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Evaluation of the effects of metals on biodegradation of total petroleum hydrocarbons☆

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ABSTRACT

In the present paper, the efficiency of biodegradation of petroleum hydrocarbons in the presence of metals in mangroves sediments was evaluated. Two models of remediation (intrinsic bioremediation and phytoremediation) were tested. The metals (Al, Fe, Pb, Cr, Cu, Zn and Ni) were determined employing Flame Atomic Absorption Spectrometry (FAAS). The total petroleum hydrocarbons (TPHs) were analyzed using Gas Cromatograph with Flame Iron Detector (GC-FID). The physical–chemical parameters were monitored for a 90-day period at the pilot scale. The results showed that both techniques were effective at degrading the organic compounds in oil, with phytoremediation (*Rizophora mangle*) being the most efficient (87% removal). It was also observed that the biodegradation model of intrinsic bioremediation did not have a direct correlation with the concentrations of metals; however, a positive correlation with some metals (Cu, Zn, Cr, Ni) for the model with phytoremediation was verified with the removal of hydrocarbons, showing efficiency at phytoextraction and phytostimulation. The results suggest that red mangroves, through their rhizosphere mechanisms are a promising plant that can be used for the removal of petroleum hydrocarbons in the presence of metals in mangrove sediments.

1. Introduction

Mangroves are transitional coastal ecosystems that occur between terrestrial and marine environments and are characteristic of tropical and subtropical regions. They are of great ecological and economic importance. In recent years, data ITOPF [1] have shown that the number of spills in mangrove areas has increased, especially in countries where the oil industry has recorded growth. These accidents have the potential to cause environmental and economic effects on a wide variety of natural resources and services in these regions. Studies on the application of remediation techniques in coastal regions are becoming increasingly necessary, given the importance of these ecosystems for ecological balance and because they are targets of major impacts of petrogenic origin [2,3].

Many mangrove areas that are affected by oil spills contain high concentrations of metals that are enhanced by the composition of the spilled oil. Metals at certain concentrations may limit microbial and plant activities that could degrade the organic compounds, causing serious problems for the application of bioremediation, a technique that uses microorganisms to degraded contaminants sediment, and phytoremediation, a technique that uses microorganisms to degraded contaminants in mangrove sediments [4,5].

Some studies have been conducted regarding the individual effects of toxic metals, and biotic and abiotic factors on the degradation of total petroleum hydrocarbons; however, few studies have addressed the effects of all factors convergent on biodegradation in mangrove sediments [6–8]. These sediments are rich in microbial diversity and rely on a set of interactions with the mangrove plant species to maintain conservation, productivity and recovery of this ecosystem when impacted by some type of antropic activity [9].

The decision to correct hydrocarbon contaminated areas should be based on pilot studies through the integrated assessment of the presence of metals along with the main physical, chemical and biological agents that can act positively or negatively on the biodegradation in mangrove sediments. The objective of this study was to evaluate how the presence of metals and other abiotic and biotic factors influence the degradation of total petroleum hydrocarbons in mangrove sediments using two models of remediation: intrinsic bioremediation and phytoremediation with *Rizophora mangle* L. (red mangrove) simulated at the pilot scale.

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2. Materials and methods

2.1. Sediment sampling and analysis

Mangrove sediment samples were collected at 0–30 cm deep, between the cities of Candeias and São Francisco do Conde, Todos os Santos Bay, Bahia, Brazil (a region with many activities in the petroleum industry: extraction, transportation and refining). These sediment samples were sieved through a 4-mm sieve to remove rocks, and the plant material was homogenized immediately after to ensure uniformity. Five sub-samples of sediment were collected, homogenized and freeze-dried for 72 h and sieved through a 2-mm mesh to determine the initial physical and chemical properties of the selected sediment. Soon after, sediment samples were mixed in a 1:10 ratio with oil residue found in the same area, and five samples of the mixture were collected to analyze the new concentrations of TPHs, metals and nutrients (Table 1). In the homogenized sediments the amount of metals (aluminum (Al), iron (Fe), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn) and nickel (Ni)) was determined by Flame Atomic Absorption Spectrometer (FAAS) with Varian model SpectrAA 220 (Mulgrave, Victoria, Australia). The total petroleum hydrocarbons (TPHs) were determined using a Varian CP 3800 (Varian Inc., CA, USA) gas chromatograph equipped with a DB-5 capillary column (15 m length, 0.25 mm ID, 0.25 lm film thickness) and flame ionization detector (FID). Particle-size distribution was determined after the organic matter was removed with 30% H_2O_2 using the Folk and Ward method [10]. Soil organic matter (TOM) was determined using a modified Mebius method [11]. Total N (nitrogen) was determined by the Kjeldahl's digestion, distillation and titration method [12], while available P (phosphorus) was determined by the Olsen extraction method [13]. Measurements were taken for pH and Eh by using a Potentiometer pH/mV HandyLab1 (Schott Glaswerke Mainz). The salinity was measured by the index of refraction using the portable refractometer atoga S/Mill-E, and dissolved oxygen (DO) was measured with a WTW Oximeter OXI 3151 (SCHOTT-GERÄTE).

2.2. Models for remediation

Over a 90-day period, two models were used to evaluate the remediation of TPH contamination in sediments. One type of remediation used to compare the removal of TPHs was intrinsic bioremediation (natural attenuation monitored), where the degradation by bacteria presented in the mixed sediment was monitored. The other remediation model was phytoremediation using *R. mangle* (red mangrove). This choice was based on pre-tests conducted earlier by our group and other studies that suggested the use of this species for phytoremediation [14–16]. Seedlings of *R. mangle* were collected at low tide, and their height (average of 3 months old) was used to define a standard sampling protocol to maintain comparable results. The density of the bacterial community was characterized to compare the presence of microorganisms with the intrinsic bioremediation method.

Table 1
Some selected physico-chemical properties of the sediments used in the experiment.

Sediment	Uncontaminated	Contaminated
Cu (μg g^{-1})	13.37	17.86
Zn (μg g^{-1})	12.66	22.23
Pb (μg g−1)	6.02	18.21
Cr (μg g^{-1})	7.43	11.47
Ni (μg g^{-1})	7.49	17.43
Fe (μg g^{-1})	12,014.88	10,739.67
Al (μg g^{-1})	7401.69	7925.57
pH	7.51	7.59
Eh	−12	−12.3
T (°C)	26.9	27
Salinity	27	24
DO (mg/L)	4.39	4.53
TPH (μg g^{-1})	–	33,215.16
TOM (%)	0.63	5.73
TOC (%)	0.37	3.32
TN (%)	0.06	0.36
P (mg/L)	0.90	2.70
C/N	5.74	9.26

2.3. Experimental design

The project design was based on pilot-scale simulation of mangrove dynamics, with the tidal regime in the sediment used for the application of the remediation models. This experiment was conducted in a greenhouse (laboratory deployed to conduct research developed within the network RECUPETRO/UFBA — Cooperative Network Recovery in Areas Contaminated by Petroleum Activities, linked to the Federal University of Bahia) near the mangroves, with environmental conditions very close to the original ecosystem, with an average temperature of 24 °C ± 1. Simulation units were made of glass (50 × 30 × 40 cm). Within each unit of simulation, 6 tubes of glass were added (30 × 10 × 10 cm) and applied to the two models of remediation compared in this study. These tubes of glass were suspended in the unit simulation, allowing the simulation of the tidal regime with water runoff. The tubes of glass were closed at the bottom to prevent loss of chemical residue when watering. All units received the daily tidal regime simulation with an adequate amount of water (approximately 10 L) to maintain the constant humidity of the sediment, as in the mangrove ecosystem. The experiment contained three replicates of each treatment and three samples from each repetition.

2.4. Statistical analysis

Principal components analysis (PCA) was applied to the average concentrations of all the data analyzed in the surface sediments for each type of remediation. PCA was used to determine the primary variables that influence the degradation of TPHs. Other statistical tests that were applied include the K-means, the Kolmogorov–Smirnov test for multiple parameters, the Tukey–Kramer test and the Pearson correlation (Considering extremely positive values between 0.7 and 1.0). All statistical evaluation was performed using the STATISTICA 6.0 and GraphPad software.

3. Results and discussion

3.1. Initial chemical properties of the sediments

Table 1 presents the physical and chemical analyses of the sediments contaminated and not contaminated by waste oil. The analytical results showed that, except for Fe, all other metals increased after the homogenization of the sediment and residue of oil in the two models, corroborating other studies [8,10]. However, the average values for the metals in the two sediments were below the TEL (Threshold Effect Level) [11], and it was previously found that these concentrations do not influence the biota negatively. The pH, which was between 7.51 and 7.59, of the two sediments was consistent with estuarine waters. These pH data are favorable to the majority of the biodegrading microorganisms in the mangrove sediments and did not compromise the principle bioremediation processes [12]. The temperatures of the two sediments were also very close to those expected for optimal biodegradation, where there is a higher enzyme activity between 25 °C and 38 °C [13]. The initial salt concentrations in the sediment confirmed the brackish characteristics of the medium and selected for particular biodegrading microorganisms but did not adversely affect the application of the remediation techniques [14]. Concentrations of macronutrients (N and P) and organic matter in the sediments were also expected for degradation by the biota in sediments impacted by organic compounds [12]. After homogenization of the sediment and residue oil, the initial concentration of TPHs was approximately 33 μg g^{-1}, which is considered a moderate contamination of the sediment [15].