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Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity

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Summary. Polycyclic aromatic hydrocarbon (PAH) biodegradation was investigated in contaminated soils from two different industrial sites under simulated land treatment conditions. Soil samples from a former impregnation plant (soil A) showed high degradation rates of PAHs by the autochthonous microorganisms, whereas PAHs in material of a closed-down coking plant (soil B) were not degraded even after inoculation with bacteria known to effectively degrade PAHs. As rapid PAH biodegradation in soil B was observed after PAHs were extracted and restored into the extracted soil material, the kind of PAH binding in soil B appears to completely prevent biodegradation. Sorption of PAHs onto extracted material of soil B follows a two-phase process (fast and slow); the latter is discussed in terms of migration of PAHs into soil organic matter, representing less accessible sites within the soil matrix. Such sorbed PAHs are suggested to be non-bioavailable and thus non-biodegradable. By eluting soil B with water, no biotoxicity, assayed as inhibition of bioluminescence, was detected in the aqueous phase. When treating soil A analogously, a distinct toxicity was observed, which was reduced relative to the amount of activated carbon added to the soil material. The data suggest that sorption of organic pollutants onto soil organic matter significantly affects biodegradability as well as biotoxicity.

Introduction

The fate of polycyclic aromatic hydrocarbons (PAHs) in nature is of great environmental concern due to their toxic, mutagenic, and carcinogenic properties (LaFlamme and Hite 1978; Pahlmann and Pelkonen 1987; White 1986). Sites of closed-down coking plants and gas works are frequently contaminated with these compounds as PAHs are produced by pyrolysis of organic-carbon-based material (Blumer and Youngblood 1975; Sims and Overcash 1983).

Although PAHs may undergo chemical oxidation, photolysis, and volatilization, microbial degradation is the major process affecting the persistence of PAHs in nature (Callahan et al. 1979). At present, many microorganisms are known to have the enzymatic capacity to oxidize PAHs that range in size from naphthalene to benzo(a)pyrene (Cerniglia 1984; Gibson and Subramanian 1984; Weissenfels et al. 1990a). Given the requisite environmental conditions, microbial communities are able to readily degrade these chemicals (Morgan and Watkinson 1989; Mueller et al. 1989). Consequently, application of microorganisms for the remediation of contaminated soils has gained considerable interest in recent years (Bewley et al. 1989; Brown et al. 1985).

Experience gained from studies on biological soil remediation have shown that biodegradation may be inhibited by abiotic factors (Weissenfels et al. 1990b). It has been suggested, that one factor causing reduced biodegradability of compounds is sorption on soil particles and organic matter (Martin et al. 1978; Ogram et al. 1985). On the other hand it has been demonstrated that sorption onto activated carbon almost completely prevents dermal uptake and the toxic effects of dioxins in rats (Poiger and Schlatter 1980). Thus, bioavailability of soil-sorbed contaminants may have a bearing on the effectiveness of microbial degradation as well as on the assessment of toxicological risks. Since sufficient understanding of these phenomena concerning soil-remediation processes is lacking, the purpose of this study was to determine the soil characteristics that prevent PAH biodegradation and to investigate the correlation of biodegradability and biotoxicity of sorbed PAHs.

Materials and methods

Chemicals. Nutrient broth medium and bacto agar (Difco) were purchased from Nordwald (Hamburg, FRG) and solvents from Riedle de Haen (Hannover, FRG). Anthracene oil, a liquid distillation product of coal tar containing about 600 mg PAHs/ml, was

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a gift from Rütgerswerke (Castrop Rauxel, FRG). The major components of anthracene oil are acenaphthene (47 mg/ml), fluorene (72 mg/ml), phenanthrene (204 mg/ml), fluoranthene (114 mg/ml), and pyrene (59 mg/ml). All other chemicals and reagents were commercial products of the highest purity available.

Bacteria. The mineral salt medium (MSM) used as basal medium for degradation studies contained per litre deionized water: 1 g K_2HPO_4, 1 g NH_4NO_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.1 g NaCl, 0.01 g $FeCl_3 \cdot 6H_2O$, and 1 ml trace element solution (Pfennig and Lippert 1966) and was adjusted to pH 7.2 by adding 1 M HCl.

Besides degradation studies concerning the autochthonus microorganisms of the contaminated soil samples, investigations were carried out by inoculating test media with a bacterial mixed culture able to degrade several PAHs. This mixed culture, called M1, was enriched on anthracene oil as the sole source of carbon (Walter et al. 1990) and kindly supplied by DMT-Gesellschaft für Forschung und Prüfung (Essen, FRG).

The quantification of microorganisms in soil was carried out by plate counts on different media. To extract the microbes, a 50-g soil sample was briefly homogenized by manual break up of clumps and 5-g portions were added to 50 ml Na-pyrophosphate solution (0.28%, w/v). After mixing on a rotary shaker (100 rpm) for 1 h the suspension was filtered (paper filter, pore size 7.4 µm) and used for enumeration. In order to estimate the number of microorganisms overall, several dilutions of the filtrate were plated on nutrient broth (NB) agar and colonies counted after 3 days of incubation at 30° C. Microorganisms able to degrade salicylic acid and naphthalene were identified by the use of selective media. Serial dilutions (1:10) of 25 µl filtrate were performed with autoclaved MSM in microtitre plates with five replicates at each dilution. Salicylic acid was added sterile to the MSM prior to dilution to give final concentrations of 5 mM, respectively; naphthalene was supplied in the vapour phase by adding crystals in six empty cavities. The microtitre plates were closed tightly using adhesive film (parafilm) and incubated for 7 days at 30° C. Growth was estimated visually by comparing turbidity with microtitre plates incubated without substrate. The number of microorganisms was calculated as described by Clark and Owens (1983).

The activity of soil microbial populations was determined as the CO_2 production rate during incubation of soils at 30° C. The water content of the soil samples was adjusted to 10% (w/w) prior to incubation. The CO_2 produced was continuously removed by a stirred solution of $BaOH_2$ (0.2 M). After incubation was stopped, the residual $BaOH_2$ was titrated with HCl and the overall amount of CO_2 produced calculated. Autoclaved soil samples treated identically served as controls to determine the abiotic release of CO_2 from the soils.

Photobacterium phosphoreum NRRL B-11177, identified as *Vibrio fisheri* according to Bergey's Manual of Systematic Bacteriology (Krieg and Holt 1984), was used for the microtox assay and was obtained from Microbics (München, FRG).

Soil sampling. Contaminated material of two different sites was investigated: Soil A was a predominantly sandy soil from a former wood impregnation plant with a contamination of about 1.8 g PAH per kg soil; Soil B consisted of heterogeneous soil material from a former tar oil refinery with a contamination of about 1.0 g PAH per kg soil.

Degradation studies. The degradation potential of the original soil samples was tested using the percolator equipment of Codner (1969). The pneumatically operated percolator was filled with a homogeneous mixture of 200 g soil sample and 100 g rashig rings. The soil column was trickled with MSM at room temperature. After 2 months of incubation the soil column was quantitatively removed, extracted and the PAH content determined.

Shake-flask studies were performed to investigate the degradation of contaminants added to extracted PAH-free soil material. For that, 150 g of original contaminated soil was extracted with 300 ml toluene for 5 h in a soxhlet apparatus. The PAH-containing organic extract was retrieved into the extracted soil and toluene was removed by evaporation in vacuo at 40° C. After thorough mixing, 20 g fresh contaminated soil material was placed in 500-ml erlenmeyer flasks and suspended in 100 ml MSM.

Microbial inoculum was prepared by growing the PAH-degrading mixed culture M1 on anthracene oil as the sole source of carbon. Cells of the logarithmic growth phase (optical density at 578 nm = 0.6) were harvested by centrifugation at 10000 *g* for 10 min and washed with MSM to prevent residual concentrations of anthracene oil being carried over into the test media by inoculation.

Each flask was inoculated with 10 ml bacterial suspension (10%, v/v) and incubated tightly closed on a rotary shaker (100 rpm, 30° C). After 1, 7, 14, 28, and 42 days of incubation, respectively, the entire contents of each flask was separately extracted and analysed for the presence of PAHs. Flasks containing suspended soil samples sublimated with $HgCl_2$ (0.1%, v/v) served as sterile controls for each sampling point to determine abiotic losses of PAHs during incubation.

The influence of sorption on microbial PAH degradation was investigated by the use of XAD 2 (amberlite resin for PAH adsorption), extracted soil material, and sand as substrates with different PAH-sorption capacities. In 500-ml erlenmeyer flasks, 20 g of the different sorptive substrates were suspended in 100 ml MSM, respectively. After adding 50 µl anthracene oil (about 30 mg PAH, corresponding to a contamination of about 1.5 g PAH/kg substrate) the flasks were inoculated and incubated as described above. PAH analysis was performed following soxhlet extraction. The percentage of oil degradation was calculated, setting the content of PAHs extracted from sterile probes with the respective sorptive substrate as 100%.

Sorption studies. To characterize sorption of PAHs onto sediments, the extraction yields of anthracene oil after incubation with unpolluted soil material were estimated. Soil was freed from PAHs by soxhlet extraction with toluene and suspended with deionized water in the proportions of 10 g to 50 ml. Contamination of the soil suspension with 50 µl anthracene oil was followed by sterilization of the samples using $HgCl_2$ (0.1%, w/v). Sorption was investigated for six periods of incubation at 30° C and 100 rpm. For each incubation time the entire contents of two flasks were extracted three times with 10-ml volumes of toluene by shaking flasks thoroughly at room temperature. The organic extracts were concentrated to a final volume of 1 ml by evaporation under a gentle stream of nitrogen. Calculation of the extracted amount of anthracene oil followed gas chromatographic (GC) analysis of the organic extracts.

Microtox assay. Aqueous eluates of soil samples were used to determine the toxicity of contaminants, to be rinsed out of soils by water. The eluates were prepared according to the shaking test Deutsches Einheitsverfahren (DEV)-S4, Deutsche Industrienorm (DIN) 38414 part 4 (1984) with slight modifications: 20 g air dried soil sample was eluted with 100 ml deionized water on an overhead shaker for 24 h. The eluate was filtered (paper filter, pore size 7.4 µm) and cleared by centrifugation at 10000 *g* for 10 min.

The toxicity of the eluates was determined as the inhibitory effect on light emission of *P. phosphoreum* NRLL B-11177, according to the German standard method for the examination of water, waste water and sludge DIN 38412 part 34 (1991). This test was carried out with a Biocounter toxicity autoanalyser (mod. M 2500, Lumac, Rodgau, FRG) according to the manufacturer's specification.

The influence of the amount of soil organic carbon on the biotoxicity of soil eluates was investigated by intermixing increasing amounts of activated carbon D-45/2 into soil A. As usual, a contact time of 24 h was selected to obtain equilibrium by stirring samples at room temperature (Suffet and McGuire 1980). Aqueous eluates and the microtox assay were performed as described above.

Analytical procedures. PAH analysis of soil suspensions (see degradation studies) were performed after centrifugation at 10000 *g* for 10 min. The air-dried solid was weighed into a soxhlet thimble that had been previously rinsed with toluene. The sample was extracted for 4 h with 150 ml toluene on a Soxtec extractor unit (Tecator, Höganäs, Sweden). The extracts were concentrated under a gentle stream of nitrogen and passed through a silica gel column (Merck, Darmstadt, FRG), deactivated with 15% (w/w) deionized water. The cleaned extract was taken down to dryness in a gentle stream of nitrogen and made up to the required volume with acetonitrile prior to analysis by HPLC. The PAHs were separated on a LiChrosorb RP 18 column 5 µm in diameter, 250 mm × 4.6 mm (Merck, Darmstadt, FRG) with a 40:60 acetonitrile/water – 100% acetonitrile gradient, run over 30 min at a flow rate of 1 ml/min using a liquid chromatograph HP 1090 A with autosampler and photodiode array detector (Hewlett Packard, Waldbronn, FRG). PAH analysis of extracts, performed by shaking soil suspensions with toluene at room temperature (see sorption studies), was carried out by GC (Varian mod. 3400, Darmstadt, FRG) using a Chrompack Sil 5 capillary column (25 m × 0.22 mm) and a flame ionization detector at the following oven programme: 60° C for 3 min, 10° C/min to 275° C, which was held for 5 min.

The quantification of dissolved organic carbon (DOC) was carried out after centrifugation of the samples at 10000 *g* for 10 min using a DOC-analyser (Shimadzu mod. TOC 500, Kyoto, Japan).

The soil organic carbon content was calculated as the difference between total carbon content and soil inorganic content. Total soil carbon content was determined as CO_2 release during ignition of soil above 1000° C (Perkin Elmer, CHN elemental analyser, Überlingen, FRG). Soil inorganic carbon content was estimated as the amount of carbonates released as CO_2 during incubation of soil samples with H_2SO_4.

Results

Biodegradation of PAH in soils

Contaminated material of two different sites was investigated, characterized by a different composition of polycyclic aromatic hydrocarbons (PAHs). Soil A was from a former wood impregnation plant and of sandy nature; soil B consisted of heterogeneous material obtained from a former tar-oil refinery. Comparing the concentrations of individual PAHs, soil A contained higher amounts of low molecular mass PAHs whereas in soil B high molecular mass PAHs occurred in a greater proportion (Table 1).

Table 1. Polycyclic aromatic hydrocarbon (PAH) analysis of contaminated soil samples

PAH	Soil A (mg/kg soil)	Soil B (mg/kg soil)
Naphthalene	4.2	16.7
Fluorene	82.4	5.2
Phenanthrene	242.0	62.7
Anthracene	90.4	22.4
Fluoranthene	540.0	160.0
Pyrene	458.0	123.0
Benz(a)anthracene	105.8	95.7
Chrysene	68.4	58.1
Benz(e)pyrene	48.6	64.4
Benz(b)fluoranthene	59.6	86.1
Benz(k)fluoranthene	41.0	48.5
Benz(a)pyrene	44.0	98.3
Dibenz(ah)anthracene	1.1	26.2
Benzo(ghi)perylene	16.6	66.1
Indenoyprene	13.0	94.1
Total amount of PAH	1815.1	1027.5

After 8 weeks of incubation in a percolator PAH degradation to an overall average of 62% occurred in soil A with the autochthonous microbial population (Fig. 1a). Highest degradation rates were achieved for pheanthrene, fluorene, fluoranthene, and pyrene while dibenz(ah)anthracene and benz(a)pyrene were degraded much more slowly. By treating soil B analogously, no significant degradation of contaminants was obtained even after inoculation with the bacterial mixed culture M1, which was demonstrated to degrade PAHs effectively (Fig. 1b).

Microbiological characterization of the soil samples led to the detection of numerous bacteria with a considerable activity concerning soil A (Table 2). Additionally, in soil A the amount of bacteria able to degrade aromatic compounds was found to be significantly increased, whereas only a sparse bacterial population with low activities of near basal respiration was found in soil B. Nevertheless, as shown by the occurrence of viable autochthonous microorganisms, biodegradation of PAHs in soil B was not inhibited by toxic effects. Experiments were undertaken to determine the princi-

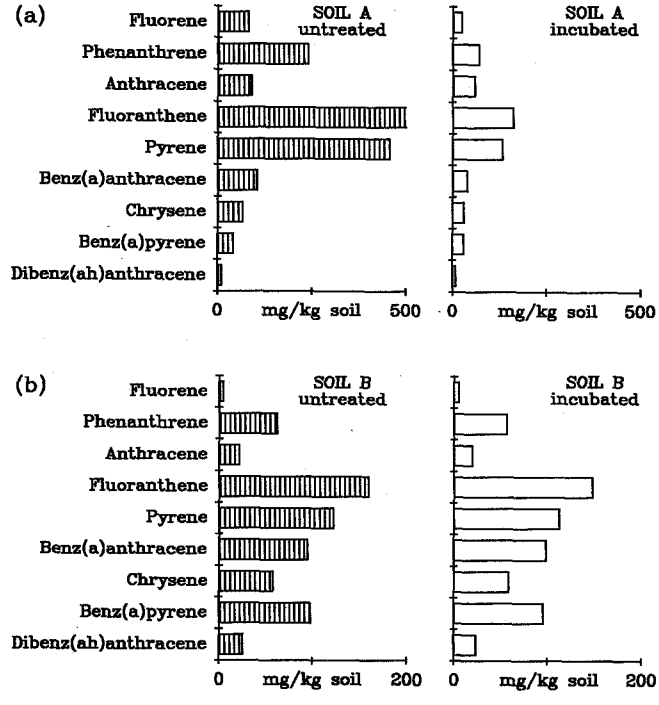

Fig. 1a, b. Polycyclic aromatic hydrocarbon (PAH) degradation in soils from different contaminated sites after 8 weeks of incubation in a percolator. Conditions: 200 g soil material were intermixed with 100 g rashig rings and trickled with mineral salt medium (MSM), pH 7.2, by four circulations per minute at room temperature. **a** Uninoculated sample of soil A. **b** Sample of soil B, incubated after inoculation with the PAH-degrading mixed culture M1

Table 2. Microbiological characterization of contaminated soils from different sites

Parameter	Soil A	Soil B
Material	Subsurface sand	Heterogeneous pouring: rubble, stones, loam
Contamination (mg PAH/kg soil)	1815.2	1027.5
Site of sampling	Impregnation plant	Tar-oil refinery
PAH biodegradability	Yes	No
Colony forming units (on NB per g soil)	$2.5 \cdot 10^7$	$2.2 \cdot 10^6$
Salicylic-acid-degrading bacteria (per g soil)	$3.5 \cdot 10^5$	$9.0 \cdot 10^3$
Naphthalene-degrading bacteria (per g soil)	$1.3 \cdot 10^5$	$9.0 \cdot 10^2$
CO_2 production rate (mg CO_2/kg soil · h)	21.7	2.6

NB, nutrient broth

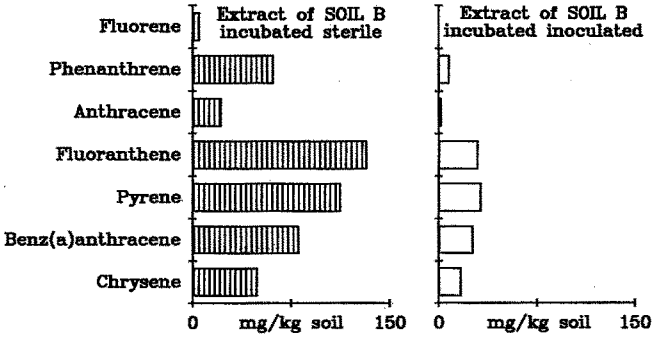

Fig. 2. Microbial degradation of sand-sorbed PAHs extracted from soil B. Conditions: 20 g sand were contaminated with organics extracted from 20 g soil B, and incubated for 4 weeks in 500-ml erlenmeyer flasks at 30° C and 100 rpm, after suspension in 100 ml MSM, pH 7.2, and inoculation with the PAH-degrading mixed culture M1

ple biodegradability of the contaminants present in soil B. After soxhlet extraction with toluene, sand was contaminated with organic compounds of soil B. Incubation of the contaminated sandy material led to microbial PAH degradation to an average of 80%, concerning those PAHs that serve as substrates for the mixed culture M1 used as inoculum (Fig. 2). In sterile control flasks no abiotic PAH degradation was observed.

The results imply that the inhibition of PAH biodegradation is due to a kind of PAH binding within the material of soil B. To verify the experimental data, the organic contaminants of soil B were extracted and retrieved onto the extracted soil material. Immediately incubation of the short-term contaminated material with the PAH-degrading mixed culture M1 yielded a considerable decrease in PAH concentration (Fig. 3). However, biodegradation was by no means complete and resulted in a residual PAH fraction of about 28%. This residual fraction was proved to be as undegradable as

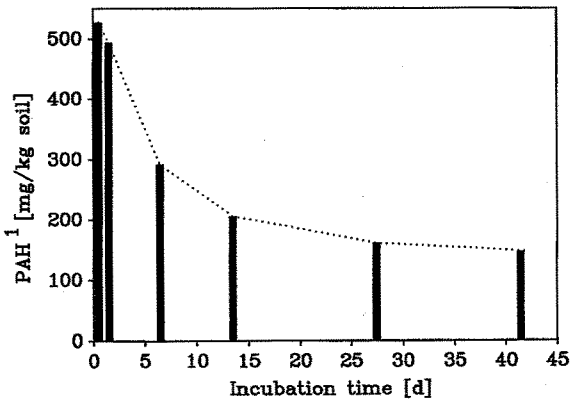

Fig. 3. Time course of microbial PAH degradation following recontamination of toluene-extracted soil B with the original organic extract. Conditions: 20 g samples of soxhlet-extracted material of soil B were contaminated with their own toluene extracts. Suspension in 100 ml MSM, pH 7.2, and inoculation with the PAH-degrading mixed culture M1 was followed by incubation in 500-ml erlenmeyer flasks at 30° C and 100 rpm. [1]PAH: total of PAH degradable by the mixed culture M1, i.e. naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, and chrysene

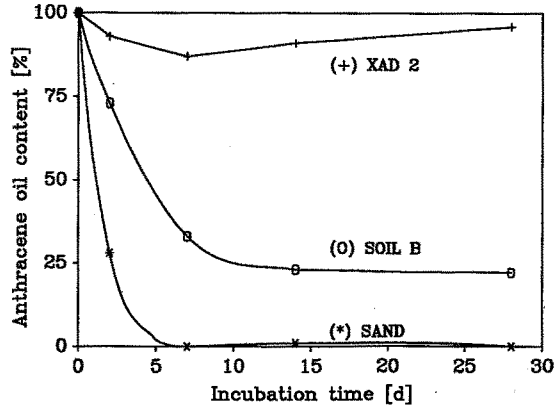

Fig. 4. Microbial degradation of anthracene oil initially sorbed onto sand (*), decontaminated material of soil B (O), and XAD 2 (+). Conditions: 50 µl anthracene oil were added to 20-g samples of the different sorptive substrates suspended in 100 ml MSM, pH 7.2, respectively. Incubation was carried out in 500-ml erlenmeyer flasks at 30° C and 100 rpm

the whole PAH content of soil B prior to extraction and recontamination.

Effect of sorption on PAH bioavailability

To elucidate the influence of sorption on microbial PAH degradation, anthracene oil was added onto different sorptive substrates. PAH analysis was performed after samples were incubated with the mixed culture M1. The percentage of anthracene oil degradation was calculated in regard to the amount of PAHs recovered from sterile probes with the respective sorptive substrate taken as 100%. Within 7 days of incubation, sand-sorbed PAHs were reduced by the bacteria below measurable levels (Fig. 4). The degradation of soil-sorbed

PAHs was considerable delayed and resulted in a residual, non-biodegradable PAH fraction of about 23% of the initially added anthracene oil. Anthracene oil sorbed on XAD 2 was not degraded by the acclimated bacteria (Fig. 4). XAD 2 is an amberlite resin with excellent PAH sorption characteristics, used for removal of PAHs from water and air. Inhibition of microbial growth caused by XAD 2 was excluded, since the mixed culture M1 showed normal growth in the presence of XAD 2 on complex media (NB added to a solution of 3 mM salicylic acid) by maintenance of PAH degradation potential.

Several physicochemical properties of the soils were characterized, in order to estimate the factor responsible for PAH sorption (Table 3). The amount of organic carbon was estimated to be the most significant difference of soil A and soil B, indicating a correlation of the extent of PAH sorption onto soil material and the content of soil organic carbon. The effect of time on PAH sorption was investigated by estimation of the PAH extractability at room temperature in relation to the time that soils were exposed to anthracene oil. Within the first hours of incubation, the amount of extractable anthracene oil components dropped to approximately 60% of the initially applied oil, demonstrating rapid and extensive sorption. However, during further exposure the degree of PAH extractability decreased continuously, indicating further sorption (Fig. 5). Thus, the initial rapid sorption process was followed by a second sorption process that occurred at an increasingly slower rate over a long period of time.

Biotoxicity of soil-sorbed PAHs

Laboratory studies were undertaken to determine the toxicity in eluates of the contaminated soils in comparison to unpolluted garden-mould (Table 4). Microtox assays demonstrated a G_L value (lowest dilution factor that exhibits an inhibition of light emission of *P. phosphoreum* <20%) of 1.0 for eluates of garden mould and soil B. In contrast, eluates of soil A exhibited a strong toxic effect on the bacteria (G_L value = 39, i.e. a solution containing 2.6% of eluate reduced bioluminescence by 20%).

For further verification, soil A was intermixed with increasing amounts of activated carbon to give final soil organic carbon contents of 1%, 5%, 10%, and 20%, respectively. As shown in Table 5, the toxicity of soil eluates decreased significantly with increasing amounts of soil organic carbon. The decrease in toxicity was accompanied by a decrease in the amount of water-elua-

Table 3. Physicochemical characterization of contaminated soils from different sites

Parameter	Soil A	Soil B
Contamination (mg PAH/kg soil)	1815.2	1027.5
PAH biodegradability	Yes	No
Water content (% w/w)	10.4	13.8
pH value	6.7	7.8
Specific surface (m^2/g soil)	1.8	3.6
Organic carbon (% w/w)	1.0	13.6

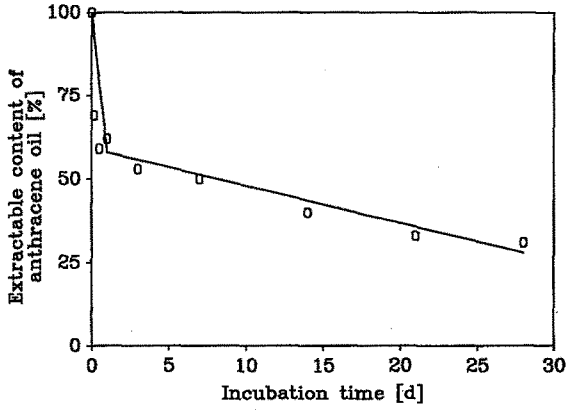

Fig. 5. Influence of sorption processes on the recovery of anthracene oil added to decontaminated material of soil B. Conditions: 20-g samples of soxhlet extracted material of soil B were suspended in 100 ml MSM, pH 7.2, and incubated with 50 µl anthracene oil in 500-ml erlenmeyer flasks at 30° C and 100 rpm under sterile conditions. Extraction with toluene was carried out by shaking the samples with toluene at room temperature

Table 4. Inhibitory effect of soil eluates on the light emission of *Photobacterium phosphoreum* NRRL B-11177

Dilution factor	Garden mould	Soil A	Soil B
G1	7%	n.d.	11%
G2	0%	89%	6%
G3	0%	n.d.	2%
G4	0%	77%	0%
G8	n.d.	59%	n.d.
G12	n.d.	48%	n.d.
G20	n.d.	36%	n.d.
G_L	1	39	1

n.d., not determined; G_L, lowest dilution factor that exhibits inhibition of light emission <20%

Table 5. Influence of addition of activated carbon on the toxicity of soil eluates measured as the inhibitory effect on the light emission of *P. phoshoreum* NRRL B-11177

Dilution factor	Soil A without a.c.	Soil A +4% a.c.	Soil A +9% a.c.	Soil A +19% a.c.
G1	n.d.	63%	50%	29%
G2	89%	51%	39%	28%
G4	77%	38%	31%	19%
G8	59%	28%	22%	18%
G12	48%	n.d.	n.d.	n.d.
G20	36%	n.d.	n.d.	n.d.
G_L	39	12	10	4

a.c., activated carbon

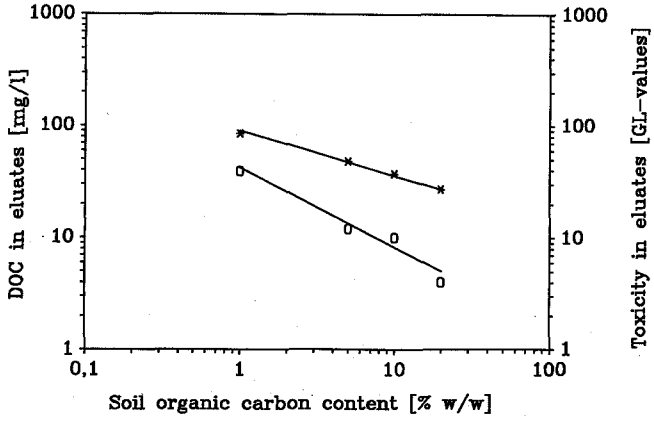

Fig. 6. Dependence of soil elutable dissolved organic carbon (DOC; *) and toxicity (O) on soil organic carbon content for one defined contamination. Conditions: addition of activated carbon to soil A to obtain contents of about 1%, 5%, 10% and 20% soil organic carbon, respectively, was followed by 24 h of elution with water (according DIN 38414). Aqueous eluates were used for determination of DOC and microtoxicity (according to DIN 38412)

ble DOC (data not shown), indicating the adsorption of organic pollutants onto the added amount of activated carbon. On the basis of the apparent exponential relationships observed, the results were plotted using logarithmic coordinates (Fig. 6). The data showed a reasonable degree of linearity, confirming the relationship of soil organic carbon content to soil-eluable DOC and eluate toxicity by a given concentration of organic pollutants.

Discussion

Because of the known toxicological effects of PAHs, restoration of PAH-contaminated sites is a major environmental goal (Dipple 1976; Kingsbury et al. 1979; Thakker et al. 1985). Although many microorganisms are known to degrade PAHs rapidly in submersed cultures (Cerniglia 1984; Gibson and Subramanian 1984), concentrations of inorganic nutrients less than the optimum for microbial growth as well as O_2 limitation may result in resistance of PAHs towards biological attack. Nevertheless, the data showed that the degree of PAH biodegradation in different soils may differ significantly even under the same optimum growth conditions concerning temperature, nutrients, O_2 supply, and occurrence of PAH-degrading bacteria.

Of particular interest is the finding that PAHs present in soil B are definitely undegradable by PAH-degrading bacteria with exceptional extraction and restoration into the original soil material. These results imply that the inhibition of PAH biodegradation in soil B is caused solely by the kind of PAH-binding within the soil material. It has been suggested that the physical state of a compound can greatly influence its bioavailability, as microbial degradation of adsorbed compounds was shown to be greatly diminished (Martin et al. 1978; Ogram et al. 1985; Manilal and Alexander 1991). Thus, sorption might be an important factor in pollutant degradation and may determine the fate of hydrophobic organic compounds in natural environments. The effect of sorption on PAH bioavailability was demonstrated by the reduction of PAH-degradation rates with increasing sorption capacity of the sorptive substrates used (sand, soil B, and XAD 2).

Among several studies concerning the sorptive behaviour of organic pollutants, the sorption of a number of PAHs on soil and sediment samples has been described (Karickhoff et al. 1979; Means et al. 1980). It has been suggested that the organic carbon content of soil is the single most important factor determining the sorption of hydrophobic molecules such as PAHs. From theoretical and experimental evidence it was concluded that the affinity for association of a contaminant with the organic material of a soil (expressed as the organic carbon normalized partition coefficient K_{OC}) is a function of the hydrophobicity of the compound (expressed as the octanol-water partition coefficient K_{OW}) (Dzombak and Luthy 1984; Voice et al. 1983; Walton and Anderson 1988). As PAHs are characterized by extremely high K_{OW} values and low vapour pressures (Callahan et al. 1979), naturally occurring organic matter is an excellent sorbent for these compounds (McCarthy and Jimenez 1985; Chiou et al. 1986). These facts are reflected in the comparison of soil organic carbon content and PAH biodegradability of soil A and soil B.

Two kinetically distinct processes were found to be associated with PAH binding onto soil material, a "fast" and a "slow" process. The initial fast adsorption process is thought to reflect rapid adsorption of the hydrophobic pollutants onto hydrophobic areas of soil surfaces, whereas the following slow adsorption process is proposed to be based on migration of the hydrophobic contaminants to less accessible sites within the soil matrix (Karickhoff 1980; Robinson et al. 1990). Thus, longer incubation times result in migration of an increasingly large fraction of the pollutant into the organic soil material. This process should last until the incorporation capacities of the soil organic matter are exhausted and equilibrium is reached. The fraction of incorporated pollutants is supposed to represent the non-bioavailable and non-biodegradable part of contamination in soils.

These suggestions are confirmed by the finding that microbial degradation of PAH added to extacted material of soil B stopped at residual PAH concentrations of about 28% (PAHs added with original organic extracts) and 23% (PAHs added with anthracene oil) respectively. The data implies that PAH biodegradation in short-term contaminated soil samples competes with migration of PAH into non-accessible soil compartments. On the whole, the results have shown the impact of the time of exposure of contaminants to soils with high organic carbon contents ("age" of contamination) on the degree and rate of PAH biodegradation.

The effectiveness of biological soil remediation is usually valued based on the removal of selected con-

taminants. In the case of PAH-contaminated environments, 16 EPA-PAHs were analysed to prove site restoration (VROM/NL 1988; Richter et al. 1988). On the one hand little attempt had been made to correlate site restoration, i.e. the successful dissappearance of parent material, with a discernible decrease in the health hazards associated with biotreated material. On the other hand, a commercial biotreatment of soils containing non-bioavailable PAHs in excess to the given boundary values is not practicable, independent on the health hazards associated with the untreated material.

To address these issues, microtox assays were performed to determine the biotoxicity in aqueous eluates of soils contaminated with non-bioavailable PAHs. Toxicity of soil eluates was determined as inhibition of bioluminescent bacteria shown to be sensitive towards influents of refinery sewage plants (Hambsch et al. 1990) and creosote-contaminated groundwater (Mueller et al. 1991). Microtox assays demonstrated negligible toxicity in eluates of soil B in contrast to aqueous eluates of soil A. Intermixing of activated carbon into the material of soil A resulted in stronger adsorption of organic hydrophobic compounds to particles and in decreasing toxicity in aqueous eluates of the soil. In conclusion, the results implicate that soil-borne PAHs, if immobilized onto soil organic matter, are non-biodegradable and not to be released by rinsing soils with water.

Similar results have already been reported concerning the influence of soil sorption and biotoxicity of dioxins. The degree of dermal uptake of soil-borne 2,3,7,8-terachlorodibenzo-*p*-dioxin (TCCD) was highly reduced, compared with that of free (solvent-borne) TCCD in rabbit (Bonaccorsi et al. 1984) and rat (Shu et al. 1988). Furthermore, sorption onto activated carbon almost completely prevents dermal uptake of TCCD by rats (Poiger and Schlatter 1980). Thus, adsorption of organic pollutants onto soil organic materials must be taken in account when evaluating the possible health hazards of contaminants distributed in the environment. The strong adsorption of toxicologic critical compounds onto activated carbon merits special attention as decontamination procedures might be based on these features.

Further investigations are needed to discover if the observed phenomena are applicable to other soil samples from different contaminated sites. Furthermore, for evaluation of the health hazards of contaminated soil more detailed studies are required. Biotoxicity must be measured in water samples seeping through contaminated soils under natural conditions including dryness and frost as well as assaying biotoxicity not only in eluates but in the original soil matrix.

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References

Bewley R, Ellis B, Theile P, Viney I, Rees J (1989) Microbial clean-up of contaminated soil. Chem Ind 23:778–783

Blumer M, Youngblood WW (1975) Polycyclic aromatic hydrocarbons in soil and recent sediments. Science 188:53–55

Bonaccorsi A, Domenico A di, Fanelli R, Merli F, Motta R, Vanzati R, Zapponi GA (1984) The influence of soil particle adsorption on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin biological uptake in the rabbit. Arch Toxicol Suppl 7:431–434

Brown KW, Donnelly KC, Thomas JC, Davol P, Kampbell D (1985) Degradation of soil applied organic compounds from three petroleum wastes. Waste Manage Res 3:27–39

Callahan MA, Slimak MW, Gabel NW, May IP, Fowler CF, Freed JR, Jennings P, Durfee RC, Whitmore FC, Maestri B, Mabey WR, Holt BR, Gould C (1979) Water related environmental fate of 129 priory pollutants, vol II. Halogenated aliphatic hydrocarbons, halogenated ethers, monocyclic aromatics, phthalate esters, polycyclic aromatic hydrocarbons, nitrosoamines and miscellaneous compounds. EPA-440/4-79-029 b

Cerniglia CE (1984) Microbial metabolism of polycyclic aromatic hydrocarbons. Adv Appl Microbiol 30:31–71

Chiou CT, Malcolm RC, Brinton TI, Kile DE (1986) Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environ Sci Technol 20:502–508

Clark KR, Owens NJP (1983) A simple and versatile micro-computer program for the determination of most probable number. J Microbiol Methods 1:133–137

Codner RC (1969) Solid and solidified growth media in microbiology. Methods Microbiol 1:427–454

DIN-Entwurf 38414 Teil 4 (1984). VCH Weinheim

DIN-Entwurf 38412 Teil 34 (1991). VCH Weinheim

Dipple A (1976) Polynuclear aromatic carcinogens. In: Searle CE (ed) Chemical carcinogens, ACS Monograph 173. American Chemical Society, 173, pp 245–314

Dzombak DA, Luthy RG (1984) Estimating adsorption of polycylcic aromatic hydrocarbons on soils. Soil Science 137:292–308

Gibson DT, Subramanian V (1984) Microbial degradation of aromatic hydrocarbons. In: Gibson DT (ed) Microbial degradation of organic compounds. Dekker, New York, pp 181–252

Hambsch B, Pelouin C, Werner P (1990) Bioluminescence test as a surrogate parameter for the toxicity of hazardous substances in refineries. Vom Wasser 74:65–74

Karickhoff SW (1980) Sorption kinetics of hydrophobic pollutants in natural sediments. In: Baker RA (ed) Contaminants and sediments, vol 2. Ann Arbor Science, Ann Arbor, pp 193–205

Karickhoff SW, Brown DS, Scott TA (1979) Sorption of hydrophobic pollutants on natural sediments. Water Res 13:241–248

Kingsbury GC, Sims RC, White JB (1979) Multimedia goals for environmental assessment. EPA-600/7-79-176 b

Krieg NR, Holt JG (1984) Bergey's manual of determinative bacteriology, vol 1, Williams and Wilkins, Baltimore

LaFlamme RE, Hite RA (1978) The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochim Cosmochim Acta 42:289–303

Manilal VB, Alexander M (1991) Factors affecting the microbial degradation of phenanthrene in soil. Appl Microbiol Biotechnol 35:401–405

Martin JP, Parsa AA, Haider K (1978) Influence of intimate association with humic polymer on biodegradation of ^{14}C-labelled organic substances in soil. Soil Biochem 10:483–486

McCarthy JF, Jimenez BD (1985) Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: binding and dissociation. Environ Sci Technol 19:1072–1076

Means JC, Wood SG, Hassett JJ, Banwart WL (1980) Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environ Sci Technol 14:1524–1528

Morgan P, Watkinson RJ (1989) Hydrocarbon degradation in soils and methods for soil biotreatment. Crit Rev Biotechnol 8:305–333

Mueller JG, Chapman PJ, Pritchard PH (1989) Creosote contaminated sites: their potential for bioremediation. Environ Sci Technol 23:1197–1201

Mueller JG, Middaugh DP, Lantz SE, Chapman PJ (1991) Biodegradation of creosote and pentachlorophenol in contaminated groundwater: chemical and biological assessment. Appl Environ Microbiol 57:1277–1285

Ogram AV, Jessup RE, Ou LT, Rao PSC (1985) Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl Environ Microbiol 49:582–587

Pahlmann R, Pelkonen O (1987) Mutagenicity studies of different polycyclic aromatic hydrocarbons: the significance of enzymatic factors and molecular structures. Carcinogenesis 8:773–778

Pfennig N, Lippert KD (1966) Über das Vitamin-B-12-Bedürfnis phototropher Schwefelbakterien. Arch Microbiol 55:245–256

Poiger H, Schlatter C (1980) Influence of solvents and adsorbents on dermal and intestinal absorption of TCCD. Food Cosmet Toxicol 18:477–481

Richter B, Krämer D, Schramek ER (1988) Gefährdungsabschätzung bei ehemaligen Kokereistandorten. Müll Abfall 20:302–307

Robinson KG, Farmer WS, Novak JT (1990) Availability of sorbed toluene in soils for biodegradation by acclimated bacteria. Water Res 24:345–350

Sims RC, Overcash MR (1983) Fate of polynuclear aromatic compounds (PNA's) in soil-plant systems. Residue Rev 88:1–68

Shu H, Teitelbaum P, Webb AS, Marple L, Brunck B, deiRossi D, Murray FJ, Paustenbach D (1988) Bioavailability of soil-bound TCCD: dermal bioavailability in the rat. Fund Appl Toxicol 10:335–434

Suffet IH, McGuire MJ (1980) Activated carbon adsorption of organics from the aqueous phase, vol 1. Ann Arbor Science, Ann Arbor

Thakker DR, Yagi H, Levin W, Wood AW, Cooney AH, Jerina DM (1985) Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In: Anders MW (ed) Bioactivation of foreign compounds. Academic Press, New York, pp 177–192

Voice TC, Rice CP, Weber WJ Jr (1983) Effect of solids concentration on the sorptive partitioning of hydrophobic pollutants in aquatic systems. Environ Sci Technol 17:513–518

VROM/NL (1988) Leidraad Bodensanering, Deel II, Technisch-Inhondelijk Deel. In deutscher Übersetzung: Leitfaden Bodensanierung, BMU, vol 2

Walter U, Beyer M, Klein J (1990) Biodegradation of polycyclic aromatic hydrocarbons by a bacterial mixed culture. In: Christiansen L, Munck L, Villadsen J (eds) Abstract of the 5th European Congress on Biotechnology. Muncksgaard, Copenhagen, p 213

Walton BT, Anderson TA (1988) Structural properties of organic chemicals as predictors of biodegradation and microbial toxicity in soils. Chemosphere 17:1501–1507

Weissenfels WD, Beyer M, Klein J (1990a) Degradation of phenanthrene, fluorene, and fluoranthene by pure bacterial cultures. Appl Microbiol Biotechnol 32:479–484

Weissenfels WD, Beyer M, Klein J (1990b) Rapid testing system for assessing the suitability of the biological reclamation for PAH-contaminated soil. In: Christiansen L, Munck L, Villadsen J (eds) Proceedings of the 5th European Congress on Biotechnology. Muncksgaard, Copenhagen, pp 931–934

White KL (1986) An overview of immunotoxicology and carcinogenic polycyclic aromatic hydrocarbons. Environ Carcinogen Rev C4:163–202