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Structure-Biodegradability Relationships of Polycyclic Aromatic Hydrocarbons in Soil

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The potential of some polycyclic aromatic hydrocarbons (PAH) to be metabolized to mutagenic and/or carcinogenic compounds (Cerniglia 1984, Gibson & Subramanian 1984) has caused concern for environmental pollution by this class of residues. In industrialized countries, pollution by PAH is primarily associated with the processing, combustion and disposal of fossil fuels (Bartha & Bossert 1984, Hites & LaFlamme 1977), but low levels of PAH have also been found in pre-industrial sediments (LaFlamme & Hites 1978). These are believed to represent incomplete combustion products from spontaneous grassland and forest fires and some may be biogenic in origin (Blumer 1976, Suess 1976). Though not novel to the biosphere, the more highly condensed PAH show considerable persistence in the environment; the rates and mechanisms of their recycling are only partially understood (Gibson & Subramanian 1984, Suess 1976). In low concentrations, PAH are present in waste sludges originating from treatment of refinery effluents (Bartha & Bossert 1984). In a previous study on oily sludge disposal by land treatment (Bossert et al. 1984), PAH as a class were found to be biodegraded more rapidly than the total solvent-extractable hydrocarbons of the sludge. This somewhat surprising result was apparently due to a predominance of low molecular weight PAH (≤ 3 rings) in the sludge sample. The higher molecular weight PAH (≥ 4 rings) exhibited persistence that seemed to increase with the number of rings and the degree of ring condensation. In order to verify and extend these observations, we have undertaken here a systematic study on the structure-biodegradability relationship (SBR) of PAH in soil. Since it was expected that the majority of the PAH would be degraded cometabolically rather than in the substrate utilization mode, 1-phenyldecane was added as primary substrate in combination with all the PAH tested. In preliminary tests, 1-phenyldecane proved to be more effective in stimulating the biodegradation of 1,2-benzpyrene (benzo[a]pyrene) than either n-hexadecane, naphthoic acid or sewage sludge. The presence of an additional hydrocarbon substrate made this SBR study also more relevant to our previous work on the fate of PAH during the disposal of oily sludges by land treatment (Bossert et al. 1984).

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MATERIALS AND METHODS

The chemical structures and names of the PAH used in this study are listed in Fig. 1. Completely linear tetra- and pentacyclic PAH such as tetracene and pentacene were not included in this study due to their chemical lability and unfavorable solubility characteristics. All PAH compounds, as well as 1-phenyldecane were purchased from Aldrich Chemical Co. (Milwaukee, WI) and had a purity of 98% or higher. In order to ensure uniform distribution of the hydrocarbons in soil, 25 mg of each PAH and 0.3 mL of 1-phenyldecane were ground with 5 g acid-washed sand. Subsequently, these hydrocarbon-sand mixtures were thoroughly mixed with 30.8 g fresh Nixon sandy loam (Bartha & Bordeleau 1969) equal to 25.0 g by dry weight. Replicate samples were prepared for each PAH and were individually placed in 100-mL glass beakers. Nitrogen and phosphorus in the samples were adjusted to an optimal C : N : P ratio (Dibble & Bartha 1979) by adding a NH_4NO_3/K_2HPO_4 fertilizer solution to provide 340 nmoles of N and 26 nmoles of P per sample. All samples were moistened to 50% of the soil water holding capacity and the beakers were covered with thin polyethylene film to minimize evaporation. Incubation was in the dark at 20°C for a total of 16 mo. Beakers were opened for aeration in 2-3 day intervals and evaporated water, monitored by weight loss, was replenished as needed to maintain conditions favorable for biodegradation. Control samples for determining abiotic PAH losses were prepared in an identical manner but contained, in addition, 2% $HgCl_2$ on dry soil basis. After 4 mo of incubation, a second application of 0.3 mL of phenyldecane was made to ensure the continued availability of a growth substrate for PAH biodegradation.

On day 0 and at each subsequent sampling point (4, 11 and 16 mo), duplicate samples for each PAH were analyzed for residues. Poisoned samples were analyzed at the last time point only. After air-drying of the samples at room temperature, they were exhaustively extracted by using dichloromethane in a Soxhlet apparatus. The extracts were concentrated under a nitrogen stream at 40°C. Care was taken to protect the extracts from prolonged exposure to light. Analysis for residual PAH was performed by gas chromatography with flame ionization detection (Hewlett Packard 5710A), using a 183 X 0.31 cm stainless steel column packed with 5% Dexsil on Chromosorb W (Applied Science, Inc.). Carrier flow was 30 mL N_2 per min. The retention times (min) and corresponding operating oven temperatures for each PAH were as follows: phenanthrene (2.7), 200°C; anthracene (2.2), 210°C; acenaphthylene (2.5) and pyrene (3.3), 230°C; 1,2-benzanthracene (4.1), 250°C; chrysene (5.8), 260°C; perylene (4.8), 280°C; 1,2,3,4-dibenzanthracene (5.0) and 1,2-benzpyrene (4.5), 300°C; and 1,2,5,6-dibenzanthracene (4.7), 320°C. The detector temperature was 350°C for all PAH. Detection limit was one percent or less of the added PAH.

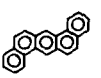
PAH	RECOVERED FROM SOIL(%) TIME 0	4mo	11mo	16mo	P.C.
 ANTHRACENE	100	nd	49	7	40
 PHEN-ANTHRENE	97	0	0	0	62
 ACE-NAPHTHYLENE	51	0	0	0	0
 1,2-BENZ-ANTHRACENE	100	98	74	64	82
 CHRYSENE	95	107	81	84	95
 PYRENE	97	49	20	3	73
 1,2,5,6-DIBENZ-ANTHRACENE	98	80	90	106	91
 1,2,3,4-DIBENZ-ANTHRACENE	100	91	93	83	97
 1,2-BENZ-PYRENE	91	97	81	72	89
 PERYLENE	100	102	109	113	89

Figure 1. Recovery of ten PAH with time from incubations of biologically active and poisoned control (PC) soils containing 1-phenyldecane as primary substrate. The PC soil was analyzed after 16 mo of incubation. The recovery values represent the average of duplicate samples. The coefficients of variation for duplicates did not exceed a maximum of 8 and 5% for biologically active and poisoned samples, respectively, except as follows. PC for chrysene had a coefficient of variation of 25%; for pyrene at 4 and at 16 mo, the coefficients of variation were 19 and 34% respectively. "nd" stands for "not determined" due to sample loss.

RESULTS AND DISCUSSION

Figure 1 summarizes the biodegradative and abiotic losses of PAH with time of incubation in soil. An expected inverse correlation between the number of PAH rings and their loss from soil was supported by the results. Tricyclic PAH disappeared rapidly, tetracyclic PAH more slowly, and pentacyclic PAH only

marginally or not at all. On the other hand, a general pattern of an inverse correlation of disappearance with the degree of ring condensation (clustered versus linear arrangement of the same number of rings) did not emerge. For the purpose of discussion, biodegradation assessed in these studies was measured as the loss of parent PAH from the soil. This may include partial metabolism to oxidized products or incorporation into the soil humus, as well as mineralization to CO_2. Toxicity of PAH towards degrading microorganisms as a determining factor in SBR was not studied in detail, but two observations prompted us to disregard this factor as explanation. In a previous land treatment study (Bossert et al. 1984), no inhibition of hydrocarbon utilization was evident after repeated applications of PAH-containing sludge. Also, in a preliminary study, 1,2-benzpyrene (benzo[a]pyrene) concentrations as high as 5% on a dry soil basis failed to inhibit the endogeneous CO_2-evolution of soil (data not presented). The correlation of the observed SBR with some other structural and physico-chemical characteristics is discussed below.

The SBR of the tricyclic PAH are somewhat obscured by strong abiotic losses, primarily by volatilization, both during incubation and sample concentration. Volatility resulted in low recovery of acenaphthylene even at time 0, and at 16 mo it was undetectable even in poisoned soil samples. Therefore, it is possible that acenaphthylene was lost to a greater extent by volatility than by biodegradation. Phenanthrene and anthracene were primarily lost by biodegradative processes, though substantial abiotic losses contributed to their disappearance. Phenanthrene was biodegraded faster than anthracene. Although the angular ring arrangement of phenanthrene is considered thermodynamically more stable than the linear arrangement of anthracene (Blumer 1976), the so-called "bay region" created by the angular arrangement has been found to favor enzyme attack on PAH (Cerniglia 1984, Gibson & Subramanian 1984). The greater water solubility (Klevens 1950) of phenanthrene (1,600 gL^{-1}) as compared to anthracene (75 gL^{-1}) offers an additional explanation for the faster biodegradation of phenanthrene. Wodzinski & Coyle (1974) concluded that phenanthrene and probably other PAH are utilized only in aqueous solution.

Among the tetracyclic PAH, surprisingly, the most condensed pyrene which lacks a "bay region" was subject to the fastest biodegradation. 1,2-Benzanthracene was subject to slow biodegradation, but the biodegradation of chrysene was marginal. The observed biodegradation pattern corresponds best to water solubilities (Klevens 1950): 175 gL^{-1} for pyrene, 10 gL^{-1} for 1,2-benzanthracene and 6 gL^{-1} for chrysene.

The pentacyclic PAH are practically insoluble (less than 1 gL^{-1}) in water (Klevens 1950). Of these compounds, only

1,2-benzpyrene showed slow but significant biodegradative losses which could be confirmed by $^{14}CO_2$ evolution from the radio-labeled compound (data not shown). For the other three pentacyclic PAH biodegradative losses were either not evident at all, or they just barely exceeded experimental error. Apparent "increases" were due, of course, to a combination of analytical and dosing errors.

Our findings are in general agreement with SBR studies on PAH conducted at other laboratories. Recent reviews (Cerniglia 1984, Gibson & Subramanian 1984) document that several tricyclic PAH are able to serve as microbial growth substrates or are at least readily cooxidized in the presence of other hydrocarbons. Groenewegen and Stolpe (1976) compared PAH disappearance from biologically active and poisoned soil percolation units and obtained semi-quantitative evidence for biodegradation of phenanthrene, fluoranthene and pyrene, while fluorene, 1,2-benzanthracene, chrysene, 1,2-benzpyrene and coronene were not measurably biodegraded during a one-month incubation period. n-Hexadecane and biphenyl were present as primary substrates in the described study. Herbes and Schwall (1978) compared the rates of biodegradation in aquatic sediments of radiolabeled PAH and found biodegradation to decrease in the order of naphthalene anthracene 1,2-benzanthracene 1,2-benzpyrene. A similar biodegradation rate relationship between naphthalene and anthracene biodegradation in estuarine sediments was found by Bauer and Copone (1985). Schocken and Gibson (1984) reported on the extensive cooxidation of acenaphthylene by a Beijerinckia strain.

In most respects, our present study is also in agreement with the pattern of PAH persistence observed in our earlier study on land treatment disposal of oily sludge (Bossert et al. 1984). A notable exception is the fate of the completely condensed tetracyclic pyrene that appeared relatively persistent in the land treatment study but was found to be quite biodegradable in the present one. Since our present findings and the results of Groenewegen and Stolpe (1976) mutually confirm each other, we now believe that the persistence of pyrene in the land treatment study (Bossert et al. 1984) was caused by the sparing of pyrene in the presence of other more biodegradable substrates, rather than by the inherent recalcitrance of this PAH.

In conclusion, the biodegradation of PAH incubated in soil in presence of 1-phenyldecane as a primary substrate was inversely affected by the number of aromatic rings and correlated positively with water solubility. No other general rule was applicable to all of the ten PAH compared in this study.

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