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# HYDROCARBONS AND BACTERIAL ACTIVITY IN MANGROVE SEDIMENTS FROM GUANABARA BAY, BRAZIL

Julio César Wasserman[1,*], André Luiz Magalhães Botelho[1], Mirian Araujo Carlos Crapez[2], Maria das Graças Silveira Bispo[2], Frederico Sobrinho da Silva[2], Conceição Maria Filgueiras[3]

[1]Departamento de Análise Geo-Ambiental (LAGEMAR),
Instituto de Geociências, Universidade Federal Fluminense (UFF),
[2]Programa de Pós Graduação em Biologia Marinha, Universidade Federal Fluminense (UFF),
[3] Programa de Geoquímica, Universidade Federal Fluminense (UFF),
Outeiro de São João Batista s/n, Campus do Valonguinho, Centro, Niterói, RJ - CEP 24.020-150
*E-mail: geowass@vm.uff.br

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## ABSTRACT

In this study, a very extensive law protected mangrove area in the Eastern part of the Guanabara Bay was screened for petroleum hydrocarbons (PAHs) and bacterial activity. The aim was to understand the processes that control oil contamination in the mangrove sediments and if there is a relationship between this contamination and bacterial activity. Surface sediment samples were collected from 23 stations within the mangrove shoreline. The parameters pH, Eh, salinity and temperature were measured in situ. The sediments were immediately analyzed for bacterial activity, including: 1) electron transport system activity (ETSA); 2) activity of the esterase enzyme (EST); and 3) bacterial biomass. Analyses of the total PAHs contents were performed by UV-visible spectrofluorescence, total organic carbon was analyzed by titration and total phosphorus was analyzed by photocolorimetry, after wet oxidation. The results show that, in spite of the significant amounts of hydrocarbons loaded into the bay, Guapimirim mangrove still presents low concentrations. The presence of elevated concentrations of organic matter and nutrients provides sufficient energy for the development of an important bacterial biomass. The quality of the organic matter is determining the activity of the bacteria as indicated by ETSA and EST. When the organic matter is easily metabolized there would be predominance of esterase enzyme. The ETSA seems to be active most of the time, because bacteria is using readly available organic matter an PAHs. The results indicated that although there is a significant supply of easily metabolized organic matter, bacteria obtain part of its energy from the refractive organic matter. It is suggested that during the degradation of refractive organic matter, some petroleum hydrocarbons are also degraded.

## RESUMO

Neste estudo uma extensa Área de Proteção Ambiental (APA) de manguezal, localizada na porção Leste da Baia de Guanabara, foi mapeada para hidrocarbonetos poliaromáticos de petróleo (HPAs) e para a atividade bacteriana. O objetivo foi compreender os processos que controlam a contaminação por óleo em sedimentos de manguezal e se há relação entre esta contaminação e a atividade bacteriana. Amostras de superfície dos sedimentos foram coletadas em 23 estações, na linha de costa que separa o manguezal da baía. Os parâmetros pH, Eh, salinidade e temperatura foram medidos *in situ*. Os sedimentos foram imediatamente analisados para atividade bacteriana incluindo: 1) atividade de sistema transportador de elétrons (ETSA); 2) atividade da enzima Esterase (EST) e 3) biomassa bacteriana. As análises de HPAs foram realizadas por espectrofluorescência UV-visível, o carbono orgânico total foi analisado por titulação e o fósforo total foi analisado por fotocolorimetria, após oxidação úmida. Os resultados mostraram que, apesar dos volumes de hidrocarbonetos lançados na Baia de Guanabara serem consideráveis, o manguezal de Guapimirim apresenta concentrações relativamente baixas. A biomassa bacteriana é elevada devido à elevada disponibilidade de matéria orgânica e nutrientes. O tipo de matéria orgânica presente no sedimento provavelmente determina o tipo e o nível de atividade bacteriana. Se a matéria orgânica é muito facilmente degradável (biopolímeros), a presença de enzima esterase é dominante. A ETSA está ativa e presente todo o tempo, porque as bactérias tanto usam a matéria orgânica disponível como também os HPAs. Os resultados indicam que embora exista um suprimento significativo de matéria orgânica de fácil degradação, as bactérias ainda obtém parte de sua energia de matéria orgânica refratária. Provavelmente, junto com a matéria orgânica refratária, os hidrocarbonetos de petróleo também são degradados.

## INTRODUCTION

Mangroves are environments where oil pollution introduces unrecoverable damages. The most noticeable damage is death of the mangrove trees that have their pneumatophores (*Avicennia schaureiana*), or lenticels (*Rizophora mangle* and *Laguncularia racemosa*) covered with oil leading to asphyxia. Other species like mangrove crabs and birds that breed in the trees are also heavily affected. Odum e Johannes (1975), suggest that the critical value for mangrove survival is 200 mL $m^{-2}$. On the short term birds, crabs, mollusks and other invertebrates can be soaked with oil and die very rapidly because of the privation of movement. On the long term, the organisms can suffer from intoxication with the petroleum poly-aromatic hydrocarbons that remain in the sediments for very long periods. The mangrove destruction will affect the whole food chain of the region, because it is a breeding area for coastal and oceanic fishes (Chapman, 1976), many of them important economic species that support thousands of fishermen's families (Figueiredo, 2002).

Once spilled oil reach the mangrove area, impregnation in fine grained sediments renders it very difficult to clean and therefore, the only path for the recovery of such environments is long term bacterial degradation (Atlas, 1981) and photo degradation (Nicodem *et al.*, 1997). Mangrove recovery periods can be extremely long, if the degradation of the oil and the growth of the trees for the re-establishment of the original community is considered. In the South of Florida, the oil spill of a bunker was surveyed and after one year, plants were still dying (Lewis III, 1980). Until the 10$^{th}$ year, the colonization of the area was still difficult because of the presence of toxic substances that increase stress and reduce competition capacity. After 10 years the mangrove started to grow normally again, reaching its ecological maturity within 40 years (Lewis III, 1980).

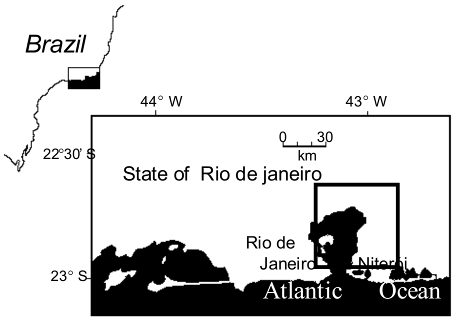
In the present work, the sediments of a very extensive law protected mangrove area in the Eastern part of the Guanabara Bay (Guapimirim mangrove) were screened for poly-aromatic hydrocarbons content and for bacterial activity. The aim was to understand the processes that control oil contamination in the mangrove sediments and if there is a relationship between contamination and bacterial activity. Considering that the presence of important amounts of organic matter and phosphorus in the sediments may interfere with the rate of biological degradation of hydrocarbons, these parameters were also measured.

## METHODS

### *Study Area*

The Guapimirim environment (Figure 1) is presently a law protected mangrove (APA) that constitutes the preserved reminiscent of the very extensive mangroves that originally covered the entire margins of the Guanabara Bay, Rio de Janeiro, Brazil. On a recent digital mapping of the area, based on Landsat satellite images Pires (2003) indicated that the whole APA-Guapimirim covers an area of 138.25 km$^2$. The author could identify 7 categories of mangrove radiation emmission, covering 61,80 km$^2$, and among them, the best preserved stands were observed in the limits of the Guanabara Bay waters (16.72 km$^2$). Within the limits of the APA-Guapimirim, he could also identify 27.07 km$^2$ of anthropic areas and the remaining 49.56 km$^2$ were classified as Guanabara Bay waters.

The climate of the region is classified as hot and humid. Monthly average temperatures range between 18.6°C (minimum average in August) to 30.5°C (maximum average in February). The average temperature of a 30 years series is 23.6°C. Annual rain-fall average of a 30 years period (1961-1990) is 1151 mm, but, as the northernmost part of the mangrove is closer to the mountain chains Serra do Mar and Serra dos Órgãos, a gradient can be observed and annual rain-fall of 2000 mm can be observed (Barbieri e Coelho-Neto, 1999).



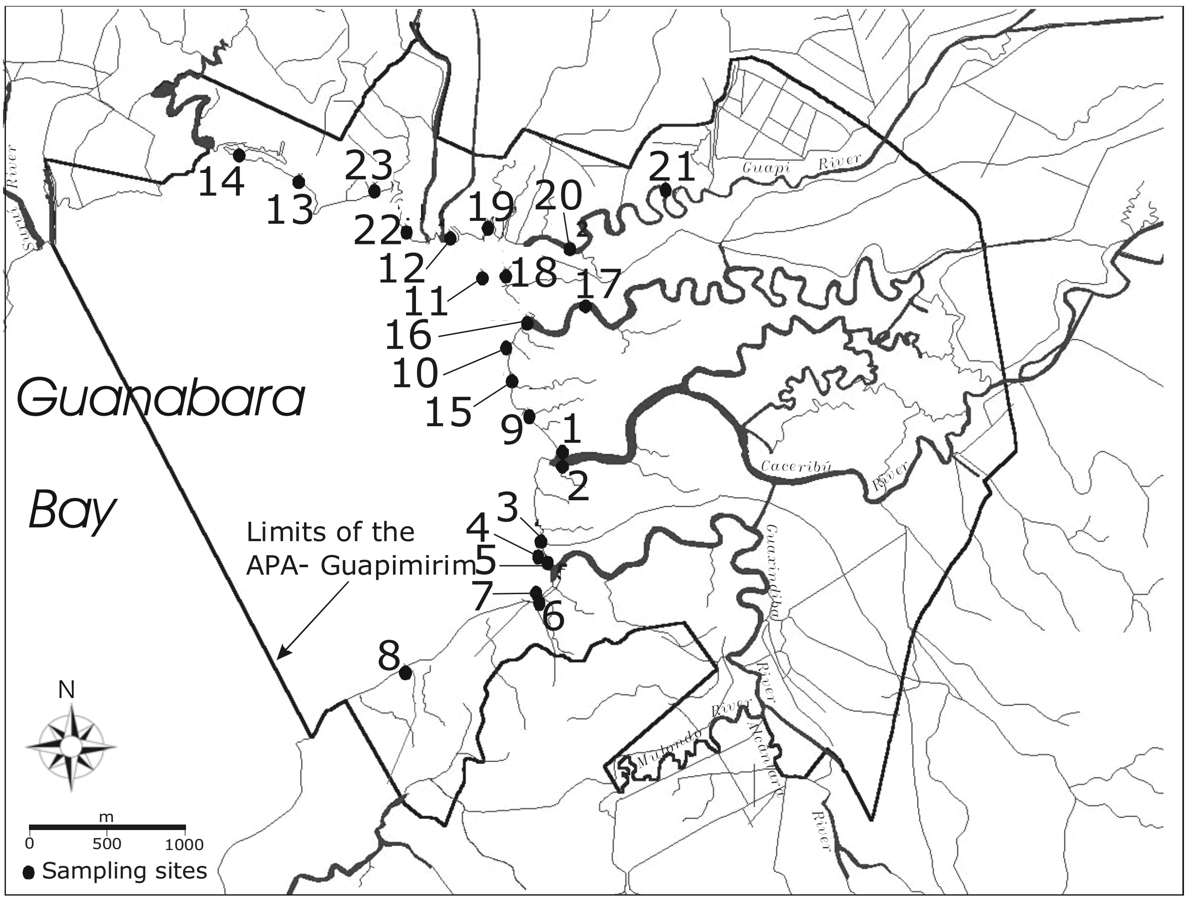




**Figure 1**: Study area showing the surface sediment sampling sites.

Although the area is law protected, traditional and artisan fishing and crab or shellfish collection is still allowed, since these activities were shown to be more or less sustainable (Pereira-Filho, 2003).

The contaminant loads flowing into the Guanabara Bay are considerable. The organic loads dumped from the untreated domestic sewage reaches 465 tons day$^{-1}$, and the presence of more than 14,000 industries in the region also contribute with important organic loads, mixed with heavy metals, and synthetic organic pollutants. The oil pollution is also a major problem. The Duque de Caxias Refinery alone contributes with almost 600 kg oil day$^{-1}$, while other reported sources contribute with a further 700 kg day$^{-1}$ (Information Center from the Guanabara Bay, 2002). More than one thousand service stations that are installed throughout the watershed should also contribute with unknown but considerable amounts of oil to the bay. Periodic oil spills resulting from accidents in the refineries, commercial harbors, dockyards and service stations can also produce considerable damages. The last major petroleum spill occurred in January 2000, when a transfer pipeline in the Duque de Caxias Refinery blew off, and discharged estimated 1.3 million liters of thick oil to the bay (Information Center from the Guanabara Bay, 2002).

### *Sampling Procedures*

The surface sediment samples were hand collected from 23 stations, as shown in the Figure 1, covering the whole mangrove area. Most of the samples were taken from under the mangrove trees, very close to the tidal flat that permitted an approximation with an outboard boat. At each station, position was determined with a portable Garmim 12 Global Positioning System (GPS) and direct measurements of pH and Eh, were carried out with a portable WTW potentiometer. Salinity of the interstitial water was measured with a Shibuia optical refractmeter and temperature was measured with a precision mercury thermometer. After collection, the samples were transported in ambient temperature, protected from the sun and in the laboratory, two aliquots were separated, one for the biological assays and the other for chemical analysis. The first aliquot was immediately analyzed while the second was frozen.

### *Analytical Procedures*

The biochemical indicators of the bacterial activity in the sediments were immediately analyzed. The bacterial activity indicators were: 1) electron transport system activity (ETSA) (Trevors, 1984; Houri-Davignon e Relexans, 1989); 2) activity of the Esterase enzyme (EST) (Stubberfield e Shaw, 1990); 3) bacterial organic carbon (BOC) was measured with the method described in Carlucci *et al.* (1986) and Kepner Jr. e Pratt (1994).

An aliquot of each sample was left frozen and another aliquot was mildly dried on a ventilated oven (not more than 40°C). Defrosted samples were extracted with n-hexane for 30 minutes in an ultrasound bath and the phases were separated by centrifugation. The extracts were analyzed for poly-aromatic hydrocarbons (PAHs), in a UV-visible spectrofluorometer (Perkin–Elmer LS-3). The chosen wavelength was 404 and 428 nm and a polyaromatic hydrocarbon mix standard (Supelco 47327) was used as calibrating solution. The procedure is an adaptation of the procedures described in Dejonge *et al.* (1997). Concentrations were expressed on a dry weight basis.

Oven dried samples were analyzed for total organic carbon (TOC) with the wet oxidation and titration procedure described in Strickland e Parsons (1972). Total phosphorus was measured on oven dried sediments with the persulfate oxidation procedure and analyzed in a photocolorimeter (Strickland e Parsons, 1972).

## RESULTS AND DISCUSSION

The results of *in situ* physico-chemical parameters measured in the sediments are presented in Table 1. It can be observed that pH values are close to neutral, which is attributed to the buffer effect of the saline tidal flooding waters. On the other hand, low hydrodynamism, a common feature of mangrove environments, favors the sedimentation of very fine grained sediments that hinders gas exchanges between free air and sediment surface. The lack of oxygen in the surface layers result in very low redox potentials (table 1) and in such conditions organic acids tend to be preserved, pulling the pH slightly down to values bellow seawater's. A number of coastal environments, regardless the environmental conditions tend to present pH values close to neutrality and extremely negative Eh values (*e.g.*: Clark *et al.*, 1998; Beiras *et al.*, 2003). The temperature showed a strong range of variation because sampling was carried out in two different days, a cooler day when temperatures measured were around 25°C (1 – 8) and a warm day when air temperatures reached 32°C (samples 9 – 23). Salinity in the interstitial water presented a considerable variability within the area that can be related to tidal flooding, and to the presence of freshwater inputs. The highest values (that can reach 36, Table 1) also indicate an evaporation process; that can be attributed to topographic features of each sampling site.

**Table 1**: Sediments physico-chemical parameters.

| Station | pH | Eh (mV) | Temp. (°C) | Sal. |
|---|---|---|---|---|
| 1 | 6,55 | -137 | 24 | 27 |
| 2 | 7,25 | -239 | 24 | 29 |
| 3 | 6,87 | -191 | 24 | 31 |
| 4 | 7,06 | -300 | 24 | 30 |
| 5 | 6,72 | -102 | 24 | 34 |
| 6 | 7,36 | -100 | 24 | 35 |
| 7 | 7,07 | -158 | 24 | 31 |
| 8 | 6,69 | -040 | 24 | 36 |
| 9 | 6,80 | -001 | 31 | 34 |
| 10 | 7,38 | -266 | 32 | 34 |
| 11 | 7,43 | -216 | 32 | 30 |
| 12 | 7,25 | -227 | 30 | 31 |
| 13 | 7,47 | -289 | 32 | 32 |
| 14 | 7,22 | -214 | 31 | 34 |
| 15 | 6,76 | -197 | 31 | 31 |
| 16 | 6,69 | -194 | 32 | 15 |
| 17 | 6,47 | -047 | 32 | 8 |
| 18 | 6,81 | -180 | 32 | 18 |
| 19 | 6,47 | -146 | 32 | 18 |
| 20 | 6,95 | -233 | 31 | 11 |
| 21 | 6,31 | -155 | 31 | 35 |
| 22 | 7,17 | -263 | 31 | 30 |
| 23 | 6,40 | -174 | 31 | 33 |
| **Av.** | 6.92 | -177 | 28.8 | 28 |
| **S.D.** | 0.35 | 79 | 3.6 | 8 |
| **R.** | 6.31- 7.47 | -300 - -1 | 24 - 32 | 8 - 36 |

Sal. = Salinity, Av. = Averrage, R. = Range

Petroleum hydrocarbons (PAHs), total organic carbon (TOC) and total phosphorus concentrations are presented in figures 2A and 2B and 2C, respectively. The results indicate that in spite of the very important diffuse sources of chronic petroleum pollution present in the region, only a limited contamination affects the Guapimirim mangrove area. An important oil spill occurred in the year 2000 (Information Center from the Guanabara Bay, 2002) that damaged very extensive areas of the bay's shore, however, Pires (1992) observed that the tidal conditions in the moment of the spill did not favored the drift of the oil spot to the Guapimirim mangrove. This absence of impact of the accident of 2000 in the Guapimirim Protected Area, was confirmed by local population, including fishermen and crab collectors. Nevertheless, stations 4 and 7 show values considerably higher than the average, which should attributed to a local restricted contamination. In fact, the Guanabara Bay is constantly subjected to localized oil spills from tankers compartments cleaning procedures, and small oil spots may randomly reach the margin. It is further interesting to note that, although stations 3, 5 and 6 (Figure 1) are close to stations 4 and 7, they did not show high values.

Stations 1 to 14, located in the tidal flats of the mangrove tend to present higher concentrations, while stations 15 to 23 (except 18), mostly located within the outcoming rivers, tend to present lower concentrations. The stations located in tidal flats are probably subjected to reduced hydrodynamics, and therefore should retain hydrocarbons longer in the sediments. In the outcoming rivers sediments, freshwater fluxes and tidal currents are constantly leaching hydrocarbons, explaining lower concentrations.

Total organic carbon (TOC, Figure 2B) presented very high concentrations that are typical for this kind of environment (Wasserman *et al.*, 2001; Kehrig *et al.*, 2003). Guapimirim mangrove trees contribute a considerable amount of autochthonous organic matter to the sediments, but the also receives sewage contaminated waters from the river system that drains residential and industrial areas. The mangrove sediment is also flooded by the highly contaminated waters of Guanabara Bay, that receives sewages from over 7 million people living in the city of Rio de Janeiro. The concentrations of organic carbon did no varyed sigificantly, but peak concentrations were observed on stations 4 and 8. The organic carbon compete with petroleum biodegradation process. Bispo (2000) showed that the bacterial degradation of Benzene, Toluene and Xylene is considerably reduced in the presence of elevated organic matter concentrations, because natural organic matter are biopolymers rich, a preferred bacterial carbon and energy source. The biopolymers are oxidized by bacterial metabolic pathways, while petroleum hydrocarbons require new bacterial enzymatic synthesis (Tiehm, 1994).

Total sedimentary phosphorus also showed elevated concentrations (Figure 2C), with higher values in the southernmost part of the study area. As discussed for organic matter, beside the autochthonous sources, the anthropic inputs are considerable. Anoxic sediments were shown to be important trapping environments for nutrients (Knoppers *et al.*, 1999), including phosphorus. Together with nitrogen, phosphorus should considerably influence bacterial growth in mangrove sediments, therefore, increasing consumption of petroleum hydrocarbons (Ramsay *et al.*, 2000).

Figure 3A presents the results of Electron Transport System Activity (ETSA) that indicate bacterial activity within the sediment. After the external breakdown of the organic matter, performed by the esterase, dehydrogenase enzymes and the ETSA are included in the process of organic matter metabolism. Activities were shown to be more elevated in the left side of the graphs (stations 1 to 14), presenting a similar behavior to hydrocarbon concentrations, that was not confirmed by correlation coefficients (Table 2). The ETSA presented in Figure 3A indicate that bacteria is actively metabolizing organic matter in the sites 1 to 14, whereas stations 15, 16, 17, 19 and 21 presented a very low activity, that only can be explained by site specific characteristics. The ETSA values indicate that although there are extremely high concentrations of organic matter available for the bacteria (figure 2B), some of the petroleum hydrocarbons are simultaneously degraded.

The esterase activities (EST, Figure 3B) are associated with the breakdown of organic matter polymers like cellulose, starch, proteins, and lipids (Stubberfield e Shaw, 1990), therefore, where organic matter is abundant, the concentrations of EST will be high. In the Figure 3B, it can be observed that except for station 4, EST generally presents high values of activity, coherent with the high organic matter contents. Low correlation coefficients between EST and TOC are attributed to the very high concentrations of organic matter that largely overcome the energy needs of bacteria to produce esterase. Low esterase activity in station 4 is probably associated to the present of high loads of PAH, which is consumed by ETSA's bacteria.

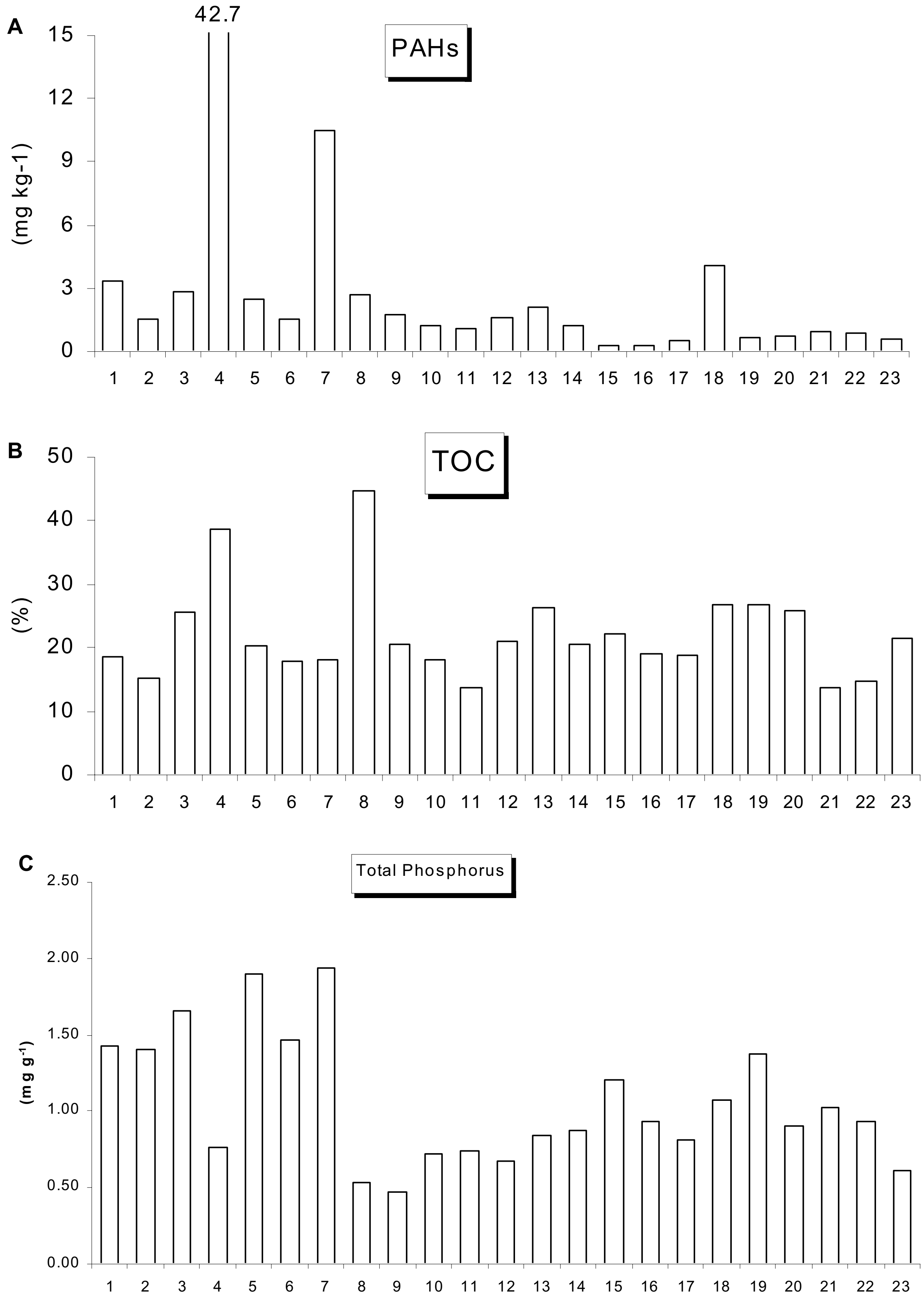



**Figure 2**: Petroleum hydrocarbons (PAHs - A), total organic carbon (TOC -B) and total phosphorus (TP - C) concentrations along the distinct sampling points in Guapimirim mangrove sediments.

| | COP | PAHs | ETSA | EST | TP | pH | Eh | TºC | Sal. | Coccus | Spir. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PAHs | -0.22 | | | | | | | | | | |
| ETSA | -0.29 | 0.17 | | | | | | | | | |
| EST | -0.12 | **0.39** | 0.07 | | | | | | | | |
| TP | -0.25 | 0.31 | 0.15 | **0.78** | | | | | | | |
| pH | -0.13 | 0.18 | **0.63** | 0.16 | -0.06 | | | | | | |
| Eh | 0.06 | -0.11 | -0.28 | 0.16 | 0.06 | **-0.54** | | | | | |
| TºC | -0.27 | **-0.39** | -0.29 | **-0.59** | **-0.57** | -0.05 | -0.17 | | | | |
| Sal. | 0.00 | 0.05 | **0.37** | 0.26 | 0.03 | 0.31 | -0.03 | **-0.37** | | | |
| Coccus | 0.24 | **0.38** | 0.17 | **0.63** | **0.45** | 0.20 | 0.12 | **-0.88** | 0.29 | | |
| Spir. | 0.27 | **0.47** | 0.10 | **0.60** | **0.46** | 0.20 | 0.01 | **-0.83** | 0.30 | **0.89** | |
| Rods | 0.23 | **0.41** | 0.22 | **0.69** | **0.59** | 0.12 | 0.18 | **-0.96** | 0.29 | **0.96** | **0.90** |

Sal= Salinity, Spir. = Spirillum

**Table 2**: Correlation matrix of the variables in the present study (n=23). Bold values are significant correlations a p<.05.

The bacterial biomass of the three morphologic groups showed a very sharp negative correlation with temperature (Table 2), indicating that this parameter may affect bacteria production. However, as correlation between ETSA and temperature was not significant, there should be no decrease in PAHs degradation under higher temperatures. The biomass values obtained in the majority of the stations (Figure 3C) are considerably higher than those observed in sandy beaches (Bispo, 2000), what can be attributed to the fact that mangrove fine grained sediments are considerably richer in organic matter. Beyond the fact that organic matter constitute a significant source of energy, this type of environment tend to retain considerable amounts of nutrients like phosphorus (Figure 2C). Furthermore, humidity provided by shadowed environment and flooding periods observed in mangroves are more suitable for bacteria development than sandy beaches. In the Saquarema lagoon (Rio de Janeiro, Brazil), Crapez *et al.* (1993) also identified elevated bacterial production, but still lower than the values presented in this work. In that lagoon, the anthropic pressure was not as important and lower loads of nutrients and organic matter were identified (Carmouze e Vasconcelos, 1992), than the values obtained in Guapimirim mangrove.

Bacteria belonging to the morphologic group of rods were shown to be dominant all over the area, and the bacterial biomass was low in stations 9 to 23. Bacterial carbon, ETSA and EST are significantlyhigher in the stations 1 to 8 (Southeastern part of the area, Figure 3), although ETSA presented low correlation coefficients (Table 2) indicating that the biomass, in these 8 sites is not actively metabolizing organic matter. Furthermore, the rate of organic matter consumption seems not to be affected by the reduction on bacterial biomass observed in the remaining sites, as shown by EST throughout the system. Furthermore, low ETSA and PAH indicate that only organic matter was degraded.

Although the dominance of the rods morphological group in the sediments was expected, the elevated values observed indicate that besides the saprophytic bacteria, probably entero-bacteria are also largely represented. These bacteria are associated with the domestic sewage dumped in the area.

The EST concentrations of the sediments seem to be good indicators of the bacterial biomass as shown by correlation coefficients between the three bacterial forms and EST (Table 2). On the other hand, ETSA is a better metabolic indicator that shows how actively bacteria are feeding. The correlation coefficients (Table 2) indicate that the presence of petroleum hydrocarbons favors the development of rod communities, while cocci and spirillum communities seem to be inhibited by the presence of this pollutant.

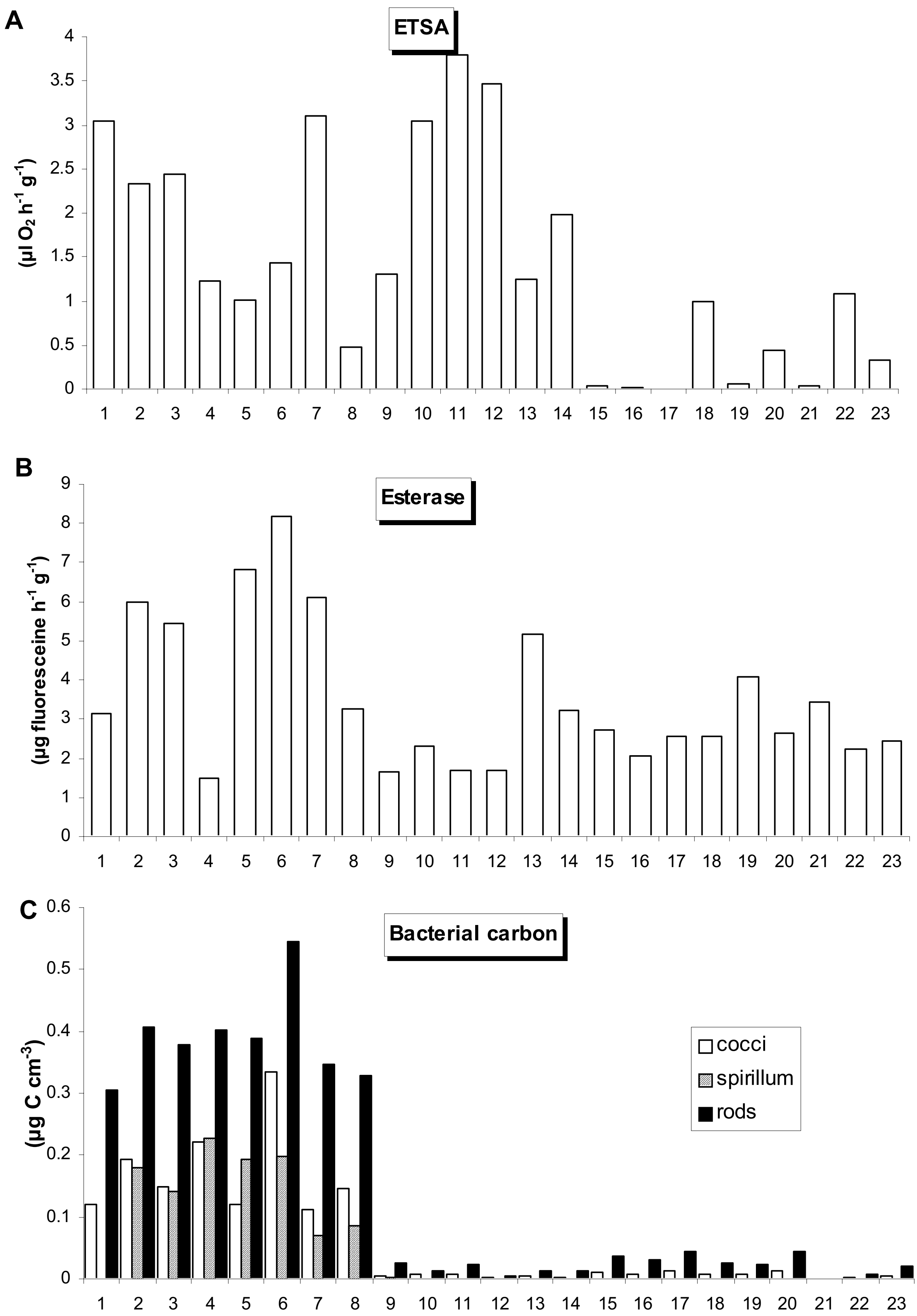



**Figure 3**: Bacterial enzymatic activity (ETSA, EST) and bacterial biomass in the sediment, showing three different morphologic groups.

Besides bacteria, the effects of the petroleum hydrocarbons on the biota were not yet identified, but it is possible that crabs, species of economic interest, are starting to decline. In a recent study Pereira-Filho (2003) showed that the populations of *Ucides cordatus*, a heavily predated crab fish of the APA-Guapimirim are consistently decreasing, reducing the yield of the fishermen of the region. This author attribute the reduction to over fishing, but it is possible that the presence of high concentrations of PAHs inhibit larval settling and organism reproduction.

## CONCLUSIONS

In spite of the heavy loads of petroleum dumped in the region, contamination of the sediments of the Guapimirim law protected mangrove is still mild. Bacterial communities were shown to be important degradators of PAHs. In the present work, the bacteria morphologic group of rods presented a better capacity to resist PAHs, but coccus and spirillum were eliminated by the presence of the oil. The rods dominated bacterial communities in all stations.

Esterase concentrations in the sediments were shown to be good indicators of the bacterial biomass. On the other hand, ETSA seems to be active most of the time, because bacteria is using readily available organic matter.

Under tropical climatic conditions the processes that relate bacterial activity and oil degradation seem very complex. Organic matter concentrations can be extremely elevated and bacteria would rather use energy from readily available compounds, therefore reducing PAHs degradation rates. In consequence, petroleum spills remediation using hydrocarbonoclastic species would be effectiveless if environmental conditions are not taken into account.

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(Footnotes)

[1]To whom the correspondence should be sent: geowass@vm.uff.br.