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Note

A case study of *in situ* oil contamination in a mangrove swamp (Rio De Janeiro, Brazil) ☆

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ABSTRACT

Mangroves are sensitive ecosystems of prominent ecological value that lamentably have lost much of their areas across the world. The vulnerability of mangroves grown in proximity to cities requires the development of new technologies for the remediation of acute oil spills and chronic contaminations. Studies on oil remediation are usually performed with *in vitro* microcosms whereas *in situ* experiments are rare. The aim of this work was to evaluate oil degradation on mangrove ecosystems using *in situ* microcosms seeded with an indigenous hydrocarbonoclastic bacterial consortium (HBC). Although the potential degradation of oil through HBC has been reported, their seeding directly on the sediment did not stimulate oil degradation during the experimental period. This is probably due to the availability of carbon sources that are easier to degrade than petroleum hydrocarbons. Our results emphasize the fragility of mangrove ecosystems during accidental oil spills and also the need for more efficient technologies for their remediation.

1. Introduction

Mangrove wetlands are intertidal ecosystems that grow in tropical and sub-tropical regions along the coastlines, constituting important nurseries for fishes, crustaceans, birds and small mammals. In these systems, sediments behave like a sink, retaining pollutants. Thus, the toxicity of pollutants will be magnified, intensively affecting the organism's health. Among these pollutants, the petroleum compounds are the most harmful, producing immediate damages to the organisms, particularly during acute spills (Nansingh and Jurawan, 1999). In order to mitigate the damage caused by accidental oil spills, the environmental impact, extent of damage caused by the spill and time for the ecosystem to naturally self-recover have been widely studied (Garrity et al, 1994; Duke and Pinzon, 1997; Abuodha and Kairo, 2001). The mortality and/or damage to plants and animals will depend not only on the type, quantity, quality and weathering state of the oil, but also on the prevailing climatic and tidal conditions. Furthermore, agrochemical environmental characteristics of the substrate and seasonal variations (such as hydrodynamics that splash oil on the root system and trunk) can contribute to the persistence of oil over or inside the sediments, increasing the environmental impact (Garrity et al, 1994; Burns and Codi, 1998). In such situations, recovery may be extremely long (up to 50 years), or the damage may be definitive.

Guanabara Bay (close to Rio de Janeiro City, Brazil) has few remaining mangrove areas, one of which is the Guapimirim Mangrove (GM). It is a protected and well-preserved mangrove area, part of the large mangrove stands that originally covered over 285 km^2 of Guanabara Bay littoral land. Presently, an area of only 135 km^2 remains. In January 2000, the rupture of a heavy oil pipeline within the northwestern portion of Guanabara Bay spilled a catastrophic 1.3 million tons of oil, contaminating large swaths of beaches and affecting the Environmental Protected Area of Guapimirim Mangrove. Furthermore, the periodic oil spills that originated in the large petrochemical complexes within the area constitute a significant threat. Guanabara Bay also receives

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discharges of untreated domestic effluent, wastewater input and other industrial discharge (Godoy et al, 1991).

Two years after the Guanabara Bay accident, we investigated the microbial diversity of culturable hydrocarbonoclastic bacteria isolated from this site (Brito et al, 2006). During the isolation process, we obtained a hydrocarbonoclastic bacterial consortium (HBC) with high degradation capacity. We report here a study to evaluate the *in situ* ability of the HBC to degrade oil under natural conditions. *In situ* microcosms were set up in the same contaminated site. They were seeded with the HBC, and oil degradation was followed over a three month period.

2. Materials and methods

2.1. The hydrocarbonoclastic bacterial consortium

The experimental area is located at the GM (Fig. 1), in Guanabara Bay. Initially, surface sediment samples (0–2 cm) were collected at low tide in order to isolate the bacterial consortium able to grow in petroleum-enriched media (Brito et al, 2006).

Growth media was prepared with 500 ml of sterile seawater (filtered through a 0.45 μm membrane and autoclaved at 1 bar for 30 min), supplemented with sucrose (15 g l^{-1}), urea (10 g l^{-1}) and Arabian Light® petroleum (3 ml) and then inoculated with 50 g of sediment. The culture was incubated under constant magnetic stirring, at room temperature. After 30 days of incubation, the consortium obtained was tested for its bioremediation capacity on the *in situ* microcosms.

2.2. In situ microcosm experiments

Nine 700 cm^2 surface area microcosms were installed in GM (Fig. 1), between 5 and 30 m from the edge of the mangrove fringe. The installation sites were randomly chosen, allowing variable exposure to tides, sunlight, run-off, and proximity to red and black mangrove trees. The microcosms were constructed with 40 cm long PVC cylindrical tubes (30 cm internal diameter). To better simulate the natural conditions inside the microcosms, the lower 15 cm of the PVC cylinders that were buried in the sediment were perforated.

Three different treatments (all in triplicate) were applied to the microcosms: (a) addition of 350 ml of petroleum and 500 ml of bacterial consortia (microcosms B1, B2 and B3), (b) single addition of 350 ml of Arabian Light® petroleum (P1, P2 and P3), and (c) control microcosms, without petroleum or bacterial consortia (C1, C2, and C3). The experiment was followed for 3 months; every 7 days, we obtained *in situ* measurements of pH, Eh and temperature, and we also collected surface sediments (using a solvent-cleaned stainless spoon) for microbiological and chemical analysis (Dehydrogenase Activity, Electron Transport System and Residual Hydrocarbons).

2.3. Characterization of bacterial diversity

The T-RFLP (Terminal Restriction Fragment Length Polymorphism) was used to verify the initial microbial structure of HBC. DNA was extracted with an Ultra Clean Soil DNA Isolation Kit® (Mo Bio Laboratories), following Precigou et al, 2001, verified by 1% agarose gel electrophoresis in Tris-Acetate-EDTA buffer (TAE). DNA solutions were preserved at −20 °C.

The 16S rRNA genes were amplified by PCR using primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 926R (5′-CCGTCAATTCCTTTRAGTTT-3′), labeled with the fluorochromes TET (5-tetrachloro-fluorescein) and HEX (5-hexa-chloro-fluorescein), respectively. The reactions were cycled in a PTC 200 Thermo-cycler (MJ Research) with an initial denaturation step at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1.5 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 15 min. PCR-products were purified with the GFX PCR DNA purification kit (Amersham) and digested with 10U

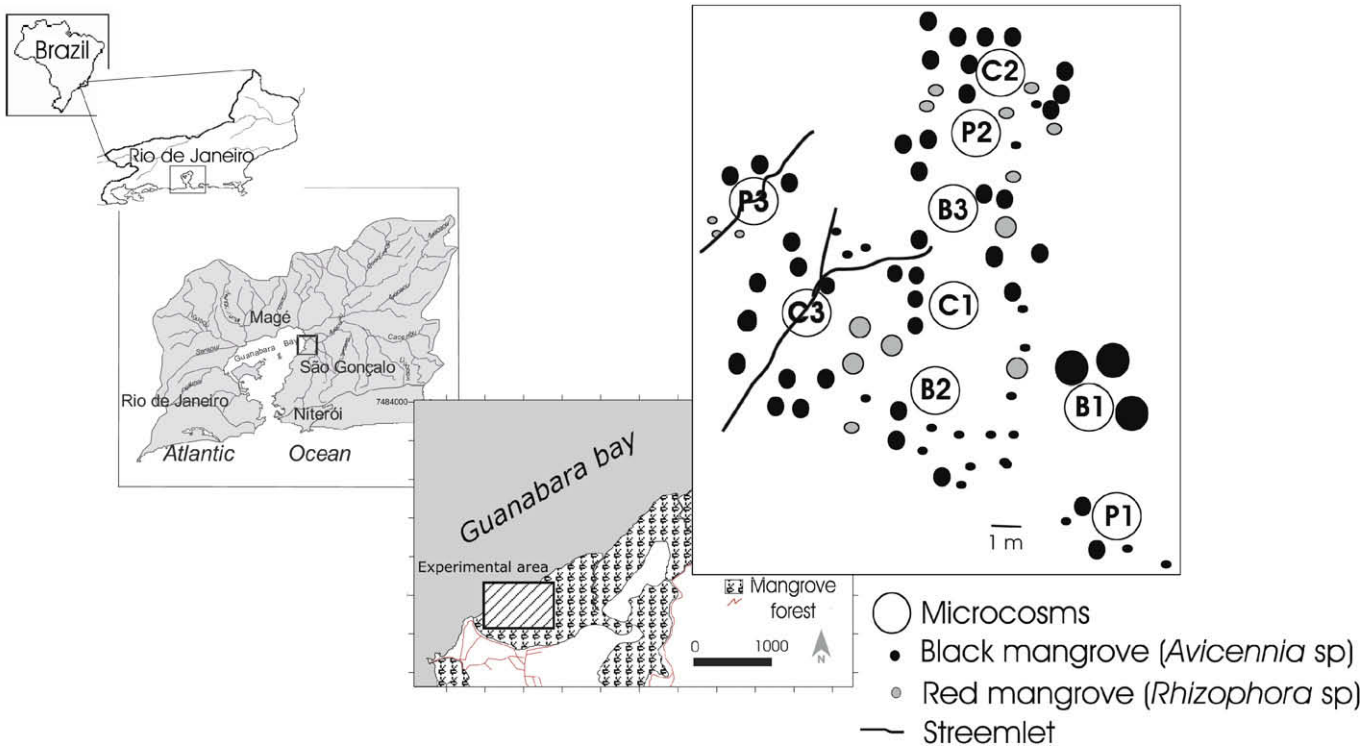

Fig. 1. Location of the experimental area in Guanabara Bay (Guapimirim Mangrove: 22°44'S, 43°02'W), where the position of the *in situ* microcosms are represented.