PERGAMON

Chemosphere 40 (2000) 7–10

CHEMOSPHERE

Plant-promoted pyrene degradation in soil

Hans-Holger Liste, Martin Alexander*

Department of Soil, Crop and Atmospheric Sciences and Institute of Comparative and Environmental Toxicology, Cornell University, Ithaca, NY 14853, USA

Received 6 October 1998; accepted 14 April 1999

Abstract

A study was conducted to investigate the capability of nine plant species to promote the degradation of pyrene in soil. The test method allowed for analysis of the entire sample of soil. More pyrene was degraded in the presence of roots of all nine species than in unplanted soil. Within approximately 8 weeks, as much as 74% of the pyrene disappeared from vegetated soil compared to 40% or less from unplanted soil. The data suggest that some of the test species may be especially useful for phytoremediation of soils contaminated with PAHs.

Keywords: Bioremediation; PAHs; Phytoremediation; Pyrene; Rhyzosphere

1. Introduction

Plants enhance the remediation of soils containing organic pollutants by various processes (Shimp et al., 1993; Cunningham et al., 1996). Phytoremediation may largely result from the enhanced degradation of organic compounds in the rhizosphere as a result of the higher densities and greater activities of microorganisms than in the surrounding soil (Cunningham et al., 1996). In addition, plant transpiration may result in the transport of contaminants dissolved in the water from areas outside the root zone towards the root (Ferro et al., 1994). Remediation of contaminated soil therefore may be enhanced by plants because they function like a solar-driven pump that has degradative activity in the rhizosphere (Cunningham et al., 1996). The more soluble the organic compound, presumably the greater is the probability that plants will reduce the concentration (Nyer and Gatliff, 1996). Recent studies suggest that the pump-and-treat concept that has been hypothesized applies even to hydrophobic compounds such as PAHs. Plants accumulate considerable amounts of pyrene and phenanthrene in rhizosphere soil by removing a portion of the compounds from the surrounding soil. With time, the PAHs that have moved into the rhizosphere soil quickly disappear but not below concentrations in unplanted soil (unpublished data).

The objective of the present study was to investigate which plant species promote the disappearance of PAHs from soil. The approach used involved analysis not just of the rhizosphere soil but also of the surrounding soil and the root system.

2. Materials and methods

Lima silt loam (pH 6.3, 2.2% organic carbon) from Aurora, New York, USA, was air dried and passed through a 2-mm sieve. Portions (5 g) soil were placed in 50-ml screw-cap test tubes and amended with 0.5 ml of acetone containing pyrene (99% purity, Aldrich Chemical) to give a final concentration of 100 mg/kg. The soil samples were shaken twice daily for 10 s with a vortex mixer in a three-day period to mix the compounds with the soil and to allow the acetone to volatilize, and deionized water then was added to bring the moisture level

* Corresponding author.

0045-6535/00/$ - see front matter
PII: S0045-6535(99)00216-7

to about 80% of the water-holding capacity. The tubes were tightly capped with Teflon-lined closures, and the soils were stored in the growth chamber for 10–14 days.

Two seeds of each plant species were sown in each tube, and the seedlings were then thinned to give 1 plant per tube. The seeding date was considered as 0 day. Tubes containing planted and unplanted soil were transferred to a growth chamber maintained at 25°C during a 16-h day and at 19°C during an 8-h night. The light intensity was 11000 lx. The soil was watered as needed and fertilized every two weeks with an inorganic salts solution (Reilley et al., 1996). Each test was conducted in triplicate, and the tubes containing the plants were randomized in the growth chamber. The plants that were used were field crops (oat, *Avena sativa*; lupine, *Lupinus polyphyllus*; and rape, *Brassica napus* var. *radicola*), horticultural crops (dill, *Anethum graveolens*; pepper, *Capsicum annuum*; and radish, *Raphanus sativus*) and tree seedlings (jack pine, *Pinus banksiana*; red pine, *Pinus resinosa*; and white pine, *Pinus strobus*).

The shoots were excised, and the soil and roots remaining in the test tubes was mixed with 15 ml *n*-butanol, and the mixture was vigorously shaken with a vortex mixer for 2 min and then filtered with Whatman no. 1 filter paper. The tubes were washed with another 10 ml *n*-butanol, and this liquid was also filtered. The soil and filter paper were placed in cellulose extraction thimbles (Whatman, 25×80 mm^2) and subjected to a 3-h Soxhlet extraction with 90 ml hexanes and 2 ml *n*-butanol. The Soxhlet extracts were evaporated to remove the hexanes. Both extracts were passed through 0.22-μm Teflon syringe filters. Based on the studies of Kelsey et al. (1997), the amount of a PAH extracted with butanol appears to be correlated with the bioavailable portion of the PAH.

The butanol and Soxhlet extracts were analyzed with a high-performance liquid chromatograph (series 1050, Hewlett-Packard, Avondale, PA, USA) fitted with a Spherisorb ODS-2 octadecyl-bonded silica column (5 μm, 250×4 mm) using acetonitrile-water (86:14) as the mobile phase at a flow rate of 0.8 ml/min. Pyrene was detected by its absorbance at 240 nm. The values for butanol and Soxhlet extractions were summed to obtain total concentrations in soil.

An analysis of variance followed by Student–Newman–Keul's multiple comparison test was used to compare means.

3. Results

3.1. Field crops

Analysis of the soils at 0 days gave values of 78.4 mg of pyrene/kg that was extracted with *n*-butanol and 83.6 mg of total pyrene/kg soil.

Three species were tested: oat, lupine and rape. The concentration of pyrene (both total and butanol extractable) declined with time in the unplanted soil (Table 1). A more marked rate of disappearance was evident if plants were present. In contrast with the 18% loss of total extractable pyrene in plant-free soil in 56 days, 55% and 74% were lost from soil containing oats and rape, respectively. Similarly, in contrast with the loss of 25% of the butanol-extractable pyrene after 56 days in the absence of plants, 62% and 78% had disappeared in the presence of oats and rape, respectively.

3.2. Horticultural plants

A study was conducted with three horticultural plants: dill, pepper and radish. The test period was 28 days, and analysis of the soil at 0 days showed that it contained 78.7 mg of pyrene per kg that was extracted with butanol and 85.2 mg of total extractable pyrene per kg.

Table 1
Pyrene concentration in unplanted soil and soil planted to field crops

Fraction	Plant	Conc in soil (mg/kg)		
		14 days	32 days	56 days
Total	None	81.3Aa[a]	79.0Aa	68.5Ab
	Oat	78.0Aa	38.1Cb	37.3Bb
	Lupin	77.2Aa	65.1ABb	ND[b]
	Rape	68.1Aa	54.4BCb	22.2Cc
Butanol-extractable	None	72.9Aa	69.8Aa	58.5Ab
	Oat	68.4Aa	31.9Cb	29.9Bb
	Lupin	67.5Aa	56.0ABb	ND
	Rape	60.1Ab	47.0BCc	17.3Cd

[a] Values in columns followed by different capital letters are statistically different, and values in rows followed by different lower-case letters are significantly different ($P < 0.05$). The statistical comparisons in rows include the 0-day values.
[b] ND, not done.

Table 2
Pyrene concentration in unplanted soil and soil planted to horticultural species

Fraction	Plant	Conc in soil (mg/kg)	
		14 days	28 days
Total	None	81.4Aa	78.0Aa
	Dill	73.1Aa	40.8Bb
	Pepper	75.9Aa	27.7Bb
	Radish	77.6Aa	31.9Bb
Butanol-extractable	None	73.7Aa	64.0Aa
	Dill	65.4Aa	32.2Bb
	Pepper	67.9Aa	22.1Bb
	Raddish	69.7Aa	25.5Bb

In this instance, a statistically significant loss of total pyrene was not evident in plant-free soil in 28 days (Table 2). On the other hand, a rapid disappearance occurred in soils with plants. After 28 days, 52–68% of the total extractable pyrene had disappeared. Similarly, after 28 days, 59–72% of the butanol-extractable fraction of pyrene had disappeared. The data correspond to 8% and 19% decline in 28 days of total and butanol-extractable fractions, respectively, in soil containing no root systems.

3.3. Pine seedlings

Analysis of the soils at 0 days gave values of 75.2 mg of pyrene/kg that was extracted with *n*-butanol and 79.8 mg of total pyrene/kg of soil.

In these samples, pyrene disappearance from unplanted soil was more rapid than in the samples previously tested (Table 3). The reasons are not known. Nevertheless, a statistically significant effect of all three pine species was evident on both the butanol-extractable and total pyrene. This stimulation was even evident after 14 days in soil planted to red and white pine and at 28 days in soil beneath jack pine. In contrast with the 40% and 43% disappearance of total and butanol-extractable pyrene from plant-free soil after 57 days, approximately 60% had disappeared from soil beneath jack and red pines.

4. Discussion

High-molecular-weight PAHs constitute a group of pollutants whose biodegradation in contaminated soil often has not been successful (Wilson and Jones, 1993). The PAHs are characteristically very persistent in soils (Shuttleworth and Cerniglia, 1995). Accelerating the biodegradation of those compounds is thus a major challenge.

The present study was conducted to investigate the capability of different plant species to promote the destruction of pyrene in contaminated soil. The plants were grown in small amounts of soil to allow for an analysis of the entire soil rather than using only rhizosphere soil or random samples of soil. This is particularly important because the PAH content of samples of the same soil may vary from 42% to 128% in the field (Qui et al., 1994). This variation may prevent definitive conclusions on possible effects of phytoremediation.

The nine plants tested enhanced degradation as compared with unplanted soil. In eight weeks, as much as 74% of the aged pyrene disappeared from vegetated soil compared to 40% or less from unplanted soil. Much of the disappearance was from the butanol-extractable fraction of the PAH, the size of which is correlated with bioavailability (Kelsey et al., 1997). The enhancement may result from a plant-stimulated microbial transformation (Reilley et al., 1996). Some plants, particularly the tree seedlings, reduced the concentration of the fraction of pyrene not extracted by butanol, which suggests that they may be able to mobilize PAHs not available to other species. It is noteworthy that no relationship was evident between the mass of roots or shoots and the ability of the plants to bring about pyrene degradation (data not shown). This is consistent with the report that correlations between shoot or root

Table 3
Pyrene concentration in unplanted soil and soil planted to pines

Fraction	Plant	Conc in soil (mg/kg)		
		14 days	28 days	57 days
Total	None	81.5Aa	71.6Ab	47.9Ac
	Jack pine	83.6Aa	59.0Cb	34.5Bc
	Red pine	70.8Bb	66.8Bb	32.2Bc
	White pine	71.9Bb	65.5Bc	ND[a]
Butanol-extractable	None	73.2Aa	62.3Ab	42.9Ac
	Jack pine	75.1Aa	50.4Bb	30.4Bc
	Red pine	64.0Bb	59.6Ab	28.4Bc
	White pine	65.4Bb	57.4Ac	ND

[a] ND, not done.

weight and the degradation of petroleum hydrocarbons are poor (Wiltse et al., 1998).

All plant species increased the extents of pyrene degradation after eight weeks, and this effect was especially marked with rape. Although the extents did not differ significantly among the other species, the rates of degradation were different. Pepper and radish brought about the fastest decrease in pyrene concentration in 28 days, persumably because of faster biodegradation.

5. Conclusions

The data show that plants enhance the rate and extent of destruction of pyrene in soil. However, the effects of plants may not be detected because of the heterogeneity of distribution of PAHs in soil in the field and the possible temporary high pollutant concentration of these compounds in soil near the roots. The latter possibility will be shown in a separate paper from this laboratory. Therefore, preliminary plant-screening tests should be conducted either by use of small amounts of soil for complete extraction or by soil-sampling protocols that consider the possible movement of PAHs to plant roots.

Acknowledgements

This work was made possible by grant no. Li 740/1-3 from the Deutsche Forschungsgemeinschaft to the senior author and by funds provided by the DuPont Committee on Educational Aid and the Mellon Foundation.

References

Cunningham, S.D., Anderson, T.A., Schwab, A.P., Hsu, F.C., 1996. Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 56, 55–114.

Ferro, A.M., Sims, R.C., Bugbee, B., 1994. Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. J. Environ. Qual. 23, 272–279.

Kelsey, J.W., Kottler, B.D., Alexander, M., 1997. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. Environ. Sci. Technol. 31, 214–217.

Nyer, E.K., Gatliff, E.G., 1996. Phytoremediation. Ground Water Monitoring Remediation 16, 58–62.

Qiu, X., Shah, S.I., Kendall, E.W., Sorensen, D.L., Sims, R.C., Engelke, M.C., 1994. Grass-enhanced bioremediation for clay soils contaminated with polynuclear aromatic hydrocarbons. In: Anderson, T.A., Coats, J.R. (Eds.), Bioremediation Through Rhizosphere Technology. American Chemical Society, Washington, DC, pp. 142–157.

Reilley, K.A., Banks, M.K., Schwab, A.P., 1996. Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual. 25, 212–219.

Shimp, J.F., Tracy, J.C., Davis, L.C., Lee, E., Huang, W., Erickson, L.E., Schnoor, J.L., 1993. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. Environ. Sci. Technol. 23, 41–77.

Shuttleworth, K.L., Cerniglia, C.E., 1995. Environmental aspects of PAH biodegradation. Appl. Biochem. Biotechnol. 54, 291–302.

Wilson, S.C., Jones, K.C., 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs). Environ. Pollut. 81, 229–249.

Wiltse, C.C., Rooney, W.L., Chen, Z., Schwab, A.P., Banks, M.K., 1998. Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. J. Environ. Qual. 27, 169–173.