m<sup>3</sup> s<sup>-1</sup> of the total mean annual freshwater input. Nowadays, 11 million inhabitants live in the greater Rio de Janeiro metropolitan area, which discharges tons of untreated sewage directly into the bay. Brazil's second largest industrial site is found in this area. There are more than 12,000 industries located in the drainage basin, which account for 25% of the organic pollution released into the Bay (FEEMA, 1990). The bay also hosts two oil refineries along its shore, which process 7% of the national oil. At least 2,000 commercial ships dock in the port of Rio de Janeiro every year, making it the second biggest harbour in Brazil. The bay is also the homeport to two naval bases, a shipyard, and a large number of ferries, fishing boats, and yachts (Kjerfve *et al.*, 1997).

Jurujuba Sound is located in Niterói, on the eastern margin of Guanabara Bay. The sound ranges in depth from 5 to 7 m at its entrance to 3 to 4 m at the centre. Since the linking of Niterói to Rio de Janeiro by a road bridge in 1974, there have been extensive deforestation and rapid urbanization of the catchment which triggered widespread erosion. A further unfortunate side-effect of this urbanization has been the widespread, uncontrolled discharge of untreated sewage and street runoff directly into the nearshore environment (Baptista-Neto *et al.*, 2000).

### 3 Material and Methods

In July and October 2005, two sediment cores varied in length from 2.45 m (T1, 22°55'25.6"S and 043°06'34.6"W) to 2.15 m (T2, 22°55'11.2"S and 043°06'11.9"W) were collected in Jurujuba Sound, Guanabara Bay, Rio de Janeiro State, SE Brazil (Figure 1). The sampled cores were sliced into the following intervals (T1: 0-10, 30-35, 70-75, 100-105 and 240-245 cm; T2: 0-10, 30-35, 40-45, 50-55, 70-80, 100-110, 140-145 and 210-215 cm). These samples were stored in sealed polythene bags, conditioned in ice and taken to the laboratory, where the following analyses were carried out.

The particle size analyses were carried out using a laser particle size analyzer CILAS1064L, after organic matter destruction, and classified according to the textural classification proposed by Flemming (2000) at Table 1.



Figure 1 Location of the study area, Jurujuba Sound, with location of the cores (Guanabara Bay – Rio de Janeiro – SE Brazil).

Code	Textural Class	Code	Textural Class
S	Sand	D-I	Extremely silty slightly sandy mud
A-I	Slightly silty sand	D-II	Very silty slightly sandy mud
A-II	Slightly clayey sand	D-III	Silty slightly sandy mud
B-I	Very silty sand	D-IV	Clayey slightly sandy mud
B-II	Silty sand	D-V	Very clayey slightly sandy mud
B-III	Clayey sand	D-VI	Extremely clayey slightly sandy mud
B-IV	Very clayey sand	E-I	Silt
C-I	Extremely silty sandy mud	E-II	Slightly clayey silt
C-II	Very silty sandy mud	E-III	Clayey silt
C-III	Silty sandy mud	E-IV	Silty clay
C-IV	Clayey sandy mud	E-V	Slightly silty clay
C-V	Very clayey sandy mud	E-VI	Clay
C-VI	Extremely clayey sandy mud		

Table 1 Letter-number codes and descriptive terminology for the 25 textural classes of the ternary diagram for a revised textural classification of hydrodynamic subdivisions on the basis of sand/silt/clay ratios.

Following acidification to remove carbonates, the elementary tests (TOC and S) were made for all samples with a LECO SC 144 device. The adopted methods were ASTM D 4239 (American Society for Testing and Materials - ASTM, 2008) and NCEA-C-1282 (United States Environmental Protection Agency-US EPA, 2002).

Protein (PRT) analyses were carried out after extraction with NaOH (0.5 M, 4 h) and were determined according to Hartree (1972), modified by Rice (1982) to compensate for phenol interference. Concentrations are reported as albumin equivalents. Carbohydrates (CHO) were analysed according to Gerchacov & Hachter (1972) and expressed as glucose equivalents. The method is based on the same principle as the widely used method of Dubois *et al.* (1956), but is specifically adapted for carbohydrate determination in sediments. Lipids (LIP) were extracted by direct elution with chloroform and methanol and analysed according to Marsh & Wenstein (1966). Lipid concentrations are reported

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as tripalmitine equivalents. For each biochemical analysis, blanks were made with the same sediment samples previously treated in a muffle furnace (450°C, 2 h). All analyses were carried out in 3–5 replicates. Protein, carbohydrate and lipid concentrations were converted to carbon equivalents by using the following conversion factors: 0.49, 0.40 and 0.75  $\mu\text{g}$  of C  $\mu\text{g}^{-1}$ , respectively. The sum of protein, carbohydrate and lipid carbon was referred to as biopolymeric carbon (BPC) (Fabiano *et al.*, 1995).

Esterase enzyme activity was analysed according to Stubberfield & Shaw (1990). It is based on fluorogenic compounds which are enzymatically transformed into fluorescent products that can be quantified using spectrophotometric assay. These enzymes act on biopolymers and transform them into low-molecular-weight organic carbon. The results are given in  $\mu\text{g}$  fluorescein/h/g of sediment.

Electron transport system activity was done using the Trevors (1984) and Hourri-Davignon & Relexans (1989) methods, based on dehydrogenase enzyme activities. These enzymes provide equivalents for ATP synthesis (third phosphate adenosine) in the electron transport systems. Results from this assay are given in  $\mu\text{L O}_2$ /h/g of sediment.

The metabolic bacterial activity such as aerobic, facultative anaerobic, denitrification and sulfate-reduction, was analysed using methodology described by Alef & Nannipieri (1995) adapted in Silva *et al.* (2008).

The most probable number (MPN) method was used to estimate abundances of total coliforms (TC) and faecal coliforms (FC). A sample of sediment from each sample was weighed aseptically and serial dilutions were prepared using phosphate buffered water. The MPN method used ten dilution series with five tubes in each dilution. Dilutions were inoculated in different culture media according to the target microbial indicator. The number of positive results from each dilution series was recorded and converted to MPN using a standard reference table (APHA, 1995). The 95% confidence intervals for each MPN value were also obtained. Final results for all indicators were reported as MPN per 1g of dry sediment. Colony counts of heterotrophic bacteria (HB) were incubated on the plates at 35°C for 48 h. Colony counts were converted into CFU/g (APHA, 1995).

HBC – heterotrophic bacteria were enumerated by epifluorescent microscopy (Axiosp 1, Zeiss, triple filter Texas Red – DAPI – fluorescein isotiocyanate, 1,000 X magnification) and using the fluorochrome fluorescein diacetate and UV-radiation (Kepner & Pratt, 1994). Carbon biomass ( $\mu\text{g C/g}$ ) data were obtained using the method described by Carlucci *et al.* (1986).

The statistic analyses utilized the core sediment samples and the parameters. Ward's method with city-block (Manhattan) distance differs from all other methods because it uses an analysis of variance approach to evaluate the distances between clusters. In short, this method attempts to minimize the sum of squares (SS) of any two (hypothetical) clusters that can be formed at each step. This distance is simply the average difference across dimensions. In most cases, this distance measurement yields results similar to the simple Euclidean distance. However, note that in this measurement, the effect of single large differences (outliers) is dampened (since they are not squared). The analyses were performed on all analyses used in this study.

## 4 Results

The core T1, 245 cm long, is formed by a black fluid mud from the top to a depth of 20 cm and a grey mud below this depth. A compact grey mud with rare presence of shell fragments were found before about 50 cm depth. The presence of shell fragments occurs between 50 to 245 cm depth. The core T2, with length of 215 cm, was also formed by very black fluid mud from the top to a depth of 30 cm. A bioturbation process seems to occur at 35 cm and more compact grey mud occurs between 35 to 50 cm depth. A dark-green inclined mud layer occurs between 50 and 60 cm in the middle of the light gray mud. Plan-parallel laminations occurs from under this depth to the bottom of the core (Figure 2).

The core sediment samples ranged from clay to sand, sedimentary textures comprised from 79.76 to 85.63% of silt, 7.9 to 10.35% of clay and 4.46 to 11.46% of sand. In core T1, silt average was  $84.13 \pm 0.11\%$ , clay average  $8.9 \pm 0.51\%$ , and sand average  $6.96 \pm 0.48\%$ . In core 2, averages were  $83.02 \pm 2.42\%$  for silt,  $9.39 \pm 0.87\%$  for clay and  $7.58 \pm 3.12\%$  for sand.



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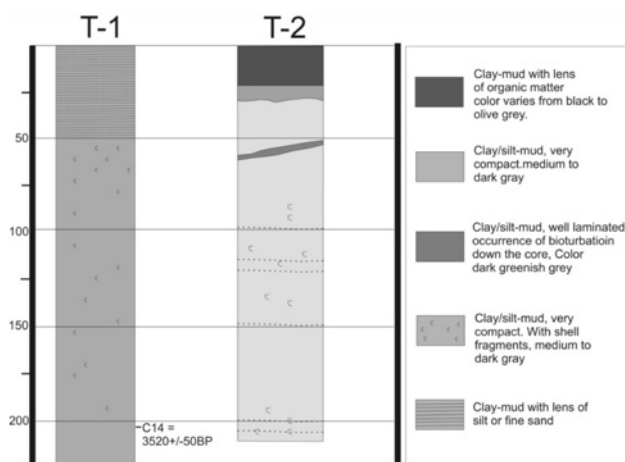


Figure 2 Description of the cores T1 and T2 from Jurujuba Sound, RJ – Brazil.

According to the classification by Flemming (2000) (Table 1), the samples from the cores were classified as very silty slightly sandy mud (DII) and slightly clayey silt (EII) (Figure 3).

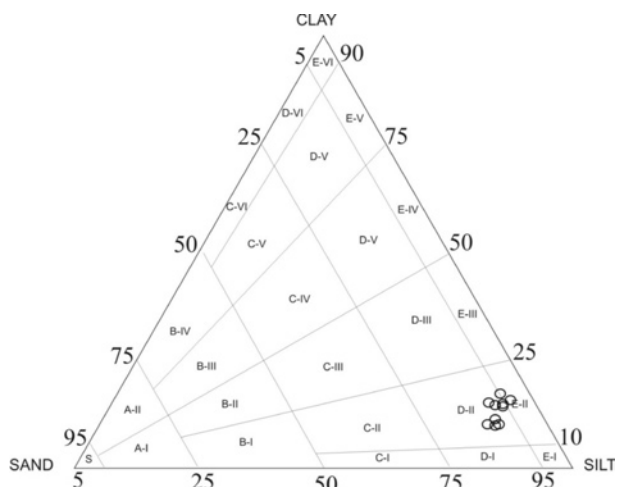


Figure 3 Particle size distributions in the cores sediments (T1 and T2) from Jurujuba Sound in Guanabara Bay, based on the classification proposed by Flemming (2000).

The total organic carbon (TOC) ranged from 1.71 to 5.04%, the sulfur (S) from 0.54 to 1.35%. In core 1, TOC was  $3.18 \pm 1.69\%$  and S  $0.72 \pm 0.15\%$ . In core 2, the values were  $2.86 \pm 1.16\%$  for TOC and  $0.86 \pm 0.32\%$  for S. The C:S ratio ranged between 2.20 to 6.94. the core T1 (slide 240-245, on bottom) and the slide 40-45(at core T2) have ratios below 3 (Figure 4). In both cores, total organic carbon and sulfur had a decrease tendency with the depth (Figure 5).

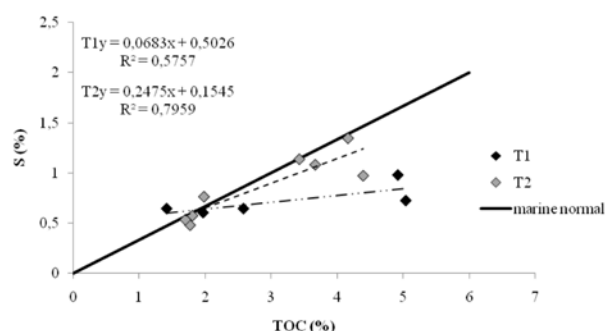


Figure 4 Distribution of C:S ratio determined in the cores sediments (T1 and T2) from Jurujuba Sound. The solid line represents marine normal ratio of  $(2.8 \pm 1.5)$  and the dotted line represents the trends of the samples.

The carbohydrates in the sediment core T1 ranged between 280 and 855  $\mu\text{g/g}$ . The concentration was the same at the center of the core and decreased between 240 – 245 cm (Figure 5). A decrease in carbohydrates was observed at the top of core T2 (0-10 cm), where the average concentration was  $446 \pm 124.72 \mu\text{g/g}$  below 10 cm of depth (Figure 5).

Protein concentrations in core T1 ranged from 264  $\mu\text{g/g}$  in the bottom to 401  $\mu\text{g/g}$  on top, with an average value of  $333 \pm 333.72 \mu\text{g/g}$ . The protein profile in the sediment core T2 had a strong decreasing trend and the concentration in the first 10 cm was two times higher than at the top of core T1 (Figure 5).

Lipid concentrations in core T1 ranged from 192  $\mu\text{g/g}$  at the top (0-10 cm) to the bottom value of 20  $\mu\text{g/g}$  (240-245 cm), with an average concentration of  $80 \pm 71.10 \mu\text{g/g}$  (Figure 5). Core T2 showed a decreasing trend in two steps, the first from the top to 40-45 cm, and the second from 50-55 cm to the bottom of the core. The average lipid concentration in core T2 was  $90 \pm 99.75 \mu\text{g/g}$  (Figure 5).

The highest values for biopolymeric organic carbon were observed at the top of both cores, 660 and 802  $\mu\text{gC/g}$ , in T1 and T2, respectively (Figure 5). The average concentrations were  $447 \pm 148.91$  and  $445 \pm 160.31 \mu\text{gC/g}$  in cores T1 and T2, respectively.

The PTN:CHO ratio ranged between 0.39 to  $1.24 \pm 0.25$  and CHO:TOC ratio ranged between 1 to  $3.15 \pm 0.74$  (Figure 5).

The biochemistry representation of biopolymers in core T1 was 57% for carbohydrates, followed by proteins (36%) and lipids (9%). The

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same representation was found in core T2, except for the content of proteins (43%), which was as high as the carbohydrates (49%), followed by lipids (8%).

The esterase enzymes were responsible for the breakdown of biopolymers. The enzymatic activity showed a decreasing trend from the top of core T1 (4316 µg fluorescein/h/g) to the bottom (0.73 µg fluorescein/h/g), with an average of 1.74 µg fluorescein/h/g (Table 2). The enzymatic activities in core T2 were not as high as in core T1 and showed the same decreasing trend in two steps of lipid profile. The activity of the transporting electron system was not detected at any depth of either core.

The bacterial respiratory activity was representative of the organic matter degradation present at each depth of both cores, and was indicative of bacterial physiology and metabolism. Aerobic and anaerobic processes, like fermentation, sulfate-reduction and denitrification, occurred in core T1 from the top to 35 cm depth. From the top to 105 cm depth facultative aerobic bacteria were found in this core (Table 2). In core T2, anaerobic processes occurred at all depths. Aerobic and fermentative processes started at 30-45 cm to the depth of the core and sulfate-reduction was not detected below 100-105 cm depth (Table 2).

Total coliforms (TC) ranged from  $0.001 \times 10^3$  to  $5 \times 10^3$  MPN/g, faecal coliforms (FC) were not detected. In core T2, TC ranged from  $0.008 \times 10^3$  to  $9 \times 10^3$  MPN/g. FC was detected from the top to 40-

45 cm depth at the same concentration of 0.2 MPN/g (Table 2).

The number of viable cells that were metabolically actable in the environment varied between  $4 \times 10^5$  and  $3 \times 10^6$  cell/g in core T1. In core T2, cell number ranged from  $5 \times 10^5$  to  $1 \times 10^6$  cell/g, with a higher number of viable bacteria from the depth of 70-80 cm. The viable bacterial carbon (BC) ranged between 0.004 and 0.036 µg/g in core T1, with an average of  $0.016 \pm 0.01$  µg/g. In core T2, it varied from 0.005 to 0.051 µg/g, with an average of  $0.029 \pm 0.01$  µg/g (Table 2).

Negative correlations ( $p < 0.005$ ;  $n = 13$ ) was observed between depth and some parameters (TOC, S, PTN, LIP, EST and TC). These parameters had a significant and positive correlation too (Table 3). The analyses of core samples utilizing Ward's method and Manhattan distance yielded two groups. Mainly distinguished by depth. One group was formed only by the samples from the top of cores. The other group contain the samples below 70cm of cores T1 and T2, respectively (Figure 6).

### 5 Discussion

Grain size was characterized according to the Flemming classification (2000) in two groups: very silty slightly sand mud and slightly clayey silt. Baptista-Neto *et al.* (2000) showed that sediment characteristics indicate clear differences in the sedimentary dynamics of Jurujuba Sound, which can

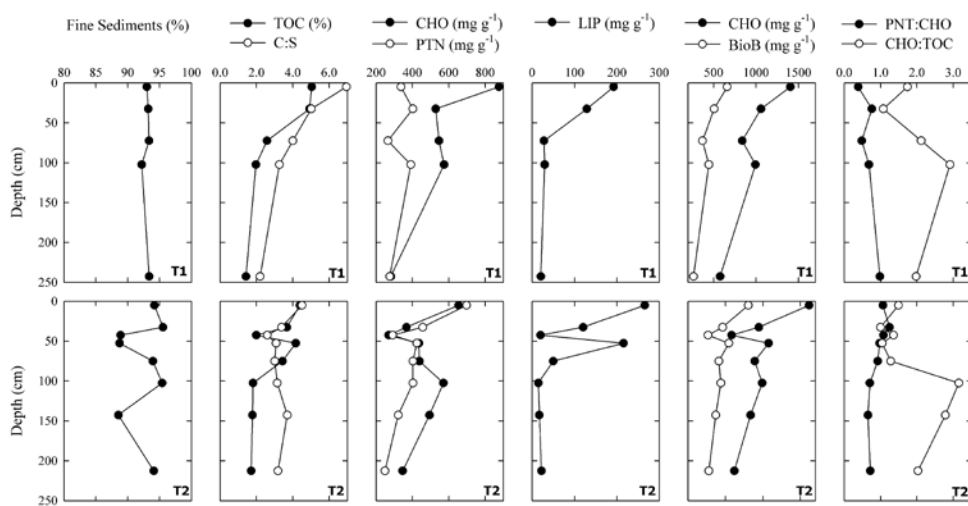


Figure 5 Profiles of the core T1 and T2 of total organic carbon (TOC), C:S Ratio, Carbohydrate (CHO), Protein (PTN), Lipid (LIP), Total biopolymers (TB) and Biopolymeric Carbon (BioB).

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Core	Sample (cm)	BC (ug/g)	TC (MPN/g)	FC (MPN/g)	MBA				EST (ug fluorescein/h/g)
					A	F	DN	SR	
T1	0-10	0.013	5x10 <sup>3</sup>	ND	P	P	P	P	4316.13
	30-35	0.036	0.9x10 <sup>3</sup>	ND	P	P	ND	P	2.40
	70-75	0.013	0.02x10 <sup>3</sup>	ND	P	P	ND	ND	2.37
	100-105	0.012	0.16x10 <sup>3</sup>	ND	P	P	ND	ND	1.46
	240-245	0.004	0.001x10 <sup>3</sup>	ND	P	ND	ND	ND	0.73
T2	0-10	0.019	9.0x10 <sup>3</sup>	0.2	ND	ND	P	P	5.49
	30-35	0.023	3.0x10 <sup>3</sup>	0.2	V	V	P	P	1.92
	40-45	0.015	0.9x10 <sup>3</sup>	0.2	V	V	P	ND	1.44
	50-55	0.005	0.9x10 <sup>3</sup>	ND	P	P	P	V	3.39
	70-80	0.051	1.7x10 <sup>3</sup>	ND	P	P	P	V	3.46
	100-105	0.043	0.09x10 <sup>3</sup>	ND	P	P	P	ND	0.82
	140-145	0.037	0.024x10 <sup>3</sup>	ND	P	P	V	ND	0.13
	210-215	0.040	0.008x10 <sup>3</sup>	ND	P	P	ND	ND	0.00

Table 2 Bacterial carbon, total coliform, faecal coliform, metabolism bacterial activity and esterase activity in T1 and T2 core samples from Jurujuba Sound. BC – bacterial carbon; TC – total coliforms; FC – faecal coliforms; MBA – metabolism bacterial activity; EST – esterase enzymes; A – aerobic bacteria; F – facultative anaerobic bacteria; DN – denitrification; SR – sulfate reduction; P – positive; V – variable; ND – not detected.

	Depth	TOC	S	CHO	PTN	LIP	EST	BC	TC	FC	TOC:S	PNT:CHO	CHO:TOC
Depth	1.00												
TOC	-0.93	1.00											
S	-0.63	0.71	1.00										
CHO	-0.40	0.48	-0.12	1.00									
PTN	-0.55	0.58	0.63	0.32	1.00								
LIP	-0.73	0.84	0.72	0.37	0.57	1.00							
EST	-0.81	0.91	0.72	0.54	0.57	0.84	1.00						
COB	0.08	-0.08	-0.13	0.01	0.15	-0.27	-0.16	1.00					
TC	-0.87	0.84	0.69	0.38	0.76	0.71	0.82	0.04	1.00				
FC	-0.54	0.24	0.34	-0.20	0.39	0.20	0.15	0.00	0.54	1.00			
TOC:S	-0.58	0.60	-0.06	0.73	0.15	0.47	0.44	0.07	0.36	0.00	1.00		
PNT:CHO	-0.20	0.05	0.57	-0.59	0.43	0.18	0.01	-0.04	0.32	0.73	-0.47	1.00	
CHO:TOC	0.58	-0.62	-0.90	0.36	-0.47	-0.64	-0.51	0.06	-0.60	-0.44	0.02	-0.66	1.00

Table 3 Spearman Correlation Coefficients between analyzed parameters in 13 samples (TOC – total organic carbon; C:S – Ratio; CHO – Carbohydrate; PTN - Protein; LIP - Lipid; BC – bacterial carbon; TC – total coliforms; FC – faecal coliforms; EST – esterase enzymes).

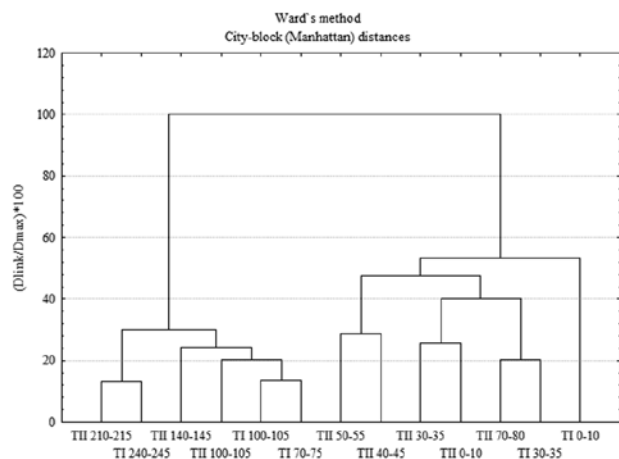


Figure 6 Cluster of samples using Ward's method and Manhattan distance.

be used to divide it into four distinct zones. The cores were sampled in zone three, classified as protected from waves and tidal current action, with very low-energy hydrodynamics characterized mainly by clays. Baptista-Neto *et al.* (2006) showed that Guanabara

Bay is dominated by organic-muddy sediments. Jurujuba Sound is similar to other Guanabara Bay areas, where the sediments are primarily clayed-silt and silt-clays deposited as a function of the SSW waves and the tidal current, characterized by the presence of muddy sediments (Kjerfve *et al.*, 1997; Quaresma *et al.*, 2000; Catanzaro *et al.*, 2004; Baptista-Neto *et al.*, 2006).

In cores T1 and T2, the TOC ranged from 1.42 to 5.04%, which are similar to surface sediments values found in the literature. Eichler *et al.* (2003) analyzed 26 superficial sediment samples all over Guanabara Bay and found values of TOC from 0.018 to 5.763%, with the highest values found in areas of lower circulation. Vilela *et al.* (2003) found values of TOC ranging from 0.05 to 4.81% in 42 superficial sediment samples from Guanabara Bay. Mendonça-Filho *et al.* (2003) found TOC varying between 0.04 and 6.1% in 25 superficial sediment samples from Guanabara Bay and 4.14% in Jurujuba Sound. The same authors suggested that the superficial sediment with TOC similar to 4%

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characterize a desoxic-anoxic environment with a high preservation tax in Guanabara Bay. Burone *et al.* (2003) found TOC values between 0.12 and 2.81% in Ubatuba Bay. Higher values (>2%) were found in the water column under 5-10 m, where the silt grain size predominated.

Sulfur ranged between 0.48 and 1.35%. These values were higher than the values found in 38 samples from Ubatuba Bay – São Paulo State (0.01-0.48%). Values higher than 0.20% occurred in the central area of the bay below > 5 m depth and were correlated to the TOC (Burone *et al.*, 2003).

The C:S ratio when is above 3 indicates oxic environment, and below (<3) indicates reduction environment (Berner, 1995; Borrego *et al.*, 1998). These samples showed values above 3, just in the slice 240-245 (core T1) and 40-45 (core T2) that is below 3. Siqueira *et al.* (2006), in the superficial sediments of Santos estuary – São Paulo, the C:S ratio was between 0.09-3.90 with average  $1,86 \pm 1,26$ . Uehara *et al.* (2007) found in sediments of Cananéia Estuary – São Paulo a C:S ratio ranged 1,75 - 5,03 and oxic condition between 324 and 290 cm depth. Borrego *et al.* (1998) in the superficial sediments of Odiel River – Spain, ranged between 2,6 – 7,03. These authors suggested that the processes of anoxic conditions are conditioned by organic matter input.

Considering that few published data is available and none of them are from tropical environments, the biopolymers concentrations found in this core samples were compared to those from temperate environments and were similar. Pusceddu *et al.* (1999) found carbohydrate, protein and lipid concentrations ranging from 0.76-70.53 mg/g, 2.16-12.1 mg/g and 0.26-4.47 mg/g, respectively, in the western Mediterranean. Dell'Anno *et al.* (2002) found similar carbohydrate, protein and lipid values in the Apulian Coast (Italy). Biopolymeric carbon was also a thousand times lower than reported in the literature. Pusceddu *et al.* (1999) found values varying between 2.5-36.1 mg C/g. However, Dell'Anno *et al.* (2002) found values between 0.9-6.9 mg C/g.

The biochemical relationship representative of biopolymers in both cores (carbohydrates > proteins > lipids) was equivalent to that reported in the literature for superficial sediments (Pusceddu *et al.*, 1999; Dell'Anno *et al.*, 2002). These authors also established a relationship between protein and carbohydrate levels indicative of coastal systems'

eutrophication: meso-oligotrophic (proteins <1.5 mg/g; carbohydrates <5 mg/g), eutrophic (proteins <1.5-4 mg/g; carbohydrates 5-7 mg/g) and hypertrophic (proteins >4 mg/g; carbohydrates >7 mg/g). Lee *et al.* (2004) demonstrated that carbohydrate and lipid levels were normally preserved up to 12 cm of the sedimentary column in the Equatorial Pacific Ocean, reaching 20% and 25%, respectively. In relation to the functional role of proteins, Dell'Anno *et al.* (2002) related their high values to primary productivity, while Pusceddu *et al.* (1999) define them as a limiting factor to benthic organisms. Lee *et al.* (2004) linked protein to bacterial activity in the particulate materials, and their concentration can increase with depth because they have been preserved in lipidic matrices.

The average available and unavailable carbon was 50%. It is a function of organic matter that initially escapes remineralization due to rapid sinking, encapsulation, and aggregation or surface-association that may be enzymatically inaccessible in depths for the bacterial communities (Lee *et al.*, 2004). This occurs with the adsorption of organic compounds onto a mineral matrix, and it has been suggested that organic matter in association with mineral material beyond that equivalent to a monolayer coating might be due to its isolation from oxygen (Crapez, 2009; Lee *et al.*, 2004).

The biopolymers hydrolysis was done by aerobic and anaerobic bacteria at the top of both cores. At the bottom of the cores, the aerobic bacteria were in latency and biopolymer hydrolysis was done by aerobic and facultative anaerobic bacteria. Production of monomers and oligomers by hydrolyzing was carried inside the cell, being available for the oxide-reduction reactions that produce energy (Fenchel *et al.*, 1988; Meyer-Reil & Koster, 2000). Esterase presented high values in the top of the core, demonstrating intense microbiological activity. Studying the amorphous organic matter in the sediments of Guanabara Bay, Mendonça-Filho *et al.* (2003) also demonstrated the occurrence of intense microbial activity. The results of esterase and its averages in the core were similar to the results found in the literature for eutrophic environments. Electron transport system activity was not detected, which could be explained by the anaerobic source of metabolic energy utilized by the bacteria, such as anaerobic facultative bacteria, denitrification and sulfate reduction, which produce 50, 100 and 170 kJ/mol, respectively. The bacterial aerobic



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process produces 500 kJ/mol (Edwards *et al.*, 2005). Crapez *et al.* (2001) found 0.54 µg fluorescein/h/g of esterase activity and 0.31 µL O<sub>2</sub>/h/g of electron transport system activity in sand beach sediments from Boa Viagem Beach (Guanabara Bay). Crapez *et al.* (2003) showed that esterase activity presented a different pattern once it reached a maximum of 0.17 µg fluorescein/h/g in the winter and electron transport system activity reached a maximum of 7.48 µL O<sub>2</sub>/h/g in the summer in sediments from Boa Viagem Beach (Guanabara Bay). Esterase activity and electron transport system activity were highest in samples from Niterói Harbour (Guanabara Bay/S.E.), 3.63 µg fluorescein/h/g and 3.38 µL O<sub>2</sub>/h/g, respectively (Baptista-Neto *et al.*, 2005). Esterase enzymes varied between 1.25 and 4.69 µg fluorescein/h/g in 30 surface sediments samples from Guanabara Bay, and the average was 3.20 µg fluorescein/h/g (Silva *et al.*, 2008).

Total coliforms ranged from 0.001x10<sup>3</sup> to 9.0x10<sup>3</sup> MPN/g and faecal coliforms only occurred between the top and 40-45 cm in core T2. Costa & Carreira (2005) showed that the distribution of *E. coli* in Botafogo Sound sediments in Guanabara Bay was 240 MPN/g. Total coliforms and faecal coliforms presented the highest amount in the Mangue Channel outlet, which is one of Rio de Janeiro's main sewage outlets (10<sup>5</sup> MPN/g and 10<sup>3</sup> MPN/g, respectively) (Silva *et al.*, 2008). The largest concentration of total coliforms (654 MPN/g) and *E. coli* to date have been detected from the sand under seaweed in Biscayne Bay (Miami) (Shibata *et al.*, 2004). Total coliforms were 10<sup>3</sup> MPN/g in Admiralty Bay (Antarctica). The absence of faecal coliforms far from the sewage outfall may be attributed to low survival rates (Martins *et al.*, 2005). Total coliforms are made up of several enterobacteria that can occur not only in the intestinal tract of homothermal animals, but also in soils and waters. Although the population density was small, the large amount of total coliforms found in the inner part of the estuary lead us to believe that many of these enterobacteria occurred as autochthonous microflora in this environment, or developed in soils and waters and were taken into the estuary mainly by rivers. On the other hand, the repression of water inside the bay caused by flood and currents tides could also propitiate an increase in the number of these organisms in the region. Faecal coliforms, on the contrary, are organisms that are obligatorily symbiotic with homothermal animals and occur exclusively in their intestinal tract, and

can survive for more or less time in the aquatic environment (Kolm *et al.*, 2002).

Bacterial carbon values varied between 0.004 and 0.051 µgC/g, which was low when compared to other studies carried out in Guanabara Bay. Crapez *et al.* (2001) found a bacterial carbon variation between 1.962 and 2.640 µgC/g in Boa Viagem Beach. However, it is interesting to compare the numbers of viable and metabolically active bacteria (10<sup>5</sup>-10<sup>6</sup>) with heterotrophic bacteria (10<sup>4</sup>-10<sup>5</sup>). The latter were an order of magnitude lower than metabolically active bacteria and total coliforms. The viable bacteria comprised in the group of facultative anaerobic bacteria were responsible for the diagenesis of organic matter along the cores, and more of them exist in latent form.

## 6 Conclusion

The fine grain size of the bottom sediments from Jurujuba Sound was linked to the low water circulation as in the case of other areas in Guanabara Bay with similar characteristics. The total organic carbon characterizes the environment of the Sound as desoxic-anoxic, with high levels of sulfur indicating the major presence of viable anaerobic microbial web along the cores.

Organic matter diagenesis, measured by active esterase enzymes that degraded the biopolymers along the two cores, was performed by anaerobic bacteria, which represented the most expressive biomass among bacteria communities. The presence of the total and faecal coliforms from the top to 45 cm depth indicated that resuspension was an important process disponibilizing this community for the water column. It would thus be appropriate to establish it as a parameter for balneability analysis.

Biopolymer distributions were similar to the results found in the northern hemisphere. However, the carbon biopolymer concentrations were 10 times lower and evidence the need for establishing new trophic evaluation indices for tropical coastal systems.

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