Sequential Supercritical Fluid Extraction (SSFE) for Estimating the Availability of High Molecular Weight Polycyclic Aromatic Hydrocarbons in Historically Polluted Soils

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ABSTRACT
Sequential supercritical fluid (CO₂) extraction (SSFE) was applied to eight historically contaminated soils from diverse sources with the aim to elucidate the sorption–desorption behavior of high molecular weight polycyclic aromatic hydrocarbons (PAHs). The method involved five extraction phases applying successively harsher conditions by increasing fluid temperature and density mobilizing target compounds from different soil particle sites. Two groups of soils were identified based on readily desorbing (available) PAH fractions obtained under mildest extraction conditions (e.g., readily desorbing fractions of fluoranthene and pyrene significantly varied between the soils ranging from <10 to >90%). Moreover, extraction behavior strongly correlated with molecular weight revealing decreasing available PAH fractions with increasing weight. Physicochemical soil parameters such as particle size distribution and organic dry mass were found to have no distinct effect on the sorption–desorption behavior of PAHs in the different soils. However, PAH profiles significantly correlated with readily available pollutant fractions; soils with relatively less mobile PAHs had higher proportions of five- and six-ring PAHs and vice versa. Eventually, biodegradability corresponded well with PAH recoveries under the two mildest extraction phases. However, a quantitative relationship was only established for soils with biodegradable PAHs. Out of eight soils, five showed no biodegradation including the four soils with the lowest fraction of readily desorbing PAHs. Only one soil (which was found to be highly toxic to Vibrio fischeri) did not match the overall pattern showing no PAH biodegradability but large fractions of highly mobile PAHs, concluding that mass transfer limitations may only be one of many factors governing biodegradability of PAHs.

Polycyclic aromatic hydrocarbons are ubiquitous environmental pollutants that are predominantly generated during fossil fuel and forest combustion (Edwards, 1983). Sixteen of them are listed on the CERCLA Priority List of Hazardous Substances (Agency for Toxic Substances and Disease Registry, 2001). Some of the high molecular weight congeners are of particular concern due to their mutagenic and carcinogenic properties as well as their recalcitrance in the environment (Shaw and Connell, 1994; Shuttleworth and Cerniglia, 1995).

With natural solids, hydrophobic organic contaminants exhibit strong chemical–physical interactions such as sorption (in)to different classes of soil organic matter as well as diffusion into microvoids, both considerably reducing their mobility and thus their availability to environmental receptors (Brusseau and Rao, 1991; Weber and Huang, 1996; Luthy et al., 1997; Pignatello and Xing, 1996). In addition, with time, these processes become even more pronounced, a phenomenon generally referred to as “aging,” making part of the pollutants at historically contaminated sites inaccessible for bioremediation measures (Luthy et al., 1997; Bosma et al., 1997, Weissenfels et al., 1992). On the other hand, the more strongly pollutants are sequestered by soil or sediment particles, the less toxic the polluted solids are. As a consequence, toxicity prediction (e.g., in risk assessment models using total chemical concentrations from exhaustive extraction of contaminated material) significantly overestimates the harm that is posed to the environment (Alexander, 2000).

Thus, over the last years several methods have been developed with the aim to assess the (bio)available pollutant fraction. The majority of these methods applied either solid phase or liquid extraction using a “mild” organic solvent and related extraction yields to biodegradation rates or body burden data from invertebrates such as *Eisenia fetida* (Cornelissen et al., 1998; Morrison et al., 2000; Tang et al., 1999, 2002; Kelsey et al., 1997; Liste and Alexander, 2002; Breedveld and Karlsen, 2000). Furthermore, cyclodextrin-based aqueous extraction (Cuypers et al., 2002; Reid et al., 2000), persulfate oxidation (Cuypers et al., 2000), and semipermeable membrane devices (Leppänen and Kukkonen, 2000; MacRae and Kenneth, 1998) were suggested for the prediction of organic pollutant (bio)availability.

Supercritical fluid extraction (SFE) using carbon dioxide has recently been suggested for the characterization of the sorption–desorption behavior of organic contaminants in natural solids. Several studies were presented on phenanthrene-spiked sorbents (Weber and Young, 1997; Young and Weber, 1997), polychlorinated biphenyl (PCB)-contaminated sediments (Björklund et al., 1999; Pilorz et al., 1999; Nilsson et al., 2002), and PAH-contaminated soils (Loibner et al., 1997, 1998, 2000; Hawthorne and Grabanski, 2000). The advantage of SFE is that the properties of the fluid and thus its extraction strength can be significantly varied over a wide range by simply changing fluid temperature and density, allowing the assessment of different soil–pollutant interactions by sequential extraction (readily to slowly desorbing sites). Moreover, it was suggested that, compared with conventional solvent extraction, pure carbon dioxide does not notably affect the integrity of soil organic matter during extraction, a major requirement for desorption experiments (Björklund et al., 1999). Eventually, it was argued that models describing SFE behavior of organic compounds may be directly

Abbreviations: PAH, polycyclic aromatic hydrocarbon; (S)SFE, (sequential) supercritical fluid extraction.
related to the models used to elucidate sorption-desorption processes in natural solids, making SFE a valuable tool for investigations of soil–pollutant interactions (Björklund et al., 1999). An in-depth review on the latest developments in SFE for sorption–desorption studies of organic pollutants in soil was published recently (Björklund et al., 2000).

In the present work, for the first time, a variety of PAH-contaminated soils from diverse sources were subjected to SSFE using carbon dioxide as extraction fluid. After a detailed characterization of the sequential extraction procedure, readily desorbing PAH fractions extracted under mild SSFE conditions were related to typical physicochemical soil parameters and PAH profiles, as well as biodegradation behavior of PAHs in all soils.

MATERIALS AND METHODS

Materials

A standard mixture of the 16 USEPA priority PAHs with 10 mg/L of each compound dissolved in acetonitrile for high performance liquid chromatography (HPLC) analysis was obtained from Supelco (Bellefonte, PA). Solvents purchased from J.T. Baker (Deventer, the Netherlands) were at least of “Baker analyzed” grade. Carbon dioxide 5.5 SFC/SFE for extraction and CO₂ 3.0 for cooling were used as received from Messer Austria GmbH (Gumpoldskirchen, Austria). Water for HPLC analysis was obtained from a Millipore Q (Millipore, Billerica, MA) plus PF system with a specific resistivity of 18.3 MΩcm. Extraction thimbles for automated Soxhlet extraction (Ederol) were purchased from Stölzl-Oberglas AG (Vienna, Austria). Anhydrous sodium sulfate obtained from J.T. Baker was heated overnight at 640°C before use.

Contaminated soil samples were received from former manufactured gas plants (ES1, WG1, WG2), an integrated steel making site (BS1), an industrial site contaminated with coal tar during World War II (TA1), and a former railroad sleeper preservation plant (AS1, AS2, AS3a). Soils were sieved to <2 mm and stored at 4°C in the dark before further manipulation. Relevant physicochemical parameters for all soils used in this study are shown in Table 1.

Sequential Supercritical Fluid Extraction

For supercritical fluid extraction, an HP 7680T SFE module was used with an HP 1050 series modifier pump (Hewlett-Packard, Palo Alto, CA). Soil samples (approximately 2 g) were thoroughly mixed with approximately 7 g of anhydrous sodium sulfate completely filling a stainless steel extraction thimble (Hewlett-Packard). Glass fiber filters (Schleicher & Schuell, Dassel, Germany) were placed on both ends of the extraction thimble to avoid clogging by small soil particles. The flow rate of the extraction fluid (CO₂) was set to 2.5 mL. Analytes were trapped on C18 sorbent material (Grace Vydac, Hesperia, CA) after the fluid was expanded through the nozzle. During extraction, the nozzle temperature was set to 5°C above the respective extraction chamber temperature, whereas the trap was held constant at 10°C except for the last extraction phase (68°C) to avoid clogging from liquid methanol used as modifier. For rinsing analytes from the trap, 1.5 mL THF and acetonitrile 1:1 (v/v) was used. During rinsing (1 min), nozzle and trap temperatures were set to 50 and 35°C, respectively.

The sequential supercritical fluid extraction involved five successive extraction phases with increasing extraction strength (from “very mild” to “very harsh”) by subsequently enhancing temperature and density of the fluid (Phase I, 120°C; Phase II, 120°C; Phase III, 180°C; Phase IV, 120°C; and Phase V, 120°C plus 5% v/v methanol as modifier). Except for the last, all phases were subdivided into four fractions collected after 6, 12, 24, and 48 min of extraction providing time-dependent desorption kinetics at each phase. However, the final “very harsh” extraction phase (45 min) was applied to exhaustively extract PAHs from soil samples. Before each of the 17 extraction fractions, the system was allowed to equilibrate for 5 min.

Biodegradation Experiments

For aerobic biodegradation experiments, a PAH-degrading consortium was enriched at 20°C from a source comprising diverse PAH-contaminated soils, activated sludge, and compost residues. The medium used for cultivating consisted of 1 g/L K₂HPO₄, 0.5 g/L KH₂PO₄, 0.5 g/L NaNO₃, 0.1 g/L MgSO₄·7H₂O, and 0.1 g/L NaCl with 1 ml/L of a trace element solution containing 0.5 g/L H₃BO₃, 0.04 g/L CuSO₄, 0.1 g/L KI, 0.2 g/L anhydrous FeCl₃, 0.1 g/L (NH₄)₂MoO₄·4H₂O, and 0.4 g/L ZnSO₄·7H₂O. This medium was amended with 130 mg/L of anthracene oil (voestalpine Stahl; Linz, Austria). Every second week, 1 mL of the microbial suspension was centrifuged, washed, and resuspended in fresh mineral medium containing 15% glycerol, yielding a final concentration of 1.22 × 10⁹ colony forming units (cfu)/mL (working cell bank). Before further manipulation, PAH degraders were stored in cryo-vials at −80°C.

Table 1. Relevant physicochemical soil parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ES1</th>
<th>BS1</th>
<th>WG1</th>
<th>WG2</th>
<th>TA1</th>
<th>AS1</th>
<th>AS2</th>
<th>AS3a</th>
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<tr>
<td>DM†, %</td>
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<td>76</td>
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<td>ODM*, %</td>
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<td>59</td>
<td>15</td>
<td>59</td>
<td>84</td>
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<td>80</td>
<td>87</td>
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<td>Sand, %</td>
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<tr>
<td>Clay, %</td>
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<td>0.82</td>
<td>0.74</td>
<td>0.63</td>
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<td>7.9</td>
<td>7.6</td>
<td>8.5</td>
<td>7.7</td>
<td>7.1</td>
<td>7.0</td>
<td>7.3</td>
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<td>7.7</td>
<td>7.1</td>
<td>7.0</td>
<td>7.3</td>
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<td>ΣPAH¶, mg/kg dry mass</td>
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<td>462</td>
<td>4429</td>
<td>387</td>
<td>245</td>
<td>623</td>
<td>2089</td>
<td>676</td>
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</tbody>
</table>

† Dry mass.
‡ Organic dry mass.
§ Maximum water holding capacity.
¶ Sum of polycyclic aromatic hydrocarbons (PAHs) including acenaphthene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, phenanthrene, and pyrene.
All soils used in biodegradation experiments were inoculated with this mixed culture to yield a final concentration of 4 × 10^6 cfu/g dry mass and adjusted to 65% of their maximum water holding capacity. The water content was controlled twice a week and adjusted accordingly; added water was evenly distributed using as stainless steel spatula, which also provided a homogeneous system and allowed a regular introduction of oxygen to avoid anaerobic conditions. Except for soils AS1 and AS2, all soils were amended with NH_4NO_3 and KH_2PO_4 to yield 2.5 g/kg dry mass N and 0.5 g/kg dry mass P. After two months of incubation at 20°C in the dark, soils were extracted and analyzed for remaining PAHs. Abiotic controls with 2% (w/w dry mass) sodium azide were included for each soil and used for the calculation of biodegraded PAHs. All experiments were performed in triplicate.

**Analysis**

For exhaustive extraction of PAHs from soil, an automated Soxhlet (Soxtherm extractor Model 2000 automatic; Gerhardt, Bonn, Germany) was used. Samples of 2.5 to 5 g (dry mass) were extracted in triplicate using ethyl acetate as extraction solvent. Eventually, the obtained crude extracts were directly diluted up to 20% (v/v) in acetonitrile. A detailed description and evaluation of this method was presented previously (Szolar et al., 2002).

All extracts were analyzed using an HP 1050 series HPLC (Hewlett-Packard) interfaced to an HP 1100 series three-dimensional fluorescence detector (Hewlett-Packard). An HP 1050 autosampler (Hewlett-Packard) was used for the injection of sample aliquots and calibration standards (20 μL) onto an ODS Hypersil guard column (20 × 4 mm, particle size 5 μm; Hewlett-Packard) followed by a C-18 separation column (250 × 4.6 mm, particle size 5 μm; Grace Vydac). The guard column was replaced routinely approximately every 250 injections to prevent potential clogging of the separation column. The flow rate was set to 1.5 mL/min. The temperature of the column was 26°C. A water–acetonitrile gradient was used as mobile phase starting with 60% acetonitrile for 2.5 min; a linear gradient (9.5 min) to 90% acetonitrile and an 8-min linear gradient to 100% acetonitrile (held for 2.5 min) was followed by a 2.5-min linear gradient back to starting conditions. Eventually, a 5-min pre-run was employed to equilibrate the separation column before each subsequent run. The three-dimensional fluorescence detector enabled a quasi-simultaneous recording of four wavelength traces. Excitation was set to 260 nm and emissions to 350, 420, 440, and 500 nm, taking (after 6, 12, 24, and 48 min) in each of Phases I to IV into account the various fluorescence properties of the individual PAHs. For quantification of PAHs, a multilevel calibration was performed ranging from 10 to 800 μg/L. For quality control, a 100 μg/L standard was analyzed every 15 samples and the system was recalibrated after about 60 to 70 samples. In this study, we analyzed the PAHs acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene.

For toxicity testing, aqueous soil elutriates were prepared according to Hund and Traunspurger (1994). The bioassay (NRRL-B-11177, LUMIStox luminescent bacteria test; Dr. Lange, Düsseldorf, Germany) was performed using *Vibrio fischeri* as test organism. Soil eluates were amended with 2% (w/w) NaCl. Luminescence was measured before and after 30 min of incubation (bacterial solution and elutrate 1:1 v/v) at 15°C (LUMIStherm LTG 053; Dr. Lange) using the LUMIStox luminometer (LPG 259; Dr. Lange). A 7.5% NaCl solution was included as positive control in each test. Results are presented as percent bioluminescence compared with a 2% NaCl solution (negative control).

Heavy metals were measured using inductively coupled plasma–atomic emission spectroscopy (ICP–AES) (JY 50 P; Jobin-Yvon, Edison, NJ) according to DIN 38406-E22 (Deutsches Institut für Normung, 1988) and screening for organic semivolatiles was accomplished using gas chromatography–mass spectrometry (GC–MS) with a method described previously (Szolar et al., 2002). However, instead of single ion monitoring (SIM), scan mode was used to identify unknown compounds and the oven temperature program was slightly modified to 50°C for 2 min followed by a 10°C/min ramp to 150°C (1-min hold) and completed with a ramp of 18°C/min to a final temperature of 320°C (15-min hold). Particle size distribution was determined according to ÖNORM L 1061 (Österreichisches Normungsinstitut, 1988).

### RESULTS AND DISCUSSION

#### Performance Assessment of the Sequential Supercritical Fluid Extraction

To evaluate the performance of the supercritical fluid extraction, triplicate subsamples of soil WG1 were subjected to the sequential extraction process comprising all five extraction phases (different conditions) with a total of 17 fractions. Summing up the four fractions (after 6, 12, 24, and 48 min) in each of Phases I to IV resulted in relative standard deviations (RSDs) of less than 10% for all PAHs investigated in this study (Table 2). The summation of all 17 extraction fractions (“total” recovery) resulted in even lower variations. Deviations of the final extraction Phase V (Fraction 17) were found to be approximately 20% (Table 2). These significantly higher RSDs are assumed to be due to the relatively low relative extraction yields for most of the PAHs in this final exhaustive extraction phase, a phenomenon that was also observed for individual extraction fractions in some of the other phases. However, since high RSDs were only noticed in the case of low PAH concentrations, no appreciable impact on the overall extraction performance was detected. Actually, these errors were counterbalanced when relative extraction yields were calculated cumulatively as shown above. Furthermore, the SSFE (sum of all fractions) was compared with Soxhlet with respect to extraction efficiencies.
for all PAHs and soils investigated in this study (Table 3). Supercritical fluid extraction yields for individual four- to six-ring PAHs ranged from 70 to 128% of the Soxhlet values, proving the capability of the sequential extraction method to exhaustively extract target analytes from soil.

### Polycyclic Aromatic Hydrocarbon Availability in Historically Contaminated Soils

To evaluate the availability and mobility of high molecular weight PAHs, eight historically contaminated soil samples from different sources were subjected to SSFE employing successively harsher extraction conditions. Figure 1 shows the extraction behavior of four- to six-ring PAHs for all soils calculated as relative yields for the different extraction phases. Phase I was assumed to remove the most readily desorbing fraction, whereas PAHs recovered under harsher extraction conditions (Phases II–V) were supposed to be gradually less mobile (slowly desorbing). In general, soils ES1 and BS1 revealed the lowest Phase I extraction yields for all PAHs closely followed by soils WG1 and WG2. Significantly higher Phase I yields, however, were observed for soils TA1, AS1, AS2, and AS3a with relative extraction yields for fluoranthene and pyrene (for example) of >73 and >67%, respectively, compared with only 10% for the same PAHs in soil ES1. Six-ring PAHs were not detectable in the majority of soils in Phases I and II; however, the same mobility pattern, only shifted toward harsher phases, was observed as outlined above [see benzo(ghi)perylen e; Fig. 1].

Relative Phase I extraction yield for the diverse PAHs in all soils highly correlated with their molecular weight, with lighter PAHs exhibiting increased extractability. This phenomenon may predominantly be attributed to lower diffusion coefficients and/or lower solubilities for compounds with higher molecular weight. However, in the case of general solubility limitations, soils with the highest PAH concentration would be expected to show lowest relative Phase 1 extractabilities (Björklund et al., 1999). Such a distinct trend could not be observed since soils with low concentrations (e.g., BS1; Table 1) also exhibited low relative extraction yields under mildest conditions and vice versa (e.g., AS2; Table 1), strengthening the hypothesis of diffusion rather than solubility limitations being the governing mechanisms in the sequential PAH extraction process. Correlation coefficients ($r^2$) of linear regressions between molecular weight and decadic logarithm of relative Phase I extraction yield computed for all soils were >0.96. Figure 2 shows the relation between molecular weight and extractability under mildest conditions (readily desorbing fraction) exemplarily for soils ES1 and AS3a.

Furthermore, heavier PAHs were found to exhibit higher relative extraction yields in the last phase employing methanol as modifier. Considerable final phase recoveries of up to 32% for indeno(1,2,3-cd)pyrene and 26% for benzo(ghi)perylen e (Fig. 1) were noticed for soil ES1, leading to the assumption that large amounts of these heavy, hydrophobic PAHs were strongly sequestered by soil organic matter. However, modifying supercritical $CO_2$ with a polar cosolvent was supposed to soften the organic matrix and eventually release this fraction, which was suggested previously (Luthy et al., 1997).

As observed by Björklund et al. (1999) for PCBs and Hawthorne and Grabanski (2000) for PAHs, desorption profiles for PAHs investigated in this study followed a typical time-dependent extraction behavior in each phase revealing successively decreasing extraction rates with time (biphasic). Thus, a total of 90 min of extraction per phase was found to be sufficient to quantitatively recover potentially extractable PAHs under corresponding conditions.

### Influence of Physicochemical Soil Parameters on Sequential Supercritical Fluid Extractability

The relationship between typical physicochemical soil parameters as shown in Table 1 and the extraction behavior of PAHs in the different soils was investigated. Particular focus was given on particle size distribution and soil organic matter (SOM) content. Especially the latter has been reported to significantly influence the desorption behavior of apolar organic contaminants by
Fig. 1. Relative extraction yields of four- to six-ring polycyclic aromatic hydrocarbons (PAHs) in the various extraction phases in percent of the total concentration (sum of all phases). Harsher extraction conditions are indicated by successively darker stacks (Phases I–V). The term * indicates that benzo(ghi)perylene was not detectable in soil TAI.
reducing their availability and mobility due to slow diffusion processes (Weber and Huang, 1996; Luty et al., 1997; Pignatello and Xing, 1996; Weissenfels et al., 1992; Cornelissen et al., 1998; Weber and Young, 1997; Karickhoff et al., 1979). Soils BS1 and ES1, which exhibited the lowest relative Phase I extraction yields, also showed the highest organic dry mass content with 33 and 12%, respectively. On the other extreme, soil TA1 with only 1.6% organic dry mass was among the soils with the highest relative extraction yields under mildest conditions supporting the hypothesis of soil organic matter significantly governing sorption–desorption processes. For the remaining soils, however, no correlation between organic dry mass and mild extractability could be established concluding that slow sorption may not simply be controlled by one single parameter especially in complex matrices such as soil.

With respect to particle size fractions, Krauss and Wilcke (2002) recently reported that PAHs were predominantly located in silt and clay. These fractions also exhibited the highest $K_{OC}$ (soil organic carbon–water partition coefficient) values, indicating low availability. However, no such trend was observed in the current study. On the contrary, soils with the highest silt content (AS soils) were among those with the highest relative Phase I extraction yields and vice-versa (Table 1). Similar findings were reported previously (Björklund et al., 1999), confirming the above assumption of more than one factor being responsible for diffusion limitations in soil.

**Relating Polycyclic Aromatic Hydrocarbon Profiles to Sequential Supercritical Fluid Extractability**

The analysis of three- to six-ring PAHs revealed appreciable differences in the distribution of these pollutants in the diverse soils investigated in the current study. This may have been a consequence of the different contamination sources; however, those soils that were associated with a higher fraction of rapidly desorbing PAHs (AS1, AS2, AS3a, and TA1) showed a significantly lower percentage of five- and six-ring PAHs (<5.5%) compared with soils ES1, BS1, WG1, and WG2 (Fig. 3). The latter soils, which exhibited comparably lower relative Phase I extraction yields, revealed considerable proportions of five- and six-ring PAHs ranging from 25 to 38% for soils ES1 and BS1, respectively.

Since five- and six-ring PAHs are known to be more recalcitrant (less degradable) and less mobile (strongly sequestered by soil particles) compared with lower-ring PAHs, their accumulation in soils such as ES1, BS1, WG1, and WG2 may indicate advanced weathering of these soils during several years or decades preferably affecting lower molecular weight PAHs (Luty et al., 1997).

Thus, the profile of PAHs in soils may be used as a fast indicator for the degree of organic pollutant availability, an important requirement for the definition of site-specific soil quality criteria and remediation cleanup goals.

**Relating Sequential Supercritical Fluid Extractability to Biodegradability**

Eventually, all soils in this study were used in biodegradation experiments. Figure 4 shows the behavior of selected four- and five-ring PAHs after 64 d of incubation using a PAH degrading consortium enriched on anthracene oil. Within this time, significant reduction of most PAHs was observed for soils AS1, AS2, and AS3a, which also revealed comparably high fractions of readily available PAHs (Fig. 1). As already shown for relative Phase I extraction yields obtained with SSFE (Fig. 2), biodegradation followed the same pattern, with molecular weight governing the extent of biodegradation. Fluoranthene and pyrene (molecular weight = 202) were degraded to a residual concentration of 4 to 27% compared with the abiotic control, followed by benz(a)anthracene and chrysene (molecular weight = 228), which were down to 14 to 42% after two months of
Fig. 4. Polycyclic aromatic hydrocarbon (PAHs) remaining in soil (in percent of an abiotic control) after 64 d of incubation with a PAH-degrading consortium. Error bars are standard errors of the mean \((n = 3)\).

On the other hand, soils ES1, BS1, WG1, and WG2 with a notably lower percentage of readily desorbing PAHs exhibited no significant biodegradation for any of the PAHs (Fig. 4). For these soils, even the three-ring PAHs were not degraded by microorganisms within 60 d of incubation. However, most surprisingly, the same behavior was observed for soil TA1, which was among the soils with the highest fractions of readily desorbing PAHs (Fig. 1). Thus, soil TA1 was screened for heavy metals and organic semivolatile other than PAHs possibly inhibiting the metabolism of (added) PAH-degrading microorganisms; however, the analysis revealed only low contamination with heavy metals (Table 4), which were not assumed to affect the soil microflora since concentrations in soil of orders of magnitude higher than those in TA1 were reported to have no effect on PAH-degrading microorganisms (Baldrian et al., 2000; Smreczak et al., 1999). Furthermore, gas chromatography–mass spectrometry analysis of ethyl acetate extracts of soil TA1 identified no appreciable amounts of semivolatile other than PAHs. However, since chemical analysis is not capable of identifying “all” potential toxicants, soils
were also tested for elutriate toxicity using the marine microorganism *Vibrio fischeri*. With this bioassay, soil TA1 revealed the highest toxicity with a bioluminescence inhibition of 91%, whereas inhibitions in the other soils ranged from 12 to 73%. This extremely high toxicity is assumed, at least in part, to be responsible for the lack of PAH biodegradation in soil TA1, and especially recolonization by the augmented microbial consortium may have been dramatically hampered.

As a consequence, for soils ES1, BS1, WG1, WG2, and TA1, no quantitative relationship between extraction and biodegradation was attempted. Similar phenomena were observed by Scow et al. (1995) who found no correlation between extent and rate of biodegradation and the sorption behavior of phenanthrene in six soils.

**CONCLUSIONS**

These findings confirm the perception that at least in some cases, mass transfer may only be one of many parameters controlling the biodegradation of PAHs in soil (Shuttleworth and Cerniglia, 1995; Cornelissen et al., 1998; Cuypers et al., 2000). It is concluded that pollutant availability is a major requirement, but not necessarily a guarantee for the biodegradation of organic pollutants such as PAHs. Factors related to uptake and metabolism of contaminants by microorganisms have been reported to significantly influence the biodegradation performance; toxic compounds, inhibitory effects from soil constituents or other substrates, and cometabolism effects may adversely affect the intrinsic microbial activity and, therefore, degradation of target compounds (Shuttleworth and Cerniglia, 1995; Cuypers et al., 2000; Dean-Ross et al., 2002). Thus, for the prediction of bioremediation of PAH-contaminated soils, the authors support a two-step evaluation process suggested previously (Cuypers et al., 2000), which involves a fast screening of the availability and mobility of pollutants using SSFE followed by biodegradation experiments on those soils that exhibit significant pollutant availability.

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