Validation of *Ucides cordatus* as a bioindicator of oil contamination and bioavailability in mangroves by evaluating sediment and crab PAH records

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Abstract

This study is aimed at verifying the relevance of *Ucides cordatus* as a bioindicator of oil contamination and PAH bioavailability in mangrove sediments. For this, crabs and sediment cores were sampled from five mangroves, including an area suspected of contamination derived from an MF380 oil spillage, and analyzed for the 16 PAH in the USEPA priority list as well as for the five series of alkylated homologues. Concentrations in sediments varied from 35 μg kg⁻¹ in the lower core layer of the control area to 33,000 μg kg⁻¹ in the upper layer of the most contaminated area. Total PAH contents in crabs varied from 206 to 62,000 μg kg⁻¹ and were closely correlated to that in sediments. In general, individual PAH profiles in both matrices were in good agreement. Phenanthrenes, however, were more predominant in crabs making up to 30–46% of the Total PAH. Accumulation factors found in the range of 0.7 to 35 were highly variable even after normalizing concentrations for organic carbon and lipid content. Survival in highly contaminated environment and reliable record of environmental contamination in the tissue provide evidence that *U. cordatus* is an excellent bioindicator for oil in mangroves.

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Keywords: *Ucides cordatus*; Crabs; PAH; Oil contamination; Sediments; Monitoring

1. Introduction

The majority of Brazilian oil production derived from offshore fields, mainly from the Campos Basin, in the state of Rio de Janeiro. This area supplies nearly 80% of the oil exploited in the country. The oil produced is transported, transferred and/or processed in coastal areas, often populated with mangrove forests which have been threatened by oil spills and illegal oil releases. There is a need to develop adequate means to monitor oil derived contaminants in these ecosystems, as well as their effects on the mangrove distinctive biota. The polycyclic aromatic hydrocarbons (PAH) present in oils are of special concern because many of its constituents are persistent, toxic and show mutagenic activity.

PAH are widely disseminated in aquatic environments as a result of natural and anthropogenic processes. Petroleum, incomplete combustion of fossil fuels (Neff, 1979) and forest fires are important sources of this group of substances. PAH from pyrolytic sources are predominantly non-substituted parental polyaromatic hydrocarbons, while those present in oils are largely alkylated homologues (Steinhauser and Boehm, 1992). Several papers have shown that marine organisms are prone to bioaccumulate these substances, particularly in lipid-rich tissues (Neff, 2002; Carls et al., 2004; Francioni et al., 2005; Dugan et al., 2005).

PAH present in sediments or food are assumed to be less bioavailable than in water, because bioaccumulation seems to involve release in solution before uptake (Pruell et al., 1987). The bioaccumulation of certain PAH by organisms in sediments...
depend, therefore, on their water solubility, water/solid phase partitioning and \( K_{ow} \) (Bierman, 1990; Lamoureux and Brownwell, 1999), as well as on the solid phase properties, as for instance, organic carbon content and speciation (Rust et al., 2004; Sundelin et al., 2004; Cornelissen et al., 2005). Crustaceans and bottom dwelling organisms can absorb toxins by ingesting contaminated food. Biotic (reproductive stage, age, sex, metabolism, lipid content, etc.) and physical (temperature and salinity) factors also influence bioaccumulation (Meador et al., 1995; Neff, 2002) and are responsible for the concentration variability observed within a population.

Organisms that accumulate contaminants but are resistant to their toxicity can be used in monitoring contamination in the marine environment (Cossa, 1989). Among various aquatic organisms, invertebrates have been preferred for environmental assessment. They are the major components in all ecosystems and, because of the usually numerous population, they can be sampled for analyses with little damage to population dynamics (Depledge and Fossi, 1994). Mussels are most frequently used as sampled for analyses with little damage to population dynamics and, because of the usually numerous population, they can be organisms, invertebrates have been preferred for environmental

forest restoration may take several decades (Tam et al., 2001). The most vulnerable to oil contamination (Grundlach and Hayes, contamination in mangroves which are among the ecosystems ly exposed to contaminants deposited in this environment. Such suitability of and depends on the properties of the mangrove area. Bioaccumulation of PAH in contaminated sediments by crab species has been reported (Pancirov and Brown, 1977; Hellou et al., 1994; Kayal and Connell, 1995; Axys Group, 1995; Baumard et al., 1998; Eichhoff et al., 2003; Dugan et al., 2005). Nevertheless, very few address bioaccumulation of alkylated homologues and none of these reports target the validation of crabs as a biomonitor for oil contamination. Also, there are no reports of monitoring oil contamination in mangrove ecosystems by using crabs.

Crabs are more efficient than mussels in biotransforming PAH into polar metabolites, facilitating excretion (Fillmann et al., 2004). The crab hepatopancreas plays a role similar to the liver in vertebrates and is the organ where biotransformation takes place. Therefore, analyses of contaminants in hepatopancreas provide information primarily on recent exposure and should reflect actual levels in the environment.

The crab Ucides cordatus is ubiquitous in inter-tidal habitat along the Brazilian coast. In mangroves, during low tide, U. cordatus escapes to sediment burrows where it can be directly exposed to contaminants deposited in this environment. Such a species is a good candidate to be used as biomonitor for PAH contamination in mangroves which are among the ecosystems most vulnerable to oil contamination (Grundlach and Hayes, 1987).

Once an oil spill reaches a mangrove, degradation is slow and forest restoration may take several decades (Tam et al., 2001). The persistence of oil in mangrove sediments is also associated to the activity of crustaceans by which the contaminant may be carried down to lower layers of the sediment, where degradation is slow due to reduced molecular oxygen concentrations. According to Irvin et al. (1997) this effect is more significant for the high molecular weight PAH. It is recognized that the fate of oils in mangrove and the associated impact will vary for different oils and depends on the properties of the mangrove area.

The goal of the present study was to investigate the suitability of U. cordatus as a biomonitor of oil contamination in mangrove sediments and as an indicator of oil bioavailability. For this, PAH concentrations as well as individual distribution in crab tissue (hepatopancreas) and in sediments were obtained and compared, as a means to determine: (1) the degree of proportionality between environmental contamination and body burden; and (2) how closely crabs reproduce PAH source fingerprinting found in sediments. This work also aimed to determine PAH levels in a mangrove contaminated by a major oil spill that took place in January 2000 in Guanabara Bay.

2. Materials and methods

2.1. Study area

Guanabara Bay is an urban estuary surrounded by a densely populated area of the city of Rio de Janeiro and of other seven smaller municipalities. This bay was selected to test the possibility of using U. cordatus as a biomonitor for oil in mangrove sediments owing to: (1) the presence of mangrove forests, some of which were contaminated in January 2000 by a major spill of MF 380 oil; (2) the presence of several potential oil sources (two oil refineries, an oil terminal, the second largest harbor in the country, several ship yards and marinas, intense ship traffic, urban runoff, oil transport and transfer); (3) the role played as a service area to the oil exploitation fields in Rio de Janeiro, providing infrastructure and ancillary activities for the petroleum industry.

2.2. Sampling

The strategy was to obtain sediment cores and crab samples from mangrove areas in Guanabara Bay thought to have different PAH contamination levels. Sediments and crabs were collected from five mangrove areas (Fig. 1). Four areas are in Guanabara Bay (Peteca Channel (PT) sampled in 2004; Nova Orleans (NO); Suruí (S); and Piedade (P) sampled in 2003 and 2004) and one, taken as control, is in Guaratiba (G, sampled in 2004). Guaratiba is a rather pristine area located 60 km west of Guanabara Bay (Fig. 1). The Suruí mangrove, situated between two branches of the Suruí River, is frontally and laterally flooded during high tide. In all mangrove areas, except for Piedade, cores and crabs were collected at two stations: station 1 was near the fringe, where higher contamination levels were expected; and station 2 was located about 200–300 m inland from the fringe. In the Peteca, station 1 is in a site crisscrossed by underground oil pipelines while station 2 is at the bay rim.

Sediment samples (S) were collected by using a piston corer (Husky Duck Equipments) especially designed for sampling mangrove sediments and equipped with 1.0 × 0.07 m sharp edged aluminum tubes. In each station samples were taken from 5 different points located 10 m apart. A total of five cores were then available at each station. Each core was sliced into three layers labeled as: layer 1: 0–28 cm; layer 2: 28–58; and layer 3: 58–90 cm. Thereafter, sediment layers from corresponding depths were mixed together to produce three composite samples for each station. The thickness of the first two layers were defined based on the depth intervals from where crabs were collected. Crabs were usually harvested from burrows up to 40–50 cm deep. In spite of this, a sediment layer below this depth was sampled to cautiously check for possible influences of the PAH content on the crabs. Cores from Guaratiba and Peteca had shorter lengths and were sliced into two layers of 0–28 cm and 28–58 cm. Samples were stored in clean aluminum boxes at −20 °C. A separate set of cores was used to measure \( \rho_{H_2O} \), redox potential and to sub-sample for organic carbon, grain size and water content determinations as described in Section 2.3.

Crabs were collected from the same sites as for sediments with the help of artisan fishermen. The number of harvested crabs varied from 15 to 30 as a result of the availability at each site. Animals were removed manually from the burrows and only adult males with carapace size in the range of 60–70 mm were taken. Females were not sampled to avoid the uncertainties imposed by the reproductive cycle. In the laboratory, mud was removed from each animal before weighing and measuring the carapace width with vernier calipers. Hepatopancreas (H) were removed with the help of surgical tools (decontaminated with detergent, acetone and dichloromethane) after opening the carapace with scissors. The material collected from the set of 15–30 crabs was lumped together
before homogenization in an UltraTurrax (Ika Labortechnik-T25 basic), and storage in clean glass containers at −20 °C. Sub- aliquots for lipid content and dry weight determination were stored separately.

2.3. Determination of ancillary data in sediment

$E_h$ and pH were measured in fresh sediments, immediately after sampling, with a platinum electrode and a combined glass electrode, respectively. Sediment fraction <63 μm was determined gravimetrically after treating the samples with hydrogen peroxide, drying and sieving through a 63 μm sieve. Organic carbon determination was performed in 10 mg aliquots of the 63 μm fraction, after eliminating carbonates with 2 M HCl (Suprapur Merck) and drying at 60 °C to constant weight. Measurements were carried out in a TOC-5000A Shimadzu equipped with an SSM-5000A module for solid samples. Water content was determined gravimetrically.

2.4. Determination of PAH in sediments

PAH determinations were based on the USEPA (US Environmental Protection Agency) methods 3540C (USEPA, 1996a) and 8270C (USEPA, 1996b). Aliquots of 10 g of fresh sediment were treated with anhydrous Na₂SO₄ (Merck) previously decontaminated at 450 °C. To this mixture, 100 ng of $p$-terphenyl-d₁₄ (AccuStandard) were added as surrogate standard before Soxhlet extraction over 24 h with a 1:1 mixture of dichloromethane-acetone (UltimAR-Mallinckrodt). The extract was rotary evaporated and concentrated to about 1 mL under nitrogen stream before quantitative transference to a 1 mL class A volumetric flask. The volume was completed with hexane after adding 100 ng of an internal standard mixture containing naphthalene-d₈, acenaphthene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂ (AccuStandard).

2.5. Determination of PAH in hepatopancreas

PAH determination in the hepatopancreas homogenates was performed in triplicate according to the procedure described below. To aliquots of 3 g of tissue homogenate, 30 g anhydrous Na₂SO₄ previously decontaminated at 450 °C were added followed by 100 ng of $p$-terphenyl-d₁₄, used as surrogate standard. The mixture was Soxhlet extracted with 200 mL dichloromethane over 24 h. The obtained extract was rotary evaporated and concentrated to approximately 1 mL under N₂ stream. The 70% lipid content in the extracts were reduce in two steps: (1) the extract was first cleaned by adsorption chromatography in a 2.2 diameter × 30 cm length glass column packed with 20 g of 2% deactivated neutral alumina. After elution with 100 mL of dichloromethane, the extract was rotary evaporated and concentrated under N₂ stream to approximately 1 mL. The 70% lipid content in the extracts were reduce in two steps: (1) the extract was first cleaned by adsorption chromatography in a 2.2 diameter × 30 cm length glass column packed with 20 g of 2% deactivated neutral alumina. After elution with 100 mL of dichloromethane, the extract was rotary evaporated and concentrated under N₂ stream to approximately 1 mL. Before solvent exchange to acetone-cyclohexane (3:7) (HPLC/Spectro, Tedia); (2) the pre-cleaned extract was further purified by gel permeation using a Shimadzu system equipped with a LC-10AD pump, a UV–VIS detector Shimadzu and Shodex CLNpack columns. Acetone-cyclohexane was used for eluting the analytes of interest. The elution volumes where calibrated with a GPC EPA mixture (AccuStandard) containing corn oil, bis(ethylhexyl) phthalate, methoxychlor, perylene and sulfur. The purified extract was rotary evaporated and concentrated under gentle N₂ stream to about 1 mL. After volume correction the extract was treated as described in item 2.4 to obtain the aromatic fraction.

2.6. GC-MS analysis, quantification and quality assurance

Determination using a full scan mode proceeded in a Finnigan Trace GC gas chromatograph coupled to a Finnigan Polaris Q Ion Trap mass spectrometer fitted with a J and W DB-5 MS ITD capillary column (30 m × 0.25 mm × 0.25 μm). Helium, the carrier gas, was adjusted at 1.2 mL min⁻¹ and the column
temperature was programmed as follows: 5 min at 50 °C, 50 °C min⁻¹ up to 80 °C, 6 °C min⁻¹ from 80 °C to 280 °C, and a final hold of 25 min at 280 °C. The injector temperature was at 250 °C (interface at 300 °C and ion source at 250 °C; electron impact 70 eV and emission current 250 mA), and the injected volume in splitless mode was 2 μL.

Quantification included the following 37 compounds and/or groups of alkylated compounds: (1) The 16 USEPA PAH: naphthalene (N), acenaphthylene (Acen), acenaphthene (Ace), fluoranthene (F), phenanthrene (Ph), anthracene (Ant), fluoranthene (Fl), chrysene (Ch), pyrene (P), benzo(a)pyrene (BaP), benz(a)anthracene, (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-c,d)pyrene (IP), dibenz(a,h)anthracene (DBA), benzo(ghi)perylene (BghiP), and (2) pyrene (P); dibenzothiophene, dibenzothiophenes (CBT, CDBT), chrysenes (C1Ch and C2Ch), and pyrenes (C1P and C2P).

Standard solutions in two concentration ranges were used for quantification: one for the low concentration levels (5, 10, 20, 50, 100 ng mL⁻¹) and another for the higher concentration levels (50, 100, 200, 400 and 1000 ng mL⁻¹). They were prepared including 100 ng mL⁻¹ of each deuterated standard (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and pyrene-d12), the 16 USEPA PAH, 2-methylnaphthalene, 1-methylnaphthalene, dibenzothiophene, and pyrene (AccuStandard). Quantification of the alkylated PAH was based on the calibration curve of the non-alkylated homolog except in the case of 1 and 2 methylnaphthalene, for which authentic standards were available. Baselines for both variables as well as the smallest fraction of fine sediments (fraction <63 μm: Surui, 20–60%; Piedade, Nova Orleans and Peteca, 70–98%; Guaratiba, 50–80%). As expected, Corg decreased with depth in the sediment cores. As for pH and Eh, most samples from the first sampling period were slightly anoxic (Eh around 0 V up to −0.150 V) or oxic (Eh of 0.700 V in Peteca, for example) and pH was 6.86 ± 0.52. In the second sampling period pH was 6.78 ± 0.18.

Sediments layers from Guanabara Bay mangroves showed a wide range of PAH concentration (Table 1). In the upper sediment layer the Total PAH ranged from 615 μg kg⁻¹ in Piedade to 33,000 μg kg⁻¹ in Surui, while in Guaratiba (the control area) it was 175 μg kg⁻¹ (Table 1).

The nonparametric Spearman test used to verify possible correlations among PAH concentrations, pH, Eh, and organic carbon gave negative results. The lack of correlation between PAH and Corg can be explained assuming that phase distribution of such compounds is effectively controlled by specific fractions of Corg, for instance black carbon and humic material (McCreddy et al., 1996; Gustafsson et al., 1997).

### Table 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Mangroves</th>
<th>Sediments (0–28 cm)</th>
<th>Sediments (28–58 cm)</th>
<th>Sediments (58–90 cm)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Total PAH Σ16 PAH</td>
<td>Total PAH Σ16 PAH</td>
<td>Total PAH Σ16 PAH</td>
</tr>
<tr>
<td>(September 03) Piedade 1</td>
<td>303 ± 87</td>
<td>85 ± 14</td>
<td>615</td>
<td>305</td>
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<tr>
<td></td>
<td>6551 ± 363</td>
<td>445 ± 81</td>
<td>1135</td>
<td>234</td>
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<tr>
<td></td>
<td>925 ± 82</td>
<td>58 ± 4</td>
<td>1098</td>
<td>217</td>
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<tr>
<td></td>
<td>62297 ± 6314</td>
<td>2290 ± 773</td>
<td>31500</td>
<td>1001</td>
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<tr>
<td></td>
<td>62398 ± 8291</td>
<td>1744 ± 247</td>
<td>33082</td>
<td>913</td>
</tr>
<tr>
<td>(October 04) Piedade 1</td>
<td>330 ± 27</td>
<td>41 ± 4</td>
<td>792</td>
<td>421</td>
</tr>
<tr>
<td></td>
<td>2319 ± 172</td>
<td>187 ± 41</td>
<td>924</td>
<td>171</td>
</tr>
<tr>
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<td></td>
<td>46272 ± 3209</td>
<td>1519 ± 39</td>
<td>23540</td>
<td>972</td>
</tr>
<tr>
<td></td>
<td>3316 ± 81</td>
<td>164 ± 13</td>
<td>6601</td>
<td>320</td>
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<tr>
<td></td>
<td>412 ± 45</td>
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<td>206 ± 33</td>
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<td>5727 ± 391</td>
<td>516 ± 46</td>
<td>2550</td>
<td>337</td>
</tr>
</tbody>
</table>

Total PAH and Σ16 PAH are given in μg kg⁻¹ dry weight.

a Standard deviation for the hepatopancreas analysis in triplicate.
which may be present in the studied sediments as different proportions of total Corg. These fractions were not quantified in the present work.

The mangroves in Guanabara Bay can be ranked according to the PAH contamination level as follows: Surui > Peteca > Nova Orleans > Piedade. Satellite images obtained after the oil spill of January 2000 demonstrated that Peteca, in the proximity of the oil spill, and especially Surui had been severely contaminated (Bentz and Miranda, 2001). The high concentrations found three to four years after the spill in Surui provide indications of the oil persistence in the mangrove as will be shown later.

In layers 1 and 2 of both Surui stations, alkylated naphthalenes (ΣC1N=15,700 μg kg⁻¹; ΣC2N=14,500 μg kg⁻¹), alkylated pyrenes (ΣC1P=3000 μg kg⁻¹; ΣC2P=2000 μg kg⁻¹), alkylated phenanthrenes (ΣC1Ph=4700 μg kg⁻¹; ΣC2Ph=2300 μg kg⁻¹), alkylated fluorenes (ΣC1F=3200 μg kg⁻¹; ΣC2F=1500 μg kg⁻¹) and alkylated chrysenes (ΣC1Ch=2000 μg kg⁻¹; ΣC2Ch=1100 μg kg⁻¹) are the major components, as should be expected for sediments contaminated with oil. A decrease in concentration with depth is generally observed though more accentuated in station 2 (Fig. 2) in 2003. At the fringe, tidal inflow added to crustacean activity may contribute to downward transport of
contaminants, leading to the high PAH concentrations observed in sediments from 58–90 cm depth (11,600 μg kg⁻¹ station 1, first sampling).

In Surui, Total PAH declined from 2003 to 2004 in all sediment layers of both stations 1 and 2. In layer 1 the decrease was of 75%. As shown in Fig. 2, the increasing concentration of naphthalene and fluorene homologues from C0 to Cn is typical of weathered oil. Phenanthrenes and dibenzothiophenes in layer 1, however, show predominantly bell-shaped concentration profiles, usually an indication of recent oil inputs. The ratios C2DBT/C2Ph and C3DBT/C3Ph were then used to investigate the source of the oil residues present in these sediments. Such ratios are considered robust tools (Wang et al., 1999; Readman et al., 2002) to identify the source of oils present in sediments. The C2 pair, as well as the C3 pair, shows similar solubility and weathering rates, which cause the concentration ratio to remain constant in time. The calculated ratios for sediments from the two upper layers of stations 1 and 2 in Surui (0.53 < C2DBT/C2Ph < 0.65; 0.53 < C3DBT/C3Ph < 0.58) are similar to those of the MF380 oil (C2DBT/...
C2Ph = 0.59; C3DBT/C3Ph = 0.64), in spite of the chronic oil contamination in the bay (derived from illegal releases and frequent spillage of diverse magnitudes), which interferes with the process of identification of a single event. In the deeper sediment layers such association is less straightforward, possibly due to the lower contributions of MF380 to the mixture of earlier contaminations.

In light of the above results, the bell-shaped concentration distribution of Ph and DBT homologues may be ascribed to a relatively higher persistence of these compounds in the mangrove environment. The sandy acidic soil in Surui (among the studied areas only with less than 35% of fine grains in surface sediments) may contribute to stagnation of pollutant concentration.

Absorption inside organic particles or occlusion in other sediment components may render the contaminants inaccessible to bacteria responsible for degradation. Under these conditions half lives turn out to be longer than expected for degradable substances. The formation of tar droplets with low surface to volume ratios, that occurs in the case of massive contamination, also limits the bacteria access to the PAH. Also, it has been shown that the sequential metabolism of PAH by marine microorganisms leads to preferential utilization of substrates. For instance, Foght et al. (1989), and Stringfellow and Aitken (1995) observed that naphthalene (N) undergoes preferential biodegradation in comparison to phenanthrene (Ph); the presence of fluorene (F) also retards Ph degradation. An attempt to verify possible hindrance of
phenanthrene degradation by these compounds was made here by searching for trends between phenanthrene ($\Sigma$Ph) and naphthalene ($\Sigma$N)+fluorene ($\Sigma$F) concentrations. The Spearman test gave good correlation coefficients for log $\Sigma$Ph versus log ($\Sigma$N+$\Sigma$F) in Surui ($r^2=0.835$; $p<0.05$; $n=12$) and also when grouping all examined mangrove areas ($r^2=0.882$; slope of 0.85; $p<0.05$; $n=26$). The possibility that these trends represent an in situ evidence of interdependent PAH degradation must be better investigated. However they, in principal, cannot be ascribed to a common origin of the compounds, since the degradation rates of each single PAH considered separately differ significantly and, therefore, should cancel out such correlations.

As for the differences in total concentration found in 2003 and 2004 (Table 1) degradation may not be the only cause, but they may rather result from the combination of two factors: the difficulty in positioning with high accuracy the sampling stations under the mangrove forest, and the heterogeneous distribution of the oil in sediments. In some cores, droplets of oil were visibly present and may have contributed to the higher values in the 2003 sampling, despite the use of sediment homogenate prepared using 5 cores from each sampled station. This aspect underlines the complexity associated with environmental impact assessment in mangrove areas. It should be emphasized, however, that individual PAH composition found in sediments collected in 2004 concurs with those obtained for samples from 2003 (Fig. 2).

The Total PAH concentration found in Surui three to four years after the oil spill is of the order of magnitude of those reported for sediments from Caribbean coast mangrove in Bahia de Las Minas four years after the Galeta Oil Spill (Burns et al., 1994) and from Prince William Sound after the Exxon Valdez spill (O’Clair et al., 1996). Comparison with other regions, as for instance those given under Readman et al. (2002), is difficult because many of the cited reports address only parental PAH which in the present case are not the prominent components.

Individual PAH distribution in Peteca (Fig. 3) differs from that in Surui. The major compounds are alkyalted phenanthrenes, pyrenes and chrysenes, while naphthalenes are at the level of some tens of $\mu g$ kg$^{-1}$. The distribution profile of homologues indicates that oil residues are further degraded than in Surui. Peteca has a known history of oil contamination due to pipeline leakages and other events. This may have resulted in adaptation of the microbial community to degrade oil more effectively than in other examined mangroves. In spite of the degradation and of the presence of other oil residues, samples from station 2 show indicative ratios (C2DBT/C2Ph=0.62; C3DBT/C3Ph=0.61) similar to those of MF380.

The Nova Orleans mangrove showed PAH concentrations more than one order of magnitude lower than in Peteca or Surui. As shown in Fig. 4, phenanthrenes are dominant in the first layer (from Ph=8.3 to maximum in C3Ph=150 $\mu g$ kg$^{-1}$) and the relative proportion of dibenzothiophenes is higher than in the other stations, in all sediment segments.

In Piedade (Fig. 5), the least contaminated mangrove in Guanabara Bay, the individual PAH profile differs significantly from the others by showing predominance of PAH heavier than fluoranthene. Guaratiba, the control area, also shows a distinct individual PAH profile (Fig. 3) in which naphthalenes, fluorene and pyrenes are dominant.

The ratio between the 16 USEPA PAH and the Total PAH can be used to compare sources of hydrocarbon contamination, as high proportions of parent aromatic hydrocarbons are characteristic of pyrogenic residues. In Nova Orleans, in the intermediate sediment layer, the significant contribution of heavier PAH of pyrolytic origin leads to a rise in the $\Sigma$16 PAH to 50% of the total (Fig. 6). In Piedade, the presence of quasi even proportions of petrogenic and pyrolytic PAH is evident since the ratio of $\Sigma$16 PAH/Total PAH ranges from 49–56%, in 2003, and from 30 (in the lowest segment)–53% in 2004. It should be noted that combustion sources are abundant in the surroundings of all mangrove areas (vehicles and industrial activities are most relevant in the area of Peteca, while biomass burning is more important in the other mangroves). However, combustion derived PAH was generally low in sediments and in crab tissue. The contribution of $\Sigma$16 PAH to Total PAH increased with decreasing contamination level; therefore, it is less prominent in Surui and Peteca as shown in Fig. 6.

Besides the fraction of $\Sigma$16 PAH, there are at least four diagnostic ratios usually applied to distinguish petrogenic sources from pyrolytic
sources (Wang et al., 1999; Readman et al., 2002), such as: Ph/Ant, Fl/P, C1Ph/Ph; and the other 3–6 rings PAH/Σ 5 alkylated PAH series (Σ 5 alkylated PAH: C0–C4 naphthalenes and phenanthrenes; C0–C3 fluorenes and dibenzothiophenes; and C0–C2 chrysenes. Σ other 3–6 rings PAH: other parental PAH with 3 to 6 rings plus perylene and benzo(e)pyrene). Because chrysene and anthracene were not found in many samples, only two ratios were applied here: Fl/P and “Σ other 3–6 rings PAH/Σ 5 alkylated PAH series”. Table 2 shows the results of these two diagnostic ratios confirming predominance of petrogenic sources in Surui. However, for Peteca, Nova Orleans, and Guaratiba (layer 1) only Fl/P indicated petrogenic predominance. The diagnostic ratios indicate inputs from both sources in Piedade.

The sediment quality benchmarks proposed by Buchman (1999) were used to evaluate the potential risk of the measured PAH concentrations to the benthic biota. In spite of the low fraction of priority USEPA PAH, in Surui several of the 16 PAH were above TEL (Threshold Effect Level); in station 2, acenaphthene was above PEL (Probable Effect Level = 88.9 μg kg⁻¹) and anthracene was close to the PEL (245 μg kg⁻¹); in stations 1 and 2, benz(a)anthracene > TEL (88.81 μg kg⁻¹), chrysene > TEL (107.77 μg kg⁻¹), fluorene > TEL (21.17 μg kg⁻¹), 2-methylnaphthalene > TEL (20.21 μg kg⁻¹). In Peteca benz(a)anthracene was also above TEL. The sum of the low molecular weight PAH (N, Acen, Ace, F, Ph and Ant) was above TEL (311.7 μg kg⁻¹) in the sediments of layers 1 and 2 of station 1, and above ERL (552 μg kg⁻¹) in layer 1 of station 2. For the sum of high molecular weight PAH (Fl, P, BaAnt, Ch, total BFl, BaP, IP, DBAnt and BghiPe) only in layer 1 of station 1 the value was higher than TEL (655.34 μg kg⁻¹). Benchmarks for alkylated homologues are not yet available, although non-planar PAH seem to be more susceptible to react in a biological system. Furthermore, the most important analytes to consider in damage assessment for oil spills are the alkylated homologues, which are more persistent and often more toxic than the parental compounds (Sauer and Boehm, 1991; Irwin et al., 1997).

3.2. PAH in crabs

Weight and carapace length of sampled crabs ranged from 114 to 173 g and from 62 to 73 mm, respectively. Lipid content in the hepatopancreas was of 50 to 74% with the lower values found in animals from Nova Orleans; humidity ranged from 50 to 74%.

Concentration trends of PAH recorded in the hepatopancreas and sediments provide, in general, (see Table 1) similar information on the different contamination status of the mangrove areas. The prevailing

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Diagnostic ratios applied to distinguish petrogenic from pyrolytic sources (Fl/P and Σ 3–6 rings PAH/Σ 5 alkylated PAH series) and to trace oil identity (C2DBT/C2Ph and C3DBT/C3Ph)</th>
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Shaded areas highlight values indicative of petrogenic contamination.

Fig. 6. Σ 16 PAH/Total PAH (%) in sediments (layer 1) and hepatopancreas of Ucides cordatus from mangroves in Guanabara Bay and Guaratiba. P11 and P21: first and second sampling in station 1 of Piedade; NO11, NO12, S11, S12: first sampling, stations 1 and 2 in Nova Orleans and Surui; NO21, NO22, S21 and S22: second sampling station 1 and 2 in Nova Orleans and Surui; PT11 and PT12: first sampling, stations 1 and 2 in Peteca Channel; G11 and G12 first sampling, stations 1 and 2 in Guaratiba.
distribution feature of PAH homologues shown in the histogram of Fig. 7 is that of increasing concentration from C0 to Cn and, as in sediments, hepatopancreas show, in most cases, predominance of the C2 or C3 homolog in the phenanthrene family.

Apart from Piedade, a rather uncontaminated area in Guanabara Bay, in all other samples there is a strong predominance of phenanthrenes ($K_{ow}$: 4.57 to 6.51 for C0 to C4) which make up 30 to 46% of the Total PAH in crabs, while in sediments phenanthrenes appeared in lesser proportion, ranging from 6% (Guaratiba — control area) to 34% (Surui). In animals from Nova Orleans, Surui and Peteca dibenzoanthiophenes, chrysenes, pyrenes and naphthalenes are the other main components. The high naphthalene concentrations found in Surui sediments are also present in the hepatopancreas. Nevertheless, there is an evident discriminative mechanism in the uptake and/or elimination that favors the higher alkylated homologues. As a result, the concentration distribution profile of the 5 alkylated PAH series in crab tissue shows closer analogy to that of weathered oil than in the sediments. This should be expected since bioaccumulation is influenced, among other factors, by $K_{ow}$s which increase from C0 to Cn in all alkylated series.

The decrease in Total PAH from 2003 to 2004 in Surui sediments is also recorded in the hepatopancreas (75%). This is a strong indication of the good $U. cordatus$ response as a biomonitor for PAH.

The diagnostic ratios (F/P and “$\Sigma$ other 3–6 rings PAH/$\Sigma$ 5 alkylated PAH series”) suggest that in crabs the petrogenic imprint is amplified in comparison to the sediments. In general, the C2DBT/C2Ph and C3 DBT/C3 Ph ratios are not as similar to the MF380 oil ratios as those found for sediments. Possibly, differences in bioavailability, uptake and metabolization rates of such compounds are responsible for the observed discrepancy. This implies that, in the application of such tools to investigate oil source by using crabs, care has to be taken. It should be noted that none of the studied mangroves suspected of contamination with the MF380 oil, were originally completely free of influences from other oil sources, since Guanabara Bay is chronically contaminated by oil. The use of these ratios to access the presence of certain oil in environments subjected to inputs from diverse sources is problematic.

The Spearman test indicated strong correlation ($r=0.934; p<0.05$) between PAH concentration in hepatopancreas and PAH in the top sediment layer (0–28 cm). For the intermediate layer (28–58 cm; $r=0.789; p<0.05$) correlation was weaker, but still significant. As already mentioned, the specific crab mobility in the sediment column contributes to push the oil front downwards. Once the contaminant is buried, aerobic degradation becomes less likely, though the burrowing act may eventually carry molecular oxygen deeper in the sediment.

![Fig. 7. PAH distribution in hepatopancreas of $Ucides cordatus$ from Guanabara Bay and Guaratiba mangroves. G11, G12: sampling of September 2003 at stations 1 and 2—control area—Guanabara); P11 and P21: samplings of September 2003 and October 2004, station 1 (Piedade); NO11, NO12, PT11 and PT12: samplings of September 2003 (Nova Orleans and Peteca); S11, S12, S21 and S22 samplings of September 2003 and October 2004 at stations 1 and 2 (Surui). PAH concentration in $\mu g \cdot kg^{-1} \cdot d.w.$ (see Fig. 2 for abbreviations).]
This aeration, however, is evidently not sufficient to promote fast elimination of the contaminant from the sediments. The result is that mangrove crabs remain exposed to oil contamination for long periods of time after a spill.

The factorial analysis of the entire data set revealed 5 factors of which the first two are responsible for 77% of the overall variance. Factor 1 groups mainly 4–5 rings PAH and some of the higher alkylated homologues; factor 2 groups the majority of the 2–3 ring alkylated and parental homologues, Ant and Ace. The 5–6 ring PAH (ID, BghiPc), Fl are grouped in factor 4 which contributes only 6% to the overall variance. The plot of the two major factors (Fig. 8) shows the great majority of sediment and hepatopancreas samples in a cluster, demonstrating that the two matrices show similar concentrations. Outside the cluster, in the upper left part of the plot, appear the Surui samples from station 1, layer 1, that differ from the cluster by factor 2 (concentration of compounds in factor 2), and from hepatopancreas samples by factor 1 (higher concentration of compounds in factor 1). In the lower part, outside the cluster, are the hepatopancreas from station 1 in Peteca, which also differ in concentration of compounds in factor 1. The analysis underlines substantive differences in Surui samples in relation to others, which are mainly due to higher concentrations of light alkylated PAH.

The percentage of parental PAH in crabs is lower than in the sediments, and varies from 11–28%, in the least contaminated areas (Guarabara and Piedade), to 3–10% in the other mangroves. There is an evident dominance of alkylated PAH both in sediments and in the hepatopancreas. Nevertheless, bioaccumulation factors (BAF was calculated using normalization of PAH concentrations to organic carbon content in sediments, and to lipid content in animals) for individual compounds are variable from area to area. In Nova Orleans and Peteca, lower molecular weight PAH show higher BAF, while in Surui, station 1, alkylated phenanthrenes and benzo(k)fluoranthene are favored (see Fig. 9). Preferred bioaccumulation of alkylated PAH should be expected from the superior \( K_{ow} \) and lipophilicity of such compounds in relation to their parent homologues (Irwin et al., 1997), although these same properties may render the compounds less available for uptake from the water phase. Lamoureux and Brownawell (1999) ascribe maximum bioaccumulation factors of hydrophobic PAH to the sorption of PAH to particulate matter in the sediments.

Melzian and Lake (1987) were possibly the first to report prevalence of alkylated homologues of napthalene and phenanthrene over parental PAH in crabs exposed to No. 2 fuel oil. Hellou et al. (1994) also report predominance of phenanthrene and alkylated phenanthrene in Chionoecetes opilio and Hyas coarctatus in relation to sediments from Conception Bay in Newfoundland. These authors found, in addition, enrichment of benz(a)anthracene and chrysene in hepatopancreas, attributing the concentration differences between crabs and sediments to the composition of the diet, to bioavailability and to the activity of MFO enzymatic system. Similar to the findings of the present paper, Dugan et al. (2005) reported that in sand crab tissues (Eremita analoga) from sandy beaches in California, 70% or more of the Total PAH was alkylated homologues.

In the present work the observed results may be explained by: the accumulation derived from dietary exposure as a result of direct food contact with free oil residues found in some sediment, as for instance, in Surui; and the variable fraction of the active organic substrate controlling bioavailability of PAH in the different areas.

Di Toro et al. (1991) and Baumard et al. (1999) report that BAF should preferably be used to predict bioaccumulation, since normalization for organic carbon in sediments and for lipids in animals ought to level out variability among sites and animals. Here the variability, expressed as relative standard deviations of BAF and of accumulation factors (AF = ratio between concentrations in sediment and hepatopancreas) calculated either using the \( \Sigma_{16} \) PAH or Total PAH were very

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**Fig. 8.** Factorial analysis of PAH distribution in crabs (U. cordatus) and sediments (all layers) from Guanabara Bay and Guarabara. Projections have been performed on the factorial plane (F1 × F2). Factor 1 represents 57% of total variance of the original data, and factor 2, 20%. Inset shows a zoom on the cluster (H = hepatopancreas and S = sediment).

**Fig. 9.** Accumulation Factor (AF) for individual PAH; Surui mangrove, station 1, samplings (■) 2003 and (□) 2004 (see Fig. 2 for abbreviations).
similar, and equal to 31% and 28%, respectively. The variation of the AF and BAF ratios for Total PAH of 0.5 to 0.05 in Piedade and 5.8 to 0.6 in Nova Orleans, showed a linear correlation characterized by the equation \( AF = 10.1BAF + 0.58 \), with a correlation coefficient \( r^2 \) of 0.941 \( (n=28; \ p<0.001) \). Therefore, normalization for lipid content (62.4±6.5%) and sediment organic carbon (1.21–8.43%) did not facilitate the observation of accumulation trends or decreased factor variability among different sites. There was no relation between AF and organic carbon content in sediments. These observations emphasize the major influence of organic carbon speciation in controlling bioavailability. The relatively low AF in crabs compared to those observed for mussels (Hellou et al., 2005) may be explained because the former present a higher level of metabolism that leads to biotransformation and excretion of PAH as polar metabolites (Nudi, 2005).

Although chemical analysis of PAH in hepatopancreas is more laborious than in sediments, the information obtained is of higher value for risk assessment. Moreover, harvest of animals can be carried out faster and requires fewer personnel in the field. This is advantageous in mangroves where effects of oil contamination can be aggravated due to the transit of persons. The decline in PAH concentration from 2003 to 2004 in sediments and crabs from Surui, which is credited to heterogeneous sites, is a strong indication that \( U.\ cordatus \) mobility must be restricted to a rather small area. This limited drift rate is an additional advantage for the use in biomonitoring, first because concentrations in tissues reliably represent the contamination level in a well specified area, and secondly because the number of harvested animals for such a purpose can be optimized, so as to reduce impact on populations. The results of this study point out \( U.\ cordatus \) as an excellent bioindicator to evaluate PAH pollution in mangrove areas.

4. Conclusions

The PAH study carried out in sediments and in hepatopancreas of \( U.\ cordatus \) from Guanabara Bay mangroves show that bioaccumulation of these substances occurs in crabs from contaminated areas such as Surui and Channel of Peteca. A preferential accumulation of parental and alkylated PAH with three and four rings was observed.

The determination of alkylated PAH homologues was essential for identifying the sources of aromatic hydrocarbons in the crabs. Except in Piedade mangrove, that is further away from oil sources, the main source of PAH contamination in the crabs is unequivocally petrogenic.

The crabs collected in the Surui show elevated concentrations of oil derived PAH, which concur with observations in the sediments. This implies an uptake of contaminants by crabs directly from the sediments, since, given the high \( K_{ow} \) of such substances, concentrations in water should be too low (some tens of ng L\(^{-1}\)) to explain the high content in the hepatopancreas. Because crustaceans are capable of metabolizing PAH and excreting the contaminants, the parity between sediment concentration and body burden suggests continuous exposure allied to a moderate efficiency of the MFO enzymatic system. The statistical treatment of the entire PAH data set indicates that the main contamination of biota occurs in the upper 28 cm of the sediment column.

Survival in a sediment environment which contains more than 30,000 \( \mu g \text{ kg}^{-1} \) PAH proves that \( U.\ cordatus \) is resilient to oil, a primary prerequisite of a good biomonitor for oil contamination in mangroves. Furthermore, both the Total PAH concentration and the individual PAH profiles found in the hepatopancreas are reliable records of different contamination levels in the environment and of contaminant source.

Finally, analysis of hepatopancreas proved that PAH, present principally in the sediments, are bioavailable to the crabs. The obtained results can also be used as a basis for evaluating risk imposed to humans due to the common practice of ingesting crab internal organs.

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