Published January, 1998

Plant and Environment Interactions

Adsorption of Naphthalene onto Plant Roots

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ABSTRACT

Higher plants are being used to enhance the remediation of soils contaminated with recalcitrant organic compounds, but the mechanisms of dissipation have not been established. One possible step in the phytoremediation process is adsorption of the organic contaminant onto the surface of the roots and subsequent uptake and/or degradation. To determine the affinity of plant roots for naphthalene, a polycyclic aromatic hydrocarbon, adsorption was quantified for tall fescue (*Festuca arundinacea* Schreber) and alfalfa (*Medicago sativa* L.). Equilibrium adsorption for naphthalene was determined for fresh roots of each species at three growth stages. For both fescue and alfalfa, adsorption was described by the Freundlich isotherm. Adsorption increased by as much as a factor of four with later growth stage of the plants. Alfalfa roots had approximately twice the affinity for naphthalene than fescue roots, despite a greater surface area per unit mass of root for fescue. Alfalfa also had a greater lipid content than fescue (10 g lipid/kg dry root vs. 4.5 g/kg), indicating that lipid content is a controlling factor in adsorption of naphthalene onto plant roots.

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic compounds with various numbers of condensed benzene rings. The physical and chemical characteristics of these compounds are dictated by the number and arrangement of the rings. All PAHs tend to be lipophilic, hydrophobic, and strongly adsorbed to hydrophobic surfaces. Although they are found in trace concentrations throughout the environment, elevated levels of PAHs can result from combustion, pyrolysis, and pyrosynthesis of organic materials. Concern arises over PAHs because of their mutagenic and carcinogenic effects on animals (Dipple et al., 1990; Thakker et al., 1985.)

Phytoremediation, the use of higher plants in the bioremediation systems, is a relatively new technology that is showing great promise (Erickson et al., 1994). The presence of plant roots and their associated microbial communities accelerates the biodegradation of various organic compounds, including pesticides, solvents, and petroleum products (Cunningham et al., 1996). However, many of the PAHs are resistant to degradation because of their high affinity for the surfaces of soil solids, low water solubility, and unfavorable energetics (Erickson et al., 1994). However, because these compounds are lipophilic, adsorption to the surfaces of roots may be an important sink for PAHs in soils and the first step in phytoremediation.

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Published in J. Environ. Qual. 27:220–224 (1998).

Plant Uptake of Polycyclic Aromatic Hydrocarbons

Uptake of PAHs has been studied for a wide variety of plant species. Concentrations of PAHs in crops were evaluated after the soil had been amended with sewage sludge (Wild et al., 1992). Adding sewage sludge for several years increased soil PAH content, and the PAHs persisted in the soils for many years (Wild and Jones, 1989). Carrots (*Daucos carota*) grown in soil amended with fresh sewage sludge accumulated low molecular weight PAHs in the outer layers of the roots (Wild and Jones, 1992), which then moved into the core, probably because of their moderate lipophilicity. However, PAH concentration in the carrot shoot was unaffected by the application of sludge with high PAH concentration.

Uptake, translocation, and metabolism of anthracene was studied in bush bean (*Phaseolus vulgaris* L.) roots grown in nutrient solution amended with ^{14}C-anthracene (Edwards, 1986). About 60% of the total ^{14}C was found in the roots during flowering and seed production. Kirchmann and Tengsved (1991) analyzed for PAHs in barley (*Hordeum vulgare* L.) grown in soil amended with sludge, a slurry of swine manure, and untreated soil. Polycyclic aromatic hydrocarbons accumulated in the grain at trace concentrations, independent of the type of amendment used. The metabolism of benzo[a]-pyrene was studied in cell suspension cultures of parsley (*Petroselinum hortense*) and soybean [*Glycine max* (L.) Merr.] (Trenck and Sandermann, 1979). From 1.0 to 2.2% of benzo[a]pyrene was sorbed by the parsley cells and 19 to 28% by soybean cells, indicating that affinity depends on plant species.

Contamination of the aboveground portions of plants by PAHs normally is the result of atmospheric deposition (Wild et al., 1992), with the pollutants originating from automobile exhaust and industrial emissions (Kirchmann and Tengsved, 1991; Edwards, 1983, 1986; Jones, 1991). Benzo[a]pyrene concentrations in the air of urban areas of the USA range from 0.1 to 61.0 μg/m^3, but concentrations in nonurban areas range from only 0.01 to 1.9 μg/m^3 (Edwards, 1983). Airborne, low molecular weight PAHs are found predominantly in the vapor phase, whereas the multiringed compounds usually are associated with suspended particulates. Those PAHs in the vapor phase can deposit on the leaf surface and become strongly adsorbed to the tissue.

Sims and Overcash (1983) reported that plant shoots contained PAH concentrations from 22 to 88 μg/kg, whereas underground vegetables had lower concentrations (0.01–6.0 μg/kg). They also found that photo-

Abbreviations: PAH, polycyclic aromatic hydrocarbons; GC, gas chromatograph; FID, flame ionization detector; RCF, root concentration factor.

decomposition, oxidation, and hydrolysis were not important mechanisms for PAH degradation in soil environments.

Adsorption of Organic Contaminants onto Plant Roots

Tames and Hance (1969) investigated the adsorption of pesticides by dead roots of several plant species including oat (*Avena sativa* L.), bean, pea (*Pisum sativum* L.), cucumber (*Cucumis sativus* L.), and radish (*Raphanus sativus* L.). Significant differences were observed among the plant species in their adsorption capacity and also among contaminants in the extent to which they were adsorbed by any one species.

Accumulation of nonionized compounds by roots can be the result of active uptake or surface adsorption, and both mechanisms are controlled by the chemical and physical properties of the compound and plant roots. Uptake has been shown to consist of two processes: (i) establishing equilibrium between the concentrations of the compound of interest in the solution within the root and the solution surrounding the root and (ii) sorption of the chemical onto the lipid and lipid-like solids of the roots (Briggs et al., 1982). Adsorption onto root lipophilic compounds is important for compounds that also are lipophilic, that is, compounds with an octanol-water partitioning coefficient (log K_{ow}) of 1.5 or greater.

Adsorption may be affected by stage of plant growth, because the chemical and physical properties of roots change significantly over the life of the plant (Harley and Russell, 1979). Older roots tend to be thicker and have less surface area per unit mass. As roots age, some of their capabilities for exudation, water uptake, and assimilation of solutes may be diminished. Environmental factors such as moisture stress or nutrient supply may have a profound effect on root development and function at all stages of growth.

Adsorption Isotherms

The Freundlich (1926) isotherm can be used to model equilibrium adsorption data:

$$q = K_f c_e^{1/n} \quad [1]$$

where q is the mass of chemical adsorbed per unit weight of solid adsorbent, K_f is the Freundlich adsorption coefficient, c_e is the concentration in bulk solution at equilibrium, and 1/n is a measure of nonlinearity. For adsorption onto roots, units for c_e may be mg/L, and 1/n is dimensionless. When adsorption is determined on a root-mass basis, the units for q are g adsorbate/kg adsorbent and the units for K_f are $(g/kg)(mol/L)^{-1/n}$. For adsorption determined on a root-surface-area basis, units of q are mg/m^2 and units for K_f are $(mg/m^2)(mol/L)^{-1/n}$.

The objective of this research was to quantify the adsorption of naphthalene, a PAH, by the roots of two plant species as a function of plant maturity. The test plants were tall fescue and alfalfa; both species are effective in phytoremediation (Reilley et al., 1996) but have quite different root architectures. Data from our experiments eventually will be used in models that predict the fate of petroleum hydrocarbons in contaminated soils.

MATERIALS AND METHODS

Plant Cultivation and Root Preparation

Tall fescue and alfalfa were grown in potting soil in a greenhouse under sodium vapor lights with 12 h of illumination and 12 h of darkness. The temperature was kept constant at 21°C. Plants were watered daily and fertilized every 14 d with 100 mL of water containing 1.25 g/L of Miracle-Gro (Stern's Miracle-Gro Products, Inc; Port Washington, NY). After 3 mo, fescue plants were transferred to a different room in the greenhouse without supplemental lighting to accelerate the flowering stage. After 4 wk, the fescue plants were returned to their original environmental conditions.

Roots were harvested at three growth stages: vegetative (84 d), flowering (approximately 115 d), and mature (approximately 150 d). Roots were removed from the soil at each stage, washed with water, dried with blotting paper, and weighed. The root surface area for each individual plant was determined with the area measurement system of the ΔT Area Meter LTD (Burwell; Cambridge, England). The system employed a video camera, video monitor, camera stand, light box, and area meter. A root sample was placed on the light box, and the video camera scanned the roots. The roots were quantified if the area meter identified the root as contrasting to the background. Output was displayed on the video monitor together with a superimposed image of the actual measured area. A digital display showed the area of the roots in view, the number of measurements made, and the total area. Individual roots were subsampled to reduce the intersection of fine root hairs with each other, and the results were summed. Roots were stored for 48 to 72 h at 4°C until used for adsorption determinations.

Naphthalene Adsorption to Plant Roots

A naphthalene stock solution was prepared by mixing the solid (99.3% purity, Sigma Chemical) with deionized distilled water. This solution was stirred overnight and then filtered through Whatman no. 42 filter. The concentration of dissolved naphthalene was determined by gas chromatography to be 18.4 mg L^{-1}.

Ten grams each of alfalfa and fescue roots were cut into 1- to 2-cm sections and placed in 250-mL Erlenmeyer flasks each containing 100 mL of naphthalene solutions. Five initial concentrations, ranging from 0.5 to 5 mg L^{-1}, were established in triplicate. Control samples containing naphthalene at each concentration without plant roots were established to monitor losses of naphthalene through volatilization and degradation. The mass of naphthalene lost in this manner is designated C_{VOL}. Blank samples (roots equilibrated with water only) were analyzed to confirm that the roots were initially free of naphthalene. All flasks were shaken on a reciprocal shaker at 120 cycles s^{-1} for 12 h at room temperature, and the solutions were filtered through Whatman no. 1 paper. Subsamples of 20 mL were analyzed for aqueous naphthalene concentration (c_e), and this mass in solution is designated C_{SOL}. The roots were shaken briefly with 15 mL of 0.01 *M* $CaCl_2$, and the solution was analyzed for naphthalene by gas chromatography (see below). This represents the naphthalene dissolved in the aqueous solution within the apparent free space of the roots (but not adsorbed), and this entrained mass is designated as C_{ENT}. Total mass adsorbed (C_{ADS}) was determined by extracting 1 to 3 g of roots with 50 mL methanol for 2 min in a stainless steel blender (Model 1003, Fisher Scientific, St. Louis, MO). The extracts were diluted with water to obtain a 20:80 methanol/water mixture from which naphthalene was recovered using solid phase extraction with C_{18} Sep-Pak car-

Table 1. Mass balance for the naphthalene adsorption experiment including C_{VOL}, volatilization; C_{ENT}, mass entrained (but nonadsorbed) solution within the roots; C_{SOL}, naphthalene in solution; and, C_{ADS}, adsorbed on the roots. Values are means for three concentrations for the vegetative stage and five concentrations for the flowering stage.

Species	Growth stage	C_{VOL}	C_{ENT}	C_{SOL}	C_{ADS}	Total
		%				
Fescue	Vegetative	36 (8)†	2.2 (0.2)	35 (12)	8.8 (1.9)	82 (14)
Alfalfa	Vegetative	36 (8)	1.7 (0.4)	31 (3)	15 (1.1)	84 (6)
Fescue	Flowering	45 (10)	1.0 (0.3)	27 (5)	15 (4)	88 (6)
Alfalfa	Flowering	45 (10)	0.8 (0.2)	14 (2)	24 (2)	84 (8)
Fescue	Mature	32 (10)	3.4 (1.0)	27 (5)	13 (4)	75 (9)
Alfalfa	Mature	32 (10)	2.1 (0.6)	15 (3)	30 (9)	79 (13)

† Mean with standard deviation in parentheses.

tridges (Waters Chromatography, Milford, MA) and analyzed by gas chromatography.

The mass balance for naphthalene can be expressed as:

$$C_{TOT} = C_{VOL} + C_{ENT} + C_{SOL} + C_{ADS} \quad [2]$$

where C_{TOT} is the total mass of recovered naphthalene.

Analytical Procedures

Naphthalene was analyzed using a Hewlett-Packard 5890A gas chromatograph (GC), equipped with an HP 3396A Integrator, an Autosampler HP7673A, J&W DB-5 capillary columns, and a flame ionization detector (FID). Zero grade air was the oxidant, H_2 was the carrier gas and fuel for the FID, and N_2 was the make-up gas. The temperature of the GC oven was programmed to run from 40 to 300°C during each analysis. Detection limits were approximately 300 μg/kg for adsorption to the roots and 30 μg/L in the aqueous phase.

Lipid concentrations in alfalfa and fescue roots were determined by ether extraction (Frankenberger and Abdelmagid, 1985; Waksman and Tenney, 1927). All the root material in the first two growth stages was used in the adsorption experiments. Therefore, only mature alfalfa and fescue roots were used in the lipid determinations. Five grams of roots were dried for 48 h at 65°C and extracted for 8 h with petroleum ether by soxhlet extraction. The extracts were evaporated at room temperature for 24 h, and the remaining mass was assumed to be lipid material.

Data Analysis

The Freundlich isotherm was found to represent naphthalene adsorption onto fescue and alfalfa roots. The analytically determined equilibrium concentrations of naphthalene in the aqueous and adsorbed phases were employed to compute K_f and 1/n by regression analysis of $\log(q)$ vs. $\log(c_e)$:

$$\log(q) = \log(K_f) + \frac{1}{n}\log(c_e) \quad [3]$$

where 1/n is the slope of the line and $\log(K_f)$ is the y intercept. All statistical analyses were performed with COSTAT (Cohort Software; Berkeley, CA).

RESULTS AND DISCUSSION

Mass Balance during Adsorption

The measurement of the adsorption of PAHs to organic solids is confounded by low aqueous solubility and high affinity for most surfaces. Naphthalene was chosen for this study because it is the most soluble PAH (approximately 35 mg/L) and has the lowest affinity for solid surfaces. Unfortunately, naphthalene also is the most volatile and easily degradable of the PAHs. A complete mass balance for naphthalene was conducted for each growth stage to help account for losses by volatilization and degradation.

For all three growth stages, the mass volatilized (C_{VOL}) was the largest component of the mass balance and ranged from 32 to 45% (Table 1). The mass in solution (C_{SOL}) usually was greater than that adsorbed to the roots (C_{ADS}), and the mass of naphthalene in the solution entrained around the roots after adsorption (C_{ENT}) was small. Total recoveries ranged from 75 to 89%. The naphthalene that was not recovered in this procedure was either degraded biologically in the presence of the roots or assimilated to the point that it could not be extracted by methanol.

In this experiment, the mass adsorbed to the roots was determined directly by extracting the naphthalene from the roots. Adsorption often is assumed to be the difference in concentration in the aqueous phase before and after exposure to the adsorbing medium. In the case of naphthalene, volatilization and degradation accounted for a significant fraction of the change in aqueous concentrations during the adsorption experiment. By quantifying these components of loss, potentially large errors in the mass adsorbed were avoided.

Naphthalene Adsorption to Roots

The Freundlich isotherm was a satisfactory choice for the adsorption model, with r^2 ranging from 0.82 to 0.97

Table 2. Freundlich isotherm parameters for adsorption of naphthalene onto fescue and alfalfa roots as a function of time, and expressed on the basis of root surface area or mass of roots.

	Root surface area basis				Root mass basis			
	Fescue		Alfalfa		Fescue		Alfalfa	
Growth stage	K_f†	1/n	K_f	1/n	K_f	1/n	K_f	1/n
Vegetative	2.43a	0.697‡	14.4a§	0.855	0.0172a	0.655‡	0.0333a§	0.787
Flowering	17.2b	1.24	35.8b	0.804	0.0361b	1.08	0.0360a	0.809
Mature	10.4b	1.11	52.7b§	1.07	0.0282b	1.13	0.0593a§	1.11

† Values of K_f in a given column followed by the same letter are not statistically different ($P < 0.05$).
‡ Values of 1/n with this designation are statistically <1.00 ($P < 0.05$).
§ At vegetative and mature stages, K_f values for alfalfa were significantly different than those for fescue ($P < 0.05$).

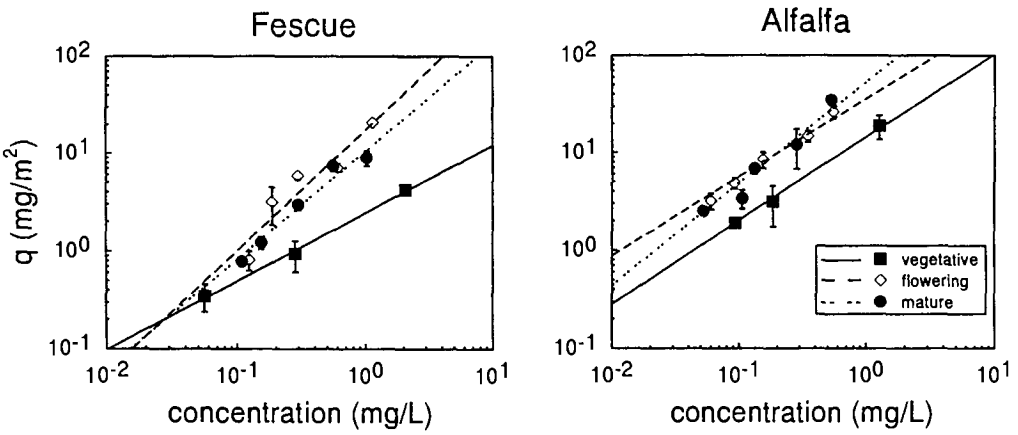

Fig. 1. Isotherms for naphthalene adsorption onto fescue and alfalfa roots expressed on a surface area basis. The symbols represent means of three experimental observations, and lines are corresponding Freundlich models for each stage of plant growth. Error bars are 95% confidence intervals for adsorption at a given initial concentration.

(data not shown). A linear adsorption model (K_d) was not appropriate, because the exponent (1/n) in the Freundlich model was significantly different than 1.0 (i.e., q was not a linear function of c_e) in two cases at $P < 0.05$ (Table 2) and in seven cases at $P < 0.10$.

The three factors that have affected previous evaluations of naphthalene adsorption to roots were root architecture, growth stage of the plants, and measuring adsorption on a root surface area basis vs. a mass basis. Alfalfa and fescue were chosen for this study because alfalfa tends to have a tap root with coarse laterals, whereas fescue has highly fibrous roots with a high degree of branching. The greater surface area associated with fescue roots (2.6 m^2/kg) was expected to enhance adsorption relative to alfalfa (1.0 m^2/kg).

The affinity of naphthalene for alfalfa roots was greater than that for fescue roots (Fig. 1, 2; Table 2). Values of K_f for alfalfa were significantly greater than those for fescue at the vegetative and mature stages. This was true on both root mass and root surface area bases. However, the ratio of $K_{f,alfalfa}/K_{f,fescue}$ was much greater on a root surface area basis (Fig. 1) than on a mass basis (Fig. 2) because of the much greater surface area per unit mass for fescue. Values of K_f for both fescue and alfalfa increased with time. Significant differences were observed for K_f at 84 and 154 d for both species on a surface area basis and fescue on a mass basis.

For the interaction of nonionic organic compounds with barley roots, Briggs et al. (1982) established the following equation describing the relationship between the root concentration factor (RCF, equivalent to a linear adsorption coefficient, K_d, for roots) and K_{ow} of the chemical:

$$\log \text{RCF} = 0.77 \log K_{ow} - 1.52 \quad [4]$$

This equation predicts that the root and solution will have equal affinity (i.e., RCF = 1) for a chemical with $\log K_{ow} = 2.0$. For naphthalene, with $\log K_{ow} = 3.4$, Eq. 4 predicts an RCF of 10 for barley roots. This equation was generalized to establish a partition coefficient (K_{RW}) on a mass basis between solution and roots of other plants to account for differences in water content (W_R, mass of water per mass of fresh root); lipid content of the roots (l_R); and density of the fresh roots (ρ_R):

$$K_{RW} = (W_R + l_R K_{OW}^b)(\rho_R/\rho_W) \quad [5]$$

where b is a correction factor for differences between plant lipids and octanol, and ρ_W is the density of the solution. The RCF in Eq.[4] can be converted directly to K_{RW} by multiplying it by the ratio ρ_R/ρ_W. Values of W_R always will be <1.0 and will contribute little to K_{RW}

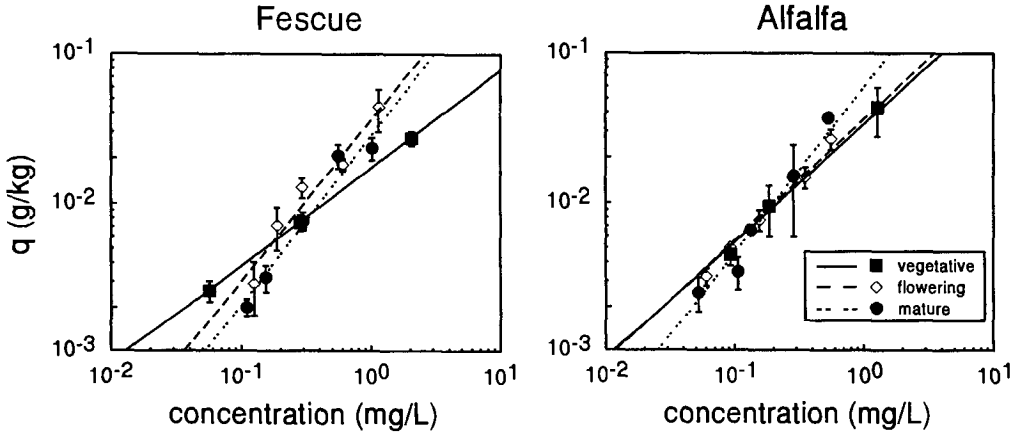

Fig. 2. Isotherms for naphthalene adsorption onto fescue and alfalfa roots expressed on a mass basis. The symbols represent means of three experimental observations, and lines are corresponding Freundlich models for each stage of plant growth. Error bars are 95% confidence intervals for adsorption at a given initial concentration.

for naphthalene. Thus, assuming that b is constant, any differences in K_{RW} between plant species or growth stages should depend only upon the lipid contents of the roots.

In the present study, *K_f (mass basis) for mature alfalfa roots exceeded K_f* for mature fescue roots by a factor of approximately two. Because 1/n was nearly identical for each species at this stage of growth (154 d), the ratio K_{RW}, alfalfa/K_{RW}, fescue would be equivalent to the ratio $K_{f,alfalfa}/K_{f,fescue}$. In the expression for K_{RW} for each species (Eq. [5]), the only difference between alfalfa and fescue would be l_R, the lipid content. Thus, we would expect the lipid content of alfalfa to exceed that of fescue by a factor of approximately two. The experimentally determined lipid contents were 10 ± 1 (g lipid/kg dry root) for alfalfa and 4.5 ± 0.8 (g lipid/kg dry root) for fescue. Therefore, the model of Briggs et al. (1982) appears to describe our system, and lipid concentration may have been the controlling factor for naphthalene adsorption to the roots.

One of the possible mechanisms by which plants are able to increase dissipation of these strongly adsorbed, recalcitrant compounds is by partitioning PAHs from the soil and onto the plant roots (Cunningham et al., 1996). The affinity of naphthalene for soil is related to its K_{oc}, the linear partition coefficient of naphthalene between the soil solution phase and soil organic carbon. Naphthalene has a K_{oc} of 980 (Knox et al., 1993), which is similar in magnitude to the $K_{oc} = 300$ for alfalfa roots (K_f converted to K_{oc} based on C content). Thus, as roots explore the soil, naphthalene affinity for roots and soil will be similar, and desorption from the soil and subsequent adsorption onto the root surfaces are possible.

Naphthalene adsorption on alfalfa and fescue roots increased with plant age (Table 2). This was due to the greater total root mass (or surface area) as well as the enhanced affinity of the roots for the naphthalene. Although naphthalene is adsorbed more strongly onto alfalfa roots than fescue per unit mass, fescue roots are present in much greater quantities in the soil (Barber, 1984), likely resulting in no difference between the two plants.

The PAHs with three or more benzene rings will be far less soluble than naphthalene and have much greater affinity for lipophilic solids. From the results in this study, we anticipate that the affinity of the PAHs for roots will be similar to that for soil solids. Partitioning of PAHs from the soil to the roots is possible, but the very high K_{ow} and low solubility suggest that the roots would have to be in very close proximity to the soil adsorption site before PAHs could be transferred to the root surface. Therefore, if desorption from soil is a critical step in the degradation of PAHs by rhizosphere microorganisms, plants with extensive rooting systems probably would be advantageous for phytoremediation of contaminated soil.

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