

## Bacterial Enzymatic Activity and Bioavailability of Heavy Metals in Sediments From Boa Viagem Beach (Guanabara Bay)

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### Abstract

This study focuses on the quality of the organic matter that reaches the sediment from Boa Viagem Beach and through the evaluation of the total bacterial count, the electron transport system activity (ETSA), the esterase activity (EST), as well as the protein and the organic matter contents. Seasonal variations of organic matter, protein content and the number of bacteria were particularly notable in the summer. ETSA reached a maximum of  $7.48 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$  in the summer. EST activity presented a different pattern once it reached a maximum of  $0.17 \mu\text{g fluorescein h}^{-1} \text{ g}^{-1}$  in the winter. The temporal variation of ETSA and EST activity indicated that biopolymers predominated in the winter, and oligomers or monomers predominated in the summer. These results suggest that organic carbon turnover is more likely to be controlled by organic matter quality. The heavy metals concentrations, especially for Cu, Zn, Ni and Cr, indicated absence of the inhibition of dehydrogenase activity, and they are not bioavailable in the EC<sub>50</sub> values

**Key-words:** Guanabara Bay, bacteria, heavy-metals, bioavailability.

### Resumo

O trabalho enfoca a qualidade da matéria orgânica do sedimento da Praia da Boa Viagem, avaliando-se a contagem de bactérias, a atividade do sistema transportador de elétrons (ETSA), a atividade de esterases (EST) e a concentração de proteína e matéria orgânica. Variação sazonal de matéria orgânica, de proteína e número de bactérias foi significativo no verão. ETSA apresentou maior valor no verão,  $7.48 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$ . A EST foi maior no inverno, chegando a  $0.17 \mu\text{g fluorescein h}^{-1} \text{ g}^{-1}$ . A variação temporal de ETSA e EST indicaram que biopolímeros predominaram no inverno e oligômeros e monômeros, no verão. Estes resultados sugerem que a transformação do carbono orgânico é controlado pela qualidade da matéria orgânica. As concentrações dos metais, especialmente Cu, Zn, Ni and Cr, indicam ausência de inibição da atividade das desidrogenase pois não estão biodisponíveis na concentração EC<sub>50</sub>.

**Palavras-chave:** Baía de Guanabara, bactéria, metais pesados, biodisponibilidade.

## 1 Introduction

Marine sediments are intensively colonized by microorganisms (bacteria, cyanobacteria, fungi, algae). Most are organized in biofilms, complex associations of microbes. Immobilized at surfaces and embedded in an extracellular organic matrix, consisting of extracellular polymeric substances (EPS) secreted by the cells. By their organization in biofilms, the 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). Their biomass is greater than the biomass of all other benthic organisms. The cell surface of the 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 (Meyer-Reil & Koster, 2000).

Coastal marine sediments have been recognized as important locations of nutrient regeneration and bacteria constitute the primary agents of the early diagenesis of organic matter. One of the most fundamental characteristics is probably the possession of unique catalytic properties by bacteria, especially extracellular enzymes that act on biopolymers, transforming them into low-molecular-weight organic carbon (Deming & Baross, 1993). An overall estimate of extracellular enzymes can be obtained by measuring the esterase activity (EST). The products of the enzymatic hydrolysis are incorporated in the cells, where the oxidation processes are carried out. Oxidation of organic matter by dehydrogenase activity occurs mostly in organisms that present respiratory chains, and an overall estimate of aerobic and anaerobic metabolism can be obtained by measuring the electron transport system activity - ETSA (Relexans, 1996). The biomass formed through microbial activities represents an important nutrient source for benthic organisms (Meyer-Reil, 1986).

The biogeochemical controls on metal behavior in aqueous environments involve complex linkages of biological, principally bacterially driven, and geochemical processes, which occur at both microscopic and macroscopic scales. Reactions controlling metal behavior are increasingly modeled, with some success. However, not yet

considered in the majority of these thermodynamic treatments of metal dynamics is that these reactions are highly influenced by biological factors, which will affect their location, magnitude and rate. The extent of this influence will be largely driven by microbial ecology, and thus, a fundamental identification and mechanistic understanding of how these factors will drive the geochemistry of a particular system is required (Warren & Haack, 2001).

This paper focuses on the quality of the organic matter that reaches the sediment from Boa Viagem Beach (Figure 1), and on the bioavailability of heavy metals in sediment samples in the coastal area of Niterói (RJ) through the enzymatic evaluation of the bacterial communities. Evaluation of the total bacterial count (MPN), the electron transport system activity (ETSA), the esterase activity (EST), the bioavailability of heavy metals, as well as the protein (PTN) and the organic matter (OM) contents are presented.

## **2 Material and Methods**

### **2.1 Organic matter quality**

Sediment samples were collected at Boa Viagem Beach, which is located inside of Jurujuba Sound (between of the longitude of 043° 08' W and 043° 06' W and latitude of 22° 54' S and 22° 56' S), during winter (BVw) and summer (BV). These collections were carried out in the infralittoral. Seven randomized sediment samples were collected (0-5cm) using core tubes ( $\phi = 10$  cm) over a delimited area of 1 m<sup>2</sup>. These samples were analyzed within 1h of collection. Seven sediment samples were collected from Boa Viagem beach in the winter (BVw) and seven in the summer (BV). 134 sediment aliquots from the two collections were aseptically separated and analyzed. The electron transport system activity (ETSA), the esterase (EST) activity (Stubberfield & Shaw, 1990), and the protein content (Lowry et alii, 1951) were determined. The ETSA was measured according to the methodology of Hourri-Davignon & Relexans (1989), without a surplus of electron donors (Trevors, 1984). During each season, the most probable number of bacteria was analyzed from one sediment aliquot obtained from the mixture of all sediment samples (Lorch et alii, 1995). The organic matter content was determined in triplicate through the change in weight after combustion at 450°C for 24 h, after mixture of all sediment samples.

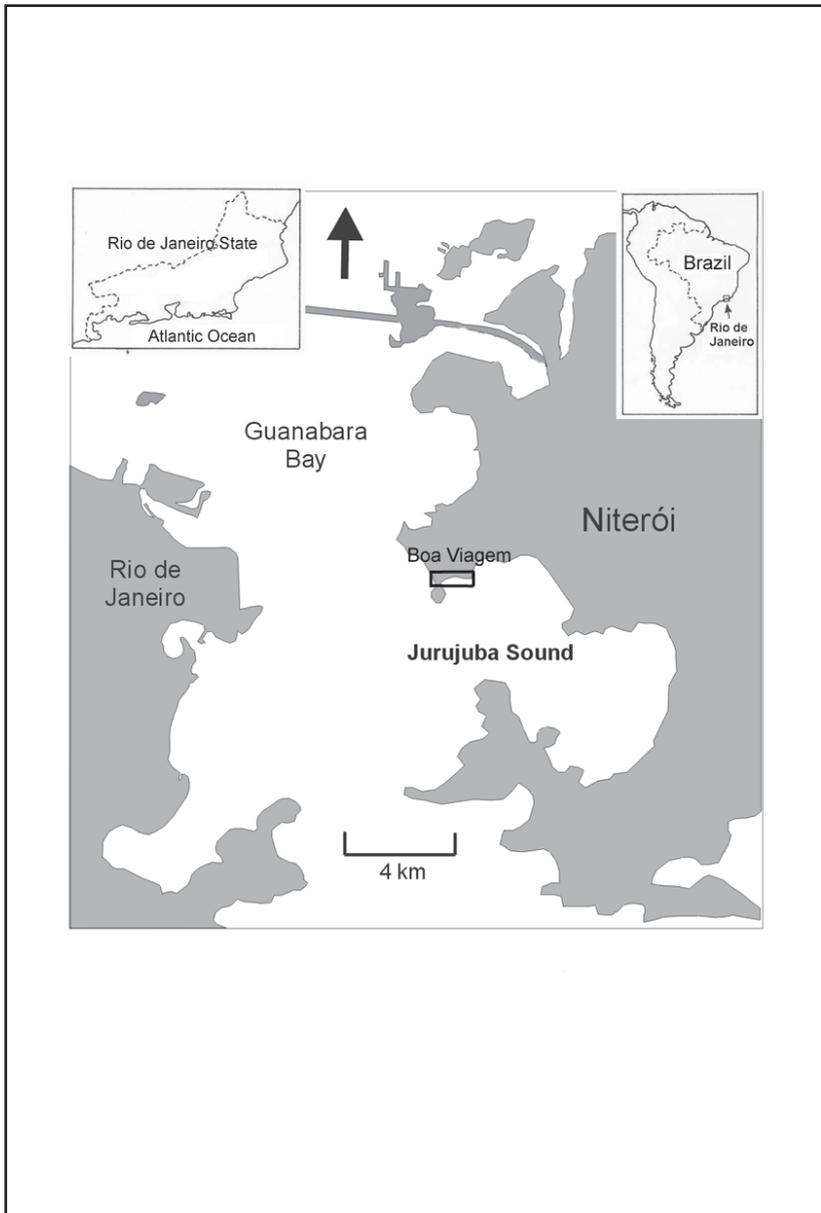


Figure 1 Map of the study area

## 2.2 Bioavailability of heavy metals in sediments

Sediments collected for heavy metals analyses were stored in sealed polythene bags, transported to the laboratory, oven dried at 105°C, and the <63µm fractions separated by passing them through a nylon mesh sieve. Sub-samples (0.1g) of these fractions were digested in 5 ml of an aqua regia solution under pressure in PTFE digestion bombs. Elemental analysis was carried out using a Perkin Elmer Model 3100 atomic absorption Spectrophotometer.

The determination of the dehydrogenase activity, in the presence of an artificial electron acceptor, (INT: 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) supplies the electron transport system activity, ETSA (Trevors, 1984; Houridavignon & Relexans, 1989). The dehydrogenase assay is an effective primary test for assessing the potential toxicity of metals to soil microbial activity. EC<sub>50</sub> values are defined as the concentration of test compound resulting in a 50% reduction in dehydrogenase activity (Rogers & Li, 1985). EC<sub>50</sub> values were obtained from a non-linear least-squares curve fit of individual data sets to the exponential equation:

$$Y = a [1 - e^{-bd}] \quad (3)$$

Where Y is the percentage inhibition observed for a given compound concentration, d is the compound concentration, a is the asymptotic value represented by 100 percent inhibition, and b is the dose dependent rate parameter. EC<sub>50</sub> values (Table 1) were calculated by setting Y equal to 50 and solving equation (3) for d.

Metals	Average (ppm)	EC <sub>50</sub> (ppm)	ETSA (µlO <sub>2</sub> . h <sup>-1</sup> . g <sup>-1</sup> )
Cu	8.0	29	2.31 - 2.79
Zn	24	177	
Ni	23	114	
Cr	16	216	

Table 1 Values of the EC50 for the sediments of Boa Viagem

### 3 Results and Discussion

#### 3.1 Organic matter quality

The distribution of the organic matter, the number of bacteria, the protein content, the ETSA and EST activity in the sediment presented seasonal variation. The highest contents were in the summer campaign. The ETSA reached a maximum of  $7.48 \mu\text{O}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ , the protein content was  $126.79 \mu\text{g} \cdot \text{g}^{-1}$  and the number of bacteria was  $10^8$  cells.  $\text{g}^{-1}$ . The availability of labile organic matter enabled significant increases in the ETSA and in the number of bacteria. Thus, the ETSA has been often used as an index of biomass (Stubberfield & Shaw, 1990).

The EST activity presented a different pattern seeing that it reached a maximum of  $0.17 \mu\text{g}$  fluorescein.  $\text{h}^{-1} \cdot \text{g}^{-1}$  in the winter, decreasing in the summer (Table 2). Models of bacterial action based on enzymatic activities in total sediment samples are supposed to predict the alteration of a wide variety of particulate and dissolved organic matter. The ETSA and the EST values varied greatly between samples probably on account of real spatial variation once attached bacteria inhabit organically rich microzones. Particles create a spatial heterogeneity in the distribution of organic matter, in the remineralised nutrients, and in the species composing bacterial populations (Azam et alii, 1993). Since bacterial membranes are not usually permeated by substrates with a molecular weight greater than approximately 600 Da (Weiss et alii, 1991), and extracellular hydrolysis of macromolecules to smaller substrates is the initial step in the degradation of organic matter.

Area	Organic matter(g. g <sup>-1</sup> )	MPN (cells. g <sup>-1</sup> )	Protein (μg. g <sup>-1</sup> )	ETSA (μl O <sub>2</sub> . l <sup>-1</sup> . g <sup>-1</sup> )	EST (μg fluorescein h <sup>-1</sup> g <sup>-1</sup> )
BV <sub>w</sub>	0.018	4.9 x 10 <sup>5</sup>	12.41 - 25.43	0.12 - 0.18	0.11 - 0.17
BV <sub>s</sub>	1.764	7.0 x 10 <sup>8</sup>	74.05 - 126.79	0.02 - 7.48	0.03 - 0.14

Table 2 Organic matter (g. g<sup>-1</sup>), MPN (cells. g<sup>-1</sup>), protein (μg. g<sup>-1</sup>), ETSA (μl O<sub>2</sub>. h<sup>-1</sup>. g<sup>-1</sup>) and EST activity (μg fluorescein. h<sup>-1</sup>. g<sup>-1</sup>) at Boa Viagem Beach (BV<sub>w</sub> and BV<sub>s</sub>).

#### 3.2 Bioavailability of heavy metals in sediments

Jurujuba Sound is considered one of the most polluted compartments of the Guanabara Bay (Baptista Neto et alii, 2000), mainly in the internal side of the sound,

where the muddy sediments and the lower hydrodynamics predominate. Boa Viagem Beach is characterized by the highest hydrodynamic in the area (Baptista Neto et alii, 1996). In this sector predominate sandy sediments, where normally it is not expected to find high levels of heavy metals. However, when the levels of heavy metals found in these sediments were compared with the concentrations found in the average sandstone see in Table 3 (Turekian and Wedepohl, 1961: Pb – 7; Zn – 16; Ni – 2; Cu – 10 and Cr – 35), it shows an enrichment of the Pb (4x), Zn (1.5x) and Ni (11.5x). Only the elements Cu and Cr shows similar and lower values, respectively, to the values of the average sandstone. However, when it is compared with the average levels of the sandy sediment from the rest of Jurujuba Sound, it shows very similar concentrations, only the element Pb shows concentration 2.2 times higher in the Boa Viagem sediments.

These data demonstrate that even Boa Viagem sediments presenting concentrations of heavy metals lower than the internal side of Jurujuba Sound, these concentrations are high when it is compared to the natural background (average sandstone).

Location	Pb (ppm)	Zn (ppm)	Cu (ppm)	Cr (ppm)	Ni (ppm)
This studyBoa Viagem Beach	10 - 135 28	20 - 30 24	5 - 15 8	10 - 25 16	10 - 25 23
Jurujuba Sound <sup>1</sup>	61	158	51	89	48
Sandy Sediment from Jurujuba Sound <sup>1</sup>	12.8	21	7.2	16.1	20.6
Muddy sediment from Jurujuba Sound <sup>1</sup>	80	211	68	118	58
Average sandstone <sup>2</sup>	7	16	10	35	2

Table 3 Concentrations of heavy metals in the study area (minimum - maximum) (Average), compared with values from the literature.

<sup>1</sup>Baptista Neto *et al.* (2000); <sup>2</sup>Turekian & Wedepohl (1961).

#### 4 Conclusion

The temporal variation of the enzymatic activities, ETSA and EST, indicated that biopolymers predominated in the winter, and oligomers or monomers, which can be transported into bacterial cells for oxidation, the terminal step of organic carbon

demineralisation, predominated in the summer at Boa Viagem. These results suggested that organic carbon turnover may be controlled more by the organic matter quality, and the ratios of ETSA and EST activities of bacterial activities might be useful indicators of changes in the sediment metabolism.

Heavy metals concentrations in the sediments of Boa Viagem is much higher than the natural background levels (average sandstone), which suggests that even being sandy sediments it can be considered polluted. On the other hands, the levels of heavy metals are not bioavailable in the EC50 values, because it does not inhibit the dehydrogenase activity.

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