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Comparison of a bioremediation process of PAHs in a PAH-contaminated soil at field and laboratory scales

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Abstract

A laboratory experiment was carried on the same initial soil and at the same time than a windrow treatment in order to compare results at field and laboratory scales for a soil mainly contaminated with PAHs. After 6 months, laboratory experiments gave similar but less scattered results than those obtained in the field indicating that the field biotreatment was well optimised. The total amount of PAHs degraded after 6 months was ca. 90% and degradation rates followed a negative exponential trend. Relative degradation rates of 3- and 4-ring PAHs were about 32 and 7.2 times greater than those of 5- and 6-ring
PAHs, respectively. With respect to the bacterial community, bacteria belonging to *Gamma-proteobacteria* persisted whereas *Beta-proteobacteria* appeared after three months of biotreatment when PAH concentration was low enough to render the soil non-ecotoxic.

**Capsule**

Comparison of field and laboratory biotreatments of the same PAH-contaminated soil gave similar results with respect to PAH concentration and bacterial diversity.

**Keywords**

Bioremediation, Contaminated soils, Polycyclic aromatic hydrocarbons (PAHs), Laboratory and field experiments, Bacterial diversity

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic pollutants generated during coke production, petroleum refining and combustion processes (Cerniglia, 1992), and these pollutants are frequently encountered in contaminated industrial sites. These organic contaminants are among the most hazardous environmental pollutants due to recalcitrance and toxic, mutagenic and carcinogenic effects (Keith and Telliard, 1979; Shaw and Connell, 1994). In the natural environment, these compounds undergo transformations involving both biotic and abiotic processes, such as volatilization, adsorption, photolysis, chemical oxidation and microbial degradation. Among them, microbial activity
makes up the primary pathway for PAH removal from soils (Yuan et al., 2000; Lors and Mossmann, 2005; Haritash and Kaushik, 2009). So, a bioremediation technique based on the optimization of biodegradation has been developed as a soil clean-up technique which is expected to be economical and efficient compared with chemical or physical remediation processes (Liebeg and Cutright, 1999; Antizar-Ladislao et al., 2004). For example, a windrow treatment applied on a soil contaminated predominantly with 2-, 3- and 4-ring PAHs resulted in 88% reduction of PAH concentration after 6 months (Lors et al., 2010b). The reduction of PAH concentration was linked to the bacterial community, which was characterized by a high diversity and the persistence of a bacterial consortium represented by Gram-negative bacterial strains during the entire biotreatment process. In particular, *Pseudomonas* and *Enterobacter* genera had a strong PAH-degrading capacity that remained throughout the whole biotreatment. Other species, such as *Beta-proteobacteria*, appeared over time, when the PAH concentration was low enough to alleviate soil ecotoxicity (Lors et al., 2010a; Lors et al., 2011). This result suggests that the presence of these bacteria could be used along with analytical methods to estimate the endpoint of biotreatment of soils containing mostly 2-, 3- and 4-ring PAHs.

In the case of PAHs, bioremediation processes, such as landfarming, biopiles, bioslurries and windrows, are based on increasing microbial activity by optimising biodegradation conditions through aeration, the addition of nutrients and control of pH, moisture and temperature (Atlas and Bartha, 1992; Namkoong et al., 2002; Sarkar et al., 2005). Thus, it is important to assess the effect of these parameters on the efficiency of the biotreatment. Laboratory experiments could be very valuable in optimising biodegradation conditions if it can be demonstrated that the biotreatment can be accurately reproduced at laboratory scale.
The present work reports on changes over time in PAH concentration and composition of the bacterial community during a laboratory experiment carried out with the same initial soil that had been subjected to a windrow biotreatment (Lors et al., 2010b). Even if the experiments were carried out at the same time, laboratory experiments enabled us to make additional samplings and better control conditions than field experiments. The comparison of present results with those obtained in the field will enable us to assess if the field experiment was well optimised and if a laboratory experiment could reproduce the results of the field biotreatment. Although many studies dealt with the monitoring of PAH-polluted soils in laboratory (Arias et al., 2008; Dandie et al., 2010), field (Ahtiainen et al., 2002; Lors et al. 2010b) and both conditions (Robinson et al., 2003), the question whether laboratory experiments can mimic field biotreatment was hardly addressed, to the exception of Diplock et al. (2009), who concluded to an urgent need for field-scale validation of laboratory methods. Additionally, this laboratory experiment could help to verify that the *Beta-proteobacteria* group appears when the soil is no longer ecotoxic, as it has been reported previously at field scale (Lors et al., 2010b).

2. Material and methods

The soil used to perform the bioremediation process was a soil contaminated by a coal tar distillation plant that was operational from 1923 to 1987 in the North of France. The contaminated soil was sieved and the fraction thinner than 6 mm was mixed with wood shavings; the characteristics of which have been given by Lors et al. (2010b). The volumetric ratio between soil and wood shavings was to 0.7:0.3. Nitrogen (agricultural urea) and phosphorus (agricultural superphosphate) nutrients were added to enhance the growth of the
microbiota. This solid matrix was sieved to 4 mm to eliminate wood shavings, and represented the starting material (called Ti soil) for both field and laboratory experiments. This substrate was characterized by different physicochemical parameters: pH, moisture, total organic carbon, total organic nitrogen, heavy metals (As, Cd, Cr, Cu, Pb, Zn) and 16 PAHs (PAHs listed by the US-EPA), using standard techniques described by Lors et al. (2010b). Heavy metals were analysed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) after hot acid digestion of the solid phase. The PAH content was determined by HPLC (High-Performance Liquid Chromatography) after ASE extraction. Moreover, microbiological investigations were performed on this substrate, including total bacteria counts and counts of PAH-degrading microbiota (Lors and Mossmann, 2005). Additionally, the diversity of total bacterial microbiota was determined by a molecular method, including total DNA extraction, PCR amplification, DGGE analysis, cloning and sequencing, which has been detailed in Lors et al. (2010b).

In situ, the PAH-contaminated substrate was placed in five windrows of approximately 5000 tons each (length = 90 m, width = 5 m, height = 2.2 m). The biotreatment started in August 2003. The solid matrix was turned each week during the first 3 months and then every 2 weeks to prevent oxygen depletion in the interstitial atmosphere. Moisture was monitored and maintained constant by periodically sprinkling water above the windrows. The temperature inside the windrows was measured at different depths (0.5, 1, 1.5 m) every 5 m along the sides of the windrows during the whole treatment. An average temperature below 30 °C for more than a week was considered improper to bacterial activity, causing the windrow to be specifically treated and eventually rebuilt. One windrow was monitored by sampling the solid matrix after 44, 60, 92 and 182 days. Each solid sample was prepared by pooling and homogenising 15 samples.
corresponding to 15 locations randomly chosen along the windrow at 0.3, 1 or 1.5 m depths. After sieving the solid matrix at 4 mm, these samples were submitted to physicochemical and microbiological analyses.

Microcosm experiments were performed under controlled conditions in the laboratory. Twenty-one 250-mL glass sterile bottles were filled with 50 g of Ti soil and closed with a porous cap which enabled oxygen to pass through. Thus, the device was in aerobic conditions and stored at 30 °C. The temperature used was representative of the temperature in the field experiment, which was around 30 °C as abovementioned. Moreover, every week, soil samples were homogenised under a sterile hood. The moisture content was measured weekly by weighing the bottles and sterile MilliQ® water was homogeneously sprayed in sterile conditions to keep the mass constant. At each sampling time (3, 7, 14, 34, 63, 92 and 182 days), three samples were sacrificed for physicochemical and microbiological analyses.

Both for field and laboratory experiments, PAH degradation was evaluated by following the decrease in PAH concentration over time. Moreover, changes over time of total microbiota were followed by the same molecular biological methods used for the initial matrix. In the laboratory experiment, sampling was done in duplicate at 14 and 92 days to determine the reproducibility of DGGE analysis.

3. Results

3.1. Chemical and bacterial characteristics of the reference soil (Ti soil)
Detailed chemical and bacterial characteristics of the Ti soil have been presented in Lors et al. (2010b) and are summarised in Table 1. The moisture content was 17%, and the soil had a C:N ratio of 56, which corresponds to favourable nutritional conditions for bacterial degradation (Meeting, 1992). Heavy metals were present at very low levels, with concentrations similar to those found in the local geochemical background (Table 1), to the exception of zinc, which exhibited a slightly higher concentration (Sterckeman et al., 2002). The Ti soil was mainly contaminated with PAHs, whose total concentration (Σ16 PAHs) was close to 3 g kg⁻¹ dry soil (Table 1). Three-ring PAHs were present at the highest concentration (1279 mg kg⁻¹ dry soil) and represented 44% of the total PAH concentration. Among them, phenanthrene was the most abundant (49% of 3-ring PAH concentration) (Table 2). Four-ring PAHs were also present at high concentrations, amounting to 28% of the total PAHs (809 mg kg⁻¹ dry soil), with fluoranthene as the main compound (accounting for 51% of 4-ring PAH concentration), followed by pyrene, accounting for 29%. The only 2-ring PAH identified was naphthalene (20% of PAH concentration). On the other hand, 5- and 6-ring PAHs were found at lower concentrations, accounting for 5% and 2% of total PAH concentration, respectively.

The total bacterial population in the Ti soil represented 4.9 \(10^8\) CFU g⁻¹ dry soil (Table 1), which is comparable to populations typically found in the superficial layer of unpolluted soils (Robert, 1996; Taylor et al., 2002). This finding indicated that there was a good biological activity in this matrix. Phenanthrene-degrading bacteria represented a large proportion of the bacteria in this soil (1.4 \(10^7\) bacteria g⁻¹ dry soil), whereas fluoranthene-degrading bacteria were present at a much lower level (7.7 \(10^4\) bacteria g⁻¹ dry soil) (Table 1). The presence of these specific bacteria indicated that the microbiota was adapted to
these compounds, probably due to historical contamination of the soil (Kästner et al., 1994; Mueller et al. 1994; Lors et al. 2004; Lors et Mossmann 2004).

3.2 Monitoring of PAH concentration during the laboratory experiment

Changes in the concentration of 16 PAHs and 2-, 3-, 4-, 5-, and 6-ring PAHs over the course of the laboratory experiment are presented in Figure 1. Data shown were average values of three replicate measurements. After 6 months of incubation, 85% of the 16 PAHs were degraded (concentrations decreased from 2895 ± 38 to 440 ± 21 mg kg⁻¹ dry soil). The overall degradation of 16 PAHs was primarily due to that of 2-, 3- and 4-ring PAHs, whereas 5- and 6-ring PAHs were hardly degraded even after 6 months of incubation. The concentration of 16 PAHs decreased rapidly over the first month and then reached a quasi asymptotic level toward the end of the experiment (Fig. 1-A). This is coherent with a negative exponential trend with regard to the remaining quantity of the 16 PAHs during the time of biotreatment that can be expressed by the following formula:

\[ [\text{PAH}] = [\text{PAH}]_0 \cdot C^t \]  

(Eq. I)

\([\text{PAH}]_0\) corresponds to the initial amount of PAH and \(C\) is a constant that is related to the degradation rate. Log \(C\) corresponds to the slope of the straight line obtained when plotting log [PAH] as a function of time. More negative values of log \(C\) indicate greater degradation rates. Exponential regression was calculated for each set of experimental data in order to compare overall degradation rates at field and laboratory scales and also according to the number of rings contained in PAHs. The determination coefficient \((R^2)\) was used to assess the accuracy of exponential regression. Values of parameters of exponential regression are reported in Table 3 except for 2-ring PAHs that were completely eliminated in 3 days and because the sampling effort was too weak to accurately calculate
exponential regression for 2-ring PAHs. The rapid elimination of 2-ring PAHs was essentially due to their volatilization. In fact, the degradation of naphthalene is expected to be very fast due to its high volatility and solubility (Cerniglia, 1992). Moreover, this compound is mainly degraded by abiotic processes as showed by Lors and Mossmann (2001) during soil biodegradation assays in sterilized and unsterilized conditions. The exponential regression of the concentration of 16 PAHs as a function of time reproduced quite accurately experimental data of the biotreatment. The best fit \((R^2 = 0.96)\) occurred by considering only the three first months and excluding the last point measured after 6 months.

The degradation of 3-ring PAHs was observed after a short induction period of 7 days (Fig. 1-C). After the induction period, the degradation rate of 3-ring PAHs occurred at the fastest rate \((\log C \text{ value } = -0.0161)\). Thus, 95% of 3-ring PAHs were degraded after 3 months of incubation, then the degradation rate slowed down relatively to what could be expected from exponential regression. As a consequence and similarly to the total 16 PAHs, the best fit for exponential regression \((R^2 = 0.94)\) was obtained considering only the first three months.

The degradation rate of phenanthrene, i.e. the main 3-ring PAH present, was similar to that of total 3-ring PAHs. Its degradation was rapid, between 7 to 34 days, leading to 84% degradation. Almost complete degradation of this compound \((94\%)\) occurred within the first three months of incubation (Fig. 2-A). As previously observed for 3-ring PAHs, 4-ring PAH concentration during the induction period was slightly higher than that of the Ti soil. Nevertheless, as 4-ring PAH concentration was lesser than 3-ring PAH concentration, the difference observed between Ti soil and soil sampled at the end of the induction period was smaller. We did not find such differences for 5-ring and 6-ring PAHs, that only represented 7% of total PAH concentration in the Ti soil.
The degradation of 4-ring PAHs displayed an induction period of 14 days was one week more than 3-ring PAHs (Fig. 1-D). Opposite to 3-ring PAHs, a greater amount of 4-ring PAHs was degraded during the last three months of the experiment (20% of 4-ring PAHs, corresponding to 163 mg kg\(^{-1}\) dry soil) (Fig. 1-D). As a consequence, exponential regression gave the best fit (R\(^2\) = 0.98) when the last point at 6 months was included in the model. The rate of degradation of 4-ring PAHs was also slower than that of 3-ring PAHs; 12%, 40%, 56% and 76% of the total 4-ring PAHs were degraded after 1, 2, 3 and 6 months of incubation respectively. This fact is numerically represented by a higher log C value: -0.0037 instead of -0.0161.

Fluoranthene, the main 4-ring PAH present, followed a similar pattern of degradation to total 4-ring PAHs; 81% of fluoranthene was degraded after 6 months of incubation (Fig. 2-B).

The degradation of 5-ring and 6-ring PAHs was even slower. After 6 months of incubation, 24% and 22% of 5-ring and 6-ring PAHs were degraded, corresponding to only 36.4 and 14.2 mg kg\(^{-1}\) dry soil, respectively. The degradation of these compounds followed a negative exponential regression, giving the best fit with the complete data set: R\(^2\) was equal to 0.7 and 0.82 for 5-ring and 6-ring PAHs, respectively. The determination coefficient was lower as a consequence of a greater relative scattering of measurements due to lower amounts of 5-ring and 6-ring PAHs. Taking into account this incertitude, it can be considered that the rates of degradation of 5-ring and 6-ring PAHs were quite similar.

### 3.3 Monitoring of bacterial diversity during laboratory experiment

The evolution of the total bacterial community during the laboratory experiment is reported in Table 4. Phylogenetic analysis of the sequences
derived from 16S rRNA gene bands in the bacterial community revealed that every clone matches for at least 94 to 99% sequence identity with 16S rRNA genes of the Genbank, except for the DGGE band number 17 (DBN 17), identified as Actinobacteria with 84% of similarity (Table 4).

A good reproducibility was observed with the replicate soil samples from 14 and 92 days. For example, duplicate soil samples collected on 14 days showed similar DGGE band profiles (Fig. 3).

As described by Lors et al. (2010b), the Ti soil contained a total bacterial community characterized by a high diversity (13 bacterial strains belonging to 9 genera), that remained throughout the experiment. The strains initially present in the Ti soil were still observed at the end of the experiment. These bacteria mainly belonged to the Gamma-proteobacteria group (Pseudomonas, Acinetobacter, Enterobacter, Klebsiella) (62%) and to a lesser extent to the Alpha-proteobacteria group (Erythromicrobium, Sinorhizobium) (15%), the Beta-proteobacteria group (Alcaligenes) (8%) and the Lactobacillales group (Aerococcus) (8%) (Table 4).

One strain, Cellulomonas variformis (DBN 17), was only detected in the Ti soil, while other strains appeared during limited periods of time. Some strains belonging to the Beta-proteobacteria group, including Brachymonas petroleovorans (DBN 13) and Uncultured Hydrogenophaga sp. (DBN 11), appeared between 92 and 182 days, whereas Alcaligenes xylosoxidans (DBN 15) was detected starting at 14 days. Pseudomonas stutzeri (DBN 16) was only detected after 34 days of incubation.

4 – Discussion

The results of the laboratory experiment can be compared with those of the field experiment, knowing that the greater number of samplings especially at the beginning of the laboratory experiments enabled us to better define the
kinetics of PAH degradation and thus may lead to more accurate exponential regression. If we compare total PAH concentrations after 6 months of biotreatment, more than 85% of the total PAHs were degraded at field and laboratory scales but with a slight advantage for the field: the concentration of 16 PAHs fell from 2894 ± 38 to 345 ± 23 and 440 ± 21 mg kg\(^{-1}\) dry soil in field and laboratory experiments, respectively. The fitness of field data with a negative exponential model was excellent (\(R^2 = 0.98\)) and the value of log C was a little more negative according to slightly more rapid degradation rate: -0.0088 instead of -0.0077. These results indicate that the field biotreatment was carried out in conditions close to optimal conditions for PAH biodegradation. Indeed, during the windrow biotreatment, the matrix was turned over periodically to allow good oxygenation. The moisture content was also kept constant by periodic water sprinkling. As a result, the temperature was always above 30 ℃, which was used as an indicator of biotreatment performance. The turning procedure was designed to minimize heat loss and to maximize gas exchange (CO\(_2\) and O\(_2\)). Indeed, temperature, moisture and aeration are the major factors associated with PAH bioremediation (Vinas et al., 2005). Moreover, additional degradation mechanisms could have occurred in the field for some PAHs. This does not seem to be the case for 3-ring PAHs as the degradation rate obtained in the laboratory was higher than in the field (Fig 1-C): log C was -0.0161 and -0.0124 for laboratory and field, respectively. The faster degradation of 3-ring PAHs at the beginning of the laboratory experiment was probably related to the fact that the experiment was carried out under better controlled and optimised conditions than in the field: for example, temperature (30 ℃) and moisture content were kept constant in the laboratory experiment. As the degradation of 3-ring PAHs is mostly controlled by biological phenomena (Lors and Mossmann, 2005), these optimal conditions allowed more rapid development of the indigenous microflora able to degrade these compounds, which were already present in the initial soil in
a considerable amount ($10^5$-$10^7$ bacteria g$^{-1}$ dry soil) (Table 1). However, the optimum conditions of the laboratory experiment did not enhance the degradation kinetics of 4- and 5-ring PAHs, which was on the contrary slightly quicker in the field (see log C values in Table 3). So, the field experiment seemed to be slightly more efficient for these high molecular weight PAHs even if the observed differences may correspond to the heterogeneity of the samples: 76%, 24% and 82%, 35% of 4- and 5-ring PAHs were degraded in laboratory and field experiments, respectively. For 6-ring PAHs, scattering of field data was high and this led to a bad fit to the negative exponential model ($R^2 = 0.34$). As the amount of 6-ring PAHs was weak, this fact can be explained by sampling and analytical heterogeneity of PAH measurements. The reported results are in agreement with those of Kästner et al. (1998) and Warmer and Peters (2005), indicating that bioremediation is more effective for low molecular-weight PAHs (Fig. 4). Indeed, the relative rate of degradation of 3-ring PAHs is about 30 times higher than 5- and 6-ring PAHs while only 7 times higher than 4-ring PAHs. Indeed, the degradation of high molecular-weight PAHs require typically more time and in some cases different microorganisms (Perry, 1979; Boonchan et al., 2000). As a consequence, the concentration of 3-ring PAHs is the lowest after 6 months while it was the highest at the beginning of the biotreatment.

Results based on the evolution of PAH concentration have to be compared with microbial consortia. First, the rate of degradation is likely to be dependent on the amount of degraders specific to some PAHs. If we consider phenanthrene, the most abundant 3-ring PAH, and fluoranthene, the most abundant 4-ring PAH, both follow the same kinetics of degradation (Fig. 2-A and 2-B), similar to those observed for the total pool of 3-ring and 4-ring PAHs, respectively (Figs. 1-C and 1-D). However, the greater amount of phenanthrene that is degraded could be related to the greater initial abundance of bacteria
specifically degrading phenanthrene ($10^7$ bacteria g$^{-1}$ dry soil) compared to the initial population of fluoranthene-degrading bacteria ($10^5$ bacteria g$^{-1}$ dry soil) (Table 1).

Second, most bacteria detected in the initial soil sample were still present at the end of the both field and laboratory experiments; bacteria present throughout the entire experiment included Strain No 1 belonging to Lactobacillales, Strains Nos 2, 3, 4, 5, 6, 8, 10 and 12 belonging to Gamma-proteobacteria and Strains Nos 7 and 9 belonging to Alpha-proteobacteria. Lors et al. (2010b) showed that bacteria belonging to the Gamma-proteobacteria group exhibited a high degrading activity towards phenanthrene and fluoranthene. As a consequence, this group could be a good bioindicator to determine the potential of PAH biodegradation of polluted soils containing predominantly 2-, 3- and 4-ring PAHs. Conversely, Alcaligenes xylosoxidans (Strain No 14) was detected in the initial soil and during the entire duration of the laboratory experiment but was no longer observed at the end of the field experiment (Table 4). Strains considered to be specific for various phases of the biodegradation process in the laboratory experiment also appeared at specific periods during the field experiment. Strains belonging to the Beta-proteobacteria group, including Brachymonas petroleovorans (DBN 13) and Uncultured Hydrogenophaga sp. clone H-3 (DBN 11), were also present starting at 92 days in the field experiment. Conversely, Alcaligenes xylosoxidans (DBN 15), which was detected starting at 14 days in the laboratory experiment, appeared at 92 days in the field experiment. The fact that samplings were performed more frequently at the beginning of the laboratory experiment allowed us to visualise more precisely the evolution of these strains between 0 and 92 days. Uncultured Hydrogenophaga sp. clone H-3 (DBN 11) and Brachymonas petroleovorans (DBN 13) appeared when the total concentration of 2-, 3- and 4-ring PAHs decreased to 427 and 307 mg kg$^{-1}$ dry soil in laboratory and field experiments, respectively. It must be noticed that the soil biotreated for 6 months
was no longer ecotoxic with respect to plant (*Lactuca sativa*) germination and
growth inhibition, earthworm (*Eisenia fetida*) mortality and springtail (*Folsomia
candida*) avoidance (Lors et al., 2010a). The occurrence of *Brachymonas*
*peloreovorans* (DBN 13) and *Uncultured Hydrogenophaga* sp. clone H-3 (DBN
11) in the laboratory experiment confirmed that these strains, belonging to the
*Beta-proteobacteria* group, could be used as indicators of the restoration of soil
quality and thus could signal the endpoint of the biological treatment of polluted
soil containing mostly 2-, 3- and 4-ring PAHs.

Third, additional microorganisms other than bacteria, such as fungi, may play a
role in PAH degradation. Li et al. (2008) revealed that microbial consortia
composed of bacteria, fungi and a bacterial-fungal mixture successfully degraded
3- to 5-ring PAHs in soil and slurry phases. It may thus be hypothesized that, in
our experiments, a colonisation of the windrow by a more complex consortium
made of bacteria and other microorganisms could eventually be one of the
reason explaining that the biotreatment worked slightly better in the field than in
the laboratory. Indeed, the change in the bacterial microbiota over time in the
laboratory experiment mirrored the changes observed during the field experiment
(Table 4) (Lors et al., 2010b) and thus the observed differences should come
from other organisms than bacteria that were not determined experimentally.

5. Conclusion

Laboratory experiments carried out on the same polluted soil than a
windrow treatment and at the same time led to results similar to those obtained
during the windrow biotreatment. The decrease of the amount of 3-, 4-, 5- and 6-
ring PAHs can be fitted to an exponential model. However, the kinetics of PAH
degradation could be defined more precisely in the laboratory, due to more
samplings, leading to higher $R^2$ values. The relative rate of degradation of 3-ring
PAHs was about 30 times higher than 5- and 6-ring PAHs while only 7 times higher than 4-ring PAHs. The optimum conditions at the laboratory just enabled us to slightly speed up the degradation of 3-ring PAHs while the degradation of 4- and 5-ring PAHs was a little faster in the field. As a consequence, the amount of degraded PAHs after 6 months was around to 85% in the laboratory instead of 90% in the field. Thus, conditions of the field biotreatment were close to optimum, mostly thanks to the optimisation of the chemical conditions (moisture, O\textsubscript{2}, temperature) and additional degradation mechanisms for 4-, 5- and 6-ring PAHs. The evolution of bacterial microbiota in the laboratory was also comparable to that obtained in the field. The bacterial consortium was represented by Gram-negative bacteria strains belonging mainly to *Gamma-proteobacteria*, in particular *Enterobacteria* and *Pseudomonas*. This consortium, which persisted throughout the biotreatment, is considered to be a good bioindicator to estimate the potential of biodegradation of soils polluted predominantly with 2-, 3- and 4-ring PAHs. The occurrence of strains belonging to *Beta-proteobacteria*, specifically *Brachymonas petroleovorans* and *Uncultured Hydrogenophaga* sp. clone H-3, was observed in both laboratory and field experiments when the PAH concentration was low enough not to cause soil ecotoxicity. Therefore, the detection of these strains could be used to estimate the endpoint of the biotreatment of PAH-contaminated soil even if some PAHs remain in the soil. Indeed, the bioavailability of PAHs should also be considered in addition to results of chemical analyses of the soil. Some additional experiments could be undertaken to study the microstructure of the soil after 6 months of biotreatment in order to find a relationship between the distribution of remaining PAHs and the bioavailability of PAHs. Indeed, the knowledge of the evolution of PAH distribution could also help to understand the effect of additional treatments, such as phytoremediation, in order to reach an even greater level of remediation (Gerhardt et al., 2009).
Finally, the ability to perform representative laboratory biodegradation experiments is very attractive because it allows the assessment of the impact of different parameters that can modify the effectiveness of a biotreatment, such as temperature, moisture, oxygenation and nutrient availability. This type of experiment could allow us to optimise the duration of the biotreatment and to reduce the cost of the process. Moreover, performing a laboratory experiment at the same time as a field biotreatment is a valuable way to monitor if the biotreatment process is performing as expected.

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References


addition, carbon supplementation and monitored natural attenuation.

Environmental Pollution 136, 187-195.


Figure captions

Fig. 1. Changes in the concentrations of all 16 PAHs (A) and of 2- (B), 3- (C), 4-(D), 5- (E) and 6- (F) ring PAHs concentrations (mg kg\(^{-1}\) dry soil ± S.E.) over time during the laboratory (L) and field (F) experiments.

Fig. 2. Changes in the concentrations of phenanthrene (Phe) (A) and fluoranthene (Flt) (B) concentrations (mg kg\(^{-1}\) dry soil ± SE) over time during the laboratory (L) and field (F) experiments.

Fig. 3. DGGE analysis of the bacterial communities during the laboratory experiment.

lane M: DGGE marker - lane 1: 0 day; lane 2: 3 days; lane 3: 7 days; lanes 4 and 5: 14 days; lane 6: 34 days; lane 7: 63 days; lane 8: 92 days; lane 9: 182 days

Fig. 4. Values of log C to function the number of rings for 3-, 4-, 5- and 6-
ring PAHs during the laboratory and field biodegradation
experiments.
Table 1. Chemical and bacterial characteristics of the Ti soil.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Moisture</th>
<th>pH</th>
<th>C:N</th>
<th>16 PAHs</th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
<th>Total bacterial microbiota</th>
<th>Phe bacterial degraders</th>
<th>Flt bacterial degraders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti soil</td>
<td>17.7 ± 0.1</td>
<td>7.9 ± 0.2</td>
<td>56 ± 38</td>
<td>2895 ± 6.5</td>
<td>&lt; LD</td>
<td>32.9 ± 0.3</td>
<td>19.3 ± 0.4</td>
<td>23.9 ± 0.8</td>
<td>92.7 ± 0.8</td>
<td>4.9 (10^8)</td>
<td>1.4 (10^7)</td>
<td>7.7 (10^4)</td>
<td></td>
</tr>
<tr>
<td>G.B.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.9 ± 1.2</td>
<td>0.4 ± 0.03</td>
<td>48.8 ± 2.7</td>
<td>16.7 ± 1.8</td>
<td>38.4 ± 5.6</td>
<td>73.7 ± 6.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Phe: Phenanthrene; Flt: Fluoranthene; Limit of detection (LD); Carbon-Nitrogen ratio (C:N).

Geochemical background (G.B.) of trace elements in soils around smelters in the North of France was determined by Sterckeman et al. (2002).
Table 2. Concentration\(^a\) of each of 16 (EPA) PAHs (reported in mg kg\(^{-1}\) of dry soil) in the Ti soil (the same soil was used in field and laboratory experiments).

<table>
<thead>
<tr>
<th>PAH</th>
<th>Concentration (mg kg(^{-1}) dry soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>594.22 ± 7.96</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>3.12 ± 0.03</td>
</tr>
<tr>
<td>Acenaphylene</td>
<td>217.42 ± 0.71</td>
</tr>
<tr>
<td>Fluorene</td>
<td>226.81 ± 1.64</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>629.31 ± 2.40</td>
</tr>
<tr>
<td>Anthracene</td>
<td>202.50 ± 18.31</td>
</tr>
<tr>
<td>Fluoranethene</td>
<td>414.34 ± 0.71</td>
</tr>
<tr>
<td>Pyrene</td>
<td>233.44 ± 0.22</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>85.72 ± 0.50</td>
</tr>
<tr>
<td>Chrysene</td>
<td>75.43 ± 0.50</td>
</tr>
<tr>
<td>Benzo(b)fluoranethene</td>
<td>56.18 ± 0.17</td>
</tr>
<tr>
<td>Benzo(k)fluoranethene</td>
<td>25.80 ± 0.18</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>60.36 ± 3.88</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>6.86 ± 0.14</td>
</tr>
<tr>
<td>Benzo(g,h,i)perylene</td>
<td>32.45 ± 0.56</td>
</tr>
<tr>
<td>Indeno(123-cd)pyrene</td>
<td>30.79 ± 0.15</td>
</tr>
</tbody>
</table>

\(^a\) Mean values ± S.E. for three replicates.
Table 3. Values of the parameters of the exponential regression applied to the kinetics of PAHs biodegradation.

[PAH]₀, C and R² corresponded respectively to the initial amount of PAH, a constant related to the degradation rate and the determination coefficient of the exponential regression.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log C</td>
</tr>
<tr>
<td>16 PAHs</td>
<td>-0.0077</td>
</tr>
<tr>
<td>3-ring PAHs</td>
<td>-0.0161</td>
</tr>
<tr>
<td>4-ring PAHs</td>
<td>-0.0037</td>
</tr>
<tr>
<td>5-ring PAHs</td>
<td>-0.0005</td>
</tr>
<tr>
<td>6-ring PAHs</td>
<td>-0.0005</td>
</tr>
</tbody>
</table>
Table 4. Diversity of the bacterial communities in the soil sampled during the field and laboratory experiments.

<table>
<thead>
<tr>
<th>DBN</th>
<th>Phylogenetic affiliation</th>
<th>Similarity (%)</th>
<th>Identified bacteria</th>
<th>Field</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 day</td>
<td>92 days</td>
</tr>
<tr>
<td>1</td>
<td>Lactobacillales</td>
<td>96</td>
<td>Aerococcus viridans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Gamma-proteobacteria</td>
<td>99</td>
<td>Enterobacter sp. GOBB3-C104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Gamma-proteobacteria</td>
<td>98</td>
<td>Pseudomonas sp. HI-B7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Gamma-proteobacteria</td>
<td>99</td>
<td>Pseudomonas stutzeri ATCC 17685</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Gamma-proteobacteria</td>
<td>97</td>
<td>Pseudomonas sp. C54A</td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>Gamma-proteobacteria</td>
<td>98</td>
<td>Acinetobacter johnsonii</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>Gamma-proteobacteria</td>
<td>94</td>
<td>Erythromicrobium ramosum DSM 8510</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Gamma-proteobacteria</td>
<td>99</td>
<td>Pseudomonas stutzeri ZWLR2-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Alpha-proteobacteria</td>
<td>92</td>
<td>Sinorhizobium sp. L1</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>Gamma-proteobacteria</td>
<td>99</td>
<td>Klebsiella planticola ATCC 33531T</td>
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<td></td>
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<tr>
<td>11</td>
<td>Gamma-proteobacteria</td>
<td>98</td>
<td>Uncultured hydrogenophaga sp. Clone H-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Gamma-proteobacteria</td>
<td>98</td>
<td>Enterobacter amnigenus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Beta-proteobacteria</td>
<td>94</td>
<td>Brachymonas petroleovorans CHX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Beta-proteobacteria</td>
<td>98</td>
<td>Alcaligenes xylosoxidans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Beta-proteobacteria</td>
<td>98</td>
<td>Alcaligenes xylosoxidans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Gamma-proteobacteria</td>
<td>94</td>
<td>Pseudomonas stutzeri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Actinobacteria</td>
<td>84</td>
<td>Cellulomonas variformis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1.

A  
F-16 PAHs  
L-16 PAHs

B  
L-2-ring PAHs  
F-2-ring PAHs

C  
L-3-ring PAHs  
F-3-ring PAHs

D  
F-4-ring PAHs  
L-4-ring PAHs

E  
F-5-ring PAHs  
L-5-ring PAHs

F  
F-6-ring PAHs  
L-6-ring PAHs
Fig. 2.

A

B

[PAHs] (mg/kg)

Time (d)

F-Phe
L-Phe

F-Flt
L-Flt
Fig. 4.

Number of rings for PAHs

-0.018
-0.016
-0.014
-0.012
-0.01
-0.008
-0.006
-0.004
-0.002
0
3 4 5 6

log C

Field
laboratory