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Differences in Sequestration and Bioavailability of Organic Compounds Aged in Dissimilar Soils

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Phenanthrene and atrazine were aged for 200 days in sterilized samples of 16 soils that differed greatly in physical and chemical properties. At regular intervals during the aging period, the extent of sequestration was determined by measurement of biodegradation of the compounds by bacteria and the amounts extracted by a mild extraction procedure. Both compounds became sequestered in each of the soils, but the rate and extent of sequestration varied markedly among the soils. Sequestration was largely complete in some soils in 120 days, but it extended for longer periods in others. The extent of sequestration of the two compounds in the 16 soils was not highly correlated. The declines in bioavailability in the soils were not highly correlated with the decreases in extractability or the amounts of unextracted compounds, although soils in which the declines in bioavailability were greatest showed the greatest declines in extractability. Because of the marked differences among soils, generalizations about the rate and extent of sequestration in soils are not yet possible.

Introduction

The rates of disappearance from field soils of slowly biodegradable organic compounds decline with time, and after several years, little or no loss can be detected. Because microorganisms able to metabolize those compounds are present in these field soils, the chemicals appear to become less bioavailable with time. This aging process, which represents a sequestration of the molecules to make them somehow no longer available, occurs with a number of organic pollutants (*1*). The addition of 1,2-dibromoethane (*2*) or simazine (*3*) to field soils containing the aged pesticides also showed that the aged chemicals were unavailable to indigenous microorganisms able to metabolize the unaged pesticides. Aging of phenanthrene, 4-nitrophenol, and atrazine in the laboratory confirmed that these compounds become less available with time to bacteria, earthworms, or both (*4*−*6*). Evidence for sequestration is not limited to investigations of bioavailability since the persistence of test compounds is accompanied by a decline in their recovery by mild extractants (*4*−*7*).

Early studies by Edwards et al. (*8*) demonstrated marked differences among three soils in the rate of time-dependent decline in bioavailability of lindane; thus, the discrepancies between chemical and bioassays for the insecticide were highly dissimilar in the test soils. More recent investigations have confirmed that sequestration and the time-dependent decline in bioavailability of phenanthrene and 4-nitrophenol to bacteria (*4*) and of phenanthrene to earthworms (*9*) were quite different in soils with dissimilar properties. Such findings are of considerable importance in attempts to predict the loss in bioavailability and the extent to which organic pollutants may become resistant to bioremediation. Moreover, because aging leads to a loss in acute toxicity, to insects (*8*; Robertson and Alexander, unpublished data) if not to higher organisms, the ability to predict the extent of sequestration in soils of different properties is of great importance for risk analysis.

A study was therefore conducted using 16 soils with widely different physical and chemical properties. Sequestration was measured by the loss in bioavailability to bacteria and the decline in recovery by mild extractants.

Materials and Methods

Soil Samples. Samples of soils 1−8 and 16 were provided by the National Soil Survey Laboratory (Lincoln, NE), and soil samples 9−15 were collected from various parts of New York. The soils were passed through a 2-mm sieve, air-dried, and amended to give 2% (w/w) of $CaCO_3$. $CaCO_3$ was added to the acid soils to allow for more active microbial degradation, but it was added to all other soils to provide the same amendment for all samples. The soils then were sterilized with 2.5 Mrad of γ-irradiation from a Co^{60} source and stored for 14 days before use. Sterility was indicated by the absence of growth on Trypticase−soy agar inoculated with samples of each soil.

The properties of the soil are presented in Table 1. For the analysis of soils 9−15, pH was measured in a 1:1 (w/w) soil−water suspension, the particle-size distribution was by the pipet method (*10*), organic carbon was determined by combustion at 1000 °C, and field capacity was determined as described by Casse and Nielsen (*11*). The properties of the other soils were provided by the National Soil Survey Laboratory.

Aging of Chemicals. [9-^{14}C]Phenanthrene (13.3 mCi/mmol, >98% pure), unlabeled phenanthrene (>96% pure), and [U-^{14}C]atrazine (17.7 mCi/mmol, 95% pure) were purchased from Sigma Chemical Co. (St. Louis, MO). Unlabeled atrazine (98% pure) was obtained from Ciba-Geigy (Greensboro, NC). Sterile samples (10 g) of each soil were aseptically added to 50-mL screw cap tubes. Phenanthrene or atrazine in 100 μL of acetone was added to the soils to a final concentration of 1.0 and 6.0 μg/g dry soil for phenanthrene and atrazine, respectively. Approximately 1.0×10^5 dpm of labeled phenanthrene or atrazine mixed with the unlabeled compound was added to each sample. The tubes were placed in a hood for 1.5 h, and the samples were vigorously mixed for 6 s every 30 min to allow the acetone to evaporate and mix the chemicals with soil. Sterile deionized water was then added to each sample to bring the moisture level to approximately 80% of field capacity. The tubes were tightly capped with silicone-backed Teflon liners and kept in the dark at 21 ± 2 °C. Samples aged for 0, 20, 60, 120, and 200 days were prepared in the same way. Three replicate soil samples of each chemical were aged for each incubation period. Sterile water was added to soils 8−10, 12, 13, and 16 at 120 days because these soils had lost some moisture.

Mineralization. After the compounds had aged for 0, 20, 60, 120, or 200 days, triplicate samples of each of the soils were inoculated with 1×10^8 cells/g of soil of a phenanthrene-

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TABLE 1. Properties of Soils

soil	series	particle size (%)			organic carbon (%)	pH[a]	field capacity
		sand	silt	clay			
1	Fuquay sandy silt loam	77.7	1.8	20.5	0.07	4.9/7.3	8
2	Clarence clay	1.5	33.9	64.6	0.30	8.0/7.6	33
3	Dothan sandy clay loam	58.7	10.7	30.6	0.34	5.1/6.9	16
4	Palouse silty clay loam	10.1	69.5	20.4	0.34	7.3/7.4	23
5	Catalina silty clay	6.5	47.7	45.8	0.41	4.8/6.5	46
6	Amor silty clay loam	8.8	55.7	35.5	0.62	8.4/8.2	28
7	Halii sandy loam	66.2	26.2	7.6	0.99	4.5/6.3	27
8	Yolo silt loam	19.5	56.0	24.5	1.34	7.4/7.4	24
9	Madrid loam	49.3	37.2	13.5	3.58	6.3/6.8	24
10	Ross silt loam	19.3	61.2	19.5	3.71	6.0/6.5	31
11	Adjidaumo clay	3.6	35.9	60.5	4.49	6.0/6.5	46
12	Angola silty clay loam	12.1	57.2	30.5	4.64	6.1/6.7	47
13	Longford silt loam	23.0	55.3	21.7	4.73	6.6/6.9	36
14	Madalin clay	30.0	22.0	48.0	6.95	6.1/6.6	42
15	Lima loam	34.2	44.3	21.5	7.16	7.2/7.2	43
16	Quillayute silt loam	21.0	57.0	22.0	11.0	5.1/6.4	38

[a] pH before addition of $CaCO_3$/pH after addition of $CaCO_3$.

mineralizing bacterium, strain P5-2 (from W.-C. Tang of this laboratory), or an atrazine-mineralizing bacterium, strain M91-3 (obtained from M. Radosevich, University of Delaware, Newark, DE). Strain P5-2 was grown in 250-mL Erlenmeyer flasks containing 500 mg/L of phenanthrene in an inorganic salts solution (*4*), pH 7.0. The flasks were incubated at 30 °C on a rotary shaker operating at 120 rpm. After 60 h, the culture was passed through a glass frit (40-μm pore) to remove the remaining phenanthrene crystals. The cells were collected by centrifugation at 10400*g* for 10 min, suspended in sterile 0.85% NaCl solution, and centrifuged again. Strain M91-3 was grown in 250-mL Erlenmeyer flasks containing 21.5 mg of atrazine and 200 mg of glucose/L in an inorganic salts solution (*7*). The culture was incubated for 60 h at 30 °C on a rotary shaker operating at 120 rpm, and the culture was washed as described above without use of the glass frit. The cells were suspended in 0.2 mL of a solution containing NH_4NO_3 and KH_2PO_4 to provide 1×10^8 cells and 200 μg each of NH_4NO_3 and KH_2PO_4/g of soil. The soil was then mixed with a sterile spatula.

Each tube was closed with a Teflon tape-wrapped silicone stopper through which was inserted an 18-gauge needle and a cannula (*6*). A 4.0-mL glass vial containing 1.5 mL of 0.5 N NaOH was attached to the end of the cannula. The soils were incubated at 21 ± 2 °C for 55 days. The alkali was removed periodically through the cannula and replaced with fresh NaOH. The alkali that was removed was mixed with Liquiscint scintillation cocktail (National Diagnostic, Atlanta, GA), and the radioactivity was measured with a liquid scintillation counter (model LS 7500; Beckman Instruments Inc., Irvine, CA).

Extraction. After the various aging periods, triplicate uninoculated samples of each of the soils were transferred to 50-mL Teflon centrifuge tubes, and 20 mL of 71% ethanol in water for phenanthrene or 95% ethanol for atrazine was added to each tube. The tubes containing soil amended with phenanthrene were mixed for 1.5 h on a reciprocating shaker operating at 200 rpm, and the tubes containing atrazine were mixed for 10 s with a Vortex mixer at 21 ± 2 °C. The tubes then were centrifuged at 7600*g* for 10 min. Portions of the supernatant were removed to determine the radioactivity.

The supernatant was removed by decanting from soils 1 and 15, and the soils were subjected to two 6-h Soxhlet extractions with 100 mL of methanol. The resulting extract was reduced in volume to approximately 3 mL in a rotary evaporator. The concentrated extract was mixed with 19 mL of scintillation cocktail, and the radioactivity was determined.

TABLE 2. Effect of Aging on Mineralization of Phenanthrene in 16 Soils

soil	% mineralized				
	0 day	20 days	60 days	120 days	200 days
1	55.8A[a]	54.7AB	50.9B	41.5C	38.0C
2	59.2A	55.2B	49.7C	49.0C	49.6C
3	45.7A	47.2A	37.9B	33.1C	33.4C
4	66.3A	60.6B	57.7B	52.9C	48.8C
5	53.8A	49.2A	32.4B	32.2B	34.7B
6	62.0A	58.9A	51.2B	42.2C	42.9C
7	46.6B	54.3A	41.3C	41.3C	44.1BC
8	66.4A	48.1B	41.7BC	40.1BC	36.0C
9	62.8A	64.5A	52.6B	47.2BC	45.6C
10	51.6A	45.9B	41.9C	38.2D	27.7E
11	43.9A	42.9A	36.9AB	33.1BC	27.7C
12	55.7A	55.9A	49.5B	35.6C	34.1C
13	61.7A	60.1A	50.9B	43.6C	41.9C
14	50.0A	47.8A	38.5BC	44.1AB	33.7C
15	55.9A	44.9B	38.3C	27.7D	27.9D
16	65.2A	57.2B	45.4C	40.9C	30.2D

[a] Values in rows followed by the same letter are not statistically different ($P < 0.05$).

Results

The extents of bacterial mineralization of phenanthrene aged in soil for 0, 20, 60, 120, and 200 days varied markedly (Table 2). In soils in which the chemical had not been aged prior to inoculation, 43.9−66.4% of the carbon was converted to CO_2, whereas only 27.7−49.6% was mineralized if phenanthrene was aged for 200 days prior to inoculation. Each soil showed aging, and in 10 of the soils, a statistically significant decline in bioavailability was evident after 20 days. In only one soil (no. 11) was aging so slow that it required 120 days for a significant decline in availability to be evident. Because the differences in bioavailability between 120 and 200 days were not statistically significant in 13 of the 16 soils, it appears that the sequestration had markedly slowed or ceased in these soils. If the differences in extents of mineralization of unaged and 200-day-aged phenanthrene are considered as measurements of the diminished bioavailability, it is evident that the sequestration/aging effect varies markedly among the soils, the values ranging from 9.6% in soil 2 to 35.0% in soil 16. Although the former soil has only 0.30% organic C

TABLE 3. Effect of Aging on Mineralization of Atrazine in 16 Soils

	% mineralized				
soil	0 day	20 days	60 days	120 days	200 days
1	89.7A[a]	82.3B	77.4B	68.2C	65.5C
2	56.2B	62.4A	60.5AB	61.6AB	56.8B
3	75.8A	76.3A	69.9AB	65.6B	48.3C
4	67.6A	66.2AB	65.3AB	63.9B	56.6C
5	67.6A	61.4AB	60.2AB	59.7AB	58.0B
6	68.6A	66.6AB	58.8BC	52.4CD	48.7D
7	88.5A	67.3B	52.5C	45.5C	21.8D
8	70.0A	59.1B	54.3BC	50.8C	41.6D
9	77.9A	60.3B	51.0C	39.1D	31.0E
10	78.3A	58.4B	50.4C	44.4D	35.2E
11	79.3A	68.5B	59.3C	48.9D	41.2E
12	77.8A	62.9B	51.3C	43.4D	32.8E
13	80.2A	60.0B	53.0C	39.9D	34.9E
14	81.2A	61.5B	48.2C	38.9D	30.5E
15	89.8A	54.6B	42.2C	28.6D	23.1D
16	48.4A	33.5B	27.0BC	26.5BC	23.3C

[a] Values in rows followed by the same letter are not statistically different ($P < 0.05$).

and the latter has 11.0%, an effect of organic matter content on the rate or extent of sequestration among all 16 soils is not immediately evident.

The extents of mineralization of atrazine aged for 0, 20, 60, 120, and 200 days also differed markedly among the test soils (Table 3). The extents of mineralization ranged from 48.4 to 89.8% in soils with atrazine that was not aged to 21−65.5% for atrazine aged for 200 days. Fifteen of the 16 soils showed statistically significant aging, but little or no change in bioavailability was evident in soil 2. Aging was evident in 11 soils and in all soils with >0.9% organic carbon even at 20 days. Because the values in 10 of the soils were significantly less after 200 days than after 120 days, the decline in bioavailability of atrazine was continuing after 4 months. As evident by the differences in the values for soils in which atrazine aged for 0 and 200 days (little or no aging in soil 2 to 66.7% in soils 7 and 15), the extent of decline in bioavailability varied greatly from soil to soil; however, the four soils with the smallest differences in mineralization of unaged and 200-day-aged atrazine (soils 2 and 4−6) had <0.7% organic C.

To determine whether soils that sequester one compound extensively or poorly have the same effect on the aging of a second compound, the correlation between aging of phenanthrene and atrazine was determined for all 16 soils. Aging was expressed as the percentage mineralized at 0 days less the percentage mineralized at 200 days; i.e., the decline in the amount available for biodegradation. A correlation was not evident however (Figure 1A). The line in the figure represents equal sequestration of the two compounds. The values for eight of the soils fell close to that line (closed circles). However, the values for the remaining eight (open circles) were well above the line, which indicates greater sequestration of atrazine than phenanthrene; those are soils 7 and 9−15, which include all soils with >0.9% organic C, except for soil 8.

Measurements were made of the percentage of the phenanthrene aged for 0, 20, 60, 120, and 200 days that was extracted by 71% ethanol in water. The recoveries of the freshly added compound ranged from 52.9 to 91.8% and were highest in soils 1−7, which contained <1.0% organic C (Table 4). In contrast, 13.6−70.8% was extracted after 200 days of aging. Sequestration as measured by extractability was evident in each soil, and the effect was evident in 12 of the soils within 20 days. In eight of the soils, the amounts extracted after 120 and 200 days of aging were not statistically

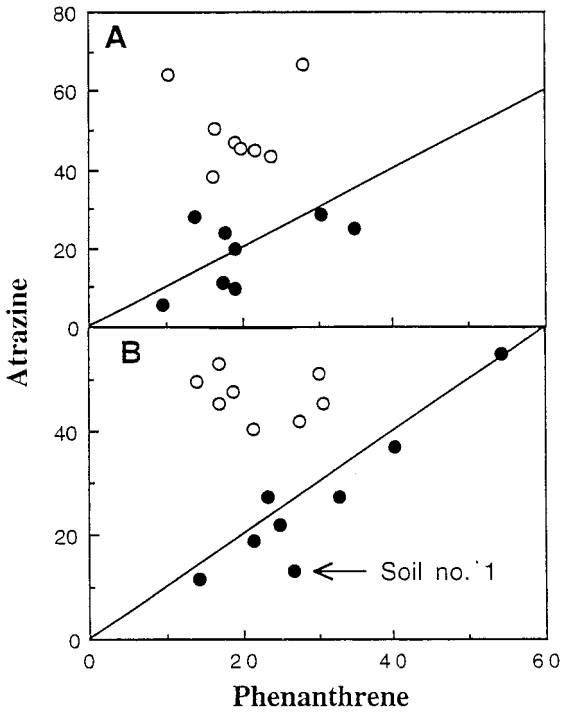

FIGURE 1. Correlation between aging of phenanthrene and atrazine in 16 soils as measured by mineralization (A) and extractability (B). Aging is expressed as the difference in percent mineralized or extracted at 0 and 200 days.

TABLE 4. Effect of Aging on Extractability of Phenanthrene in 16 Soils

	% extracted				
soil	0 day	20 days	60 days	120 days	200 days
1	91.8A	84.5B	73.7C	71.7C	65.1D
2	76.5B	85.0A	75.1B	71.5C	70.8C
3	89.5A	81.7B	75.1C	65.1D	56.7E
4	81.4A	71.9B	63.8C	61.8CD	58.1D
5	86.2A	82.6B	74.9C	67.8D	64.8D
6	87.8A	74.6B	63.8C	62.2C	62.9C
7	81.7A	74.0B	64.6C	61.0C	54.3D
8	69.6A	45.0B	41.0C	31.5D	29.3D
9	74.9A	68.9AB	61.6BC	54.6C	58.1C
10	56.5A	39.0B	33.2C	29.1D	25.9E
11	63.1A	58.7B	51.2C	48.6D	41.8E
12	65.6A	62.1B	53.7C	49.4D	48.8D
13	69.4A	71.2A	62.7B	59.9C	57.1D
14	65.4A	59.0AB	52.4BC	51.8BC	46.7C
15	52.9A	40.7B	34.3C	29.2D	22.7E
16	67.9A	41.6B	32.7C	21.1D	13.6E

[a] Values in rows followed by the same letter are not statistically different ($P < 0.05$).

different, and the values were not too dissimilar in the other eight soils; hence, the sequestration had markedly slowed and possibly stopped in some instances after 120 days. Nevertheless, sequestration was still proceeding in soils 1, 3, 7, 10, 11, 13, 15, and 16 after 4 months. In the case of extractability as a measure of sequestration, it is not certain whether the differences between 0-day and 200-day values or just the 200-day values should be considered; in either instance, the differences among the soils are marked, ranging from 5.7 to 56.3% for the differences between 0-day and 200-day values or 13.6−70.8% for the latter determinations alone.

The percentage of the atrazine added to the soils that was extracted with 95% ethanol was also determined after the herbicide had been in soil for 0−200 days. From 39.7 to

TABLE 5. Effect of Aging on Extractability of Atrazine in 16 Soils

	% extracted				
soil	0 day	20 days	60 days	120 days	200 days
1	88.4B[a]	92.6A	89.7AB	81.5C	79.5C
2	39.7AB	45.6A	37.8AB	27.0BC	34.2BC
3	75.4B	86.1A	75.9B	56.0C	58.8C
4	82.2B	85.2A	71.3C	65.8D	57.8E
5	81.2A	81.0A	75.1B	66.2C	62.3C
6	73.2A	75.2A	64.5B	52.1C	53.4C
7	61.9A	49.1B	38.7C	29.3D	19.9E
8	58.2A	41.4B	37.0C	31.3D	21.2E
9	81.1A	55.1B	44.4C	33.4D	28.1E
10	66.8A	39.9B	34.0C	25.0D	21.4D
11	62.0A	42.6B	38.8C	28.0D	21.6E
12	69.2A	49.7B	42.2C	24.9D	24.0D
13	74.8A	46.3B	36.9C	31.2CD	25.2D
14	61.6A	40.7B	33.3C	21.0D	14.0E
15	66.5A	37.6B	37.3B	18.4C	15.2C
16	66.1A	38.6B	23.8C	16.1D	11.1E

[a] Values in rows followed by the same letter are not statistically different ($P < 0.05$).

88.4% of the freshly added compound was recovered with the ethanol, but the extractability declined with persistence time so that only 11.1−79.5% was recovered at 200 days (Table 5). This decline occurred in most or possibly all the soils, but the extent of diminution in extractability ranged from very low values in the two soils with the least organic C (soils 1 and 2) to >50% decline. Sequestration was evident after 20 days, and the amount extracted after 200 days was least in soils with >0.9% organic C (soils 7−16). In seven of the soils, sequestration was not complete after 120 days, as evident by the significant differences in the values at 120 and 200 days.

As presented above for mineralization, the correlation between aging of phenanthrene and atrazine was determined for the 16 soils, but aging in this instance is expressed as the difference between the percentage extracted at 0 days less the percentage extracted at 200 days. With seven of the soils (closed circles), similar amounts of the two compounds were extracted (Figure 1B). These were soils 7 and 9−15; i.e., all soils with >0.9% organic C, except for soil 8. In one soil (no. 1), a higher percentage of phenanthrene than atrazine was sequestered. The diagonal line in the figure again represents equal sequestration of the two chemicals. In eight of the soils (open circles), all of which have <1.4% organic C, a higher percentage of atrazine than phenanthrene became sequestered.

The quantity of a compound not removed at 200 days by the mild extractant may be a measure of aging rather than, or possibly in addition to, the decline in quantity of the compound that is extracted. The latter parameter excludes the quantity immediately sorbed and becoming less extractable from the quantity assumed to be sequestered, while the former parameter considers that quantity also to have been sequestered. An assessment of whether the amounts of phenanthrene and atrazine not extracted from the 16 soils were correlated gave an r^2 values of 0.457 ($P < 0.005$). If five soils (soils 1 and 3−6, which have <0.7% organic C) were excluded from the calculation, the r^2 value was 0.616 ($P < 0.001$).

In addition, the percentage not extracted might be a means of predicting the loss in bioavailability to microorganisms as a result of aging. However, the r^2 values for plots of percentage not extracted vs percentage decrease in mineralization gave values of 0.656 ($y = 3.24 + 0.323x$; $P < 0.001$) and 0.397 ($y = 2.05 + 0.555x$; $P < 0.01$) for phenanthrene and atrazine, respectively. Nevertheless, as indicated in Figure 2, the soils in which the decline in bioavailability was greatest contained the least extractable chemical. Thus, if the decrease in mineralization of phenanthrene and atrazine was >35%, <80% of the phenanthrene and <70% of the atrazine were not extracted at 200 days.

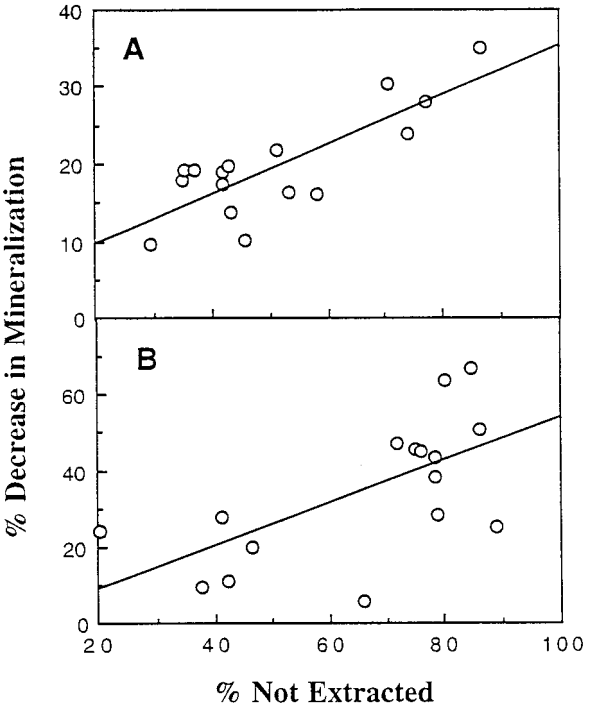

FIGURE 2. Correlation between decrease in mineralization in 200 days and the percentage of phenanthrene (A) and atrazine (B) not extracted at 200 days.

The decreases in extractability in the 200-day period might be a means of predicting the decline in bioavailability to microorganisms as a result of aging. However, the r^2 values for plots of the percentage decrease in extractability vs percentage decrease in mineralization in 200 days gave values of 0.475 ($y = 8.24 + 0.449x$; $P < 0.01$) and 0.575 ($y = -0.616 + 0.958x$; $P < 0.001$) for phenanthrene and atrazine, respectively. Nevertheless, as shown in Figure 3, if the decline in extractability was <30%, the decline in bioavailability was <25%, and if the decline in extractability was >40%, the decline in bioavailability was nearly always large.

On the other hand, the percentage not extracted at 200 days might be a means of predicting the percentage that was not available to microorganisms as a result of aging. The relationship is shown in Figure 4. The r^2 values for plots of percentage not extracted vs percentage not mineralized gave values of 0.511 ($y = 46.9 + 0.308x$; $P < 0.005$) and 0.773 ($y = 21.9 + 0.568x$; $P < 0.001$) for phenanthrene and atrazine, respectively. Thus if the percentage of phenanthrene and atrazine that was not mineralized was <60% and <55%, <50% and <70% were not extracted at 200 days, respectively.

To determine whether there were any losses of the test compound during the aging periods, sterile samples of soils 1 and 15 were amended with phenanthrene (1.0 μg/g) or atrazine (6.0 μg/g) and brought to a moisture level of 1/3 bar. At 0 and 120 days, each of the three replicates was extracted with 71% ethanol in water (phenanthrene) or 95% ethanol (atrazine). All samples were then subjected to two Soxhlet extractions with methanol for 6 h. All of the phenanthrene and atrazine added to soil 1, which contains 0.07% organic C, was recovered after 0 and 120 days of aging (Table 6). The differences between the values at the two time periods were not statistically significant. However, in soil 15, which contains 7.16% organic C, somewhat less phenanthrene and

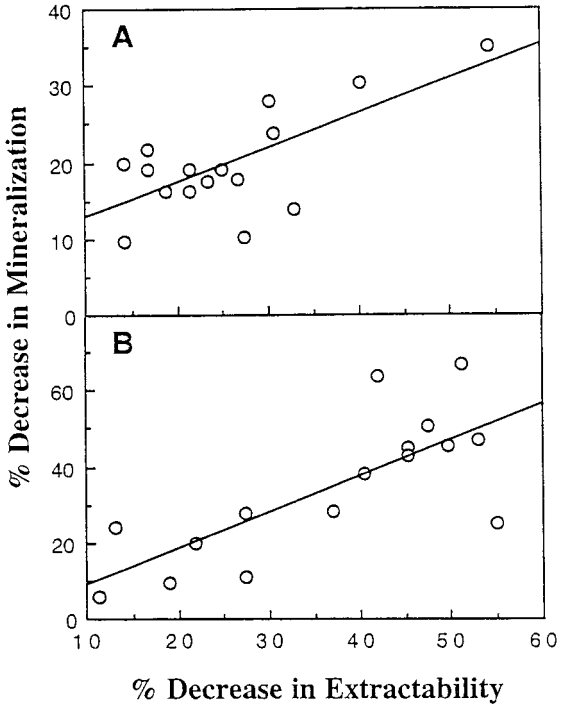

FIGURE 3. Correlation between decrease in mineralization and decrease in extractability as a result of aging of phenanthrene (A) and atrazine (B) for 200 days in 16 soils.

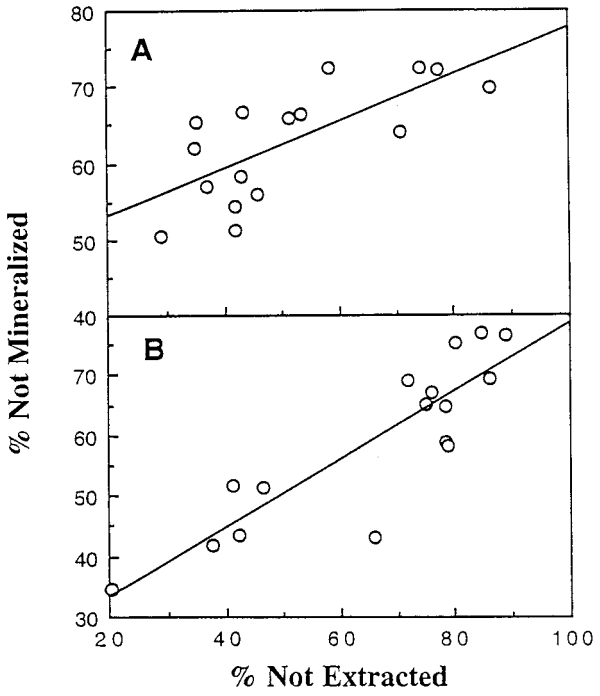

FIGURE 4. Correlation between percentage of phenanthrene (A) and atrazine (B) not mineralized and percentage not extracted at 200 days.

approximately one-fifth less atrazine was recovered at 120 days than at 0 days; these differences are statistically significant ($P < 0.05$).

Discussion

The exposure of humans, animals, or plants to a toxicant that is sequestered in soil is less than to the same concentration of the compound that is fully available, and the risk from the compound is consequently less. If the rates and extents of sequestration differ among soils with different properties, it is therefore necessary either to determine the bioavailability of aged compounds in each soil type or to find generalizations allowing a prediction of the effect of soil characteristics on the diminution in bioavailability as a function of time.

TABLE 6. Recovery of Phenanthrene and Atrazine by Mild Followed by Vigorous Extraction

		% recovered	
soil	aging time (days)	phenanthrene	atrazine
1	0	107.5	101.4
15	120	104.3	105.7
	0	107.1	100.6
	120	97.2	79.0

The results of this study clearly show marked dissimilarities in the rate and extent of sequestration. This is not unexpected based on the previous studies of a very small number of soils (*4, 8, 9*). By extending the earlier observations to a wide range of soils, however, some initial generalizations are possible. Sequestration was found to be rapid in some soils and slow in others, and the maximum extent was reached at different times in different soils. Moreover, a high degree of correlation in the percentage sequestration of two dissimilar compounds was not observed, which may result from dissimilar mechanisms of sequestration, dissimilar properties of the two compounds, or both.

It has been known for some time that extractability with mild extractants diminishes with increasing time of aging (*12*). By such methods, it is possible to obtain one estimate of sequestration. Nevertheless, although the greatest diminutions in bioavailability to a bacterium were evident in soils showing the greatest diminution in extractability, a strong correlation was not observed among all 16 soils. This suggests that the mild extractants that were used in this investigation are not appropriate surrogates to predict bioavailability. It is possible that the rates and extents of decline in availability to a particular solvent and to bacteria occur at different rates or that they differ among bacteria and animals (*6*). If so, the search for a surrogate assay for the effect of sequestration on bioavailability will be difficult.

Although vigorous extraction resulted in quantitative recovery of phenanthrene after 120 days of aging in two soils and of atrazine in a soil low in organic matter, approximately 20% of atrazine was not recovered from soil 15. This may result from the formation of a bound residue by complexation with soil organic matter (*13*). This view is supported by the fact that soil 15 has appreciably more organic C (7.16%) than soil 6 (0.62%).

Even though a large portion of chemical was mineralized in the initial stage of bioassays, the assay was slow so that a portion of chemical was being sequestered and became less available to the microorganism even during the assay.

In view of the diversity in rates and extents of sequestration among soils and the importance of sequestration in determining the bioavailability and thus the risk of persistent pollutants in soil, research is needed to determine the role of soil properties in sequestration. Correlations are needed to assess the role and contribution of organic matter, clay content, nanoporosity, surface area, or other soil properties in governing the rate and extent of decline in bioavailability, so that predictions of diminished exposure will be possible. Such studies are currently in progress.

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