Changes and variations of polycyclic aromatic hydrocarbon concentrations in fish, barnacles and crabs following an oil spill in a mangrove of Guanabara Bay, Southeast Brazil

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**A B S T R A C T**

On April 26th, 2005, an accident caused a leak of 60,000 L of Diesel Oil Type “B”, freighted by train wagons upstream on a mangrove area within Guanabara Bay, Southeast Brazil. After the accident, samples from animals with different biological requirements were collected in order to monitor polycyclic aromatic hydrocarbons concentrations for the following 12 months. Fish, mobile, carnivorous, omnivorous, organic detritus feeders, planktivorous and suspension feeders were some of the attributes compared. Concentrations of PAHs did not vary in relation to different dietary habits and the best response was from the sessile suspensivorous barnacles. A background level of <50 μg kg⁻¹ was suggested based on the reference site and on values observed in the following months after the accident. The highest values of PAH concentrations were observed in barnacles in the first month immediately after the spill, decreasing to background levels after few months. Barnacles are suggested as a sentinel species.

**Keywords:**
- Polycyclic aromatic hydrocarbons
- Oil spill
- Crabs
- Barnacles
- Fish
- Sentinel
- Guanabara Bay
environmental contamination through biota using species with distinct ecological niches. This approach allowed an assessment of environmental contamination from different perspectives, allowing a better evaluation of the accident and its consequences.

Guanabara Bay is one of the most important embayments of the Brazilian coast and various forms of pollution threaten its estuarine environment. The bay includes two refineries, 16 oil terminals, 6000 industries and two shipyards, and receives an estimated daily oil input around 9.5 tons, among other pollutants (Francioni et al., 2005). The PAH contamination of the bay sediment was documented by several studies (Hamacher, 1996; Lima, 1996; Meniconi et al., 2002) and a detailed description of the bay has been presented by Kjerfve et al. (1997). In spite of being located in a highly urbanized area and thus considered heavily polluted (Kjerfve et al., 1997), the bay supports important local fisheries (Jablonski et al., 2002) and still retains 90 km² of fringing mangroves (Pires, 1992) of which ca. 50% are relatively preserved inside the Guapimirim Environmental Protection Area (APA de Guapimirim).

After the oil spill, monthly samples were taken in the affected area. The first sampling was conducted between 6th and 10th days after the accident on May 2nd to 6th 2005. The second was also carried out in May and after that, monthly samplings were performed until March 2006, totalling 12 surveys. Each sampling survey was performed within the affected area (i.e. were the oil slick was observed in the first campaign), and in each survey different species of fish, the crab *Ucides cordatus* and the barnacle *Fistulobalanus citerosum* were collected. Fish were randomly collected, whereas crabs and barnacles were sampled at six fixed stations (Fig. 1). Specimens were maintained in ice in an isotherm box until tissue extraction in the laboratory. Muscle tissue (10 g) of each collected fish and crab was extracted and approximately 10 barnacles were pooled per station in order to obtain enough mass for analysis. Specimens of crabs and fishes from reference areas (Angra dos Reis and Itaipú) and from a fish market (São Pedro Municipal Market, Niterói) were also analysed for comparison.

PAH extraction was carried out based on the extraction method employed by Meniconi et al. (2002). Each sample was freeze-dried and approximately 1 g of the homogenate was extracted in Tedia methylene chloride pesticide grade (50 mL) for 3 min, using a high rotation tissuemizer (11,000–16,000 rpm). The residue obtained was re-extracted and the extracts were pooled and filtered. The lipid content was reduced by cleanup on a 2% deactivated Merck aluminium oxide 90 active neutral (70–230 mesh ASTM) column (20 g), using 100 mL of methylene chloride. The extract obtained after cleanup was concentrated on a rotatory evaporator to 5 mL.

Fixed Fluorescence spectroscopy (FF) measurements were performed on a Perkin-Elmer LS 55 Luminescence Spectrometer. Spectra were recorded in 1 cm quartz cuvettes with both excitation (ex) and emission (em) slit widths set to 4 nm. Pyrene (>97%) purchased from Sigma (St. Louis, USA) was used as the standard PAH and its optimal wavelength pair for detection was identified as being 334/383 nm. As other polycyclic aromatic compounds can present fluorescent properties in the same wavelength pair used for detection, results were indicated as pyrene equivalents. The
work range was 30–150 μg L\(^{-1}\), the limit of detection was 8.274 μg L\(^{-1}\) and the limit of quantification was 25.15 μg L\(^{-1}\) for the diluted samples.

A total of 465 specimens of fish from 18 species were collected, from which eight were selected as the most representative due to their relative frequency (>50%) and abundance (>3%), accounting for 86% of the fish specimens collected (Table 1). Considering the sampling feasibility, 190 specimens of the crab (U. cordatus) and 50 barnacle samples (F. citerosum) were collected. Monthly values of PAH concentrations obtained for each selected species are presented (Figs. 2–5).

Categorization of fish tissue contamination (after Varanasi et al., 1993) was used as a reference (Table 2). Nevertheless, as these authors’ values were obtained utilising different techniques (GC/MS) than those here employed, the categorization was used with caution. For fish, all but January 2006 samples could be classified as minimally contaminated. A second contamination increase was observed in the following summer months with a peak of concentration in almost every species of January 2006 (9 months after the accident), in levels higher than those of the first sample after the accident (May 2005a), decreasing again to lower concentrations in February and March 2006. The values observed in January 2006 may be associated with the rainy season, when runoff from adjacent systems can expose the environment to another peak of oil exposure, not necessarily from the studied accident, as the Caceribu basin drains water from at least four cities (Itaborai, Tanguá, Rio Bonito and São Gonçalo) with approximately 300,000 inhabitants.

From the eight selected fishes, two were top carnivores (Centropomus parallelus and Hoplias malabaricus), three benthic omnivores (Mugil liza, Geophagus brasiliensis, and Genidens genidens), one planktivorous (Brevoortia aurea) and two benthic carnivores (Hoplosternum littorale and Micropogonias furnieri). The crab (U. cordatus) feeds on mangrove leaves and the barnacle (F. citerosum) is a suspension feeder. The different diets did not reflect PAH concentrations since a similar pattern was observed for all species during the months after the accident. One could expect that the different diets would exhibit particular contamination levels but this was not the case for the fishes studied in this investigation. This fact might be correlated with the high level of inducible MFO activity (cytochromes P450 mixed function oxygenase system) that is able to metabolize and excrete accumulated PAHs rapidly (van der Oost et al., 2003). Alternatively, the low values observed in most fishes, regardless of their diet, may be associated with their mobility or to the fact that most occur in the water column rather than the air–water interface, where most of the oil slick was located. Nonetheless, our results did not show consistent patterns of accumulation relative to the different diets of the fishes collected.

The barnacle F. citerosum was the most sensitive species to oil exposure, being the only species with PAH values above 100 μg kg\(^{-1}\) in the first two sampling periods after the spill (May 2005a and May 2005b); in the third survey (April 2005) the PAH concentration levels were similar to the other species (Fig. 2). Barnacles are sessile and F. citerosum lives in the low midlittoral zone on mangrove roots exposed to contaminants without the possibility of escaping. No mortality was observed for this species following the oil spill, and the values of PAH concentration observed for barnacles may as well be associated with its sessile habitat and feeding mode. According to Neff (2002), burrowing and filter-feeding shellfish species are more likely to accumulate PAHs from oil spills than finfish because they are more vulnerable to exposure and less efficient at metabolizing petroleum compounds once exposed. These results are in accordance with those obtained by Francioni et al. (2005) for the GC/MS analysis of mussels collected after a major oil spill in Guanabara Bay in January 2000. A similar pattern was observed by Niyogi et al. (2001) for barnacles collected...
in a PAH exposed site, with much higher concentrations than those from a reference site.

Immediately after the oil spill incident in Itaboraí, ICMBio instigated a temporary closure of seafood harvesting in the area affected by the spill and its surroundings (APA de Guapimirim). This was a preventive measure in order to collect and evaluate information on the nature of the spill, such as oil type, fate, transport and seafood resources at risk of exposure.

Table 2
Fish tissue contamination criteria (adapted from Varanasi et al. (1993)).

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<thead>
<tr>
<th>Classification</th>
<th>PAH concentration (µg kg⁻¹)</th>
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<tr>
<td>Not contaminated</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Minimally contaminated</td>
<td>10–99</td>
</tr>
<tr>
<td>Moderately contaminated</td>
<td>100–1000</td>
</tr>
<tr>
<td>Highly contaminated</td>
<td>&gt;1000</td>
</tr>
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</table>

Samples from reference sites and the fish market were classified as minimally contaminated, with values ranging from 9.8 to 47.9 µg kg⁻¹ PHA (Table 3). The background levels of oil contamination in Guanabara Bay have not been established. It is expected that a background level would lie within the range 10–50 µg kg⁻¹ based on samples from reference sites (Table 3) and from contamination values observed in the following months after the accident, when values below 50 µg kg⁻¹ were found for the majority of species (Figs. 2–5). This range is in line with Silva et al. (2007), where values between 4 and 53 µg kg⁻¹ PAHs were established for two fish species from Guanabara Bay. This range is within the “minimally contaminated” level and the background level proposed herein might suggest that Guanabara Bay is under chronic oil exposure.

According to Yender et al. (2002), diesel-like products show moderate to high risk of seafood contamination because of their relatively high content of low molecular weight, water-soluble aromatic hydrocarbons, which are very volatile. In addition, they evaporate slowly and dispersed droplets are also bioavailable. However, whether seafood is exposed and contaminated not only depends on a series of weathering processes which determine the bioavailability of contaminants but also physiological processes which determine the extent of uptake and the extent to which compounds are retained or metabolized.

Fistulobalanus citerosum appeared to be highly sensitive to oil exposure and could be effectively used in assessing post-spill impacts; along with a calibration with fish tolerance values, barnacles and certain fish species are the best choice. Barnacles and other sessile filter-feeding organisms have already been demonstrated to be appropriate sentinel species (Viñas et al., 2009), and in this work barnacles were efficient bioindicators in the early stage of the impact, providing an adequate assessment of the pollutant agent.

### References


