

A Model for the Effect of Rhizodeposition on the Fate of Phenanthrene in Aged Contaminated Soil

R. KAMATH,[†] J. L. SCHNOOR, AND P. J. J. ALVAREZ*

Department of Civil and Environmental Engineering, University of Iowa, Iowa City, Iowa 52242

Microcosm data were used to develop a deterministic model to describe how rhizodeposition affects the fate of phenanthrene in aged contaminated soil. Microbial mineralization and soil sequestration of ¹⁴C-phenanthrene were compared in microcosms amended weekly with phenolic-rich mulberry root extracts versus unamended controls. Mineralization was higher in the amended soils simulating the rhizosphere ($57.7 \pm 0.9\%$) than in controls simulating bulk (unplanted) soils ($53.2 \pm 0.7\%$) after 201 days ($p < 0.05$). Humic was the main soil sink for the residual ¹⁴C-label. Whereas the total ¹⁴C-label associated with humin remained constant in biologically active soils (at about 30%), it increased up to 80% after 201 days in sterile controls. The initial phenanthrene extraction with *n*-butanol (commonly used to assess bioavailability) slightly underestimated the fraction that was mineralized (assessed by ¹⁴CO₂ recovery). Changes in the unextractable fraction (determined by combustion in a biological oxidizer) suggested the presence of two soil sequestration domains: (1) irreversibly bound residue, and (2) an intermediate transition phase that is unextractable by solvents at a given point in time but could become bioavailable due to physicochemical or biological transformations of the binding matrix. The fate of phenanthrene was accurately modeled by considering the transfer of the ¹⁴C label between different soil compartments as first-order kinetic processes. Model simulations suggested that the system was approaching a stable end-point after 201 days of simulated rhizoremediation, and corroborated that microorganisms have a significant impact on the fate of phenanthrene in soil.

Introduction

Phytoremediation, which exploits natural plant processes to enhance the degradation and removal of environmental pollutants, is receiving increasing attention to treat soils contaminated with polynuclear aromatic hydrocarbons (PAHs) (1). Many studies have shown that rhizodeposition (i.e., the release of plant-derived substrates through exudation of soluble plant products plus root turnover (2)) enhances biodegradation by favorably altering the composition and concentration of the soil microbial community (3–5).

However, most of these PAH rhizoremediation studies were conducted with freshly contaminated soils, as a result of which the contamination was generally more bioavailable and easier to remove than in most sites where PAHs and soils have been in contact for several years. Contaminant aging decreases PAH bioavailability due to increased binding to the soil organic matter (SOM) (6–10). Specifically, increased binding with the soil humin (the insoluble fraction of humic matter) has been shown to be a main cause for decreased biodegradation (8–10, 12).

Whereas it is important to determine how plants influence PAH sequestration and biodegradation in aged contaminated soils, very few studies have addressed this issue. Yang et al. (13) showed that adding 0.01 M citrate (an organic acid found in root exudates) significantly increased phenanthrene desorption from soil. Apparently, citrate destabilized humic material by chelating polyvalent metals such as Fe, Al, and Ca that served as structural components. Furthermore, it has been suggested that acidification (pH < 5) by organic acids can modulate the size of humic molecules and their potential to sorb contaminants (14). Thus, it is possible that continuous throughput of organic acids and/or chelators during rhizodeposition could enhance PAH bioavailability. However, it is unknown how rhizodeposition affects abiotic sequestration mechanisms and the partitioning of PAHs (or metabolites) into different SOM domains, and no mathematical models exist to interpret the rhizosphere effect on PAH bioavailability.

The nature of PAH sequestration by SOM is of important consideration. Studies with pyrene-contaminated soils showed that while metabolites were covalently bound to the soil matrix, sorption of pyrene primarily involved entrapment by hydrophobic interactions following diffusion into micropores and voids in the humin fraction (10, 15). Northcott et al. (9) reported increased recovery of PAHs from aged soils following base saponification, corroborating that diffusion of PAHs into the soil matrix is an important sequestration mechanism. These results support two similar conceptual models for sorption of hydrophobic organic contaminants to SOM: the distributed reactivity model (DRM) (16) and the dual reactive domain model (DRDM) (17).

Weber et al. (16) postulated that SOM is comprised of two domains: highly amorphous and relatively condensed organic carbon. Xing et al. (17) similarly conceptualized SOM as an amalgam of rubbery and glassy carbon phases. Thus, it is generally accepted that soils exhibit at least two general sorption domains (18). PAH sorption in the “amorphous”, “rubbery”, “soft polymeric”, or “gel like” carbonaceous matrix is generally considered to be linear, fast, and reversible. In contrast, sorption onto the “condensed”, “glassy”, “black”, “hard” and/or “microporous” domains is considered nonlinear, slow, and hysteretic (16–22). Thus, PAHs sorbed in the first domain would be readily bioavailable, while PAHs sorbed in black/condensed SOM might be poorly bioavailable. PAH sequestration in this latter domain might be an acceptable treatment endpoint, although little is known about the potential for PAH release from this phase and incorporation into food webs.

In summary, microbial-plant interactions that influence PAH bioavailability and biodegradation are not fully understood, and there is a need for a model to interpret how these interactions affect the fate of PAHs in the rhizosphere. This paper presents the first model to quantify the long-term effect of rhizodeposition on the fate of a model PAH, phenanthrene, in aged contaminated soil, including mineralization and sequestration into (or release from) different soil domains.

* Corresponding author current address: Department of Civil and Environmental Engineering, Rice University, Houston, Texas 77005; phone: (713) 348-5903; fax: (713) 348-5203; e-mail: alvarez@rice.edu.

[†] Current address: Groundwater Services, Inc., 2211 Norfolk Suite 1000, Houston, Texas 77098-4054; phone: (713) 522 6300; fax: (713) 522 8010.

TABLE 1. Physical Characteristics of Amana Soil^a

pH	7.2
organic matter content (%)	3.9
particle distribution	
sand	47.5
silt	40
clay	12.5
cation exchange capacity	12.1

^aMinnesota Valley Testing Labs, Nevada, IA.

Experimental Section

General Approach. Soil microcosm experiments were conducted to investigate how rhizodeposition during phytoremediation affects the fate of ¹⁴C-labeled phenanthrene in aged contaminated soil, and to generate data for model development. Rhizodeposition was physically simulated by periodic addition of root extracts, and bioavailability, mineralization, and soil sequestration were monitored as described below. Deterministic model simulations and data analyses were also conducted to discern the relative rates of PAH sequestration and desorption caused by biological and abiotic reactions.

Collection and Characterization of Root Extracts. Previous studies showed that root extracts from mulberry trees are an effective bacterial growth substrate that is rich in phenolic compounds that promote the proliferation of aromatic degraders (i.e., analogue enrichment). Root extracts were collected as described earlier (23). Briefly, dark, fine roots (<1 mm), which are representative of the root turn over material that predominates in rhizodeposition (2), were used as source of root extract. This material was homogenized in distilled water using a hand homogenizer (Biospec Products Inc, Bartlesville, OK), centrifuged at 13 000 rpm, and filter-sterilized (0.22 μm) prior to storage at 4 °C.

Soil Characteristics and Aging with Phenanthrene. Uncontaminated soil (Table 1) was spiked with unlabeled and 9-¹⁴C-labeled phenanthrene (300 mg kg-soil⁻¹ and 10 000 dpm·g-soil⁻¹). This was accomplished by dissolving the phenanthrene in 300 mL of acetone and adding this mixture to 500 g of soil. After a few hours of shaking, acetone was evaporated in a ventilated fume hood and the soil was mixed with an equal volume of clean soil to make up 1 kg. To minimize biological activity (24), the contaminated soil was subsequently air-dried in the fume hood and aged in the dark at 20 °C for 518 days prior to initiating simulated rhizodeposition. Natural wet-dry or freeze-thaw cycles, which can promote PAH sequestration (24), were not replicated here. Whereas such (climate-dependent) cycles could accelerate the decrease in phenanthrene bioavailability, this goal can also be achieved by extended aging.

HPLC analysis with radiochromatographic detection was conducted after the aging period to determine whether the radiolabel remained associated with phenanthrene. Soil samples (10 g) were extracted with acetonitrile. Analysis was performed using an Agilent 1100 HPLC equipped with a LC-PAH column (Supelco Inc., Bellefonte, PA [4.6 × 300 mm]) and a Radiomatic, Series A-500 detector (Packard Instrument Co.). This analysis showed that all of the detectable radiolabel was still associated with phenanthrene after the 518-day aging period. Apparently, the dry conditions inhibited biotransformation.

Microcosms. The fate of phenanthrene in aged soil was investigated in 100-mL serum bottles containing 30 g of [9-¹⁴C]-phenanthrene-contaminated soil (300 000 dpm-reactor⁻¹) and 100 mL of Hutner's mineral medium (25). Four treatment sets were prepared, with 9 sacrificial replicates for each treatment. Microcosms simulating rhizodeposition (rhizosphere microcosms) were amended with 2 mL of

mulberry root extract (1000 mg-C·L⁻¹) on a weekly basis, resulting in a dissolved organic carbon concentration of 20 mg-C·L⁻¹. This concentration is on the low end of total organic carbon concentrations reported for root exudation (5, 26), to ensure root extract availability over the 30-week duration of the experiment. A similar liquid volume (2 mL) was withdrawn for analysis prior to root extract addition. Microcosms simulating unplanted soil (unamended microcosms) were treated similarly, except that distilled water was used instead of root extracts. Sterile (poisoned) controls (sterile rhizosphere and sterile bulk microcosms) had similar setup but were poisoned with HgCl₂ (50 mg L⁻¹) and NaN₃ (0.13 g L⁻¹).

To determine mineralization, a vial containing 5 mL of 1 M NaOH was added to each reactor to trap any ¹⁴CO₂ evolved during microbial metabolism of [9-¹⁴C]-phenanthrene. The traps were changed every 10 days and analyzed for ¹⁴C using the liquid scintillation counter LS6001C (Beckman Coulter, Fullerton, CA). To estimate the solution phase ¹⁴C, the 2-mL aqueous samples were centrifuged and analyzed by liquid scintillation counting (LSC) after ensuring that a minimum 2:1 signal-to-noise ratio was achieved. Microcosms were periodically sacrificed to investigate the effect of simulated rhizodeposition on the bioavailability and partitioning of the ¹⁴C-phenanthrene into different SOM domains.

Soil Extractions. Analysis of Phenanthrene Bioavailability. Extraction by *n*-butanol has been shown to be a good indicator of phenanthrene bioavailability to bacteria (27). This protocol was modified as follows: 10 g of air-dried soil samples were vigorously shaken for 2 min with 15 mL of *n*-butanol in a vortex mixer, and then centrifuged at 10 000 rpm. The extraction was repeated with 10 mL of *n*-butanol and the supernatant was stored at 4 °C until analysis by LSC.

Butanol extraction was followed by extraction with dichloromethane (DCM), which is a more hydrophobic solvent. The soil sample was sonicated three times in half-hour intervals with 10 mL of DCM. The extract was collected and evaporated to dryness to remove DCM, which interferes with LSC, and the residue was resuspended in acetonitrile. The sample was then analyzed for ¹⁴C by LSC.

The residual phenanthrene bound to the soil after butanol and DCM extraction was operationally defined as the nonlabile or unextractable fraction, and was measured by LSC following combustion of the bound residue to ¹⁴CO₂ using a Biological Oxidizer OX600 (R. J. Harvey Instrument Co.). This unextractable portion represents covalently bound metabolites as well as the phenanthrene that diffused into nanopores of the soil matrix that could not be extracted by butanol or DCM. Recovery efficiency for the biological oxidizer was measured by spiking 500 mg of mannose with 10 μL of ¹⁴C-phenanthrene. Recovery was greater than 90% throughout the course of the experiment.

Fractionation of Phenanthrene into Soil Organic Fractions. To determine whether humification of phenanthrene in the simulated contaminated rhizosphere was significantly different from that in contaminated bulk soil, the following protocol was used: 100 mL of 0.1 M NaOH was added to 10 g of air-dried soil from each reactor. The sample bottles were purged with N₂ and shaken for 24 h at room temperature. The samples were centrifuged for 10 min at 2500 rpm. The base-extracted centrifugate (28) (henceforth referred to as humin residue) was washed with distilled water and then air-dried and analyzed for ¹⁴C-content in the biological oxidizer. The supernatant was acidified to pH 2 with 2 M HCl to separate the soluble fulvic fraction from the insoluble humic fraction. The humic fraction was air-dried, weighed, and analyzed for ¹⁴C using the biological oxidizer. To extract and concentrate the ¹⁴C in the fulvic acid fraction, 30 mL of hexane was added to 100 mL of the fulvic fraction and shaken for 24 h. After centrifugation, the hexane supernatant was

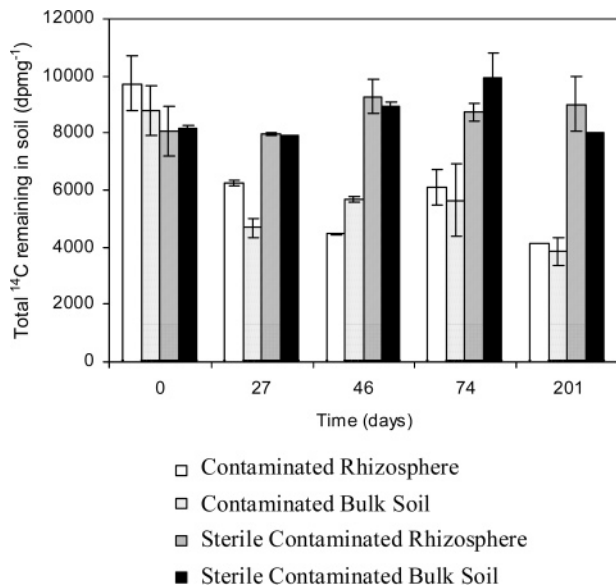


FIGURE 1. Total ¹⁴C remaining in soil (dpm-g-soil⁻¹) from microcosms sacrificed during the experiment. Data were obtained by combustion in a biological oxidizer with LSC. Loss of ¹⁴C was mainly due to mineralization. Error bars represent the range of duplicate measurements.

evaporated and the residue was resuspended in 10 mL of acetonitrile.

Results and Discussion

Unlike previous experiments that investigated the fate of PAHs in freshly contaminated soils, this study was conducted with “aged” contaminated soils. This distinction is important to delineate the capability and limitations of rhizoremediation, where plants are generally introduced years after contamination has occurred and weathering processes have decreased PAH bioavailability. Note that aging was conducted under air-dried conditions to inhibit phenanthrene biodegradation during the aging period, which was conducive to having sufficient contamination remaining for the simulated rhizodeposition experiment. Whether microcrystalline phenanthrene structures were formed during the aging period following solvent evaporation was not investigated. Crystallization could hinder phenanthrene partitioning into less bioavailable soil domains, although the high organic content of this soil ($f_{oc} = 0.039$) and the extended aging period (518 days) were conducive to extensive phenanthrene sorption.

After 518 days of aging but prior to initiating simulated rhizodeposition, an average of 87.8 (± 0.5) % of the initially added ¹⁴C (300 000 dpm/reactor) was recovered from the aged soils using the biological oxidizer (Figure 1, $t = 0$). Radiochromatographic analysis of acetonitrile-extracted ¹⁴C indicated that all of the radiolabel remained associated with a single peak corresponding to phenanthrene. The percentage of radiolabel recovered after the aging period is similar to that reported by other studies, such as 101 (± 7) % after 525 days (9); 96.6% after 100 days (8); and 77.93 (± 12.53) % after 244 days (29). The overall high recovery of ¹⁴C indicates that phenanthrene volatilization was not significant during the aging process.

Overall mass balances (based on ¹⁴C recovery from mineralization, extraction, and combustion of bound residue) were also relatively high for the simulated rhizodeposition experiment, averaging 90.2 \pm 8.3% for all treatments at the end of the 201-day incubation period (Table 2). The recovery was higher for unextracted soil, considering mineralization and combustion alone (averaging 99.7 \pm 5.0%, data not

TABLE 2. Overall Mass Balances for Rhizodeposition Experiment after 201 Days of Incubation, Expressed as Percentage of Initial ¹⁴C

microcosm	mineralized (¹⁴ CO ₂)	total extractable (butanol + DCM)	bound residue (recovered by combustion)	total recovery
rhizosphere	57.7 \pm 0.9	8.0	29.6	95.2
bulk soil	53.2 \pm 0.7	8.8	34.9	97.0
sterile rhizosphere	N.D.	72.5	6.1	78.6
sterile bulk soil	N.D.	74.8	15.4	90.2

shown), indicating that some losses occurred during the sequential extraction procedure.

¹⁴C-Phenanthrene Mineralization. Simulated rhizodeposition (20 mg-C L⁻¹ week⁻¹) enhanced phenanthrene mineralization in the aged soil relative to unamended controls, possibly due to the combined effect of a healthier and larger microbial population (30) and increased phenanthrene bioavailability. After 201 days of incubation, ¹⁴C-phenanthrene mineralization reached 57.7 \pm 0.9% in the rhizosphere and 53.2 \pm 0.7% in the bulk soil microcosms (Figure 2A). Since no other ¹⁴C-phenanthrene degradation study with aged soils has been carried out for this long, comparisons of the overall extent of mineralization in aged soils are difficult. However, after 74 days of the experiment, which covered the rapid mineralization phase (Figure 2B), approximately 34% of the ¹⁴C was recovered as ¹⁴CO₂ in the bulk soil microcosms. This is slightly lower than the 48% reported by Nam and Kim (8), 45% reported by Hatzinger et al. (7), and 46% reported by Namhyun et al. (31) for spiked soils aged for 100, 84, and 200 days, respectively. Our soils were aged for 518 days and thus, presumably had reduced bioavailability compared to the other studies, which is conducive to a lower extent of rapid mineralization. No mineralization was observed in sterile controls.

The difference between the extent of mineralization in the treatments simulating rhizosphere and bulk soils, although statistically significant at the 95% level, was relatively small (Figure 2A). This corroborates previous research showing that the benefit of plant roots on PAH removal efficiency is less pronounced in aged contaminated soils than in freshly spiked soils (3, 32–34).

Enhanced mineralization due to simulated rhizodeposition occurred only after 46 days, after the readily bioavailable (butanol-extractable) fraction had been depleted (Figure 2B). The rate of mineralization decreased subsequently, presumably as a result of decreased bioavailability. Although it is possible that the slow release of ¹⁴CO₂ originated from decaying cells that had initially incorporated radiolabel into their biomass, such radiolabel would be butanol-extractable (i.e., bacteria and cell debris would be removed from the soil during extraction with butanol), and this fraction was depleted after 46 days. Thus, the notion that slow-released ¹⁴CO₂ originated from sequestered ¹⁴C-phenanthrene is more plausible. Interestingly, some mineralization was still occurring after 201 days of incubation (7 \pm 1.01% per month), indicating that phenanthrene can be bioavailable even after nearly two years of contact with soil.

Assessing ¹⁴C-Phenanthrene Bioavailability Using Sequential Chemical Extraction. Extraction of soils with *n*-butanol prior to initiating simulated rhizodeposition gave a good indication of the PAH fraction available for mineralization (Figure 2A and B). After 518 days of aging, that is, prior to initiating the experiment, 50.4 (± 4.2) % of the initially added ¹⁴C-phenanthrene was butanol-extractable, 26.8 (± 3.6) % was subsequently DCM-extractable, and 23.7 (± 1.1) % remained unextractable (Figure 3A and B). After 46 days, the butanol-extractable fraction decreased from 50.4% to

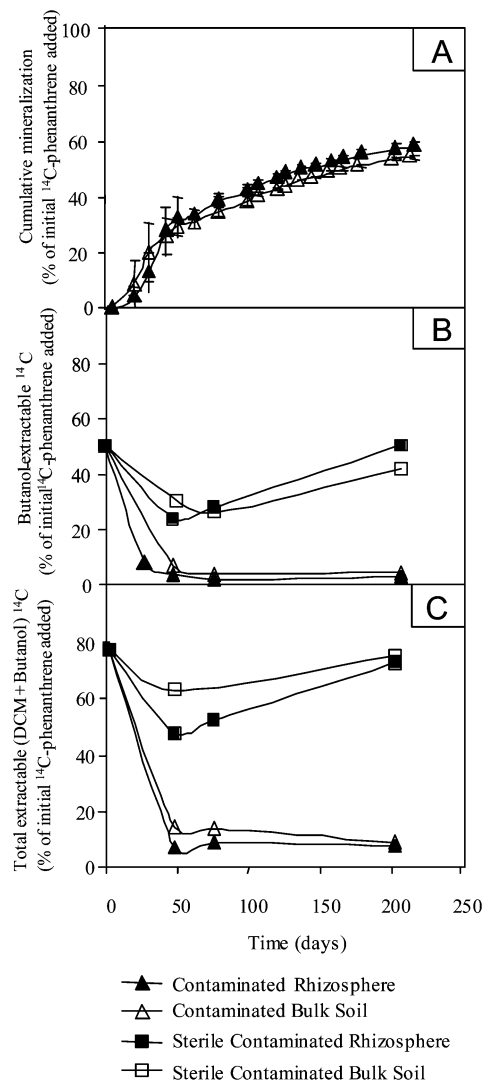


FIGURE 2. Fate of ^{14}C in different treatments. Results are reported as a percentage of ^{14}C -label initially added to the microcosms prior to the experiment. (A) Cumulative mineralization of ^{14}C -phenanthrene; (B) *n*-Butanol extractable fraction; (C) Total solvent-extractable fraction (*n*-butanol followed by DCM). Error bars represent standard deviation on 9 replicates initially (one sample was sacrificed on days 27, 47, 74 and 201). Data from extractions represent single measurements from the microcosm that was sacrificed.

3.8% in the rhizosphere microcosms, and to 7.3% in the bulk soil microcosms. This rapid decrease could not be fully accounted for by mineralization. For example, after 26 days, the butanol-extractable fraction had decreased by over 40% (as a percentage of the initially added ^{14}C -phenanthrene) in the rhizosphere microcosms (Figure 2B) whereas mineralization had reached levels of only 13% (Figure 2A). Furthermore, a sizable fraction of ^{14}C that was not butanol-extractable at that time eventually became available for mineralization (Figure 2). This suggests that a reversible transfer of ^{14}C -label occurred between the butanol-extractable fraction and an intermediate unextractable fraction (Figure 4). For example, covalent binding of hydroxylated phenanthrene metabolites (34) or dissolution and sequestration of microcrystalline phenanthrene (36) could promote the transfer of butanol-extractable into unextractable fraction. On the other hand, changes in SOM structure may facilitate desorption of phenanthrene or noncovalently bound metabolites back into the butanol-extractable fraction.

On the basis of previous studies with complexing agents (14) and organic acids (13), it was hypothesized that

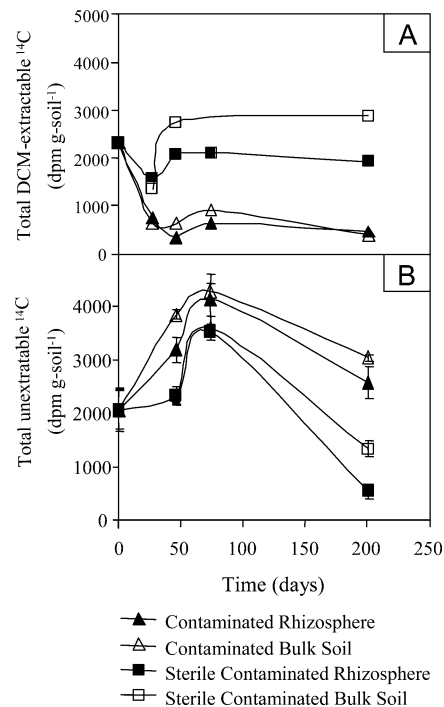


FIGURE 3. Changes in DCM-extractable (A) and unextractable ^{14}C (B) in different treatments. DCM followed butanol extraction. Error bars represent the range of duplicate samples.

intermittent addition of mulberry root-extracts containing similar compounds would enhance phenanthrene desorption and bioavailability. Results from poisoned controls showed higher concentrations of butanol-extractable ^{14}C -label in the sterile rhizosphere microcosms by day 201 (Figure 2B). However, this increase was small (8%), possibly because compounds that alter the SOM matrix and enhance phenanthrene bioavailability might not have accumulated in sufficient quantities (after intermittent addition of root extracts) to exert a more pronounced effect. The expected enhancement in bioavailability by root extracts would be lower in nonsterile microcosms, where microorganisms could degrade fatty acids and complexing agents that decrease sequestration by humic matter. Nevertheless, the enhancement in phenanthrene mineralization in the rhizosphere microcosms (relative to bulk soil microcosms) occurred after 46 days, when most of the butanol-extractable (readily bioavailable) ^{14}C had been depleted (Figure 2). This shows that plant-derived compounds could enhance the bioavailability of nonlabile phenanthrene.

The trends for DCM-extractable fraction were similar to the butanol-extractable phase, and a sharp decrease in extractable ^{14}C occurred within the first 46 days from 26.8% to 7.3 and 8.9% in the rhizosphere and bulk soil microcosms, respectively. However, the decrease in DCM-extractable fraction coincided with the increase in the unextractable fraction (Figure 3A and B), suggesting that this increase was due to sequestration of ^{14}C -metabolites and/or recently dissolved microcrystalline phenanthrene.

Note that the unextractable fraction increased during the period that phenanthrene was readily bioavailable, and decreased subsequently (Figure 3B). This trend could be explained by a dual-domain conceptual model similar to the DRM (16) and DRDM (17). Specifically, the operationally defined “unextractable fraction” might actually constitute the following: (1) an unextractable bound residue phase (B) that is strongly bound and practically unavailable for release into the labile pool (i.e., unextractable bound residue), and (2) an intermediate (transition) phase (I) that is unextractable with solvents at a given point of time, but may be eventually

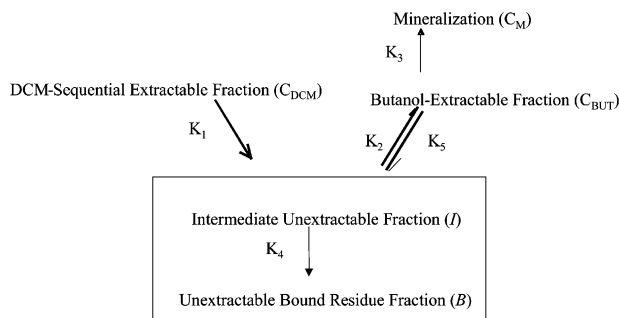


FIGURE 4. Conceptual model of ^{14}C partitioning and fate. The box represents the operationally defined unextractable fraction (I), which is the sum of intermediate unextractable (I) and unextractable bound residue (B). Letters in parentheses denote symbols used in the model.

accessible to bacteria for mineralization due to physico-chemical or biological transformations of the SOM matrix (Figure 4).

Most of the initial ^{14}C -label in the sterile microcosms was still bioavailable at the end of the experiment, with 74% of the initially added ^{14}C being solvent-extractable (Figure 2C). Similar results were observed by Guthrie et al. (37) wherein the aging of pyrene contaminated soils under sterile conditions did not lead to increased sequestration in the SOM. Thus, abiotic sequestration factors and increased contact time between a contaminant and the soil matrix (i.e., aging) may not necessarily eliminate risks associated with PAH bioavailability.

This experiment provided useful information about the relative rates of sequestration caused by biological and abiotic reactions. While the total extractable fraction (sum of butanol- and DCM-extractable fractions) decreased from an initial $77.1 (\pm 7.3) \%$ to $8.4 (\pm 0.4) \%$ in 201 days in the nonsterile microcosms, the decrease was insignificant in the sterile microcosms (Figure 2C). This suggests that phenanthrene removal by mineralization and irreversible soil sequestration (possibly following oxidative biotransformation) requires microbial activity. Thus, rhizodeposition-induced proliferation and maintenance of an active microbial consortium (30) is an important process for the cleanup of PAH-contaminated soil.

Partitioning of Phenanthrene into Humic Matter. ^{14}C was not detected in the fulvic acid fraction in any of the soil samples throughout the course of the experiment. During the course of the 201-day experiment, the amount of humic acid collected from the different soil microcosms increased steadily from an average of 6.1 ± 1.2 to 41, 46, 77, and 51 mg-humic acid-gram-soil $^{-1}$ in the rhizosphere, bulk soil, sterile rhizosphere, and sterile bulk soil microcosms, respectively. Some ^{14}C -label concentrated in the humic acid fraction of the soil. After 74 days, this sorbed fraction increased from $5.9 (\pm 2.4)\%$ to 19.4% in the rhizosphere soils, 16.9% in bulk soils, 7.2% in the sterile rhizosphere controls, and 14.7% in the sterile bulk soil controls. This steady accumulation of ^{14}C -label in the humic phase is important because the humic acids represent the soluble and therefore, mobile fraction of organic matter that may be more accessible to the food chain.

A higher percentage of the ^{14}C label ($31.2 \pm 4.5\%$) was initially associated with the humin phase. This was expected since the large surface area of humin phase allows for greater sorption of the contaminant than in the other fractions of the soil. Also, the presence of nanopores and channels allows for slow yet continuous diffusion of contaminant into the humin matrix where they are retained by sorption. Less ^{14}C -label was associated with humin in biologically active soils than that in sterile soils (Figure 5), possibly due to microbe-

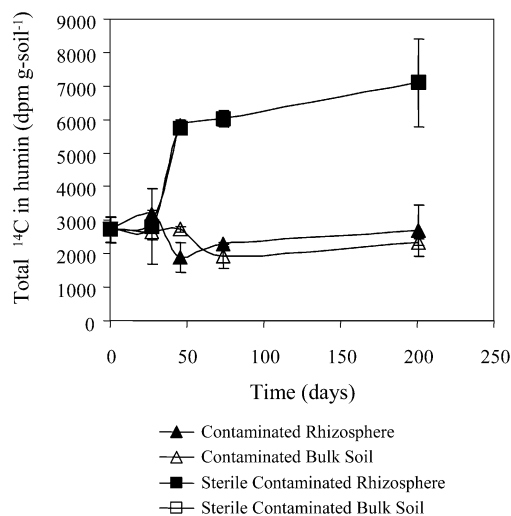


FIGURE 5. Total ^{14}C measured in humin residues from different treatments. Error bars represent standard deviation on 9 replicates initially (one sample was sacrificed on days 27, 47, 74 and 201).

mediated mineralization that represents a competing pathway to sequestration. Note that the increased ^{14}C sequestration in the humin fraction in the sterile bulk microcosms (Figure 5) had no effect on the bioavailability as determined by butanol-extractability (Figure 2B). This suggests that the sorption of different ^{14}C -labeled compounds in the humin phase is variable in nature (covalent and noncovalent). Thus, PAH accumulation in the humin phase does not necessarily represent a stable sequestration end point.

Mathematical Modeling. A model was developed to assess the fate of ^{14}C -phenanthrene over longer periods and predict the time required to reach a stable endpoint. This model assumed that transfer of phenanthrene (or its metabolites) between different soil compartments follows the law of mass action with first-order kinetics (Figure 4). The bioavailable fraction was defined as the fraction of contaminant that was extractable from the aged soils using *n*-butanol (C_{BUT}), and included any microcrystalline structures that could have been initially present but likely dissolved relatively fast in water compared to their sequestration into nonbioavailable domains.

In this model, only ^{14}C -label that was in this bioavailable fraction was considered to have the potential to be mineralized (C_{M}). However, the DCM-extractable fraction (C_{DCM}) was simulated to partition into the butanol extractable fraction through an intermediate unextractable phase (I), which is unextractable by solvents at a given point of time but might become accessible to bacteria (butanol-extractable) as a result of rhizodeposition over longer periods of time. This is plausible because the ^{14}C -fraction extracted by DCM is nonpolar and would, therefore, have a greater tendency to reversibly bind with the soil organic matter. On the basis of Figure 4, the equations that describe ^{14}C partitioning and fate are

$$dC_{\text{BUT}}/dt = K_2C_{\text{BUT}} - K_3C_{\text{BUT}} - K_5C_{\text{BUT}} \quad (1)$$

$$dC_{\text{DCM}}/dt = -K_1C_{\text{DCM}} \quad (2)$$

$$dC_{\text{M}}/dt = K_3C_{\text{BUT}} \quad (3)$$

$$dI/dt = K_1C_{\text{DCM}} - K_2C_{\text{BUT}} - K_4I + K_5C_{\text{BUT}} \quad (4)$$

$$dB/dt = K_4I \quad (5)$$

where K_1 is the rate coefficient for partitioning of ^{14}C from DMC-extractable to unextractable phase; K_2 is the reversible rate coefficient for partitioning of ^{14}C from unextractable to butanol-extractable phase; K_3 is the mineralization rate coefficient for butanol-extractable ^{14}C ; K_4 is the partitioning

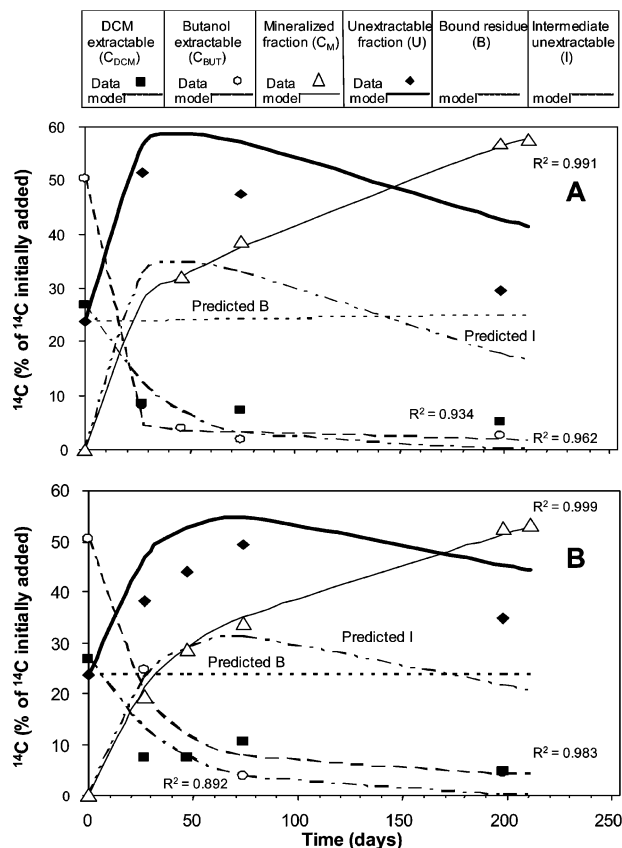


FIGURE 6. Comparison of simulated and experimental data for the bioavailability of ^{14}C -phenanthrene in a contaminated aged bulk soil through time. Unextractable ^{14}C -phenanthrene (U) = sum of intermediate unextractable and unextractable bound residue. Discrete data points represent experimental data, and lines represent model simulations (eqs 1–5). **A:** Rhizosphere contaminated soil; **B:** bulk contaminated soil.

rate coefficient from the intermediate unextractable phase to the unextractable bound residue; and K_5 is the reversible rate coefficient for ^{14}C partitioning from the butanol-extractable to the unextractable phase (Figure 4).

The unextractable phase, which was measured by combusting soils in the biological oxidizer after sequential extraction with butanol and DCM, was divided into two domains: (1) unextractable irreversibly bound residue (B), and (2) an intermediate phase (I) from which contaminants could eventually desorb and/or diffuse out.

According to this model, an acceptable posttreatment endpoint would be reached when all the ^{14}C was mineralized or retained in the unextractable bound residue phase (B). To accommodate the fast sorption/desorption kinetics occurring in the transition intermediate phase, the interactions between butanol-extractable and the unextractable phase were considered reversible. The transfer of ^{14}C from the intermediate domain to the unextractable bound residue was considered irreversible.

Initial conditions (expressed as a percentage of phenanthrene added) were set according to lab measurements, assuming that the entire unextractable fraction in the aged soil was bound residue (i.e., $C_{\text{BUT}} = 50.4\%$, $C_{\text{DCM}} = 26.8\%$, $C_{\text{M}} = 0\%$, $I = 0\%$; $B = 23.7\%$). Simulations were performed using MicroMath Scientist version 2.0 (MicroMath Inc.).

The model-fitting for mineralized, DCM- and butanol-extractable and unextractable ^{14}C fractions was generally good (R^2 ranged from 0.892 to 0.999; Figure 6). The estimated K_2 (which represents the rate of transfer from the unextractable to the butanol-extractable phase) and K_3 (which represents

TABLE 3. Parameter Fitting for Bioavailability Model (Figure 4)

parameter	rhizosphere K values (day^{-1})	bulk soil K values (day^{-1})
K_1	0.029 ± 0.007	0.027 ± 0.007
K_2	0.011 ± 0.012	0.007 ± 0.010
K_3	0.063 ± 0.039	0.024 ± 0.003
K_4	<0.0004	<0.0004
K_5	0.057 ± 0.07	0.014 ± 0.009

the rate of mineralization) were statistically indistinguishable between rhizosphere and bulk soil microcosms (Table 3). K_5 (representing the partitioning rate from the butanol-extractable into the unextractable phase) was significantly greater in the rhizosphere than the bulk soil microcosms. This could be due to faster biotransformation of phenanthrene into hydroxylated metabolites that undergo oxidative cross-coupling and/or covalent binding with SOM (34) in the rhizosphere. Extrapolation of our data using the model to day 720 (2 years) suggests that the system had almost achieved a stable endpoint in terms of the fate of the contaminant by day 201.

This is the first study to report on the effect of rhizodeposition on ^{14}C -phenanthrene humification and bioavailability in an aged contaminated soil, as well as to develop a model that simulates the fate of PAHs in rhizosphere systems. Results showed that continuous exposure to organic matter in the rhizosphere can enhance phenanthrene degradation in aged soils, possibly due to a combination of a healthier and larger microbial population and/or increased bioavailability. There was circumstantial evidence for simulated rhizodeposition enhancing partitioning of phenanthrene (or its metabolites) from an intermediate unextractable phase into the butanol-extractable (bioavailable) phase, possibly due to the presence of complexing agents and dicarboxylic acids that destabilize the humic matrix. However, more research is required to test this hypothesis.

In summary, microorganisms significantly influenced the sequestration process in the soil. In the absence of microbial degradation, the butanol-extractable fraction remained constant and very little ^{14}C partitioned into the unextractable phase. In contrast, microbial degradation depleted the total-extractable fraction within 201 days, and there was no evidence of re-mobilization of bound residues. These data as well as model simulations suggest that depletion of the total-extractable fraction of PAHs might be an acceptable treatment endpoint. Furthermore, the proliferation and maintenance of a healthy microbial population will be important for achieving this goal. Since the rhizosphere provides a favorable habitat for growth and maintenance of an active microbial consortium (38), plants should be regarded as a cost-effective and aesthetic strategy to manage the microbial community, with expected benefits depending on the age and bioavailability of the target contaminants.

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