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Relationship between Organic Matter Content of Soil and the Sequestration of Phenanthrene

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A study was conducted to determine the relationship between organic matter content of soil and the availability of aged phenanthrene. Phenanthrene was aged for 200 days in sterile samples of dissimilar soils, soils treated with H_2O_2 to reduce the content of organic matter, and sand. Sequestration as measured by the extent of mineralization of phenanthrene by an added bacterium was appreciable in samples with >2.0% organic C, and the bioavailability of the hydrocarbon declined with time of aging. Sequestration was not evident in soils or sand with <2.0% organic C. Phenanthrene aged for 200 days was more slowly degraded than the freshly added compound in soils with >2.0% organic C, but a small effect on rate was evident in soil and sand with <2.0% organic C. More of the compound remained after biodegradation of the hydrocarbon aged for 200 days than if it was not aged, with the largest amount remaining in soils with >2.0% organic C and the least in sand. Aging as measured by a decline in extractability of 1-butanol was evident in all soils, although the rate was fastest in soil with >2.0% organic C. The volume occupied by pores of <10-μm diameter was higher in soils containing more organic matter and was negligible in sand. We suggest that the organic matter content of soil is a major determinant of sequestration.

Introduction

A number of organic compounds become sequestered as they age or persist in soil. This sequestration is evident by the decline in bioavailability to bacteria (*1, 2*), earthworms (*3*), insects (*4*), and plants (*5*) and in extractability using a mild solvent (*3, 6*). Because a decline in bioavailability is also evident among toxic compounds, this sequestration has toxicological significance and is important in assessing risk from organic pollutants. Therefore, it is important to be able to predict the differences among soils in the rate and extent of loss in bioavailability.

Differences are evident among soils in both the rate and extent of sequestration, whether measured by biological or chemical means. For example, differences in bioavailability to earthworms of phenanthrene and atrazine aged in dissimilar soils has been reported (*7*). In addition, a study of 16 soils varying greatly in physical and chemical properties showed appreciable dissimilarities in the effect of aging on the availability of phenanthrene and atrazine to bacteria and to mild extractants (*8*). Because of the differences among soils, it is not now possible to determine whether sequestration will be fast or slow and whether it will be extensive or moderate.

A study was therefore conducted to determine the possible relationship of organic C content and nanoporosity of soil to sequestration. Because sequestration as reflected by slow sorption of organic compounds has been postulated to be a reflection of partitioning into soil organic matter (*9, 10*) or penetration into small pores (*11*−*13*), the possible relationship of sequestration with organic matter content and nanoporosity of the soils was investigated. The test compound was phenanthrene.

Materials and Methods

Soil Preparation. The soils used were Lima loam (2.99% organic C, pH 6.7, 32% sand, 45.5% silt, 22.5% clay), Maile loamy sand (14.7% organic C, pH 5.7, 78.6% sand, 14.3% silt, 7.1% clay), and Savannah sand (1.15% organic C, pH 6.8, 89.1% sand, 9.9% silt, 1.0% clay). A sample of sand (Sigma Chemical Co., St. Louis, MO) was found to contain 0.03% organic C.

Organic C was partially removed from Lima loam and Savannah sand by treatment with H_2O_2 as described by Kunze and Dixon (*14*) to give samples with pH values of 6.7. Iron oxides were extracted from the soils, and Fe was determined by the procedures described by Olson and Ellis (*15*). For one study, Lima loam was heated at 500 °C for 2 h to reduce the organic C content to 0.72%; because this treatment increased the soil pH, the pH was adjusted to ca. 6.0 for tests of biodegradation. Before use, soil samples were air-dried, passed through a 250-μm sieve, and sterilized with 2.5 Mrad of γ-irradiation from a ^{60}Co source.

Aging. Ten-gram portions of sterilized soil were added aseptically to sterile 50-mL test tubes, and 1 μg of [9-^{14}C]-phenanthrene (8.3 mCi/mmol, >98% purity; Sigma Chemical Co., St. Louis, MO) and 99 μg of unlabeled phenanthrene in dichloromethane were added to each sample. The soils were shaken with a vortex mixer for 10 s every 15 min in a 1-h period to allow the solvent to evaporate and to mix the compound with the soil. Sterile distilled water was then added to bring the moisture to 80% of field capacity. The tubes were closed with Teflon-lined screw caps and stored at 21 ± 1 °C in the dark to allow for aging.

Mineralization. Bacterium P5-2 was grown on a rotary shaker (120 rpm) at 30 °C for 4 days in a medium containing 1.0 g of phenanthrene, 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.01 g of $FeCl_3$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NH_4NO_3, 0.2 g of KH_2PO_4, and 0.8 g of K_2HPO_4/L of sterile distilled water at pH 7.0. The culture was passed through a sterile filter (Whatman No. 3) to remove particles, and the filtrate was centrifuged at 7650*g* for 10 min at 4 °C. The cells were resuspended and washed twice in a solution containing the inorganic salts, and 200 μL of a cell suspension containing 10^8 cells was used as inoculum for the soils.

Soil samples containing aged phenanthrene were transferred to 50-mL flasks, and 10 mL of a solution containing 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.01 g of $FeCl_3$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NH_4NO_3, 0.9 g of KH_2PO_4, and 0.1 g of K_2HPO_4/L of sterile distilled water (pH 5.7) was added to each flask. The flasks were then inoculated, and each flask was sealed with a Teflon-wrapped silicon stopper with an 18-gauge needle and with a cannula that was inside the flask. The $^{14}CO_2$ was trapped in 1.5 mL of a 0.5 N NaOH solution present in the vial. The NaOH solution was periodically replaced with fresh solution. The solution that was removed was mixed with 4.5

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TABLE 1. Porosity and Iron Oxide Content of Hydrogen Peroxide-Treated and Untreated Soils

sample	treatment	organic C (%)	pore volume (cc/g) 1−10 μm	pore volume (cc/g) <1 μm	surface area (m^2/g)	iron oxides (mg/g soil)
Maile loamy sand	none	14.7	0.276	0.265	14.8	ND[a]
Lima loam	none	2.99	0.171	0.112	6.04	4.73
Lima loam	H_2O_2	2.13	0.177	0.104	6.42	4.16
Savannah sand	none	1.15	0.020	0.009	0.89	0.51
Savannah sand	H_2O_2	0.99	0.020	0.008	1.08	0.46
sand	none	0.03	0.001	0.000	0.55	ND

[a] Not determined.

mL of Liquiscint scintillation cocktail (National Diagnostics, Inc., Somerville, NJ), and the radioactivity was measured with a liquid scintillation counter (Model LS7500, Beckman Instruments, Irvine, CA). Triplicate flasks were incubated for 30 days at 30 °C on a rotary shaker operating at 100 rpm.

Extraction. Soil samples containing aged phenanthrene were transferred to 50-mL Teflon centrifuge tubes, and 20 mL of 1-butanol was added to each tube. The contents were shaken vigorously with a vortex mixer for 1 min and then centrifuged at 18700*g* for 20 min, and the radioactivity of the supernatant was then determined.

To establish a mass balance of phenanthrene aged for 200 days, the soil samples were first extracted with 1-butanol and then transferred to cellulose thimbles, which were placed in Soxhlet extractors. The extractors were fitted with 250-mL round-bottom flasks containing 90 mL of hexane and 2 mL of 1-butanol. After 16 h of Soxhlet extraction, the hexane was removed using an evaporator. Samples from the 1-butanol and Soxhlet extractions were combined, passed through 0.22-μm Teflon filters, and analyzed with a high-performance liquid chromatograph (Hewlett-Packard series 1050, Hewlett-Packard Co., Avondale, PA) fitted with a Spherisorb ODS-2 octadecyl-bonded silica column (5 μm, 250 × 4 mm). Acetonitrile−water (86:14) was used as a mobile phase at a flow rate of 0.8 mL/min. Phenanthrene was detected by its absorbance at 254 nm.

The amount of phenanthrene remaining after mineralization was also determined using slurries with triplicate samples. The phenanthrene remaining was extracted from both the aqueous phase and the soil solids. The aqueous phase was removed by centrifugation and extracted with 5 mL of hexane. The residual soil was extracted with 1-butanol and by Soxhlet extraction, and the extracts were analyzed for phenanthrene. None was detected in the aqueous phase.

Porosity and Surface Area. Pore-size distribution and surface area were determined using a mercury-intrusion porosimeter (Pore Sizer 9300, Micromeritics Inc., Norcross, GA). A 1.5−2.0-g sample of soil was placed in a 3-mL penetrometer bulb and lightly packed. The soil was dried for 16 h at 105 °C, and the penetrometer containing the soil was filled with Hg (>99.99% pure) in a preparation chamber and transferred to a high-pressure chamber. The change in the volume of Hg was measured by increasing the chamber pressure from 14 to 31 000 psia (0.1−214 MPa). The Washburn equation (*16*) was used to calculate pore diameters by assuming that the pores are cylindrical. The equation is $P = 4\gamma \cos \theta / d$, where P is the required pressure in psia, γ is the surface tension of mercury in mN^{-1}, θ is the contact angle in degrees, and d is the pore diameter in μm. The surface tension of Hg of 480 mN^{-1} and the contact angle of mercury on soil of 140° (*17*) were used to calculate pore diameters ranging from 0.007 to 10 μm. Surface area was calculated from Hg porosimetry data using the equation (*16*):

$$\text{surface area } (m^2/g) = 1/(\gamma \cos \theta) \int P \, dV$$

where P is the pressure in pounds per square inch and V is the intrusion volume of Hg in mL.

Results

Porosity and Surface Area. The porosity and surface area of the soils and sand were determined to assess whether they were related to sequestration and whether they were affected by treating the samples with H_2O_2. The porosity varied greatly among the three soils and the sample of commercial sand tested (Table 1). In the ranges of pores measured, the porosity decreased as the percentage of C in the samples decreased. Approximately 30−50% of the volume of pores in the size range measured was accounted for by pores <1 μm in diameter. The porosity of the two soils tested was unaffected by treatment with H_2O_2. In the Savannah sand and the commercial sand, the values for porosity were quite low. The data also show that the surface area was greatest in the soil with the highest organic C level, and the area was least in the sandy soil and commercial sand, which had the lowest organic C concentrations.

Phenanthrene Bioavailability. Phenanthrene was aged for up to 200 days in sterile samples of untreated or H_2O_2-treated soils and the sand, and the bioavailability of the hydrocarbon then was determined. In samples in which the phenanthrene was not aged, the extent of mineralization was not affected by the content of organic matter in the soil (Table 2). The data from untreated and H_2O_2-treated soils and the sand are arranged in order of decreasing C content. In the samples with >2.0% C, the extent of mineralization became less with the progress of aging, but aging as measured by extent of mineralization was not evident in samples containing <2.0% C. Similarly, the reduction in rate of mineralization was marked in soils with >2.0% C, but the effect of aging on rate was small or not detected in samples with <2.0% C. The rates of biodegradation of phenanthrene aged for 200 days were also markedly less than of the unaged compound in soils with >2.0% organic C, but the effect of aging on rate was small in samples with <2.0%. Thus, when measured by bioavailability for bacterial mineralization, sequestration was pronounced or occurred only in organic matter-rich soils. It is interesting that the rates of degradation of unaged phenanthrene decreased with increasing C of the soils, a possible result of the diminished availability with increasing sorption.

The amount of phenanthrene remaining after mineralization was determined by HPLC analysis after 1-butanol and Soxhlet extraction. In all soils and sand tested, more phenanthrene remained in samples in which the compound had been aged for 200 days (100 days in one instance) than if it had not been aged (Table 3). In samples in which the compound had not aged, <1.0 μg remained if the organic C content was <2.0%, and 3.52−6.61 μg was still present in soils with organic carbon content >2.0%. In contrast, >25.0 μg remained if the compound had aged in soils with >2.0% organic C, and 1.24−7.94 μg was present in samples with <2.0% organic C. In the sample of commercial sand, more

TABLE 2. Extent and Rate of Mineralization of Freshly Added or Aged Phenanthrene in Soils and Sand[a]

organic C in soil (%)	extent of mineralization (%)				maximum rate (%/day)	
	0 days	30 days	100 days	200 days	0 days	200 days
14.7	60.8 Aa	34.9 Ba	30.8 Ba	ND[b]	17.1 Aa	2.66 Ba[c]
2.99	68.6 Ab	58.3 Bb	47.2 Bcb	41.7 Ca	49.0 Ab	16.6 Bb
2.13	68.3 Abc	57.8 Bb	44.3 Cb	45.3 Ca	40.6 Ab	18.8 Bb
1.15	69.5 Abc	64.6 Ac	65.0 Ac	63.3 Ab	51.6 Abc	44.4 Ac
0.99	65.9 Ab	60.7 Abc	63.3 Ac	63.1 Ab	51.8 Ab	43.0 Bc
0.03	71.6 Ac	72.4 Ad	69.2 Ac	66.7 Ab	59.1 Ac	46.4 Bc

[a] Values in rows and columns followed by the same uppercase and lowercase letters are not significantly different ($P < 0.05$), respectively. [b] Not determined. [c] Phenanthrene aged for 100 days.

TABLE 3. Phenanthrene Remaining after Mineralization in Soils Varying in Organic Carbon Content

organic C in soil (%)	phenanthrene remaining (μg)[a]	
	unaged	200-day aged
14.7	3.52 Aa	22.6 Ba[b]
2.99	5.62 Aa	26.2 Ba
2.13	6.61 Aa	25.1 Ba
1.15	0.43 Ab	7.49 Bb
0.99	0.92 Ac	7.94 Bb
0.03	0.54 Ab	1.24 Ac

[a] Values in rows and columns followed by the same uppercase and lowercase letters are not significantly different ($P < 0.05$), respectively. [b] Phenanthrene aged for 100 days.

TABLE 4. Effect of Aging in Various Soils on Amount of Phenanthrene Extracted by 1-Butanol and on Total Recovery[a]

organic C in soil (%)	phenanthrene extracted with 1-butanol (%)				recovery after 200 days (%)
	0 days	30 days	100 days	200 days	
2.99	80.9 Aa	63.2 Ba	49.5 Ca	46.2 Ca	108.9
2.13	82.1 Aa	66.6 Ba	55.1 Cb	47.8 Ca	99.4
1.15	87.9 Ab	81.1 Bb	62.0 Cb	53.3 Cb	105.3
0.99	84.2 Aa	78.9 Ab	60.8 Bb	56.1 Bb	104.6
0.03	88.0 Ab	81.4 ABb	83.5 Bc	82.7 Bc	97.0

[a] Values in rows and columns followed by the same uppercase and lowercase letters are not significantly different ($P < 0.05$), respectively.

phenanthrene was detected after aging than in unaged samples, but the effect was not statistically significant.

Extraction of Aged Phenanthrene. A mild extraction procedure with 1-butanol was used as a chemical assay for sequestration. The recovery of unaged phenanthrene extracted from the soils was unaffected by their C content, although more was recovered from the commercial sand (Table 4). In each of the four soil samples, the quantity removed by butanol diminished as the compound persisted. A small decrease was evident in the commercial sand. The rate of decline in extractability generally diminished with decreasing organic C content of the five samples and was fastest in soils with >2.0% C, but the values continued to fall in the four soil samples during the 200-day period, although the declines were not always statistically significant. For this study, analyses were not conducted of the soil (Maile loamy sand) with 14.7% organic C.

To confirm that the decline in availability comes from sequestration and does not reflect a loss of compounds during aging, the amount of phenanthrene recovered by butanol followed by Soxhlet extraction was determined. After 200 days of aging, essentially all the phenanthrene was recovered from all soils and the commercial sand (Table 4). The values for recovery from the six samples were not statistically different.

Combusted Soil. Phenanthrene was aged in Lima loam that had been combusted to reduce its organic matter level. The extents of mineralization of phenanthrene that was not aged and aged for 100 days were 62.2 and 50.9%, respectively, and the percentages extracted with 1-butanol after these aging periods were 77.7 and 64.5%, respectively (significant at $P < 0.05$). Thus, some sequestration had occurred even with the diminished organic C level of the soil. However, in contrast with H_2O_2 treatment, which caused little or no change in the nanoporosity and surface area of the soil, the combustion led to a 1.63-fold increase in the volume of <1 μm pores and a 1.55-fold increase in the surface area. Therefore, combustion was deemed to be too harsh a treatment to use for assessing the effect of soil C content on sequestration.

Discussion

Several lines of evidence show the relationship between sequestration and organic matter content of the soils. Sequestration is considered here as the decrease in availability of the compound for uptake by a living organism and for nonvigorous extraction by an organic solvent. The fact that a vigorous extraction procedure recovered the phenanthrene indicates that the compound was still present and not complexed by covalent linkages. The first line of evidence is the decline in extent of mineralization as phenanthrene aged in soils with >2.0% organic C but not in soils with less organic C. Second, the decline in extractability with increasing time of persistence was more rapid in soils with >2.0% organic C. Third, after biodegradation of the phenanthrene that had aged for 200 days, more of the compound remained in soils with >2.0% organic C. Fourth, the depression in the rate of biodegradation as a result of aging for 200 days was more marked in soils with >2.0% organic C. It is noteworthy that, among the soils with the higher content of organic matter, these effects did not become more pronounced with increasing levels of organic matter, suggesting that a threshold level of organic C is required for sequestration but that the aging effect is independent of additional levels of organic matter.

It has been suggested that the mechanism of sequestration of hydrophobic compounds entails their partitioning into the organic fraction of soil (*18*, *19*). Moreover, the extent of sorption of such compounds is related to the percentage of organic matter in soil (*20*). Therefore, it is not surprising that sequestration is related to the organic C content of the soil. The finding of little sequestration in soils with <2.0% organic C suggests that there may be a threshold level of organic matter below which sequestration is minor or absent. The relationship between rate of mineralization of unaged phenanthrene and organic C content (*21*), although not necessarily related to the aging effect, also points to the importance of organic matter in determining bioavailability of hydrophobic substrates. In view of the finding that more aged than unaged phenanthrene remained in organic C-poor soils and sand, sequestration is a factor even in these matrices. To the extent that desorption resistance and aging may

represent similar or identical processes of sequestration, it is noteworthy that volatile compounds became resistant to desorption in soils with 0.04−1.12% organic C (*22*).

Soils and sediments are known to have an abundance of pores with diameters appreciably smaller than 1 μm (*23, 24*), and it has been suggested that organic materials that penetrate these nanopores, which have large surface areas, become resistant to degradation (*24*). Tests with nanopore-containing beads confirmed the possible role of these small pores, provided they have hydrophobic surfaces (*13*). The data presented here suggest that soils in which sequestration was greatest had the largest nanopore volume and surface area. Although this observation may indicate that nanoporosity and surface area are determinants of sequestration, the apparent relationship may simply reflect the greater porosity and larger surface area in soils rich in organic matter. To assess the relative importance of these parameters requires a larger number of soils with differences in organic C content, nanoporosity, and surface areas.

Several lines of evidence suggest that the H_2O_2 treatment did not affect sequestration or bioavailability. The results show that the porosity and surface areas were not altered to a significant extent, if at all, by treatment with H_2O_2. A possible role of iron oxides in causing sequestration or changes in bioavailability seems unlikely because, although the H_2O_2 reduced organic C levels and affected sequestration, it did not change the iron oxide content of the soils. It is unlikely that toxins were generated by H_2O_2 addition since the rate of mineralization of unaged phenanthrene was the same regardless of treatment.

The data suggest that the organic matter content of soil is a major determinant of sequestration. However, an investigation of 16 soils suggests that other properties of the soil may also contribute to the decline in availability of organic compounds as they age in soil (*8*). Therefore, additional study is needed to assess the role of various other soil properties in determining the availability of organic compounds that are of environmental concern.

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