# Ecotoxicological Assessment of Soils of Former Manufactured Gas Plant Sites: Bioremediation Potential and Pollutant Mobility

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Analytically well-characterized soils from four different former manufactured gas plants (MGP) sites contaminated by coal tars were used in tests of extensive biodegradation of polycyclic aromatic hydrocarbons (PAHs) in stirred reactors. In all cases, the extent of biodegradation was limited to 80−100% for 2- and 3-ring PAHs, 40−70% for 4-ring PAHs, and below 20% for 5- and 6-ring PAHs. The capacities to transfer pollutants to water were compared for leachates from soils that had or had not undergone biological treatment. Leachate analysis involved determination of PAHs and bacterial tests of acute toxicity (Microtox) and genotoxicity (SOS Chromotest). For some untreated soils, PAH leaching was observed, and positive responses to the Microtox test were well correlated to the concentrations of naphthalene and phenanthrene. Biologically treated soils had lost all capacities for leaching as concluded from PAH determinations and responses to the Microtox test. All soil leachates were devoid of genotoxic effect, in accordance with the low concentrations observed of mutagenic PAHs. The results of this risk-based approach for assessment of MGP soils showed that pollutants remaining after biological treatment were unavailable for further biodegradation and that the extent of leaching had been reduced to the level that it did not represent a significant threat to groundwater.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants (*1*) that cause concern because of their genotoxic properties (*2*). Their environmental importance led the U.S. Environmental Agency (EPA) to include 16 unsubstituted PAHs in the priority list of pollutants (*3*). At coal pyrolysis sites such as the former manufactured gas plant (MGP) sites, PAHs are present as components of coal tar (*4*−*8*) and constitute persistent pollutants. Several authors have shown that only partial biodegradation of PAHs could be achieved in laboratory experiments performed with these MGP soils even under optimized conditions (*9*−*12*). In terms of risk assessment, an important question that arises is whether residual PAHs can still be transferred to water or if they can be considered as nonaccessible and presenting a negligible potential for aquifer contamination. A similar approach has been discussed under the name of biostabilization by Smith et al. (*13*) for soils contaminated by creosote PAH.

The aim of the present work was to make a detailed evaluation of the relevance of this concept to the case of MGP sites. We selected soils of different MGP sites representing a wide range of PAH concentrations and for which detailed analytical characterization of pollutants had been performed (*8*). Experiments of extensive biological degradation were performed in well-defined conditions with these soils in bench-scale stirred reactors. The capacities to transfer pollutants to water were compared for soils that had or had not undergone biological treatment. Soil leachates were prepared and characterized by analytical determination of PAHs and by evaluation with bacterial tests of acute toxicity and genotoxicity. The results obtained concerning the different points of the experimentation are described.

## Materials and Methods

**Soil Preparation.** All soil samples, designated SA, M, LH, and G, originated from different contaminated former MGP sites. Granulometric characterization and analysis of pollutants of these soils have been previously described (*8*). After thorough homogenization, the 2-mm soil fraction was used for analytical characterization and for the biotreatability and leaching studies.

**Soil Analysis.** PAH extraction from soil samples was carried out with a cyclohexane−acetone (85/15, v/v) mixture. PAH analyses in solvent extracts were carried out by gas chromatography (GC) with a flame ionization detector (FID). The operating conditions used were as previously described (*8*). The calibration was done for each PAH mentioned in the EPA list. The mean value of the response coefficient of these PAHs was used for quantifying all other (mostly substituted) PAHs for which no commercial standards were available (*8*, *14*, *15*). The concentration of PAHs was expressed for the individual PAHs of the EPA list, for the sum of them (16 EPA PAHs), and for the sum of all PAHs detected by gas chromatography (total PAHs).

The soil total organic carbon (TOC) was analyzed with a Rock Eval III apparatus (Vinci Technologies, Rueil Malmaison, France) as previously described (*8*). Heavy metal analysis of soil was performed by atomic absorption (Spectraa 600 Zeeman, Varian Instruments, San Fernando, CA) after acidic extraction. Mercury was determined separately by atomic fluorescence detection (MLD, Spectra France, Cogny, France).

**Biological Treatment of Soils.** Experiments of PAH biodegradation were carried out at 30 °C in the stirred (300 rpm) 1-L glass reactors of a Sapromat respirometer (type D, Voith, Ravensburg, Germany). In this apparatus, oxygen was kept at a constant partial pressure by electrolytic production, and its consumption was continuously recorded, whereas $CO_2$ was trapped by soda lime pellets. The minimal measurable quantity of oxygen in the Sapromat system was 0.25 mg.

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10.1021/es990203r CCC: $18.00 
Published on Web 11/04/1999

TABLE 1. Analytical Characterization of Polluted Soils from Four Former MGP Sites[a]

| | organic pollutants (g/kg of dry soil) | | | inorganic pollutants (mg/kg of dry soil) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| soil | sum of 16 EPA PAH | total PAH | TOC | Hg | Cr | Cd | Ni | Pb | As |
| SA | 6.7 (0.6) | 17.4 (2.4) | 103 (8.8) | | 5−10 | <0.8 | | 40 | |
| M | 4.7 | 10.9 | 41 (7.8) | 0.2 | nd[b] | nd[c] | nd[b] | 38 | 4.7 |
| LH | 0.9 (0.1) | 2.1 (0.2) | 30 (3.3) | <0.5 | 30 | <0.5 | 20 | 200−375 | 6−9.5 |
| G | 0.3 (0.00) | 1.1 (0.00) | 18 (0.4) | 0−2 | 10−15 | <0.5 | 8−12 | 80−150 | 10−15 |

[a] Standard deviation in parentheses. Number of independent PAH analyses: for soil SA, 18; for soil LH, 7; for soil G, 3. Number of independent TOC analyses: for all soils, 4. [b] nd, below detection limit (10 mg/kg dry soil). [c] nd, below detection limit (1 mg/kg dry soil).

The reactors contained 40 g of contaminated soil and 900 mL of mineral salt medium. The indigenous microflora was used without additional inoculum after being characterized for degraders of PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene) with the numbering technique on microtiter plates described by Stieber et al. (*16*). Incubation was carried out until oxygen consumption became negligible (50 days for soil M and 90 days for soils SA, LH, and G). Abiotic controls were run simultaneously in the same operating conditions. After treatment, the complete reactor content was filtered through a glass fiber filter (GF/F Whatman, Maidstone, GB). The soils were extracted, and hydrocarbon analysis was performed as described above for untreated soils.

**Leaching Experiments.** The method used for soil leaching was adapted from the standard method (DIN 38 414 part 4). A total of 100 g (dry weight) of soil was shaken with 1 L of distilled water for 24 h on a rotary shaker at 10 rpm. Except when mentioned otherwise, it was completed at 20 °C in the presence of 1 g/L $HgCl_2$ in order to avoid PAH biodegradation. Before analysis of the leachates, the solid fraction was systematically removed from the water by filtration on a regenerated cellulose membrane with a calibrated porosity of 0.45 $\mu$m (Schleicher & Schuell, Dassel, Germany) placed on a Teflon-coated stainless steel filter holder (toxic waste filter holder, 142 mm, Millipore, Bedford, MA).

**Leachate Analysis.** PAHs in leachates were analyzed by high-performance liquid chromatography (HPLC). PAHs were analyzed after liquid−liquid extraction of duplicate 50- or 100-mL samples with 1 mL of cyclohexane. The solvent was then evaporated to near dryness and replaced by acetonitrile. As some loss of volatile PAHs (naphthalene, acenaphthylene, and acenaphthene) occurred during cyclohexane evaporation, the concentration of these PAHs was determined by direct analysis of nonextracted leachate samples mixed with acetonitrile (1/1, v/v). HPLC analysis of PAHs was performed with a gradient pump series 410 equipped with an autosampler ISS 200 (both from Perkin-Elmer, Norwalk, CT) using a Supelcosil LC PAH column (Supelco, Bellefonte, PA). Elution conditions were as follows: 10 min with acetonitrile/water 50/50 (v/v), rise in 30 min to acetonitrile/water 90/10 (v/v), and hold 20 min. The PAHs were quantified by UV detection with a diode array detector (PDA 996, Waters, Millford, MA) at the specific wavelength of each compound and with a fluorimeter (model 470, Waters, Millford, MA) at specific excitation/emission wavelengths.

**Ecotoxicological Characterization.** Ecotoxicity tests were carried out on soil leachates prepared according to the protocol described above except that $HgCl_2$ was omitted. Prior to analysis, leachates were stored at −20 °C. Acute toxicity was determined with the bioluminescence test (Microtox) according to standard methods (DIN 38 412) using lyophilized cultures of *Vibrio fisherii* from Microbics (Carlsbad, CA). The luminescence was measured with a Lumacounter (Lumac, Schaesberg, The Netherlands) on dilution series of the leachates.

The genotoxicity was evaluated using the SOS Chromotest with the tester strain *Escherichia coli* PQ37 (*sulA::lacZ)* (*17*) kindly provided by Philippe Quillardet (Institut Pasteur, Paris, France). In this test, genotoxicity is proportional to the $\beta$-galactosidase activity induced in a growing culture of *E. coli* PQ37, growth being measured by the activity of constitutive alkaline phosphatase. The induction factor (IF) is defined as the normalized ratio between $\beta$-galactosidase activity and alkaline phosphatase activity for a given sample concentration. The SOS induction potency (SOSIP) is the slope of the linear region of the IF versus concentration. The test according to Quillardet and Hoffnung (*18*) was adapted to a protocol using microtiter plates. The 200-$\mu$L aliquots of dilutions of leachate samples were incubated in 100 $\mu$L of a *E. coli* PQ37 culture. Both enzymatic activities were determined colorimetrically by the $OD_{414\ nm}$ values recorded in an incubator/OD reader MF iEMS (Labsystems, Helsinki, Finland). Positive controls were run with 20 $\mu$L of DMSO solutions of 4-nitroquinoline 1-oxide (4NQO) for direct genotoxic compounds (assays without enzymatic activation) and of benzo[*a*]pyrene (BaP) with rat liver microsomes (S9 mix) for progenotoxic compounds. The SOSIP values determined for the positive controls were in accordance with Quillardet et al. (*19*).

**Chemicals.** For GC-FID, the calibration standard used was the TCL Polynuclear Aromatic Hydrocarbons Mix (ref 4-8905) containing the 16 EPA PAHs from Supelco (Bellefonte, PA). For HPLC, the calibration standard Custom PAH mixture (ref. CUS-1262) from J. T. Baker (Deventer, The Netherlands) was used. Distilled water with a resistivity >18 MΩ was produced by a Milli-Q purification system (Millipore, Bedford, MA). The S9 mix of rat liver induced with a phenobarbital−methylcholantrene mixture was from IFFA CREDO (l'Arbresle, France).

## Results and Discussion

**Analytical and Microbiological Soil Characterization.** Four soil samples from different former MGP sites were selected for the study. Table 1 shows that the soils studied presented a wide range of concentrations of organic pollutants but very similar characteristics: the PAHs were systematically associated to a heavy organic fraction that is typical of contamination by coal tar (*8*). In these soils, the 16 EPA PAHs represented about 40% of total PAHs and 5% of the TOC. The heavy metal concentrations were not particularly high with regard to natural soils and will not be further discussed.

All the soils were found to have a sizable although variable microflora of specific PAH-degrading bacteria (between $10^3$ and $10^7$ per gram of dry soil) that were able to grow on the wide range of 2-, 3-, and 4-ring PAHs tested (*20*). This specific microflora represented a significant proportion of the total microflora. No attempt was made to enumerate bacteria able to degrade PAHs heavier than pyrene as the method is not appropriate for these compounds that are less soluble and are probably often degraded by co-metabolism.

**Biological Treatment of Soils.** Considering the 16 EPA PAHs (Table 2), the biological treatment of the different polluted soils in stirred reactors led to similar degradation rates from one sample to another despite large differences

TABLE 2. Biodegradation of Coal Tar PAH in Stirred Reactors[a]

| | soil SA | | soil M | | soil LH | | soil G | |
|---|---|---|---|---|---|---|---|---|
| | initial PAH concn[b] (mg/kg of dry soil) | degradation[c] (%) | initial PAH concn[b] (mg/kg of dry soil) | degradation[d] (%) | initial PAH concn[b] (mg/kg of dry soil) | degradation[c] (%) | initial PAH concn[b] (mg/kg of dry soil) | degradation[c] (%) |
| naphthalene | 794 (13) | 99 (1) | 74 | 98 | 0 (3) | nd | 0 (0) | nd |
| sum of 2-ring PAH | 794 (13) | 99 (1) | 74 | 98 | 0 (3) | nd | 0 (0) | nd |
| acenaphthylene | 189 (12) | 85 (3) | 238 | 85 | 0 (4) | nd | 0 (1) | nd |
| acenaphthene | 38 (3) | 88 (7) | 180 | 98 | 1 (1) | 54 (27) | 1 (0) | 56 (67) |
| fluorene | 180 (11) | 93 (2) | 310 | 99 | 5 (3) | 84 (12) | 5 (1) | 84 (16) |
| phenanthrene | 751 (57) | 87 (2) | 1 043 | 99 | 34 (16) | 81 (3) | 12 (4) | 78 (8) |
| anthracene | 262 (23) | 84 (2) | 438 | 94 | 37 (6) | 88 (2) | 52 (8) | 97 (1) |
| sum of 3-ring PAH | 1 420 (83) | 87 (2) | 2 210 | 96 | 78 (28) | 79 (4) | 70 (13) | 89 (5) |
| fluoranthene | 1 116 (57) | 51 (1) | 938 | 90 | 133 (11) | 64 (4) | 29 (3) | 74 (5) |
| pyrene | 920 (49) | 38 (10) | 707 | 64 | 107 (6) | 58 (5) | 30 (3) | 69 (7) |
| benz[*a*]anthracene | 519 (41) | 33 (1) | 233 | 50 | 77 (7) | 51 (4) | 23 (2) | 66 (6) |
| chrysene | 444 (38) | 34 (3) | 186 | 34 | 74 (5) | 50 (4) | 26 (2) | 59 (5) |
| sum of 4-ring PAH | 2 999 (167) | 41 (3) | 2 063 | 72 | 391 (29) | 57 (4) | 108 (10) | 67 (6) |
| benzo[*b*]fluoranthene[e] | 773[e] (146) | 3[e] (8) | 77 | −26 | 152[e] (13) | 27[e] (11) | 47[e] (7) | 42[e] (14) |
| benzo[*k*]fluoranthene | | | 80 | 13 | | | | |
| benzo[*a*]pyrene | 562 (108) | 20 (7) | 81 | −61 | 108 (5) | 23 (2) | 35 (5) | 33 (6) |
| indeno[*c,d*]pyrene[f] | 304 (38) | −15 (3) | 35 | −12 | 80[f] (5) | 23[f] (3) | 26[f] (1) | 25[f] (6) |
| dibenz[*a,h*]anthracene | 83 (18) | −12 (27) | 16 | 44 | | | | |
| benzo[*g,h,i*]perylene | 286 (74) | −18 (7) | 36 | −46 | 69 (11) | 8 (2) | 24 (1) | 1 (21) |
| sum of 5- and 6-ring PAH | 2 008 (179) | 0 (7) | 327 | −19 | 409 (15) | 17 (3) | 132 (12) | 23 (5) |
| sum of the 16 EPA PAH | 6 732 (599) | 41 (3) | 4 673 | 77 | 877 (66) | 40 (3) | 310 (35) | 53 (4) |
| total PAH | 17 416 (2 422) | 27 (3) | 10 872 | 61 | 2 120 (301) | 34 (4) | 1 065 (244) | 44 (9) |

[a] nd, not detectable. Standard deviation in parentheses. [b] Number of independent PAH analyses: for soil SA, 18; for soil M, 1; for soil LH, 7; for soil G, 3. [c] Five independent experiments. [d] One experiment. [e] For the sum of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene. [f] For the sum of indeno[*c,d*]pyrene and dibenz[*a,h*]anthracene.

in PAH concentrations, i.e., a nearly complete degradation of naphthalene in the soils where it was initially present, a very good biodegradation (between 80 and 95%) of the 3-ring PAHs, and a significant degradation (between 40 and 70%) of the 4-ring PAHs. The 5- and 6-ring PAHs only underwent marginal biodegradation. Some differences between the different soil samples however could be observed in particular for soil M, which presented a lower proportion of heavy compounds and where fluoranthene in particular underwent a higher biodegradation. No significant changes in PAH concentration were observed during incubation in abiotic flasks. The reproducibility of degradation rates appeared to be quite good. Actually, it essentially reflected the reproducibility of the determination of PAH concentrations in soils that was made satisfactory by thorough soil homogenization. These results were in accordance with the reports of other teams working with MGP soils (*9*−*12*), but higher degradation rates have been reported for soils contaminated with creosote (*21, 22*), a coal tar distillate that contains more 2- and 3-ring PAHs and no heavy organic matter. The degradation of total PAHs was between 30 and 60%, which implied a significant biodegradation of substituted PAHs (19% for soil SA, 48% for soil M, 29% for soil LH, and 40% for soil G). It can be noted that the substituted PAHs that represent the major part of the total PAHs (*8, 14, 15*) were quantified less precisely because of their low individual concentrations.

As mentioned above, the end of significant oxygen consumption was taken as the end point of biological treatment. The oxygen profiles obtained, presented in Figure 1, were reproducible and were correlated to the coal tar contamination level but without the proportionality between oxygen consumed and PAHs degraded that is observed during the biodegradation of pure PAHs by pure cultures (*23*). Considering a PAH composition of 92.3% carbon and 7.7% hydrogen (average formula $C_xH_x$) results in an oxygen requirement for mineralization corresponding to a molar oxygen/carbon ratio ($O_2$/C) of 1.25. During biological treatment of soils contaminated with coal tars, more oxygen than theoretically necessary for PAH biodegradation was often observed ($O_2$/C ranged up to 5.2 for soil LH and 6.5 for soil G). This suggested the concomitant degradation of other organic compounds, a point also noted by Stieber (*24*). The absence of a strict proportionality between oxygen consumption and PAH biodegradation did not allow the reliance on the respirometric time courses to follow the progress of PAH biodegradation in MGP soils, but these data definitely showed the end point of aerobic degradative activities. In fact, it was observed that PAH biodegradation occurred in less than 3 months: after 2 weeks of treatment of soil SA in stirred reactors, 95% of the PAH degradation observed in 3 months was obtained (data not shown). In these conditions, the incomplete final biodegradation (Table 2) was quite likely to result from a lack of bioavailability of residual PAHs.

**Leaching Capacity of Soils and Bioavailability.** The leaching protocol used was found to yield quite reproducible results for all the soils studied in the conditions described (Table 3), and other experiments showed that the time of leaching (24 h) was quite sufficient to reach the equilibrium concentration of PAHs in water (data not shown), as recently reported for PAHs originating from petroleum hydrocarbons in polluted harbor sediments (*25*). The more soluble PAHs were found in highest concentrations in the leachates, but no compound reached its saturation concentration (*26*) despite the presence of enough PAHs in the soil reservoir.

In untreated soils, the highest PAH concentrations in leachates were observed for the most polluted soils, but there was no clear correlation between PAH concentration in soil and leaching capacity. For example, soils SA and M had an important PAH exchange capacity with water, and the two others (soil LH and G) had a very low one (Table 3). One of the most important points shown in Table 3 is that after biological treatment all the soils presented no more significant PAH leaching capacities though not all the pollutants had been biodegraded. As a consequence, these residual pol-

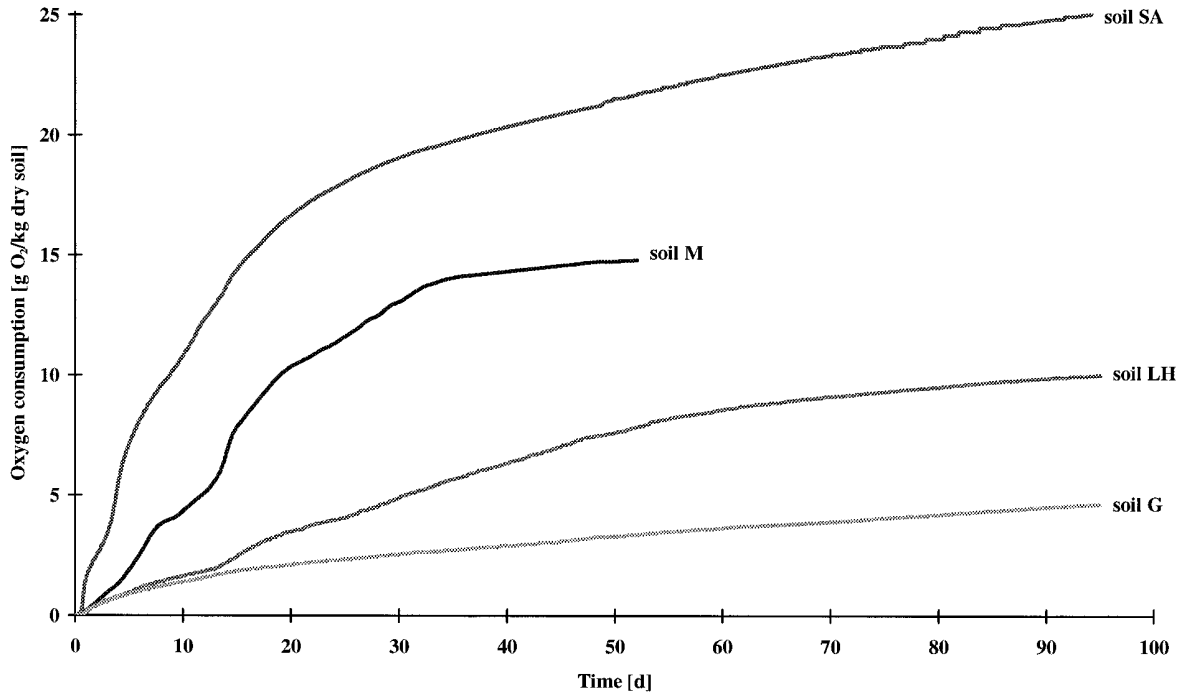


**FIGURE 1. Time course of oxygen consumption during PAH degradation in soil suspensions.**

TABLE 3. Leaching Capacities of Untreated and Biologically Treated PAH-Polluted Soils[a]

| | PAH concentration in water after 24 h soil leaching ($\mu$g/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | soil SA | | soil M | | soil LH | | soil G | |
| | untreated soil[b] | after biotreatment[c] | untreated soil[b] | after biotreatment[d] | untreated soil[a] | after biotreatment[e] | untreated soil[b] | after biotreatment[e] |
| naphthalene | 5 059 (1) | 0.09 (29) | 7 (2) | 0.05 | 2.12 (12) | 0.10 (11) | 0.08 (82) | 0.09 (14) |
| sum of 2-ring PAH | 5 059 (1) | 0.09 (29) | 7 (2) | 0.05 | 2.12 (12) | 0.10 (11) | 0.08 (82) | 0.09 (14) |
| acenaphthylene | 236 (27) | <0.01 | 99 (5) | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| acenaphthene | 37 (33) | 0.01 (12) | 28 (1) | <0.01 | 0.11 (4) | 0.02 (5) | 0.07 (26) | 0.01 (8) |
| fluorene | 112 (0) | 0.05 (55) | 124 (1) | <0.01 | 0.33 (1) | 0.09 (16) | 0.17 (7) | 0.10 (6) |
| phenanthrene | 134 (4) | 0.05 (24) | 213 (1) | 0.03 | 0.60 (3) | 0.07 (20) | 0.19 (6) | 0.07 (16) |
| anthracene | 27 (7) | 0.01 (7) | 38 (1) | 0.01 | 0.26 (6) | 0.01 (12) | 0.31 (12) | 0.01 (16) |
| sum of 3-ring PAH | 546 (8) | 0.12 (32) | 502 (0) | 0.03 | 1.30 (3) | 0.19 (15) | 0.74 (0) | 0.19 (8) |
| fluoranthene | 24 (9) | 0.08 (41) | 35 (1) | 0.03 | 0.47 (11) | 0.06 (77) | 0.09 (3) | 0.03 (14) |
| pyrene | 17 (10) | 0.05 (92) | 22 (3) | <0.01 | 0.30 (18) | 0.08 (39) | 0.10 (5) | 0.05 (64) |
| benz[*a*]anthracene | 0.26 (5) | 0.00 | 0.22 (17) | 0.02 | 0.01 (0) | <0.01 | 0.01 (12) | <0.01 |
| chrysene | 0.10 (10) | 0.02 (8) | 0.08 (27) | <0.01 | 0.03 (10) | 0.02 (28) | 0.02 (21) | 0.02 (21) |
| sum of 4-ring PAH | 41.4 (9) | 0.15 (10) | 57.3 (2) | 0.05 | 0.81 (13) | 0.16 (16) | 0.22 (2) | 0.10 (37) |
| benzo[*b*]fluoranthene | 0.25 (9) | <0.01 | 0.01 (11) | <0.01 | 0.03 (3) | 0.01 (40) | 0.03 (29) | <0.01 |
| benzo[*k*]fluoranthene | 0.10 (6) | <0.01 | <0.01 | <0.01 | 0.01 (9) | <0.01 | 0.01 (12) | <0.01 |
| benzo[*a*]pyrene | 0.15 (5) | <0.01 | <0.01 | <0.01 | 0.02 (9) | 0.01 (29) | 0.02 (4) | <0.01 |
| indeno[*c,d*]pyrene | 0.03 (13) | <0.01 | <0.01 | <0.01 | 0.01 (10) | <0.01 | 0.01 (11) | <0.01 |
| dibenz[*a,h*]anthracene | 0.04 (9) | <0.01 | <0.01 | <0.01 | 0.04 (4) | <0.01 | 0.03 (21) | <0.01 |
| benzo[*g,h,i*]perylene | 0.12 (4) | <0.01 | <0.01 | <0.01 | 0.04 (3) | <0.01 | 0.04 (20) | <0.01 |
| sum of 5- and 6-ring PAH | 0.69 (5) | <0.01 | 0.01 | <0.01 | 0.15 (3) | 0.02 (34) | 0.14 (18) | <0.01 |
| sum of the 16 EPA PAH | 5 647 (2) | 0.36 (10) | 566 (0) | 0.13 | 4.38 (3) | 0.47 (8) | 1.18 (3) | 0.38 (15) |

[a] The concentration of the 16 EPA PAH (mg/kg of dry soil) in untreated and biologically treated soils were respectively for soil SA, 6732 and 3972; for soil M, 4673 and 1056; for soil LH, 877 and 527; and for soil G, 310 and 146. Mean deviation in parentheses (given in % of the mean value). [b] Two independent experiments. [c] Three independent experiments. [d] One experiment. [e] Four independent experiments.

lutants had to be considered as no longer available for transfer to water.

Weissenfels et al. (*10*) proposed that the presence of organic matter associated with PAHs constituted a factor limiting leaching capacity. The results described here showed the existence of a leachable fraction, which was important in some samples and which completely disappeared in all cases after biological treatment in reactors. These results confirmed a lack of bioavailability. Furthermore, the results clearly indicated a link between the PAH-leaching capacity of a soil and the potential for biodegradation of these compounds.

The characteristics observed for biodegradation and leachability of PAHs in MGP soils are well in line with the presence of PAHs as constituents of coal tar that appeared to trap a large portion of PAHs (*8, 15*). Inside such a matrix, PAHs have to diffuse through the heavy organic matter of coal tar before being released in the aqueous phase and/or

TABLE 4. Acute Toxicity and Genotoxicity of Leachates of PAH-Polluted Untreated and Biologically Treated Soils

| | acute toxicity (Microtox)[b] max light inhibition (%) | | genotoxicity (SOS Chromotest)[c] induction factor[d] | | | |
|---|---|---|---|---|---|---|
| | | | with S9 mix | | without S9 mix | |
| | untreated soil | after biotreatment | untreated soil | after biotreatment | untreated soil | after biotreatment |
| soil SA | 85 | <20 | <1.5 | <1.5 | <2 | <2 |
| soil M | 50 | <20 | <1.5 | na[a] | <2 | na[a] |
| soil LH | <20 | <20 | <1.5 | <1.5 | <2 | <2 |
| soil G | <20 | <20 | <1.5 | <1.5 | <2 | <2 |
| 17.8 $\mu$g/L 4 NQO[e] | | | | | 2.9 | |
| 178 $\mu$g/L BaP[e] | | | 2.0 | | | |

[a] na, not analyzed. [b] Mean of two independent experiments. [c] Analyzed on two independent experiments. [d] Induction factor: ratio of $\beta$-galactosidase/alkaline phosphatase activities. [e] Lowest significant concentration in the test.

being taken up by degradative bacteria. The fact that only part of the PAH pool of coal tars appeared available for leaching and biodegradation may result from progressive rigidifying of the coal tar matrix after PAH release (*6*), making diffusion of remaining PAHs inside coal tar less and less efficient. Other phenomena such as sorption on the mineral matrix of soil may also contribute to limitation of mass transfer of PAH (*27*). Huesemann (*28*) discussed the reasons of incomplete biodegradation of hydrocarbons in soils in terms of bioavailability or of inherent recalcitrance. PAH accessibility rather than limited degradative capacities of microflora appears responsible for the incomplete biodegradation observed in our tests as indicated by the fact that the residual PAH fraction of the treated soils had practically lost all capacity for leaching. However, the efficiency of microflora by appropriate degradation tests involving accessible PAHs deserves to be confirmed in particular for higher PAHs, and evidence concerning this point has been obtained (*20*).

The results presented here indicate that biodegradation and leaching are also responsible for the process of natural attenuation for which evidence based on the evolution of coal tar composition in MGP soils has been reported (*8*). Such a process gradually leads to a decrease in the leaching capacities of the polluted soils. The different leaching capacities of untreated soils observed in this study thus probably reflect the different degrees of natural attenuation undergone by the studied soil samples. The residual PAH values observed in the biodegradation tests can be seen as the ultimate values that can be reached by natural attenuation or more probably noticeably below these limits, because severe attrition of coal tars occurred in degradation tests in mechanically stirred reactors. In the case of active (engineered) bioremediation, lower pollution abatement will be likely to be reached, but then residual leaching will proceed at much slower rates, and natural attenuation (postremediation) will be susceptible to prevent significant groundwater contamination. The efficiency of postremediation in relation to pollutant bioavailability has been discussed by Brown et al. (*29*) in the case of contamination by monoaromatic hydrocarbons.

**Ecotoxicological Determinations.** The results of the acute toxicity and genotoxicity tests performed on the soil leachates of untreated and biologically treated soils are presented in Table 4. Concerning untreated soils, the data show that soils SA and M, which had an important leaching capacity, were also the only ones presenting acute toxicity values in leachates higher than the significance limit of 20% light inhibition of the method. Compared to the acute toxicity values reported by Stieber (*24*) for pure PAHs, it appeared that the toxicity values observed in leachates of soil SA (85% of light inhibition) and of soil M (about 50% light inhibition) could be respectively attributed to the presence of naphthalene (5 mg/L) and phenanthrene (about 200 $\mu$g/L). The leachates from all biologically treated soils showed no more acute toxicity. This was in accordance with the PAH concentrations in the latter leachates and also showed the absence of toxic dead-end metabolites of PAH biodegradation (*30*).

Concerning the characterization of genotoxicity, the SOS Chromotest had been chosen because of its extensive validation based on the large data available in the literature (*17, 31*−*35*) of comparative studies with the Ames test. PAHs are progenotoxic compounds, which means that their genotoxicity (indirect genotoxicity) can only be measured in bacterial tests after activation with rat liver microsomal extract (*36*) that contains the enzyme cytochrome P450 (S9 mix). No indirect genotoxicity could be measured in the soil leachates of untreated and biologically treated soils. Indeed, all the responses observed were below 1.5, and as shown in Table 4, the IF value corresponding to the lowest significant benzo[*a*]pyrene concentration (*19*) was 2. This result was not surprising since an IF value of 2 corresponded to 178 $\mu$g/L of benzo[*a*]pyrene, a much higher concentration than those observed in leachates (Table 3). Furthermore, no direct genotoxicity such as observed by Belkin et al. (*30*) in the case of accumulation of PAH metabolites could be detected; the responses observed (Table 4) being also below the lowest significant IF value.

From analytical and ecotoxicological determinations, even though the corresponding PAH concentrations in biologically treated soils could still be substantial, the capacities of these soils to transfer PAHs and PAH-derived toxic metabolites appeared too low to represent a significant threat to aquifers. These conclusions are quite important for the evaluation of natural attenuation, engineered bioremediation, and postremediation in terms of risk assessment.

Recently, Salanitro et al. (*37*) assessed the bioremediation potential and the ecotoxicity of soils contaminated with crude oil hydrocarbons. Their approach was similar to that presented here, but ecotoxicity determinations were, in their case, directed to the soils themselves and not to soil leachates. A general conclusion of their work, quite in line with that of this study, was that residual hydrocarbons were found in soils after biological treatment. However, these hydrocarbons were no longer available for leaching and for biodegradation.

## Acknowledgments

We thank Véronique Bardin (IFP) for skillful participation to PAH analyses, Philippe Quillardet (Institut Pasteur, Paris) for detailed advice on SOS-Chromotest, and Michael Stieber and Dethlef Beethmann (DVGW-TZW) for very useful discussions about soil leaching.

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*Received for review February 19, 1999. Revised manuscript received September 10, 1999. Accepted September 14, 1999.*

ES990203R