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Bioremediation of a tropical clay soil contaminated with diesel oil

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ABSTRACT

The removal of polyaromatic hydrocarbons (PAH) in tropical clay soil contaminated with diesel oil was evaluated. Three bioremediation treatments were used: landfarming (LF), biostimulation (BS) and biostimulation with bioaugmentation (BSBA). The treatment removal efficiency for the total PAHs differed from the efficiencies for the removal of individual PAH compounds. In the case of total PAHs, the removal values obtained at the end of the 129-day experimental period were 87%, 89% and 87% for LF, BS and BSBA, respectively. Thus, the efficiency was not improved by the addition of nutrients and microorganisms. Typically, two distinct phases were observed. A higher removal rate occurred in the first 17 days (P-I) and a lower rate occurred in the last 112 days (P-II). In phase P-I, the zero-order kinetic parameter ($\mu g\ PAH\ g^{-1}\ soil\ d^{-1}$) values were similar (about 4.6) for all the three treatments. In P-II, values were also similar but much lower (about 0.14). P-I was characterized by a sharp pH decrease to less than 5.0 for the BS and BSBA treatments, while the pH remained near 6.5 for LF. Concerning the 16 individual priority PAH compounds, the results varied depending on the bioremediation treatment used and on the PAH species of interest. In general, compounds with fewer aromatic rings were better removed by BS or BSBA, while those with 4 or more rings were most effectively removed by LF. The biphasic removal behavior was observed only for some compounds. In the case of naphthalene, pyrene, chrysene, benzo[k]fluoranthene and benzo[a]pyrene, removal occurred mostly in the P-I phase. Therefore, the best degradation process for total or individual PAHs should be selected considering the target compounds and the local conditions, such as native microbiota and soil type.

1. Introduction

The risks of environmental contamination from the exploitation, transport and storage of petroleum represents a global concern. This contamination is mainly due to spills from storage containers and pumps or accidental leakages (Udiwal and Patel, 2010). In Brazil, gas stations are responsible for more than half of soil and aquifer contamination cases, largely due to the lack of effective monitoring and the high average lifetimes of underground storage containers of approximately 25 years (Brito et al., 2010). Nevertheless, the contamination of clay soil by petroleum derivates has not widely been reported (Bourotte et al., 2009; Al-Turki, 2009). Clay soil is very common in tropical climates and is used as a natural sealant for underground tanks in gas stations and in industrial areas (Daniel and Wu, 1993). In the Recife metropolitan area (RMA) (08°03′14″S–34°52′51″W), there is a development boom in the Suape Industrial and Portuary Zone associated with the introduction of several new industries. A petroleum refinery with the capacity to process 200,000 barrels per day will begin operation after 2012, with 60% of the production being diesel oil. Consequently, the potential for environmental impacts from the refinery and related activity are significant.

In previous studies, good results were obtained from *in situ* and *ex situ* bioremediation processes for the treatment of diesel oil-contaminated soil in temperate climates (Huang et al., 2004; Ghazali et al., 2004; Bento et al., 2005; Riffaldi et al., 2006). Each of the various techniques that were employed in these cases is indicated for remediation of a specific fraction of hydrocarbons produced in the petroleum refining process, with some preferentially removing the lightest compounds while others were better suited for the heaviest contaminants (Huang et al., 2004). However, some limitations characterize the remediation of hydrophobic PAHs, like bioavailability and adsorption (Johnsen et al., 2005).

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Therefore, the objective of this work was to evaluate the influence of the addition of nutrients and microorganisms on the PAH removal under aerobic condition. The removal of total and individual PAH compounds (specifically, 16-priority PAHs identified by the USEPA, Bruzzoniti et al., 2011) was studied in a clay soil contaminated by diesel oil.

2. Material and methods

2.1. Microbial consortium for bioaugmentation treatment

The microbial consortium used for bioaugmentation was derived from the original clay soil sample (see Section 2.3) through the addition of 12 mL of diesel oil and 20 mL of Büshnell-Haas mineral medium (1.00 g of KH_2PO_4; 1.00 g of K_2HPO_4; 1.00 g of NH_4NO_3; 0.20 g of $MgSO_4.7H_2O$; 0.05 g of $FeCl_3$; 0.02 g of $CaCl_2.2H_2O$ and 1.0 L of deionized water) to 200 g of soil. The soil was manually mixed (aerated) every two days, and 20 mL of mineral medium was added to provide moisture and nutrients every seven days. After 30 days, 10 g of soil were transferred to 500-mL glass flasks containing 100 mL of sterilized deionized water. After 20 min of agitation (200 rpm), 10 mL of liquid phase were submitted to grown through decimal serial dilution until the concentration of 10^8 cells mL^{-1} was achieved.

2.2. Bioremediation treatments

A 50-kg clay soil sample was collected at a 15-cm depth from a natural vegetation area in the municipality of Moreno in RMA (UTM Coordinates (Zone 25 L): 265,452; 9,102,499). The sample was dried in the air and sieved through a 2-mm screen. The experiments were carried out in six plastic trays (6.8 × 27.0 × 42.5 cm) with 5 kg of soil in each. The soil was combined with commercial diesel fuel in the proportion of 40 g of diesel per kg of soil (dry mass at 60 °C), based on the experiments of Barahona et al. (2004). The soil was mixed immediately after contamination and maintained at room temperature for one week to guarantee adequate binding, as described by Huang et al. (2004). Experiments were then initiated with the addition of nutrients and microorganisms to the soil ($t = 0$) for two of the experimental treatments.

Three treatments were used: landfarming (LF), in which the soil was only mixed in the tray; biostimulation (BS), in which the soil was mixed and received additions of a nutrient solution; and biostimulation and bioaugmentation (BSBA), in which the soil was mixed and received the same BS nutrient solution; additionally, 87.5 mL of microbial consortium solution containing 10^8 cells mL^{-1}, as previously described in Section 2.1, was added per kg of soil. The nutrient solution was used to correct the C:N:P ratio found in the soil after the addition of diesel fuel (100:0.375:0.0011) to 100:10:1, by adding 4.2 g $(NH_4)_2SO_4$ kg^{-1} of soil and 0.5 g K_2HPO_4 kg^{-1} of soil.

Nutrients and microorganisms were added only at the beginning of the BS and BSBA experiments. It was assumed that abiotic and volatilization losses were equal for all the treatments. Experiments were conducted in duplicate for each treatment. During the 129-day experimental period, each tray was mixed twice weekly to promote contact with the air. Deionized water was also added twice per week to maintain the soils' water-holding capacities (WHC) at approximately 50% (Trindade et al., 2005).

2.3. Soil analyses

At the days 0, 3, 10, 17, 24, 45, 73, 101 and 129, five 15-g subsamples were removed, four from the corners and one from the center of each tray. The five sub-samples collected from each tray on the same date were mixed together to represent a single composite sample for that tray. Then, the total concentration of all PAH compounds, or sum of PAHs (ΣPAH), was quantified, as were the individual concentrations of the 16-priority PAH compounds. The soil samples were stored at 4 °C until ready for analysis. An aliquot of 10 g was extracted from each sample using a soxlet extractor and dichloromethane as a solvent over an 8-h period. Afterwards, an eluent clean-up step was performed using a 40-cm silica-alumina chromatographic column with dicloromethane and *n*-hexane as solvents. The 16 PAH compounds were identified and quantified through gas chromatography-mass spectrometry (Varian 3900 plus Varian Saturn 2100T) using USEPA method 8270D (USEPA, 2008). A capillary column (Varian CPSil8 CBlb/MS, 30 m × 0.25 mm, 0.25 m PN:CP 5860) with helium as carrier gas (1.2 mL min^{-1}), was used. The oven temperatures were: 40 °C for 1 min; 100 °C (ramp of 15 °C min^{-1}) for 0.1 min and 300 °C (ramp of 5 °C min^{-1}) for 5 min. The PAH detection limit was 0.05 ng g^{-1} of dry weight of soil and the surrogate was *p*-Terphenyl-d14.

Soil physical–chemical analyses, granulometry and WHC measurements were conducted in accordance with the EMBRAPA's methodology (1997). The soil analyses were conducted in duplicate. A pH adjustment was made for the BS and BSBA treatments on day 24 based on a neutralization curve previously constructed using a stabilization period of 48 h. The relation between the $Ca(OH)_2$ concentration applied to 10 g of dry soil and the pH was obtained by increasing the $Ca(OH)_2$ concentration until the pH 7 was achieved.

Quantification of heterotrophic microorganisms such as bacteria and fungi in the soil samples was conducted using the pour plate technique. The media used to grow bacteria and fungi were Triptic Soy Agar and Sabouraud Dextrose Agar, respectively. To each bacteria or fungi plate, 50 μg mL^{-1} of the fungicide cycloheximide or the bactericide streptomycin was added, respectively, to guarantee the exclusive growth of the microorganisms to be quantified. The plates were incubated at 30°C ± 1 °C, and colonies were enumerated (CFU g^{-1}) after 24 or 48 h (Trindade et al., 2005). The plates were prepared in triplicates. The same procedure was used for the blanks. Bacteria species were identified by comparing the profile of methylic esters of cellular volatile fatty acids with that of the library of the Microbial Identification System (MIDI) using the Sherlock system. The chromatographic profiles were also compared with a library of environmental isolates (TSBA 50). The results were evaluated according to variance analysis ($p < 0.05$), non-parametric tests (Statgraf®) when appropriate and by kinetic model fitting (ORIGIN®).

3. Results and discussion

3.1. Soil characteristics

The studied soil was a yellow-red Tb podzolic soil (Argisoil) consisting of 38% sand, 17% silt and 45% clay. Thus, it can be classified as having a clay texture (35% < clay < 60%). Table 1 shows the main chemical characteristics of the soil before and after contamination. Since the organic matter and nutrients contents after contamination resulted in a C:N:P ratio of 100:0.375:0.001, a nutritional supplementation was required.

3.2. pH effect and microbial enumeration

The microbial consortium added as part of the BSBA treatment contained a wide variety of bacteria (*Acinetobacter*, *Arthrobacter*, *Bacillus*, *Paenibacillus*, *Pseudomonas* and *Staphylococcus*) and fungi species (*Aspergillus*, *Eupenicillium*, *Paecilomyces* and *Penicillium*), with the potential to metabolize hydrocarbons derived from

Table 1
Chemical characteristics of the soil before and after contamination with diesel fuel.

Characteristic	Parameter	Soil before contamination	Soil after contamination
Sorptive complex (cmol dm^{-3})	Ca^{2+}	0.03	0.03
	Mg^{2+}	0.22	0.23
	Na^{+}	0.06	0.05
	K^{+}	0.15	0.14
	Al^{3+}	0.90	0.90
	$(H + Al)^{a}$	2.21	1.88
	SB[b]	0.45	0.45
	CEC[c]	1.36	1.35
Nutrients (g kg^{-1})	TOC[d]	9.77	48.00
	OM[e]	16.85	82.80
	N	0.13	0.18
Nutrients (mg kg^{-1})	P	0.51	0.51

[a] Soil potential acidity (sum of H^{+} and Al^{3+}).
[b] Sum of bases.
[c] Cation exchange capacity.
[d] Total organic carbon.
[e] Organic matter.

petroleum products, such as PAHs (Cerniglia, 1984, 1992; Gestel et al., 2003).

Fig. 1 shows the results obtained over the experimental period for the number of microorganisms present and pH. Two distinct phases were observed, the first (P-I) lasting until the 17th day and the second (P-II) lasting from the 18th day until the end of the experiment. Regardless of the phases in general, the BS and BSBA treatments showed similar behavior with respect to the number of microorganisms (Fig. 1a and b) and the soil pH (Fig. 1c). By contrast, the LF tray showed differences from the other two treatments. In all cases, pH appeared to be a key factor that affected the overall behavior of all the treatments.

During phase P-I, the development of bacteria populations in both the BS and BSBA treatments (Fig. 1a) differed significantly (variance analysis, $p < 0.05$) from that of fungi (Fig. 1b). In general, the number of bacteria decreased sharply during phase P-I, while the number of fungi increased. The tendency of fungi to increase in concentration as soon as soil is contaminated with diesel oil has been reported previously (Gestel et al., 2003). A sharp decrease in pH values was also observed during P-I.

Differences in the number of bacteria present differed between the two treatments only during the first days of P-I, as expected due to the intentional inoculation of the soil in the BSBA condition. On day 3, the number of bacteria (6.92 log bacteria g^{-1}) for BSBA was one order of magnitude higher than that (5.88 log bacteria g^{-1}) of the BS condition (ANOVA, $F = 156.008$, $p = 0.0002$). However in the same 3-day period, fungi concentrations showed no significant difference between the two treatments, with concentrations of approximately 4.5 log fungi g^{-1} (ANOVA tests: $F = 12.044$, $p = 0.0203$). Between the 3rd and 17th days, a sharp 1 to 2 orders of magnitude decrease in the concentration of bacteria was observed for both treatments, while the number of fungi increased by more than 1.5 orders of magnitude. The similar behavior of both treatments showed that the addition of microorganisms was unnecessary, at least in the case of bacteria.

Compared to the BS and BSBA treatments, the LF condition showed different behavior with respect to microorganism concentrations and pH during the P-I phase. The decrease in the number of bacteria was less significant and occurred only until the 10th day, after which point bacterial concentrations began to increase (Fig. 1a); fungi concentrations also decreased initially until the 10th day; later on they increased, but not significantly (Fig. 1b).

3.3. Bioremediation treatments: total PAH removal

Fig. 2 shows the results obtained for total PAH removal. The highest ΣPAH degradation rates occurred in phase P-I for all three treatments. This observation can be attributed mainly to the activity of fungi because bacteria populations decreased sharply for both BS and BSBA but significantly increased for LF. The decrease in pH values observed for the BS and BSBA treatments in particular

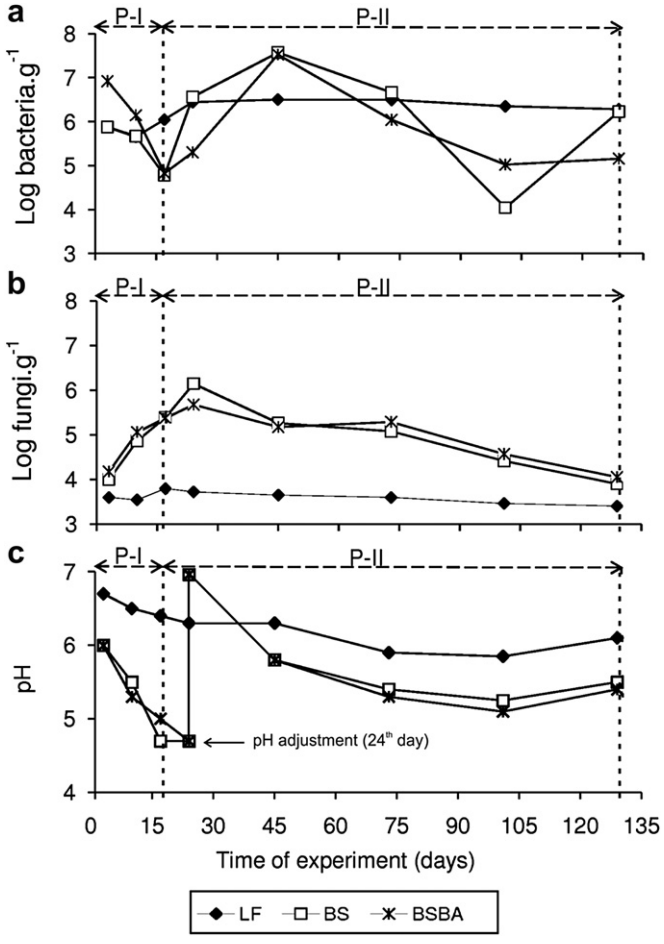

Fig. 1. Parameter variation over the experimental period for the three treatments (LF, BS and BSBA): (a) concentration of bacteria; (b) concentration of fungi; (c) pH.

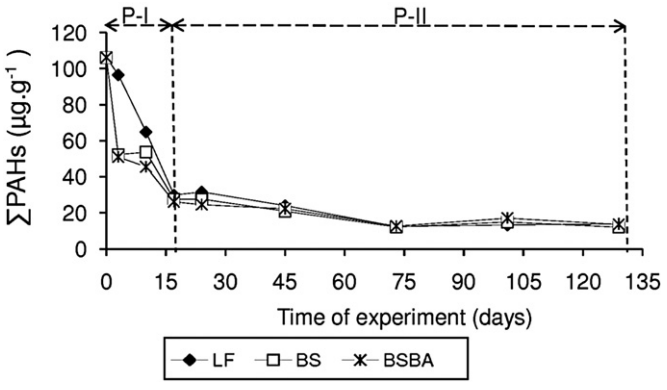

Fig. 2. Sum of the 16-priority PAH concentrations (ΣPAH) during the 129-day experimental period for LF, BS and BSBA treatments.

favored the dominance of fungi and can be related to the addition of salts (nutrient solution) to the soil samples. Interestingly, the LF condition showed similar ΣPAH removal rates at the end of the P-I phase, but removal proceeded at a lower rate during the first ten days compared with the BS and BSBA treatments, while LF removal occurred at a higher rate between days 10 and 17. This pattern can be attributed to the higher rate of increase in bacteria concentrations for LF and the lower concentrations of fungi present in the BS and BSBA trays after day 10. PAH degradation occurred predominantly by bacteria under the LF condition and predominantly by fungi under the BS and BSBA conditions; this observation showed that the addition of nutrients for process stimulation resulted in the growth of fungi rather than bacteria for the BS and BSBA treatments. The ability of fungi to biotransform xenobiotics has received attention due to their dominance, ubiquity and different metabolic pathways for detoxifying aromatic hydrocarbons (Romero et al., 2010).

During phase P-II, there was bacterial growth for all the treatments in the period from day 17 to day 24. This growth was most pronounced for the BS treatment, probably as a consequence of the microbial adaptation process. During the same period, the fungi population continued to increase, but it began to decrease after day 24, when the pH was adjusted to around 7.0. However, by day 45, the pH had already decreased to values below 6.0. The pH correction on day 24, combined with the availability of new substrates that were easier to degrade, favored the growth of bacterial populations and the decrease in fungi numbers for the BS and BSBA trays between day 24 and day 45. After day 45, bacterial populations began to decrease again following the new decrease in the pH values for the BS and BSBA conditions.

Over the course of the 129-day experimental period, the ΣPAH removal process exhibited different behavior compared with the removal of each individual PAH component. In the case of ΣPAH, removal efficiencies of 86.9%, 88.7% and 87.2% were obtained at the end of the experiments for LF, BS and BSBA, respectively (Fig. 2). No statistical significant differences were observed among the treatments (ANOVA: $F = 2.140$; $p = 0.2645$). Therefore, it can be concluded that the addition of nutrients and microorganisms did not result in any significant increase in ΣPAH removal rates. As shown above, the ΣPAH removal pattern for all treatments was characterized by a high removal velocity in P-I with a significant reduction in removal rates in phase P-II. This 2-phase behavior has been previously described as being common to petroleum hydrocarbons (Alexander, 1999). No kinetic model could be identified that adequately described all experimental data with one-phase behavior. Therefore, a kinetic adjustment was carried out to fit the data using separate zero-order reactions for the removal of ΣPAH for each of the two phases (Table 2).

Table 2 shows that there was no significant statistical difference ($p < 0.05$) for the zero-order kinetic constant values among the three treatments, either for P-I or P-II (ANOVA: $F = 1.884$ and $p = 0.2952$ for P-I; and $F = 1.046$ and $p = 0.4523$ for P-II). Therefore, it can again be concluded that the treatment type used had no influence on the degree of removal of ΣPAH. The decay velocities of ΣPAH in the first phase (P-I) were 31-, 33- and 42-fold higher than those obtained in the second phase (P-II) for LF, BS and BSBA, respectively. This fact can be attributed to many factors during the P-II phase, including (i) reduction in the bioavailability of the remaining PAH material during the treatment period (Amellal et al., 2001; Rizzo et al., 2008), (ii) accumulation of toxic intermediates (Balba et al., 1998; Barahona et al., 2004), (iii) presence of more recalcitrant compounds (Song et al., 1990) and (iv) modification of microbial diversity (Canals, 2005). The last factor represents a strong indication of the shift in the ΣPAH removal efficiency between the two phases. As discussed previously, the P-I phase was characterized by a low pH range for BS and BSBA, resulting in low bacteria populations but high fungi concentrations.

Table 2
Values of kinetic constants of zero order obtained for ΣPAH removal in the P-I and P-II phases for the three bioremediation treatments.

Treatment	Kinetic constant of zero order (μg PAH g soil^{-1} d^{-1})	
	P-I[a]	P-II
LF	4.4847 ± 0.1314 A[b]	0.1432 ± 0.0331 a
BS	4.6206 ± 0.1389 A	0.1391 ± 0.0253 a
BSBA	4.7062 ± 0.0562 A	0.1117 ± 0.0008 a

[a] The P-I phase covered the period from 0 to 17 days and the P-II period from 17th to the 129th day.

[b] The use of the same character (A, a) for different treatments in the same column indicates that there is no significant difference between those treatments: capital letters were used for P-I and lower case letters were used for P-II (Tukey Test, $p < 0.05$).

3.4. Bioremediation treatments: individual PAH removal

In the case of the 16 individual PAH compounds, five were not detected at day 0 in the contaminated soil, including acenaphthylene (ACY), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), dibenzo[a,h]anthracene (DBA) and benzo[g,h,i]perylene (BGP). Therefore, these were not considered as being present in the used diesel oil. As shown in Table 3, four components of the remaining 11 PAHs showed the same removal efficiency for all the three treatments at the end of the experimental period, and the same letter (A) was assigned to these compounds using Tukey's post-test. These four compounds were naphthalene (NAP), anthracene (ANT), fluoranthene (FLT) and indeno[1,2,3-cd]pyrene (IND). The other seven components exhibited different final removal efficiencies when compared individually among every pair of treatments. Nevertheless, a removal efficiency of 72.7% or higher was obtained for all the individual compounds with each of the three treatments.

It is also important to note that the most significant removal occurred during phase I for compounds such as NAP, FLT, BaP and IND, with final removal efficiencies of approximately 90%. These observations indicated that the duration of the experiment could potentially be shortened for some target compounds of interest.

The treatment response can be divided in two groups according to the number of aromatic rings on each PAH. The first group includes the six compounds with up to three rings (NAP, ACE, FLU, PHE, ANT, FLT). The treatment efficiency of these compounds by LF was approximately the same as (NAP, ANT, FLT) or slightly lower (ACE, FLU, PHE) than that observed for the BS or BSBA treatments. The removal efficiency for BS and BSBA was the same for all six compounds. The second group includes the remaining five compounds with four or five rings (PYR, CHR, BkF; BaP, IND), for which treatment by LF resulted in higher (4-ring components: LF > BS > BSBA) or equivalent (5-ring component, only IND) removal efficiency compared with BS and BSBA (LF = BS = BSBA). In the case of the other 5-ring compound (BaP), the removal efficiency was highest for BSBA, followed by LF and BS that had the lowest removal efficiency.

Fig. 3 shows the individual removal behavior for the three treatments. Two of the hydrocarbons with two aromatic rings, NAP and ACE, were completely removed after 10 days and 60 days, respectively. Meanwhile, fluorene, which also has two rings, was almost completely removed after the full 129 days. In general, it was observed that the three treatments showed very high and similar final removal efficiencies for all 2-ring compounds. The time required for removal was shortest for NAP, followed by ACE and, finally, FLU. As in the case of total PAHs, 2-phase removal behavior

Table 3
Removal of individual PAH compounds in soil contaminated by diesel fuel after the 129-day incubation period for the three different treatments.

PAH	Symbol	Number of rings	Removal of PAHs (%) per experiment[a]		
			Landfarming (LF)	Biostimulation (BS)	Biostimulation + Bioaugmentation (BSBA)
Naphthalene	NAP	2	100.0 ± 0.0 Aa[b]	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
Acenaphthene	ACE		99.2 ± 0.1 Ba	100.0 ± 0.0 Aa	100.0 ± 0.0 Aa
Fluorene	FLU		94.6 ± 0.9 Babc	98.2 ± 0.0 Aa	98.3 ± 0.2 Aab
Phenanthrene	PHE	3	79.6 ± 2.6 Be	87.4 ± 0.5 Abc	88.3 ± 0.3 Ab
Anthracene	ANT		87.6 ± 1.7 Ad	75.4 ± 4.0 Ad	75.0 ± 4.9 Ac
Fluoranthene	FLT		87.3 ± 0.4 Ad	85.4 ± 3.5 Abc	88.6 ± 1.1 Aab
Pyrene	PYR	4	90.3 ± 0.4 Abcd	82.1 ± 0.1 Bcd	72.7 ± 1.9 Cc
Chrysene	CHR		89.0 ± 1.9 Acd	81.4 ± 0.3 Bcd	74.2 ± 1.1 Cc
Benzo[k]fluoranthene	BkF		90.5 ± 2.5 Abcd	93.4 ± 1.3 Aab	73.9 ± 7.8 Bc
Benzo[a]pyrene	BaP	5	90.9 ± 2.0 ABbcd	83.2 ± 4.1 Bcd	95.1 ± 0.1 Aab
Indeno[1,2,3-cd]pyrene	IND		95.2 ± 1.0 Aab	92.8 ± 0.0 Aab	93.9 ± 1.9 Aab

[a] Five components (ACY, BaA, BbF, DBA and BGP) were not detected in the contaminated soil at day 0.
[b] Different characters (A, B, C, a, b, c, d) in the same line indicate significant difference among the three treatments (capital letters) or among the individual PAH (lower case letters) (Tukey Test, $p < 0.05$).

(Fig. 2) was observed for all individual PAH species. Changes in PAH concentration with time were not linear; consequently, as with total PAH degradation, a single kinetic decay constant value could not be obtained for individual PAH compounds. Such fluctuations in apparent decay rate may have been due to sorption and desorption phenomena. The soil sorptive capacity (K_{OC}) was evaluated at the 10th, 45th and 129th days of the experiments. K_{OC} was determined using the linear coefficient K_d normalized by the concentration of organic carbon (C_{org}) present in the experiment. Similar values of approximately 1300 L NAP kg^{-1} soil were obtained at day 10 for all the treatments. In the case of the LF treatment, concentrations were similar on the 10th, 45th and 129th days (Chagas-Spinelli, 2007).

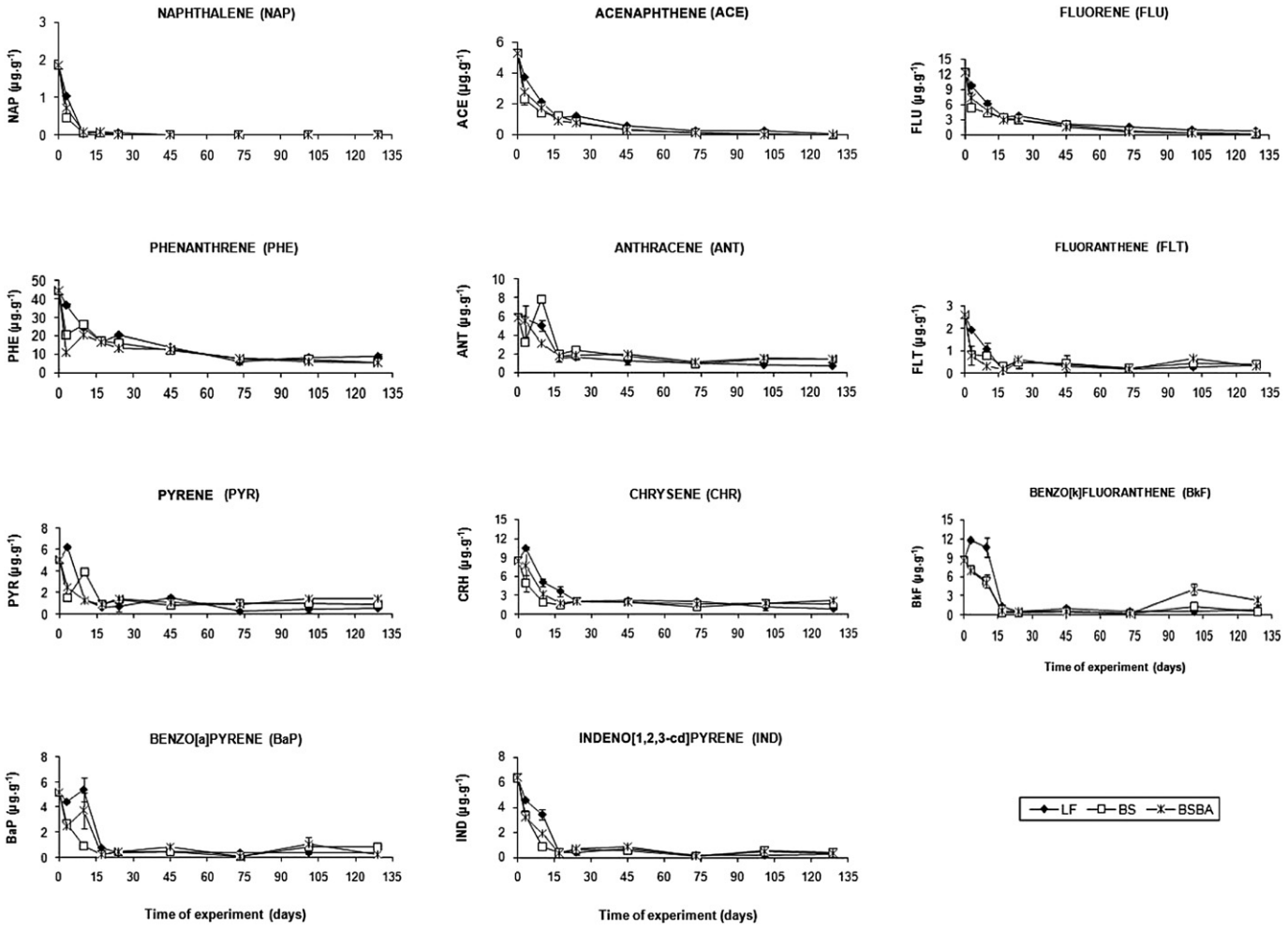

Fig. 3. Concentration of individual PAH compounds during the 129-day experimental period for LF, BS and BSBA treatments.

However, the addition of nutrients and microorganisms resulted in a continuous decrease in the soil's sorptive capacity, as shown on the 45th (900 L NAP kg^{-1}) and 129th (600 L NAP kg^{-1}) days for both the BS and BSBA treatments. Consequently, the result was that the organic matter levels decreased due to a probable change in the nature of the humification, which promoted an increase in the availability of the PAH contaminants.

In heterogeneous media like soil, PAHs may be absorbed inside of organic particles located in small pores being inaccessible for bacteria (Johnsen et al., 2005). Changes in the organic matter nature probably had influence in the PAH bioavailability.

When compared with the limits established for soils in urban and industrial areas, the removal levels of the 11 PAH compounds studied can be considered very satisfactory (CETESB, 2005; Canals, 2005). All of the compounds studied were below acceptable levels at the end of the 129-day experimental period, with the exception of benzo[a]pyrene (BaP) and chrysene (CHR). Of particular concern is benzo[a]pyrene (BaP), a highly toxic PAH compound that is considered carcinogenic, teratogenic and embryotoxic (Bento, 2005). *According to the Dutch Norm* (Canals, 2005), this compound should not be detected in urban or industrial areas. In this study, the initial concentration of 5.19 μg g^{-1} was reduced to 0.26 μg g^{-1} at the end of the experimental period for the BSBA treatment. This value was 2- to 3-fold lower than the final concentrations obtained for the LF and BS treatments.

With respect to the selection of an optimal treatment for the removal of PAHs, the overall results suggest that the method used will depend on the intended bioremediation objective. In the case of total PAH removal, the addition of nutrients or nutrients and microorganisms did not increase the degradation efficiency by the end of a 129-day period when compared with the use of aeration alone (LF). Therefore, the LF treatment appears to be the most adequate for ΣPAH removal. However, with respect to the removal of individual PAH compounds, the LF treatment was found to be best suited to the removal of compounds with four or more aromatic rings, while BS and BSBA were indicated for removal of compounds with up to three rings. However, the three treatments were nearly equivalent for the removal of most 2-ring components. Some exceptions to the above guidelines were observed, however. The 5-ring compound benzo[a]pyrene (BaP) showed the highest removal efficiency (95%) with BSBA treatment, while BS and LF removal efficiencies were 83% and 91%, respectively. Consequently, in accordance with worldwide standard practices, the method used for the degradation of the 16-priority PAH compounds should be determined based primarily on the target-compounds that present the greatest risk to the environment (Huang et al., 2004; Canals, 2005) and, secondarily on local conditions such as native microbiota and soil types.

4. Conclusions

A combination of PAH compounds were found to be efficiently removed from tropical clay soil contaminated with diesel oil using bioremediation treatments such as landfarming, biostimulation and biostimulation with bioaugmentation. Removal efficiencies of 87%, 89% and 87% were obtained for LF, BS and BSBA, respectively, at the end of a 129-day experimental period. All treatments showed a 2-phase degradation behavior typical of such bioremediation processes. However, the values of the degradation rates for each phase did not differ significantly among the three treatments. Nevertheless, the total PAH removal mechanisms probably differed between the LF method and treatments that received the addition of nutrients and/or microorganisms (BS and BSBA). The role of fungi was apparently more important than that of bacteria in the biochemical conversion of PAH during the first phase of both the BS and BSBA treatments. In the case of LF, no pH variation resulted in an increasing bacterial population with essentially non increase in the population of fungi. Thus, bacteria played an important role in total PAH degradation by the LF method.

In the case of the individual PAHs, LF showed a higher removal efficiency for those compounds with four or more aromatic rings, while BS and BSBA were more efficient for other compounds, although for 2-ring species, the three treatments were largely equivalent. One exception was the 5-ring compound benzo[a]pyrene, which was removed with the highest efficiency (95%) by the BSBA treatment. Therefore, the best degradation process for total or individual PAHs should be selected according to the target compounds of interest and the prevailing local conditions such as native microbiota and soil type.

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