Biochemical and microbiological tools for the evaluation of environmental quality of a coastal lagoon system in Southern Brazil

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(With 6 figures)

Abstract

This study aimed to evaluate the environmental quality of surface water of the Maricá Lagoon System through physicochemical, biochemical and microbiological parameters, in order to assess its environmental quality. Marine influence over the system was evidenced by the salinity and temperature gradients, where the most distant point, in Maricá Lagoon, presented the largest protein, lipid and biopolymeric carbon concentrations. Biopolymers, with predominance of lipids, presented a pattern that differs from the literature for coastal sediments. The concentration of thermotolerant coliforms characterised Maricá Lagoon and Boqueirão Channel as unfit for bathing (60.0 and 66.3 cells.mL^{-1}, respectively). The bacterioplankton in the system proved to be predominantly heterotrophic, a consumer of organic matter, with fermentative, denitrifying and sulfate-reducing metabolism. No esterase enzyme activity was detected, despite the presence of active metabolism, measured by the electron transport system (average of 0.025 μgO_2.h^{-1}.mL^{-1}). The bacterial biomass (autotrophic, heterotrophic and coliforms), bacterial respiratory activity and biopolymer parameters evinced a spatial degradation pattern in the Maricá Lagoon System, where the points with less water renewal are the most impacted.

Keywords: Maricá, biopolymers, bacteria, surface water.

Ferramentas bioquímicas e microbiológicas para a avaliação da qualidade ambiental de um sistema lagunar costeiro no sudeste do Brasil

Resumo

O estudo teve como objetivo avaliar a qualidade ambiental das águas superficiais do Sistema Lagunar de Maricá através de parâmetros físico-químicos, bioquímicos e microbiológicos, para avaliar sua qualidade ambiental. A influência marinha no sistema foi evidenciada pelo gradiente de salinidade e temperatura, e o ponto mais distante, na Lagoa de Maricá, apresentou maiores concentrações de proteínas, lipídios e carbono biopolimérico. Os biopolímeros, com predominância dos lipídios, apresentaram padrão que difere da literatura para sedimentos costeiros, sendo necessários novos estudos na coluna d'água para a proposição de novos índices. A concentração de coliformes termotolerantes caracterizou a Lagoa de Maricá e Canal do Boqueirão como impróprios para o banho (60,0 e 66,3 cels.mL^{-1}, respectivamente). O bacterioplâncton no sistema se mostrou predominantemente heterotrófico, consumidor de matéria orgânica, com metabolismo fermentativo, desnitrificante e sulfato-redutor. Não foi detectada atividade das enzimas esterases, apesar de haver metabolismo ativo, mensurado pela atividade do sistema transportador de elétrons (média de 0,025 μgO_2.h^{-1}.mL^{-1}). Os parâmetros biomassa bacteriana (autótrofa, heterótrofa e coliformes), atividade respiratória bacteriana e biopolímeros evidenciaram um padrão espacial de degradação no Sistema Lagunar de Maricá, onde os pontos com menor renovação das águas, são os mais impactados.

Palavras-chave: Maricá, biopolímeros, bactéria, água superficial.

1. Introduction

Coastal lagoons constitute 13% of the world's coastal environments. Although sharing a common origin and age, they are very unstable environments that differ in physicochemical and biological properties, related to spatial gradients in climate and human impact (Manini et al., 2003). They function as filters by retaining matter from rivers, oceans and the atmosphere, they are nurseries for many marine species and they serve various human purposes, such as feeding, energy, transport, recreation and cityscaping. Their natural balance is easily disturbed, often accompanied by socioeconomic problems. On the coast of Rio de Janeiro state, there exist many lagoons that present problems like those of Maricá, such as Itaipú, Piratininga, Saquaremaand Araruama (Lacerda et al., 1999; Lacerda and Gonçalves, 2001).

According to Jones (2001), in aquatic systems the main organic matter components available to microorganisms are lipids, carbohydrates and proteins. Such biopolymers are degraded by the interaction, in consortia, of at least 2 or 3 physiologically distinct groups of bacteria (Brock et al., 1994). When organic matter reaches the aquatic environment, under anaerobic conditions it tends to be completely degraded by the action of esterase exoenzymes and transformed into inorganic compounds. However, there is a limit to the mineralisation capacity and, if the quantity of organic matter exceeds the bacterial degradation capacity, especially under anaerobic conditions, it tends to accumulate (Marques Júnior et al., 2002).

Over the last decades, the scientific community has increasingly sought a multidisciplinary approach, where new parameters have been proposed in order to better characterise the processes taking place in the environment (Meyers et al., 1995; Volkman et al., 1998). Fabiano et al. (1995) and Dell'Anno et al. (2002) identified new descriptors for trophic state and environmental quality of coastal systems, analysing the organic composition of organic matter (O.M.) determined by the carbohydrate, lipid and protein contents. Applying the new approach based on biochemical composition, Dell'Anno et al. (2002) ascertained that systems previously classified as oligomesotrophic (by classic parameters) changed to eutrophic. Based on results reported in their study, the authors concluded that the biochemical composition of sedimentary O.M. may be considered a sensitive tool to classify the trophic state of coastal systems.

Despite constituting a promising tool in trophic state classification, most studies using the descriptors proposed have been carried out in the northern hemisphere (Fabiano et al., 1995; Danovaro et al., 1999; Dell'Anno et al., 2002; Manini et al., 2003; Pusceddu et al., 2003; Cotano and Villate, 2006), where biochemical composition quantification is proposed as a descriptor for the characterisation of trophic states of coastal environments, associated to bacterial biomass, in addition to parameters already consecrated in the literature (C, N, P). The aim of the present study was to apply biochemical and microbiological tools to evaluate the environmental quality of the coastal lagoon system in Maricá, Rio de Janeiro state, Brazil.

2. Material and Methods

2.1. Study area

The Maricá Lagoon System (Figure 1) is located on the coast of Rio de Janeiro state, Brazil. The Mombuca and Caranguejo river basins constitute the system, which has four topographically well defined lagoons, situated between a sandy *restinga* and branches of the Serra do Mar: Maricá Lagoon (19.5 km^2), Barra Lagoon (6.2 km^2), Padre Lagoon (3.1 km^2) and Guarapina Lagoon (6.5 km^2) (Lacerda et al., 1999). Maricá Municipality, which encompasses the whole lagoon system, is characterised by rapid urban growth due to its proximity to Rio de Janeiro, in addition to its increasing participation in petroleum royalties. In spite of all the changes observed, there is a great lack of basic sanitation infrastructure. Growth of the urban area, fields and pastures, has sparked many land use conflicts, especially around the lagoon system and *restinga* areas, where there are plans for the construction of a luxurious resort (TCE, 2007).

Maricá Lagoon (Figure 1) is connected to Barra, Padre and Guarapina Lagoons by Boqueirão, Ponte Preta and Cordeirinho Channels, respectively. Ponte Preta and Cordeirinho Channels have undergone siltation over the years, hampering water exchange between the lagoons. The sole communication with the ocean is accomplished through Ponta Negra Channel in Guarapina (Lacerda et al., 1999; Lacerda and Gonçalves, 2001).

These lagoons present high concentrations of dissolved organic carbon (6.2–7.2 $mg.L^{-1}$) and high primary productivity (Lacerda and Gonçalves, 2001). Hydrodynamic changes performed since the 1950's have caused problems related to low water renewal, destabilising the system (Oliveira et al., 1955), which is aggravated by the region's large demographic growth, since the lagoon system becomes the final destination for wastewater.

2.2. Sampling

The Maricá Lagoon system was sampled at four points determined by GPS at Maricá (22° 55' 32.6" S and 42° 49' 39.8" W), Barra (22° 57' 32.2" S and 42°48'49.5" W) and Guarapina (22° 57' 05.2" S and 42° 42' 04.2" W) Lagoons and Boqueirão Channel (22° 56' 58.1" S and 42° 49' 21.6" W). Salinity (rephractometer model 10419, American Optical), temperature and pH (pH-meter CG 837, Schott Gerate) and dissolved oxygen (O_2-meter CG 867, Schott Gerate) were determined. Surface water samples were collected in sterile flasks and placed in thermostable boxes for quantification of biochemical and microbiological parameters in the laboratory.

2.3. Biochemical parameters

Biopolymers: protein (PTN) analyses were carried out after extraction with NaOH (0.5 M, 4h) and were determined according to Hartree (1972), as modified by Rice (1982)

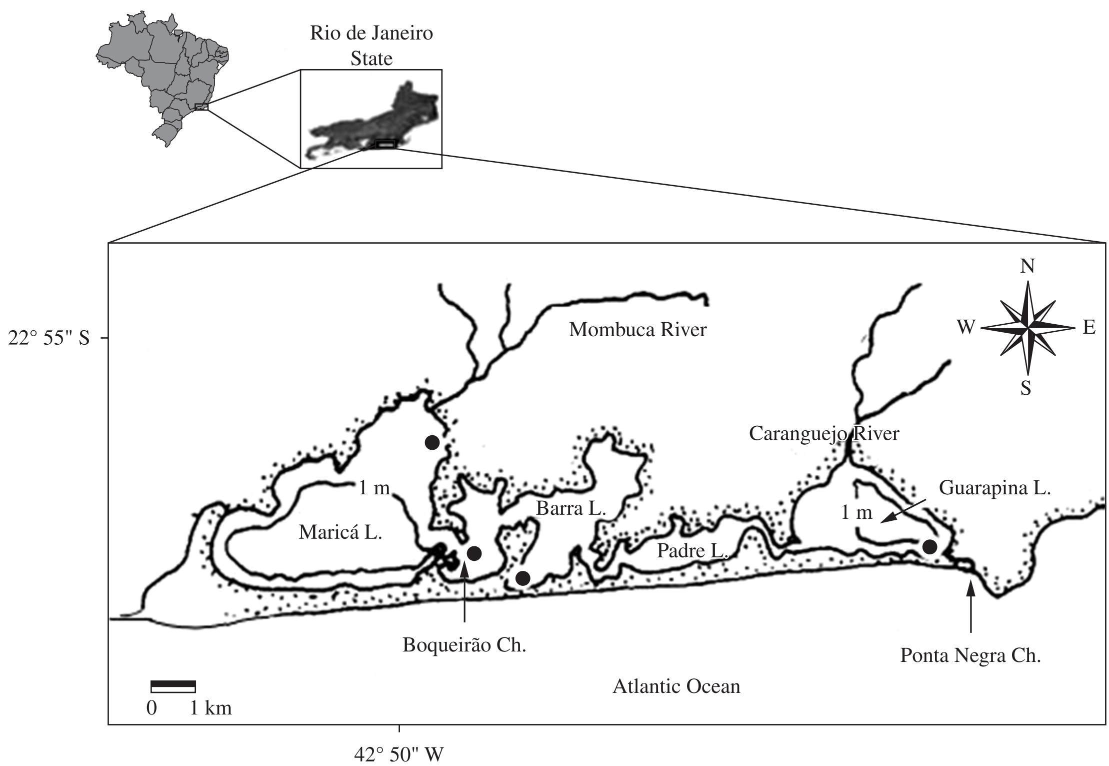

Figure 1. Maricá Lagoon System with sampling points of water (black dots).

to compensate for phenol interference. Concentrations are reported as albumin equivalents. Carbohydrates (CHO) were analysed according to Gerchacov and Hachter (1972) and expressed as glucose equivalents. Lipids (LIP) were extracted by direct elution with chloroform and methanol and analysed according to Marsh and Wenstein (1966). Lipid concentrations are reported as tripalmitine equivalents. Protein, carbohydrate and lipid concentrations were converted to carbon equivalents by using the following conversion factors: 0.49, 0.40 and 0.75, respectively (Fabiano and Danovaro, 1994). The sum of protein, carbohydrate and lipid carbon was referred to as biopolymeric carbon (BPC) (Fichez, 1991).

2.4. Bacterial parameters

Bacterial carbon: autotrophic (AB) and heterotrophic bacteria (HB) were enumerated by epifluorescent microscopy (Axiosp 1, Zeiss, triple filter Texas Red – DAPI – fluorescein isothiocyanate, 1.000 X magnification) and using fluorochrome fluorescein diacetate and UV-radiation (Kepner and Pratt, 1994). Carbon biomass (cells.mL^{-1}) data were obtained using the method described by Carlucci et al. (1986).

The most probable number (MPN) method was used to estimate abundances of total coliforms and thermotolerant coliforms. The Lauryl Triptose Broth medium is used for presumptive multiple-tube test (total coliforms), with incubation temperature at 37 °C, and the EC Broth medium is used in the confirmed phase for thermotolerant coliforms, with incubation temperature at 45 ± 0.5 C. The incubation time is 24-48 hours for both (APHA, 2001).

Bacterial enzyme activities: EST – Esterase enzyme activity was analysed using the method described by Stubberfield and Shaw (1990). It is based on fluorogenic compounds, which are enzymatically transformed into fluorescent products that can be quantified by absorption on a spectrophotometer. These enzymes hydrolyse polymeric organic matter. The results are in µg fluorescein/h/mL.

ETSA – Electron transport system activity was analysed using the method described by Houri-Davignon and Relexans (1989), without a surplus of electron donors (Trevors, 1984). It is based on dehydrogenase enzymes that are the major representatives of oxidoreductase reactions. They catalyze the oxidation of substrates producing electrons that can enter the cell's electron transport system (ETSA) and can be quantified by UV-visible absorption on a spectrophotometer. The results are in µL O_2/h/mL.

Bacterial respiratory activity such as aerobic activity, fermentation, denitrification and sulfate reduction were analysed using the methodology described by Alef and Nannipieri (1995). Aerobic, fermentation and denitrification growth media and sulfate-reduction growth medium contained peptone (0.2 g.L^{-1}) and sodium lactate (0.2 g.L^{-1}), respectively. Methylene blue solution (0.03% final concentration) and resarzurin solution (0.0003% final concentration) were used as redox indicators in fermentation and sulfate-reducing growth mediums. Durham vials and $NaNO_2$ (0.687 g.L^{-1}) were utilised in denitrification growth medium. The results were described as positive, negative or variable.

2.5. Statistical analysis

Differences among sampling points were investigated by means of one-way analysis of variance (ANOVA). When a significant difference for the main effect was observed ($p < 0.05$), a Tukey's pairwise comparison test was also performed.

In addition, a multivariate analysis of group was performed - Ward's method with City-block (Manhattan) distance, that is distinct from all other methods because it uses an analysis of variance approach to evaluate the distances between clusters. In short, this method attempts to minimise 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 measure 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 with the biochemical and biological parameters lipid (LIP), protein (PTN), carbohydrate (CHO), biopolymeric carbon (BC), heterotrophic bacterial (HB), autotrophic bacterial (AB), total coliform (TC) and thermotolerant coliform (TTC) values, and physicochemical parameters such as salinity (S), pH and dissolved oxygen (DO).

3. Results

3.1. Physicochemical parameters

Data on the location of points, salinity, temperature, dissolved oxygen and pH are given in Table 1. Salinity decreased from Guarapina (19 S) to Maricá (0 S) Lagoons. Temperature displayed an increasing gradient from Guarapina (22.7 °C) to Maricá (25.5 °C) Lagoons. Barra Lagoon presented an intermediary concentration of dissolved oxygen (3.8 mg.L^{-1}) and Boqueirão Channel a concentration of 5.4 mg.L^{-1}. The pH did not vary considerably, and was highest at Guarapina Lagoon (7.56).

3.2. Biochemical composition of organic matter

At Guarapina Lagoon, protein, carbohydrate and lipid concentrations were 0.35, 9.1 and 7.1 µg.mL^{-1}, respectively (Figure 2). These were the three lowest values found, differing significantly from the surface water in the three other lagoons ($p < 0.05$). Barra Lagoon and Boqueirão Channel had intermediary concentrations of proteins, 2.7 and 3.6 µg.mL^{-1}, respectively. The largest concentration was found in Maricá Lagoon (10.9 µg.mL^{-1}). Carbohydrate and protein distributions differed. Barra and Maricá Lagoons had intermediary concentrations, 12.5 and 10.1 µg.mL^{-1}, respectively. The largest carbohydrate concentration was found at Boqueirão Channel (14.4 µg.mL^{-1}). Lipids were the predominant and best preserved biopolymers in the surface water of all lagoons. Taking lipid concentration in Guarapina Lagoon as a reference point, concentrations were 2, 4 and 6 times larger at Barra Lagoon, Boqueirão Channel and Maricá Lagoon, respectively. Concentrations ranged from 14.6 to 42.4 µg.mL^{-1} (Figure 2).

Biopolymers at Guarapina Lagoon, Barra Lagoon, Boqueirão Channel and Maricá Lagoon reached 16.5, 29.8, 47.6 and 63.4 µg.mL^{-1}, respectively. However, levels of biopolymeric carbon or bioavailable carbon in the lagoon surface water were much lower, 9.1, 17.3, 29.7 and 41.2 µg.mL^{-1}, increasing from Guarapina to Maricá (Figure 2).

3.3. Bacterial parameters

Heterotrophic and autotrophic bacteria presented the same order of magnitude between sampling points (10^6 cells.mL^{-1}). Guarapina and Barra Lagoons yielded a smaller number of heterotrophic and autotrophic bacteria than Boqueirão Channel and Maricá Lagoon (Figure 3).

In the surface water of Guarapina and Barra Lagoons, the thermotolerant coliforms values, < 5 MPN.mL^{-1}, was within the limits established by Brazilian environmental legislation – National Environment Council (CONAMA, 2000), that review criteria for balneability of waters, establishing water quality as 'very good'. However, at

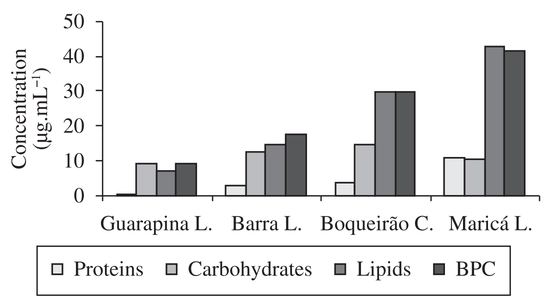

Figure 2. Proteins, carbohydrates, lipids and biopolymeric carbon concentration in surface waters of Maricá Lagoon System.

Table 1. Physicochemical parameters in surface waters of Maricá Lagoon System.

Station	Salinity (psu)	Temperature (°C)	O_2 (mg.L^{-1})	pH
Guarapina L.	19	22.7	4.8	7.56
Barra L.	9	23.8	3.8	7.18
Boqueirão Ch.	5	24.7	5.4	7.28
Maricá L.	0	25.5	1.8	7.20

Boqueirão Channel and Maricá Lagoon the values indicate that the water is unfit for bathing (> 25 MPN.mL^{-1}) (Figure 3).

Through qualitative tests, it was possible to observe a gradient of bacterial respiratory activity in relation to the points sampled (Table 2). Anaerobic processes, such as fermentation, denitrification and sulfate reduction, predominated in the surface waters of Barra Lagoon, Boqueirão Channel and Maricá Lagoon. Aerobiosis represented a predominant bacterial process only at Guarapina Lagoon.

In the lagoons' surface water no esterase enzyme activity, responsible for hydrolysis of polymers into oligomers and monomers, was detected. Activity of the electron transport system was 0.04 and 0.03 μL O_2.h^{-1}.mL^{-1} at Maricá and Guarapina Lagoons, respectively. Boqueirão Channel and Barra Lagoon reached the smallest values, with 0.020 and 0.014 μL O_2.h^{-1}.mL^{-1}, respectively (Figure 4).

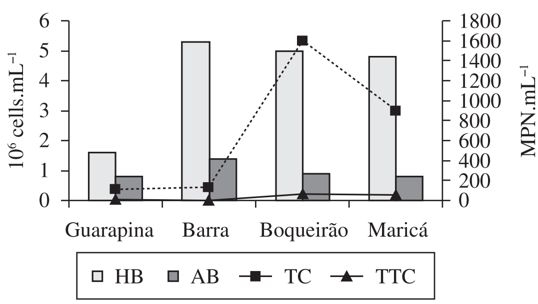

Figure 3. Heterotrophic (HB), autotrophic (AB), total coliforms (TC) and thermotolerant coliforms (TTC) in surface waters of Maricá Lagoon System.

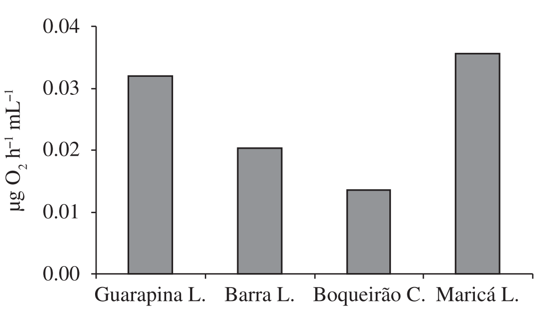

Figure 4. Bacterial electron transport system activity in surface waters of Maricá Lagoon System.

4. Discussion

The parameters monitored revealed that the Maricá Lagoon System presents a spatial gradient of increasing degradation. Guarapina Lagoon displays more preserved characteristics, as seen by the aerobic bacterial metabolism, very good balneability, and low biopolymer concentration, a result of renewal of 50% of its waters in 7 days. Maricá Lagoon appeared as a more impacted subsystem, with discharge of untreated sewage, evidenced by the presence of thermotolerant coliforms, concentration of biopolymers and the predominance of anaerobic bacterial metabolism in the surface water.

In coastal lagoons, organic matter sources originate by drainage basin, connection to the sea, sewage discharge, particle resuspension, internal cycling and autochthonous organisms. Organic matter labile compounds (e.g., carbohydrates, lipids and proteins) are oxidised and used as carbon and energy sources by bacteria, with significant implications to carbon cycling and other elements of the microbial loop in the environment (Danovaro et al., 1999; Jones, 2001). Replacing quantification of phosphorus and chlorophyll-*a*, quantification of biopolymers in the sediment has been used as an indicator of the trophic state of aquatic systems (Dell'Anno et al., 2002). However, there is no record in the literature of the use of biopolymers as indicators of water column trophic patterns. The results obtained in the Maricá Lagoon System were lipids > carbohydrates > proteins. Studies in superficial sediments on the coasts of Italy and Greece showed the predominance of carbohydrates > proteins > lipids (Danovaro et al., 1999; Manini et al., 2003; Pusceddu et al., 2003). In the superficial sediment at 30 points of Guanabara Bay, Rio de Janeiro State, the relation carbohydrates > lipids > proteins was established (Silva et al., 2008). In the Mediterranean, the available biopolymers oxidised by microorganisms are lipids, and in a subtropical estuary, such as Guanabara Bay, the more common and more oxidised biopolymers are proteins (Dell'Anno et al., 2002; Silva et al., 2008). At the above mentioned sites, carbohydrates were the best preserved polymers in the organic matter. Surface water metabolism in the Maricá Lagoon System took place differently, with greater protein consumption and greater preservation of lipids, which in the water column may (like some sterols) indicate contamination by domestic sewage (Carreira et al., 2004). The disordered population growth and the direct input of untreated sewage discharged by river runoff, coupled with weak currents, caused siltation of the channels

Table 2. Bacterial respiratory activity present in surface waters of Maricá Lagoon System.

Station	Aerobiosis	Fermentation	Sulfate reduction	Desnitrification
Guarapina L.	Positive	Variable	Variable	Variable
Barra L.	Variable	Variable	Positive	Positive
Boqueirão C.	Negative	Positive	Positive	Positive
Maricá L.	Negative	Positive	Positive	Positive

interconnecting the lagoons, favouring the maintenance of high lipid concentrations in the water column.

The high coliform values at Boqueirão Channel and Maricá Lagoon indicated untreated sewage discharge in the vicinity. Maricá Lagoon has been receiving sewage in natura from the Mombuca River (its main tributary) and partially treated sewage from the municipal treatment plant. The survival in the surface water of fermentative bacteria, such as coliforms, once again confirms the anaerobic processes of the water column particles, independently of the dissolved oxygen concentration in the aqueous system. The distribution of heterotrophic and autotrophic bacteria, total coliforms and thermotolerant coliforms followed a pattern in the Maricá Lagoon System. Bacterioplankton is predominantly heterotrophic, consuming organic matter, with fermentative, denitrifying and sulfate-reducing metabolism. Maricá Lagoon was the only site where the presence of spirilla, which are associated to microaerophilic environments (Atlas, 1997), was observed, corroborating the environment's hypoxic character (Jones, 2001). According to Crapez (2002), the particulate/dissolved material in a water column creates anoxic microzones, responsible for the microbiota's anaerobic metabolism. Only in Guarapina was the bacterioplankton aerobic, since the lagoon renews 50% of its waters in seven days through Ponta Negra Channel, which connects the lagoon to the sea (Knoppers et al., 1999). This connection generates a greater increase in organic matter, evidenced by the low biopolymer concentrations found at that site.

Hydrolysis of organic matter polymers is carried out by extracellular enzymes, called esterases. After hydrolysis, monomers and oligomers become available for oxireduction reactions, which culminate in the release of energy (Fenchel et al., 1988). Activities of esterase enzymes and of the electron transport system have been employed as indicators of microbial metabolism, in aquatic or terrestrial systems (Trevors, 1984; Houri-Davingnon and Relexans, 1989; Stubberfield and Shaw, 1990). Esterase enzyme activity was not detected in the lagoons, despite bacterial electron transport system activity being found, indicating bacterial metabolism and suggesting the availability of molecules with molecular weights up to 600 Da in the surface water (Weiss et al., 1991). According to Lacerda and Gonçalves (2001), these lagoons contain 6.2–7.2 $mg.L^{-1}$ of dissolved organic carbon, which theoretically serves as a source of carbon and energy for the bacterioplankton, which present the greatest metabolic activities which took place at Guarapina and Maricá Lagoons, the first through aerobic bacterial processes, the second with predominance of anaerobic processes.

The tree diagram for variables showed two groups (Figure 5). The first is linked to the highest temperatures, and carbon bioavailability (BPC, CHO and PTN) for heterotrophic bacteria with preferentially anaerobic and fermentative metabolism, favouring survival of total and thermotolerant coliforms. The other group is formed by autotrophic bacteria, with a preference for greater salinity, higher pH and dissolved oxygen, and with metabolic activity geared to energy production, where lipids are preserved. The tree diagram for four cases showed three groups (Figure 6). Guarapina Lagoon and Boqueirão Channel made up the first group, the two lagoons being linked by temperature. Next came Barra Lagoon, closer to the first group, and then Maricá Lagoon on its own, linked to the three other lagoons.

The Maricá Lagoon System bacterioplankton is predominantly heterotrophic and anaerobic, linked to fermentative, denitrifying and sulfate-reducing processes,

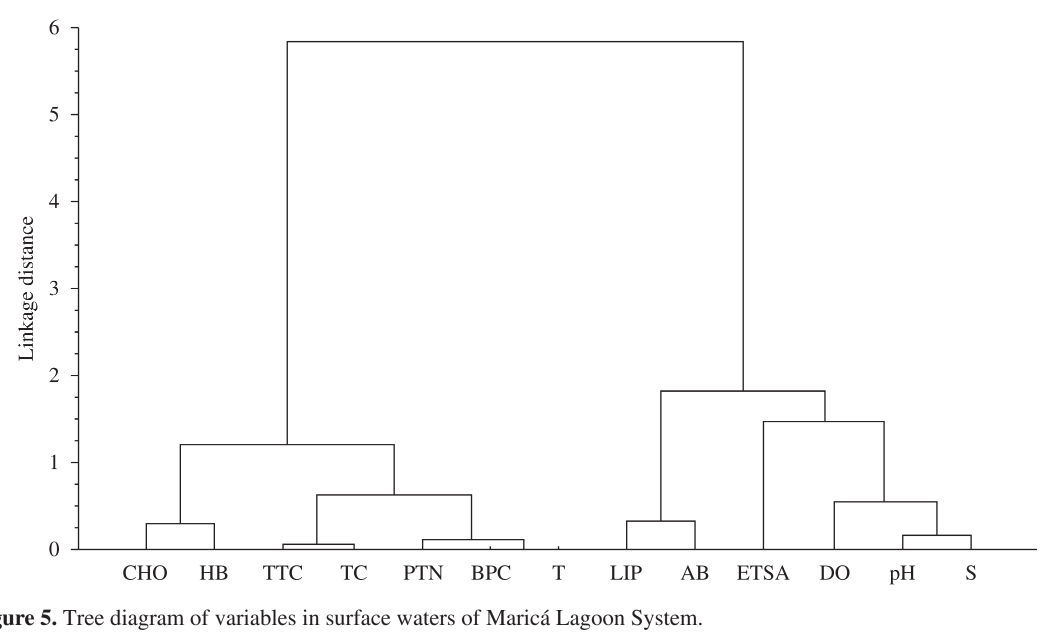

Figure 5. Tree diagram of variables in surface waters of Maricá Lagoon System.

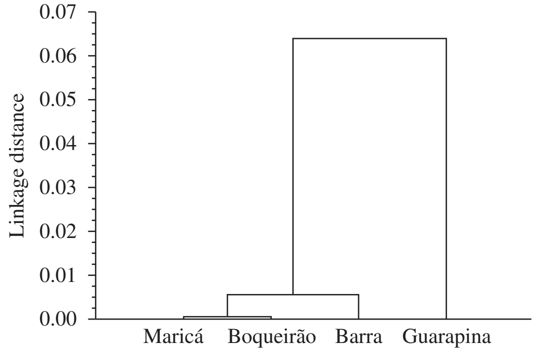

Figure 6. Tree diagram of cases (stations) of Maricá Lagoon System.

using preferentially proteins as a carbon and energy source, while preserving lipids. Parameters like biopolymers, respiratory activity and autotrophic, heterotrophic and coliform bacterial biomass appropriately indicated the studied system's heterogeneous condition and delineated the environmental degradation process, and may thus be used in environmental monitoring programs. Although many studies have already focused in the environmental quality, this study is innovative in proposing the use of new tools such as analysis of biopolymers in water. This way, the work can serve as a guide for future studies aimed at the characterisation of lentic water bodies through biochemical and microbiological parameters, which may lead to a better understanding of the processes taking place in aquatic environments, ensuring a more adequate focus for their management.

References

ALEF, K. and NANNIPIERI, P., 1995. Enrichment, isolation and counting of soil microorganisms. In Alef, K. and Nannipieri, P. (Eds.). *Methods in applied soil microbiology and biochemistry*. Academic press, London, p. 126-127. doi:10.1016/B978-012513840-6/50019-7

American Public Health Association – APHA, 2001. *Standard methods for the examination of water and wastewater*. APHA-AWWA-WPCF. 21nd ed. Washington: Victor Graphics. 1220 p.

ATLAS, RM., 1997. *Principles of Microbiology*. 2nd ed. Dubuque: Wm. C. Brown Publishers. 1298 p.

BROCK, TD., MADIGAN, MT., MARTINKO, JM. and PARKER, J., 1994. *Biology of Microorganisms*. New Jersey: Prentice Hall XVII. 909 p.

CARLUCCI, AF., CRAVEN, DB., ROBERTSON, DJ. and WILLIAMS, PM., 1986. Surface-film microbial populations die amino acid metabolism, carbon utilization and growth rates. *Marine Biology,* vol. 92, p. 289-297. doi:10.1007/BF00392847

CARREIRA, RS., WAGENERB, ALR. and READMAN, JW., 2004. Sterols as markers of sewage contamination in a tropical urban estuary (Guanabara Bay, Brazil): space–time variations. *Estuarine, Coastal and Shelf Science,* vol. 60, p. 587-598. doi:10.1016/j.ecss.2004.02.014

Conselho Nacional do Meio Ambiente – CONAMA, 2000. *Resolução nº. 274, de 29 de Novembro de 2005. "Define os critérios de balneabilidade em águas brasileiras". Diário Oficial [da] União*, Brasília, 3 p.

COTANO, U. and VILLATE, V., 2006. Anthropogenic influence on the organic fraction of sediments in two contrasting estuaries: A biochemical approach. *Marine Pollution Bulletin*, vol. 52, p. 404-414. PMid:16257013. doi:10.1016/j.marpolbul.2005.09.027

CRAPEZ, MAC., 2002. Bactérias Marinhas. In PEREIRA, RC. and SOARES-GOMES (Eds.). *Biologia Marinha*. Rio de Janeiro: Interciência. p. 83-101. vol. 1.

DANOVARO, R., MARRALE, D., DELLA CROCE., N, PARODI, P. and FABIANO, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic-benthic coupling. *Journal of Sea Research,* vol. 42, p. 117-129. doi:10.1016/S1385-1101(99)00024-6

DELL'ANNO, A., MEI, ML., PUSCEDDU, A. and DANOVARO, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. *Marine Pollution Bulletin*, vol. 44, p. 611-622. doi:10.1016/S0025-326X(01)00302-2

FABIANO, M. and DANOVARO, R., 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. *Hydrobiologia*, vol. 277, p. 71–84. doi:10.1007/BF00016755

FABIANO, M., DANOVARO, R. and FRASCHETTI, S., 1995. Temporal trend analysis of the elemental composition of the sediment organic matter in subtidal sandy sediments of the Ligurian Sea (NW Mediterranean): a three years study. *Continental Shelf Research*, v. 15, p. 1453–1469.

FENCHEL, T., KING, GM. and BLACKBURN, TH., 1988. *Bacterial biogeochemistry: the ecophysiology of mineral cycling*. 2nd ed. London: Academic Press. 307 p.

FICHEZ, R., 1991. Composition and fate of organic matter in submarine cave sediments: implications for the biogeochemical cycle of organic carbon. *Oceanologica Acta*, vol. 14, p. 369-377.

GERCHACOV, SM. and HACHTER, PG., 1972. Improved technique for analysis of carbohydrates in sediment. *Limnology Oceanography,* vol. 17, p. 938-943. doi:10.4319/lo.1972.17.6.0938

HARTREE, EF., 1972. Determination of proteins: a modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry,* vol. 48, p. 422-427. doi:10.1016/0003-2697(72)90094-2

HOURI-DAVIGNON, CH. and RELEXANS, JC., 1989. Measurement of actual electrons transport system (ETS): Activity in marine sediments by incubation with INT. *Environmental Technology Letters,* vol. 10, p. 91-100. doi:10.1080/09593338909384722

JONES, JG., 2001. Freshwater Ecosystems – Structure and Response. *Ecotoxicology and Environmental Safety*, vol. 50, p. 107-113. PMid:11689026. doi:10.1006/eesa.2001.2079

KEPNER JR., R. and PRATT, JR., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiological Reviews,* vol. 58, p. 603-615.

KNOPPERS, B., CARMOUZE, JP. and MOREIRA-TURCQ, PF., 1999. Nutrient dynamics, metabolism and eutrophication of lagoons along the east Fluminense coast, State of Rio de Janeiro, Brazil. In KNOPPERS, B.; BIDONE, ED. and ABRÃO, JJ. (Eds.). *Environmental Geochemistry of Coastal Lagoon Systems, Rio de*

Janeiro, Brazil. Niterói, Rio de Janeiro: Ambiental/UFF. p. 123-154. vol. 6, Série Geoquímica Ambiental. Programa de Geoquímica.

LACERDA, LD., ABRÃO, JJ., BERNAT, M. and FERNEX, F., 1999. Biogeodynamics of heavy metals in the lagoons of Eastern Rio de Janeiro State, Brazil. In KNOPPERS, B., BIDONE, ED. and ABRÃO, JJ. (Eds.). *Environmental Geochemistry of Coastal Lagoon Systems, Rio de Janeiro, Brazil*. Niterói, Rio de Janeiro: Programa de Geoquímica Ambiental/UFF. p. 179-195. vol. 6, Série Geoquímica Ambiental.

LACERDA, LD. and GONÇALVES, GO., 2001. Mercury distribution and speciation in water of coastal lagoons of Rio de Janeiro, SE Brazil. *Marine Chemistry*, vol. 76, p. 47-58. doi:10.1016/S0304-4203(01)00046-9

MANINI, E., FIORDELMONDO, C., GAMBI, C., PUSCEDDU, A. and DANOVARO, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. *Oceanologica Acta,* vol. 26, p. 27-38. doi:10.1016/S0399-1784(02)01227-6

MARQUES JÚNIOR, ANM., MORAES, RBC. and MAURAT, MC., 2002. Poluição marinha. In PEREIRA, RC. and SOARES-GOMES (Eds). *Biologia Marinha*. Rio de Janeiro: Interciência. p. 311-334.

MEYERS, PA., LEENHEER, MJ. and BOURBONNIERE, RA., 1995. Diagenesis of vascular plant organic matter components during burial in lake sediments. *Aquatic Geochemistry*, v. 1, p. 35-52. doi:10.1007/BF01025230

MARSH, JB. and WENSTEIN, DB., 1966. A simple charring method for determination of lipids. *Journal Lipids Research,* vol. 7, p. 574-576.

OLIVEIRA, L., NASCIMENTO, R. and KRAU, LMA. 1955. Observações biogeográficas e hidrológicas sobre a lagoa de Maricá. *Memórias do Instituto Oswaldo Cruz*, vol. 53, p. 171-225.

PUSCEDDU, A., DELL'ANNO, A., DANOVARO, R., MANINI, E., SARÀ, G. and FABIANO, M., 2003. Enzymatically hydrolyzable proteins and carbohydrate sedimentary pools as indicators of the trophic state of detritus sink systems: A case study in a Mediterranean Coastal Lagoon. *Estuaries,* vol. 26, no. 3, p. 64-650. doi:10.1007/BF02711976

RICE, DL., 1982. The detritus nitrogen problem: new observation and perspectives from organic geochemistry. *Marine Ecology Progress Series,* vol. 9, p. 153-162. doi:10.3354/meps009153

SILVA, FS., BITENCOURT, JAP., SAVERGNINI, F, GUERRA, LV., BAPTISTA-NETO, JA. and CRAPEZ, MAC., 2008. *Bioavailability of organic matter in the superficial sediment of Guanabara Bay*. Rio de Janeiro, Brazil.

STUBBERFIELD, LCF. and SHAW, PJA., 1990. A comparison of tetrazolium reduction and FDA hydrolysis with other measures of microbial activity. *Journal of Microbial Methods,* vol. 12, p. 151-162. doi:10.1016/0167-7012(90)90026-3

TREVORS, J., 1984. Effect of substrate concentration, inorganic nitrogen, O_2 concentration, temperature and pH on dehydrogenase activity in soil. *Water Research,* vol. 77, p. 285-293.

Tribunal de Contas da União - TCE, 2007. *Estudo sócio-econômico 2007, Maricá*. Rio de Janeiro: Secretaria Geral de Planejamento do Estado do Rio de Janeiro. 152 p.

VOLKMAN, JK. BARRET, SM., BLACKBURN, SI., MANSOUR, MP., SIKES, EL. and GELIN, F., 1998. Sterol biomarkers: a review of recent research developments. *Organic Geochemistry*, v. 29, no. 5/7, p. 1163-1179.

WEISS, MS., ABELE, U., WECKESSER, J., WELTE, W., SCHILTZ, E. and SCHULZ, GE., 1991. Molecular architecture and electrostatic properties of a bacterial porin. *Science,* vol. 254, p. 1627-1630.