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Microbial Abundance and Degradation of Polycyclic Aromatic Hydrocarbons in Soil

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Polycyclic aromatic hydrocarbons (PAHs) are a group of highly lipophilic chemicals that are generally formed during combustion, pyrolysis and pyrosynthesis of organic matter and are present ubiquitously in the urban environment as pollutants in very small quantities (IARC Monograph, 1983, 1984 and 1985). In industrialised countries, pollution by PAHs are primarily associated with the processing, combustion and disposal of fossil fuels (Bartha & Bossert, 1984). PAHs have also been found in pre-industrial sediments (LaFlame & Hites, 1978) and are believed to be incomplete combustion products from spontaneous fires involving grass lands and forests, and some may be biogenic in origin (Blumer, 1976). The more highly condensed PAH show considerable persistence in the environment; the rates and mechanism of their recycling are only partially understood (Gibson & Subramanian, 1984). There is little information about the fate of PAH in the soil environment and virtually nothing is known about mechanisms of its biodegradation.

Of the many options available for the remediation of waste sites, the use of microorganisms to degrade organic chemicals offers many advantages in terms of speed, efficiency and cost (Lee & Ward, 1985). Bioremediation may involve the ability of the in situ microorganisms to utilise the waste or the application of microorganisms acclimated to degrade particular wastes or combinations of wastes. Bioremediation has been used with success to treat soils contaminated by hydrocarbon products (Dibble & Bartha, 1979; Bossert et al., 1984). In this study, the indigenous contaminated soil populations were effective in removing the hydrocarbons and returning the soil to productivity.

The objective of the present study was to determine the activity of indigenous microbial (fungi and bacteria) population of hazardous waste site, their degree of

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adaptation, its ability to degrade toxic PAHs, and to study the potentials of different indigenous microbes to degrade the following selected PAHs from the polluted soil environment. PAHs selected for the study were 1.Anthracene(An), 2.Phenanthrene(Ph), 3.Crysene(Cry), 4.Pyrene(Pyr) and 5. Flouranthene(Fl).

MATERIALS AND METHODS

All PAH compounds and 1-phenyldecane were purchased from Aldrich Chemical Co. with a purity of 98%. In order to ensure uniform distribution of the hydrocarbons in soil (Collected near the banks of the Musi river which contains automobile, industrial and municipal sewage waste), 25 mg of each PAH and 0.3 ml of 1-phenyl decane were ground with 5g acid washed sand. This mixture was thoroughly mixed with 30g fresh sandy loam soil (Bartha & Bordeleau, 1969) equal to 25g by dry wt. Replicate samples were prepared for each PAH and individually placed in 100 ml glass beakers. Nitrogen and phosphorus were adjusted in the samples to an optimal C:N:P ratio (Dibble & Bartha, 1979) by adding a NH_4NO_3/K_2HPO_4 fertilization solution to provide 340 n moles of N and 26 n moles of P per sample.

All samples were moistened to 50% of the soil water holding capacity and the beakers covered with thin polyethylene film to minimize evaporation. Samples were incubated maintained at $27\pm1^{o}C$ for a period of 15 mo. During incubation the beakers were opened for a period of 24 h. aeration in 3 day intervals and evaporated water was replenished as needed to maintain conditions favourable for biodegradation. Control samples for determining abiotic PAHs losses were prepared in an identical manner but contained 1% $HgCl_2$ on dry soil basis.

After 5 mo. of incubation a second application of 0.3ml of 1-phenyldecane was made to ensure the continued availability of growth substrate for PAHs biodegradation. On day 0 and each subsequent sampling point (5, 10 and 15 mo.) duplicate samples for each PAHs were analysed for residues. After air drying of samples at room temperature, they were exhaustively extracted by using dichloromethane in a soxhlet apparatus. The extracts were concentrated under pressure at $40^{o}C$, and care was taken to protect the extracts from prolonged exposure to light.

Analysis for residual PAHs was performed by gas chromotography with flame ionization detection (Hewlett Packard 5710 A), using 183x0.31 cm stainless steel column packed with 5% Dexsil on chromosorb W.(Applied Science, Inc.) Carrier gas (N_2) flow 40 ml/min. Column temperature for each PAH maintained (Anthracene-$230^{o}C$,

Phenanthrene-220°C, Cyrsene-280°C, Pyrene-250°C, and Fluoranthene-280°C). The detector temperature was 380°C for all PAHs, for ppm recovery (0.5 ul sample, attentuation 1x16).

Soil microflora (Fungi and Bacteria) were isolated (0,5,10 and 15 mo), from the study samples, at different time intervals during 15 mo incubation period. Colony forming units (cfu) of soil microflora were estimated by Waksmans serial dilution plate method (Waksman, 1922) and soil plate method was used as a complementary method for the estimation of soil microflora (Warcup, 1957). Potato Sucrose Agar as well as CZapek (Dox) Agar medium used for fungi and Ericksons Agar medium (Clacium Nitrate-0.3g, Clacium sulphate-0.8g, Magnesium sulphate-0.7g, Potassium sulphate-0.025g, K_2HPO_4- 0.005g, $NaHCO_3$-0.1g, glucose-0.01g, yeast extract-0.0059g, distilled water 1000ml and pH=7) employed for the isolation of bacteria. Three replicates were maintained in each case. Fungi and bacteria which were dominant during the study were identified down to species level.

RESULTS AND DISCUSSION

The table-1 gives the details of biotic and abiotic losses of PAH in soil during 15 mo. incubation period. Anthracene and pyrene degradation enhanced as the incubation period increases and crysene degradation is almost nil.For the purpose of discussion biodegradation assessed in these studies was measured as the loss of parent PAH in the soil. This may include incorporation into the soil humus, as well as mineralisation to CO_2. The table-2 summarises that cfu of fungi and bacteria were increased as incubation period increases. The dominant bacteria during the study were Pseudomonas spp, Agrobacterium spp and the species of Bacillus subtilis, whereas fungi dominated by Aspergillus niger, A.flavus, Penicillium tordum, Fusarium oxysporum, Sclerotium rolfsii, Trichoderma viride and Trichoderma harzianum. In a previous pesticidal treatment study (Mahmood et al 1989), Bacillus subtilis, Sclerotium rolfsii and Trichoderma harzianum were not reported. This may attribute to the PAH presence and other physico-chemical environment of the soils. Phenanthrene and anthracene were primarily lost by microbial degradative processes, though substantial aboitic 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" due to angular arrangement might have favoured enzyme attack on PAH (Gibbson and Subramanian, 1984). The water solubility of phenanthrene

Table 1. ppm recovery of PAHs from soil.

S. No.	Name of PAH	Rt. (min)	Formulae of PAH	Mol wt.	Concentration[a]	Time in months 0	5	10	15[b]	AL
1.	An	2.0	$C_{14}H_{10}$	178	1000	986	721	430	92	552
2.	Ph	2.5	$C_{14}H_{10}$	178	1000	992	602	234	nd	751
3.	Cry	5.4	$C_{18}H_{12}$	228	1000	973	1020	774	723	855
4.	Pyr	3.1	$C_{16}H_{10}$	202	1000	998	583	152	62	805
5.	Fl	1.7	$C_{16}H_{10}$	202	1000	961	684	nd	155	936

The recovery values are average of duplicate samples. AL (abiotic losses) samples were analysed after 15 mo. nd - not detected. a - actual concentrations of PAH before incubation. b - concentrations of PAH at the end of incubation. An-Anthracene, Ph-Phenanthrene, Cry-Crysene, Pyr-Pyrene, Fl-Fluoranthene, Rt-Retention time.

(1600 gL^{-1}) as compared to anthracene (75 gL^{-1}) might have been contributed for the faster biodegradation of phenanthrene. Here cfu of Sclerotium rolfsii and Trichoderma harzianum were increased dramatically from 5 mo. onwards. On 15 mo. phenanthrene was not detected, might be due to the trace level or possibilities of experimental error. In the case of Pyrene Bacillus subtilis might be dominating the degradation process, because it was abundantly available during the process.

Anthracene and Fluoranthene degradation was dominated by Agrobacterium spp. Pseudomonas spp, Aspergillus terrus, A.flavus and Penicillium tordum. Crysene degradation was not detected and the microbial numbers of fungi as well as bacteria were parallel. No individual microbe was dominated during the incubation period of Crysene. Our present findings and the results of Bossert and Bartha (1986) more or less confirm each other. In conclusion the biodegradation of PAH in the selected soil was due to PAH degrader present in the bacterial as well as fungal communities. No other general rule was applicable to all PAHs selected for the study.

In brief, hazardous waste contaminated soils possess active population of soil micro-organisms, which in this particular study are more abundant and more active than adjacent non-contaminated soil. Abundance and activity of microbial populations varies considerably from site to site, and may be related to differences of nutrient status or the presence of inhibitory chemicals. It is proposed that PAH itself as well as other

Table 2. Microbial cfu in thousand/g dry soil of PAHs during incubation period.

S.No.	PAH in Soil	Time in months 0	5	10	15
1.	Anthracene	1680 (59)	2100 (120)	3500 (135)	5400 (110)
2.	Phenanthrene	2100 (80)	2190 (90)	2080 (140)	2100 (160)
3.	Crysene	1800 (72)	1900 (82)	1750 (92)	1800 (80)
4.	Pyrene	3200 (77)	3950 (80)	4200 (93)	5190 (120)
5.	Fluoranthene	2500 (90)	3000 (130)	2700 (150)	2600 (180)

Values which are not in parentheses are bacterial cfu and fungal cfu are in parentheses.

contaminants at the site may be exerting inhibitory effects on microbes with PAH degrading capabilities. The observed heterogeneity will have to be taken into consideration in implementing bioremediation of contaminated soils.

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