BACTERIOLOGICAL STUDY OF THE SUPERFICIAL SEDIMENTS OF GUANABARA BAY, RJ, BRAZIL*

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A B S T R A C T

Thirty sediment samples were collected in Guanabara Bay in August, November and December 2005. The material was analyzed for organic matter, total and faecal coliforms, heterotrophic bacteria, bacterial respiratory activity, esterase activity and electron transport system activity. The organic matter in the superficial sediments (2 cm) ranged from 4 to 6%. The highest level of total coliforms and faecal coliforms were 1.7×10^5 MPN/g and 1.1×10^3 MPN/g, respectively. Heterotrophic bacteria presented the highest values at station 7 (4.1×10^6 CFU/g) and the lowest values at station 3 (7×10^4 CFU/g) (northwestern part of Guanabara Bay). Esterase enzyme activity showed activity in the sediment of all 30 stations. Electron transport system activity ranged between 0.047 μL O_2/h/g and 0.366 μL O_2/h/g at six stations. The benthic microbial foodweb is anaerobic. Anaerobic processes such as fermentation, denitrification and sulphate-reduction are responsible for the biogeochemical cycles in the sediment of Guanabara Bay. The input of untreated sewage in the Mangue Channel outflow in Guanabara Bay has favored the increase of the organic load, and the maintenance of the total coliforms and faecal coliforms in the sediment. Faecal coliforms have been reported to be a good indicator of aquatic pollution and organic contamination in the water column, but total coliforms may be an ideal candidate group for sediment quality tests as well.

R E S U M O

Trinta amostras de sedimento foram coletadas na Baía de Guanabara em agosto, novembro e dezembro de 2005. Foram analisados matéria orgânica, coliformes totais e fecais, bactéria heterotrófica, atividade respiratória bacteriana, atividade de esterase e do sistema transportador de elétrons. A matéria orgânica no sedimento superficial variou entre 4 e 6%. O maior nível de coliformes totais e fecais foi 1.7×10^5 MPN/g e 1.1×10^3 MPN/g, respectivamente. Bactérias heterótrofas mostraram o maior valor na estação 7 (4.1×10^6 CFU/g) e o menor na estação 3 (7×10^4 CFU/g) (nordeste da Baía de Guanabara). A enzima esterase mostrou atividade em todos os sedimentos das 30 estações. A atividade do sistema transportador de elétrons variou entre 0.047 μL O_2/h/g e 0.366 μL O_2/h/g em seis estações. A cadeia microbiana bêntica é anaeróbia. Os processos como fermentação, desnitrificação e sulfato redução são responsáveis pelos ciclos biogeoquímicos no sedimento da Baía de Guanabara. O despejo de esgoto não tratado no Canal do Mangue da Baía de Guanabara tem favorecido o aumento da carga orgânica e a manutenção de coliformes totais e fecais no sedimento. Os coliformes fecais são bons indicadores de poluição e contaminação orgânica na coluna de água, mas os coliformes totais também poderão ser um grupo candidato para testes de qualidade de sedimento.

Descriptors: Microbial indicators, Organic matter, Bacterial respiratory activity, Electron transport system activity, Esterase activity, Guanabara Bay.
Descritores: Indicadores microbiológicos, Matéria orgânica, Atividade respiratória bacteriana, Atividade do sistema transportador de elétrons, Atividade de esterase, Baia de Guanabara.

INTRODUCTION

The complex nature of estuaries derived from their hydrodynamics, as well as the presence of strong physico-chemical gradients and intricate biological structure, impose difficulties in forecasting the extension of anthropogenic impacts. Multidisciplinary approaches, specific sampling strategies, as well as multiple analytical tools and a variety of models, have been used to tackle this problem.

During the last century, organic pollution in coastal areas has become a serious world problem. One of the major sources of stress comes from the input of excessive macronutrients, resulting in a change in the trophic status of a given body of water, which leads to eutrophication. Although the effects of eutrophication are well known, the mechanisms governing them are poorly understood. In particular, effects on microbial processes are the key to many aspects of the functioning of the ecosystem, and are usually addressed inadequately (RICHARDSON; JORGENSEN, 1996; MEYER-REIL; KÖSTER, 2000).

Bacteria are present in sediments in high numbers (about 10^{10} cells/g). Their biomass is greater than the biomass of all other benthic organisms, due to the structure and function of microbial biofilms. 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 (DEMING; BAROSS, 1993). Beside oxygen, microbes may use alternative electron acceptors (nitrate, manganese, iron, sulphate, and carbon dioxide) for the oxidation of organic material (EDWARDS et al., 2005). Combined with their logarithmic growth and short generation times, bacteria possess a high metabolic potential, which allows their use as eutrophication indicators in estuaries.

The aim of the present study was to develop a general understanding of the effects of anthropogenic activities on a eutrophic tropical estuary, studying the relationship of bacteria to organic matter in the superficial sediment in 30 points of Guanabara Bay. Organic matter, total and faecal coliform numbers, heterotrophic bacteria, bacterial respiratory activity and bacterial enzyme activities, such as electron transport system activity and esterase activity, were quantified.

MATERIALS AND METHODS

Study Area and Sampling Procedure

Guanabara Bay is one of the largest bays on the Brazilian coastline, located in Rio de Janeiro State (Fig. 1). The bay, including its several islands, has an area of approximately 384 km^2 and presents a coastline 131 km long and a mean water volume of 1.87 x 10^9 m^3 (AMADOR, 1980; KJERFVE et al., 1997).

The hydrographic basin extends over 4080 km^2 and includes 45 rivers, 6 of which are responsible for 85% of the runoff (100 ± 59 m^3/s). The mean half-water volume renewal time is 11.4 days, although in some parts of the bay it is significantly higher. Temperature (24.2 ± 2.6°C) and salinity (29.5 ± 4.8 S) profiles show a well mixed water condition at the bay mouth, extending up to 15–20 km inwards. Thereafter, the system is moderately stratified. A sandbank located on the ocean side of the bay mouth greatly influences the inner water circulation due to current channeling (KJERFVE et al., 1997).

Tides are mixed mainly semidiurnally with a range of 0.7 m, and peak spring tidal currents reach 0.5 m/s inside the bay and 1.6 m/s near the bay entrance. The central channel, with depths of 30-40 m and delimited by the 10-m depth isoline, transports the sand into the bay. The bottom topography is influenced by tidal currents that drain through the central channel, and by a strong sediment input. The increase in width after the entrance channel results in a decrease of tidal current velocities, leading to the deposition of fine sands and mud. Coarse sands predominate in the central channel and in the regions near the bay mouth. Extensive mud deposits resulting from the active transport of clastic material and from intensive primary production are found in the bay's northern area (JICA, 1994; KJERFVE et al., 1997).

A strong stratification of dissolved oxygen is observed in areas where depths are under 10 m. Surface oxygen values reach 300% oversaturation in the photic zone ($\cong$ 5 m), while bottom (4-5 m) concentrations may stay below 1 ml/L (REBELLO et al., 1990).

Approximately 11 million inhabitants live in the Greater Rio de Janeiro metropolitan area and, as a result of rapid urbanization and population growth, untreated sewage is discharged directly into the bay. This area is the second largest industrial region in Brazil and has over 12,000 industries operating along the Guanabara Bay drainage basin, and these account for 25% of the organic pollution released into the Bay (FEEMA, 1990). Two oil refineries process 7% of the national oil and approximately 2,000 commercial ships dock in the port of Rio de Janeiro every year, making

it the second largest harbor in Brazil. The Bay is also homeport to two naval bases, a shipyard, and a large number of ferries, fishing boats and yachts (KJERFVE et al., 1997).

Over the past 100 years, catchment areas around Guanabara Bay have been greatly modified by deforestation and uncontrolled settlement. These activities have increased river flow velocities and transport of sediment load, resulting in a sediment deposition of 1 to 2 cm per year (GODOY et al., 1998). Guanabara Bay is considered to be one of the most polluted environments on the Brazilian coastline (REBELLO et al., 1986; VANDENBERG; REBELLO, 1986; LEAL and WAGENER 1993; BAPTISTA-NETO et al., 2006).
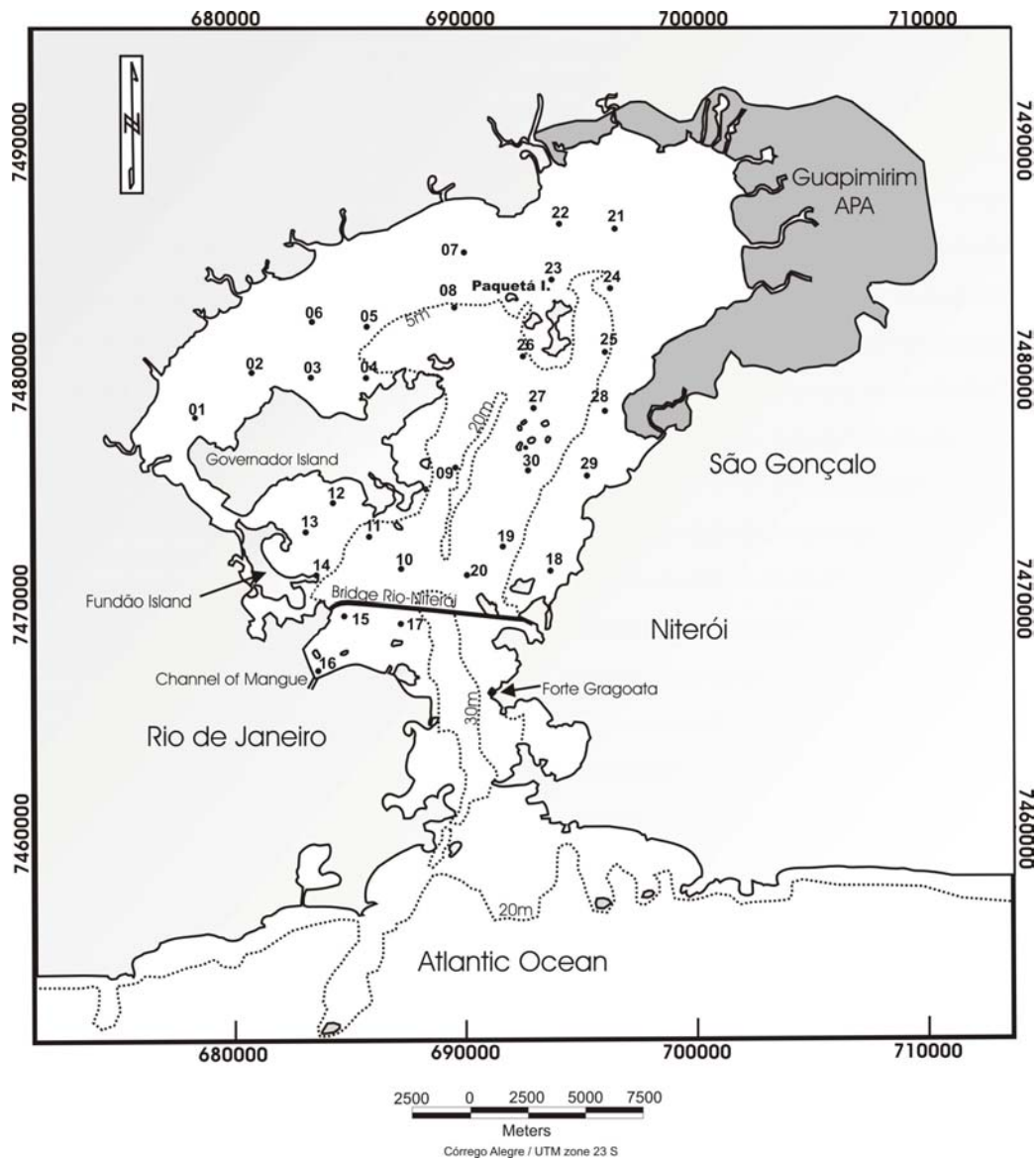

Fig. 1. Map of the study area showing location of superficial sediment samples.

Analytical Methods

In August, November and December 2005, 30 samples of undisturbed surface sediment (2 cm) in Guanabara Bay were collected along 3 transects (1st transect: points 01, 02, 03, 04, 05, 06, 08, 09; 2nd transect: points 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20; and 3rd transect: points 21, 22, 23, 24, 25, 26, 27, 28, 29, 30), using a van-Veen grab sampler for sandy sediment and an Eckman sampler for mud sediment (Fig. 1). These samples were stored during 2 h in sealed polythene bags, conditioned in ice and taken to the laboratory, where the following analyses were conducted. The analyses were performed with 2 cm of sediment samples in triplicate.

1- Total organic matter was determined as the difference between dry weight (60ºC, 24 h) of the sediment and weight of the residue after combustion at 450ºC (2 h) (BYERS et al., 1978).

2- The most probable number (MPN) method was used to estimate abundances of total coliforms (TC) and faecal coliforms (FC). The Lauryl Triptose Broth medium is used for presumptive multiple-tube test, and the Brilliant Green Lactose Bile Broth medium is used in the confirmed phase (APHA, 2001). Heterotrophic bacteria (HB) were cultured on Marine Agar 2216E, at 35ºC for 48 h. Colony counts were converted to CFU/g.

3- Esterase enzyme activity was analyzed according to Stubberfield and Shaw (1990). This analysis is based on fluorogenic compounds, which are enzymatically transformed into fluorescent products that can be quantified using a spectrophotometric assay (490 nm). These enzymes act on biopolymers (carbohydrates, proteins, lipids) and transform them into low-molecular-weight products, the assimable organic carbon fraction which is taken up by the viable bacteria. The results are in μg fluorescein/h/g (wet weight of sediment).

4- Determination of the electron transport system was made according to Trevors (1984) and Houri-Davignon and Relexans (1989). 2-(p-Iodophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) accepts electrons from dehydrogenase enzymes and is reduced to a red-colored formazan (INTF), which can be quantified by colorimetric analysis (475 nm). Trevors (1984) made an essay incubating INT in a medium with no supply of electron donors, observing the natural conditions for the quantification of the microbiota's actual enzymatic activity. Houri-Davignon and Relexans (1989) modified Trevors's method (1984), establishing a relation between O_2 consumption and INTF in bacterial cultures and in sediment samples. Results are thus expressed as electron transport system activity (μL de O_2/h/g) (wet weight of sediment).

5- Bacterial respiratory activity such as aerobic activity, fermentation, denitrification and suphate-reduction, was analyzed using methodology described by Alef and Nannipieri (1995). Aerobic, fermentation and denitrification growth media and sulphate-reduction growth medium contained peptone (0.2g/L) and sodium lactate (0.2g/L), respectively. Methylene blue solution (0.03 final concentration) and resazurin solution (0.0003% final concentration) were used as redox indicators in fermentation and sulphate reducing growth media. Durham vials and $NaNO_2$ (0.687g/L) were utilized in denitrification growth medium. Results were described as positive or negative.

6- Statistical analyses were performed using Ward's method with city-block (Manhattan) distance with the program STATISTICA© 6.0.

RESULTS

The levels of organic matter in the sediment show the highest concentration at station 28 (8.35%), and the lowest at station 30 (0.59%), with the average value of 5.62% for all 30 stations in Guanabara Bay (Fig. 2).

Total coliforms showed the highest level in the sediment at station 16 (1.7×10^5 MPN/g). The average for the other 29 stations was 1.8×10^4 MPN/g (Fig. 2). Station 16 was the only one to present faecal coliforms in the sediment, with values higher than 1.1×10^3 MPN/g. The other stations showed an average of 3.7×10^1 MPN/g.

Heterotrophic bacteria showed the highest values at station 7 (4.1×10^6 CFU/g) and the lowest at station 3 (7×10^4 CFU/g), with an average for all stations of 9.8×10^5 CFU/g (Fig. 2).

Manhattan distance analysis, utilizing total coliforms, faecal coliforms and heterotrophic bacteria numbers as parameters, formed two groups. The first group comprised 6 stations. Station 16 stood apart, because of its higher number of total and faecal coliforms. The other stations (6, 7, 8, 9 and 23) were characterized by heterotrophic bacteria numbers, with values ranging from 1.8×10^6 CFU/g to 4.1×10^6 CFU/g. The second group, comprising the other 24 stations, was also characterized by heterotrophic bacteria, whose values are below 1.3×10^6 CFU/g (Fig. 3).

Bacterial esterase enzyme activity was present in the 30 sediment samples. The highest value was obtained at station 18 (4.69 μg fluorescein /h/g), and the lowest at station 24 (1.25 μg fluorescein/h/g). The average for this activity was 3.20 μg fluorescein /h/g. Stations 1, 2, 6, 12, 16 and 18 showed levels above 4.0 μg fluorescein /h/g (Fig. 2).
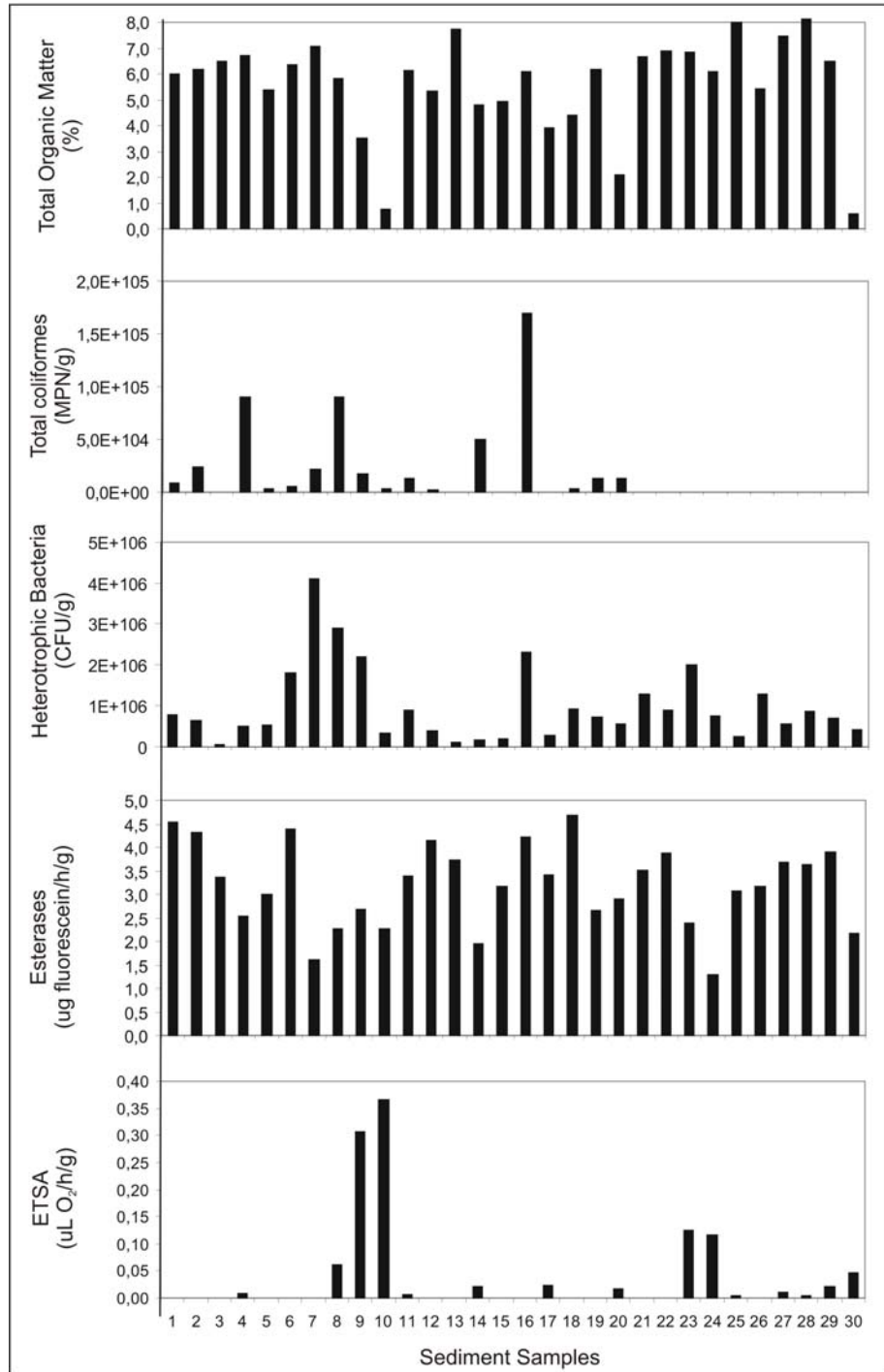
Fig. 2. Organic matter, total coliforms, cultivated bacteria, esterase enzyme and electron transport system activity in the sampled stations.
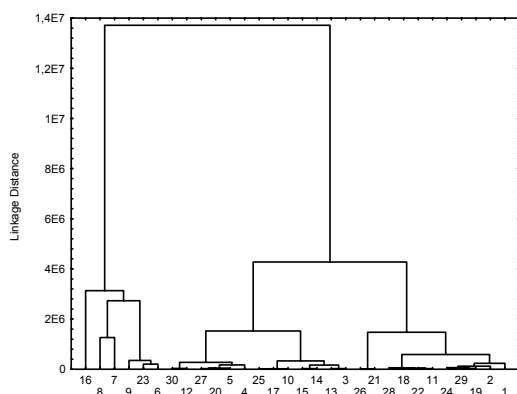
Fig. 3. Manhattan clustering of total coliforms, faecal coliforms, cultivated heterotrophic bacteria.

Electron transport system activity ranged between 0.047 μL O$_2$/h/g and 0.366 μL O$_2$/h/g at stations 8, 9, 10, 23, 24 and 30. Values were lower than 0.047 μL O$_2$/h/g at stations 4, 11, 14, 17, 20, 25, 27, 28 and 29. Activity was lower than the method's detection threshold at stations 1, 2, 3, 5, 6, 7, 12, 13, 15, 16, 18, 19, 21, 22 and 26, corresponding to 50% of all sediment samples collected (Fig. 2).

Manhattan distance analysis, utilizing esterase enzyme activity and electron transport system activity as parameters, formed two groups. The first had a higher electron transport system activity and lower values of esterase activity (stations 4, 7, 8, 9, 10, 14, 19, 23, 24 and 30). The second group is divided in two blocks: the first one had low electron transport system activity and intermediate esterase activity (stations 3, 5, 11, 13, 15, 17, 20, 21, 22, 25, 26, 27, 28 and 29). The second had high esterase activity, whereas electron transport system activity was not detected (1, 2, 6, 12, 16 and 18) (Fig. 4).
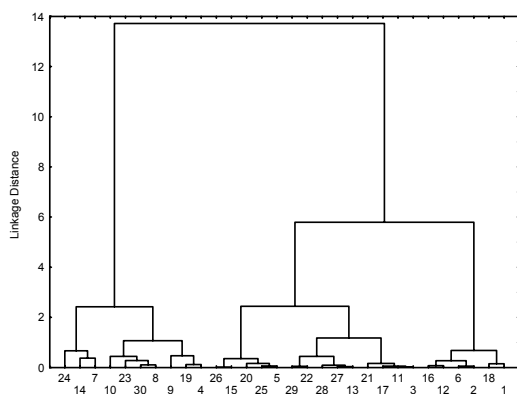

Fig. 4. Manhattan clustering of esterase enzyme and electron transport system activity.

The redox potential in the sediment samples ranged between -120 mV and -465 mV. Manhattan

distance analysis, using bacterial respiratory activity as a parameter, formed 5 groups. Group A was characterized by bacteria either growing aerobically or fermentatively (stations 3 and 5). The B-group was characterized by aerobic, fermentation, denitrification and sulphate-reduction respiratory activities (stations 2, 4, 11, 13, 14, 15, 17, 19, 26, 27, 28, 30). Groups C, D and E did not show aerobic processes. Group C was characterized by fermentation, denitrification and sulphate-reduction processes (stations 10, 16, 18, 20, 25 and 29). Group D was characterized by the denitrification and sulphate-reduction processes (stations 6, 8, 12, 21, 22 and 24). Group E was characterized only by the denitrification process (stations 1, 7, 9 and 23) (Fig. 5).
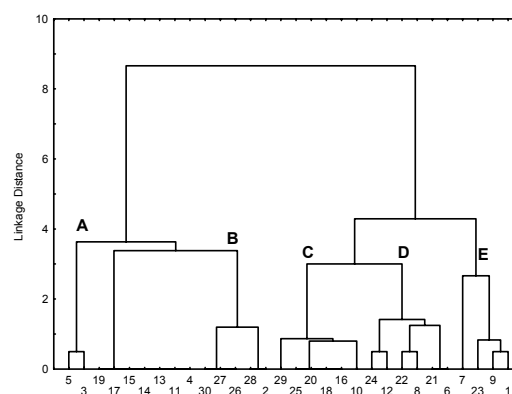

Fig. 5. Manhattan clustering of bacterial respiratory activity in the sampled stations.

DISCUSSION

Average levels of organic matter in the superficial sediments from Guanabara Bay ranged from 4 to 6%. Of all 30 sediment samples, the highest levels of organic matter (8.4%) were found in the areas close to the Guapimirim APA (a protected environmental area), which encloses one of the bay's most protected mangrove areas. This mangrove area contributed more organic matter to this area than to the northwest part of the bay, where the mangrove system was nearly wiped out by petroleum-related industrial activities. These values are similar to the results found by Mendonça-Filho et al. (2003), Catanzaro et al. (2004), and Baptista-Neto et al. (2006). However, Barbosa et al. (2004), sampling the Fundão Island Channel, near the northwestern part of the bay, found high concentrations of organic matter (~15 – 27%) and a high load of fine sediments with high plasticity. Other organic matter levels, ranging from 0.97 – 15.35 %, were found in Ubatuba Bay, Brazil, in 38 superficial sediment samples (BURONE et al., 2003). Dell'anno et al. (2002), on the Apulian coast of Italy, found total organic matter varying from 1.8 to 5.4% along one year.

In Guanabara Bay, along the line stretching from Gragoatá Fort to Santos Dumont Airport, the bay widens into the main channel, which results in a reduction of current speeds, increasing deposition of fine sediments on both sides of the channel. Sediments are primarily clayed-silt and silt-clays deposited as a function of the SSW waves and the tidal current. The north and center parts of the bay are characterized by the presence of muddy sediments. These areas are protected from the waves and tidal current action, and have very low hydrodynamic energy, accumulating mainly silty and clay sediments (KJERFVE et al., 1997; QUARESMA et al., 2000; CATANZARO et al., 2004; BAPTISTA-NETO et al., 2006).

According to Hedges et al. (1997) and Premuzic et al. (1982), in Guanabara Bay the organic compounds aggregated to clay minerals in the water column have been deposited in sediment with low oxygen tension, forming a sediment rich in organic matter and sub-oxide and with anoxic conditions. These conditions favor the adaptation, biomass increase and spatial distribution of anaerobic bacterial populations in Guanabara Bay sediments, except in the sediment samples located in the southwest and central channel, where aerobic bacteria occurred. These anaerobic populations are living in consortia in the bay sediment with physiological interactions among all groups, as already noted by Meyer-Reil and Koster (2000) for the southern Baltic Sea. The depletion of oxygen levels is one of the effects of the eutrophication impact, preventing aerobic organisms from remaining in this region (DIAZ; ROSENBERG, 1995). It can thus be stated that the microbial foodweb in the 30 anoxic sediment samples from Guanabara Bay is constituted by anaerobic bacteria.

The continuous input of organic substances in the Guanabara Bay sediment, reaching the levels mentioned above, results in the organic matter cycling being carried out by the consortia of heterotrophic bacteria, whose numbers are two orders of magnitude below the minimum values found by Osterreicher-Cunha et al. (2003), which ranged from 10^7 to 10^9 CFU/g.

Total coliforms and faecal coliforms were present in greatest numbers in the Mangue Channel outlet, which is one of the main sewage outlets for the city of Rio de Janeiro (10^5 and 10^3 MPN/g, respectively). In spite of the number of incipient studies that deal with the maintenance of faecal bacteria in sediment samples, results were significant in relation to the literature found and evidenced the contamination of an important area of Guanabara Bay, located near downtown Rio de Janeiro. Costa and Carreira (2005) showed that the distribution of *E. coli* in sediments from Botafogo Sound in Guanabara Bay was 240 MPN/g. The largest concentrations of total coliforms (654 MPN/g) and *E. coli* were detected in

the sand under seaweed in Biscayne Bay, Miami (SHIBATA et al., 2004). 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 homeothermic animals, but also in soils and waters. The large amount of total coliforms found in the inner part of the estuary leads us to believe that many of these enterobacteria occurred as autochthonous microflora in the environment, or developed in soils and waters and were taken to the inner part of the estuary mainly by rivers. On the other hand, the retention of water inside the bay caused by flood and full tides could also propitiate an increase in the number of these organisms in that region. Faecal coliforms, on the contrary, are organisms that are obligatorily symbiotic with homeothermic animals and occur exclusively in their intestinal tract, and, in marine conditions population decay starts in 5 h (KOLM et al., 2002).

Hydrolysis of organic matter biopolymers was carried out by anaerobic bacteria, whose esterase enzymes are active in the superficial sediment of Guanabara Bay. However, in 50% of the sediment samples, no electron transport system activity was detected. These results indicate that the anoxic environmental conditions of Guanabara Bay sediments are not favoring aerobic diagenesis of organic matter. Electron transport system activity in the denitrification and sulphate reduction processes was low. Such processes are energetically less efficient than aerobic respiration, and the bacteria performing them are more restricted in the carbon substrates they can utilize (RELEXANS et al., 1966; FENCHEL et al., 1988; EDWARDS et al., 2005). CRAPEZ et al. (2001) found 0.54 µg fluorescein/h/g of esterase activity and 0.31 µL O_2/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_2/h/g in the summer in the sediments from Boa Viagem Beach (Guanabara Bay). Esterase activity and electron transport system activity were highest in samples from Niterói Harbor (Guanabara Bay/S.E.), 3.63 µg fluorescein/h/g and 3.38 µL O_2/h/g, respectively (Baptista-Neto *et al.*, 2004).

The predominance of anaerobic diagenesis in Guanabara Bay sediments is also inferred from geochemical and geological studies. Mendonça-Filho et al. (2003) verified that the fluorescence coloration of the organic compounds and high levels of organic matter indicated a high level of preservation and a low level of free oxygen. The seismic record also showed

acoustic anomalies, produced by the processes of anaerobic decomposition of organic matter (QUARESMA, et al., 2000). Catanzaro et al. (2004) described this type of anomaly as "acoustic curtains" in the northwest area of the bay. Carreira et al. (2002, 2004) found high concentrations of coprostanol in the sediment samples, preserved under anaerobic conditions. Eichler et al. (2003) and Vilela et al. (2004), studying the foraminifera distribution in Guanabara Bay, found species typical of environments with low concentrations of oxygen and high levels of anthropogenic organic matter.

The environmental quality legislation (CONAMA 274/2000 and 357/2005) contemplates only the study of the water column. However, Dell'Anno et al. (2002) have explained that sediments represent a "recorder" of water column processes, and are the final storage place for the accumulation of autochthonous and allochthonous organic matter. Despite the fact that the majority of the classifications of environmental systems are established based on water quality, the authors have shown that the water column can be characterized by oligotrophic conditions while the sediment is eutrophic.

CONCLUSIONS

The high levels of organic matter in the Guanabara Bay sediment are linked to a continuous input of sewage and industrial effluents, making the superficial sediment suboxic to anoxic.

The benthic microbial foodweb is anaerobic, with a greater number of bacteria in the Mangue Channel outlet and in northwestern Guanabara Bay. In other regions, the anoxic condition of the sediment have not favored the maintenance of bacterial numbers and bacterial enzymatic activities of organic matter diagenesis.

There are aerobic bacteria in the southwest region and the central channel of the bay, raised on Marine Agar 2216E medium. These populations are responsible for the aerobic diagenesis of organic matter still occuring in the bay.

Anaerobic processes like fermentation, denitrification and sulphate-reduction are responsible for the biogeochemical cycles in the sediment of Guanabara Bay. This statement is supported by the low activity of the electron transport system, which is energetically less efficient in anaerobic respiration. Out of the 30 sediment samples studied, those from northeastern Guanabara Bay are the most worrying from the standpoint of organic matter diagenesis, since bacterial populations utilize existing carbon and energy sources only with the processes of denitrification and sulphate reduction.

The sewage input from the Mangue Channel outlet in Guanabara Bay has favored the increase of the organic load and the survival of the total coliforms and faecal coliforms in the superficial sediments. Total coliforms in the sediment are also good indicators of pollution and organic contamination.

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