

Estimation of Initial Concentrations of Phenanthrene and Atrazine from Soil Properties and Bioavailability During Aging

Namhyun Chung*

Division of Biotechnology and Genetic Engineering, College of Life and Environmental Sciences,
Korea University, Seoul 136-701, Korea

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Contaminated sites are often abandoned for an extended period of time, thus organopollutants becoming sequestered. The information on the initial concentrations of the contaminants would be helpful for the economic bioremediation of the chemicals. The present study estimated the initial concentrations of atrazine and phenanthrene through multiple regression analyses using soil properties and the amount of chemicals available *in situ*. Percentage mineralized or extracted was best correlated with organic C or logarithm of organic C, and the R^2 values were 0.548 and 0.894 for atrazine and phenanthrene, respectively. Estimation of the initial concentration of the chemicals was then calculated from both the percentage mineralized or extracted and the amount of chemicals extracted or mineralized. Results showed that the estimation of the initial concentration of the chemical at the time of contamination is feasible.

Key words: aging, phenanthrene, atrazine, multiple linear regression, extractability, bioavailability.

Bioremediation is considered as an economic technology for cleaning up sites contaminated with organopollutants. However, the polluted sites were often abandoned for a long period of time before governmental agency applied strict environmental laws.¹⁻³⁾ When the polluted fields were remediated, it was found that some fractions of chemicals in soil were very resistant to biodegradation by microorganisms. Possible reasons might be that the pollutants have been in contact with the soils for an extended period of time, become weathered, and been complexed with the soil components.¹⁻³⁾ During recent years, evidences have accumulated that there are certain slow processes that reduce the availability of the pollutants and thus the toxicity of the compounds in soils with time.¹⁻⁹⁾ Aging or weathering is presently the terminology that describes the slow processes and is supported by a host of publications, indicating that organic molecules are slowly sequestered but not complexed (i.e., chemically bonded) within the soil matrix.³⁾ The sequestered compounds are not easily bioavailable by microorganisms or extracted by mild extractants. Thus, the aging process of organopollutants in soil is characterized by the declining bioavailability and sequestration, affecting the potential risk posed by the pollutants and the appropriate remediation technologies.¹⁻³⁾ Regulations for the cleanup of sites containing hazardous chemicals assume that all soil organopollutants recovered by vigorous extractions are considered as bioavailable.¹⁰⁾ The general acknowledgement on the sequestration of chemicals in soil and

thus declining bioavailability expedites the reevaluation of the current risk assessment.

Bioavailability and sequestration appear to be affected by different types of soils and chemicals, and various environmental factors such as water content.^{1,5)} Chung and Alexander⁵⁾ showed that the loss in the availability of phenanthrene and atrazine varied among 16 dissimilar soils, which were contaminated with known amounts of various chemicals and aged, and that the degree of sequestration with the same soil differed greatly between the two compounds, which might be resulted from dissimilar mechanisms of sequestration and/or properties of the two compounds. In another study, they showed that the change in bioavailability and sequestration of the compounds with time could be predicted using soil properties (personal communication). However, the initial concentration of a chemical in contaminated soil is rarely known in a real situation since soil samples are collected at the time of the sites pollution. The present study suggests that the initial concentration of a chemical in soil might be estimated, at some degree, from soil properties and the availability of the chemical. In view of the implications with risk assessment, the estimation may be useful in resolving related problems.

Materials and Methods

Measurements of soil properties. Soils were chosen based on dissimilar physicochemical properties. Soil preparation was performed as previously described.⁵⁾ The particle-size distribution was assessed using the pipet method, organic C through combustion at 1000°C, the cation-exchange capacity (CEC) through the procedure described by Rhoades, and sur-

*Corresponding author
Phone : 82-2-3290-3026, FAX : 82-2-3290-3503
E-mail : nchung@korea.ac.kr

Abbreviations: CEC, cation exchange capacity.

face area was measured using ethylene glycol monoethyl ether (EGME).¹¹⁻¹³⁾

Determination of sequestration. The procedure for determining sequestration and the full set of sequestration data were presented elsewhere.⁵⁾ In essence, the procedure involves aging process of phenanthrene (10.0 µg/10 g of dry soil) or atrazine (60.0 µg/10 g of dry soil) containing 10⁵ dpm (disintegrations per minute) of ¹⁴C for 0, 20, 60, 120, and 200 days under a sterile condition. The concentrations of the chemicals were chosen since the characteristics of sequestration were observed best at the indicated concentrations. Moisture level of the soils was brought to 80% of field capacity. Triplicate

samples of soil containing each chemical were aged at room temperature for each incubation period, after which each of triplicate samples was subjected to the measure of the extent of sequestration. Sequestration represents decline in the amount of compounds mineralized by bacteria or extracted by a mild extractant as a function of time. Bioavailability was measured based on the release of ¹⁴CO₂ from the labeled compounds. The extractants were 70% and 95% ethanol for phenanthrene and atrazine, respectively.

Statistical analyses. Linear or multiple linear-regression models were used to assess possible relations between the sequestration of each chemical and soil properties. Simple

Table 1. Values measured for extents of sequestration.

Soil	Series	Phenanthrene				Atrazine			
		% Mineralized		% Extracted		% Mineralized		% Extracted	
		0 day	200 days	0 day	200 days	0 day	200 days	0 day	200 days
1	Fuquay sandy silt loam	55.8	38.0	91.8	65.1	89.7	65.6	88.4	79.5
2	Clarence clay	59.2	49.6	76.5	70.8	56.2	56.8	39.7	34.2
3	Dothan sandy clay loam	45.7	33.4	89.5	56.7	75.8	48.3	75.4	58.8
4	Palouse silty clay loam	66.3	48.8	81.4	58.1	67.6	56.6	82.2	57.8
5	Catalina silty clay	53.8	34.7	86.2	64.8	67.6	58.0	81.2	62.3
6	Armor silty clay loam	62.0	42.9	87.8	62.9	68.6	48.7	73.2	53.4
7	Halii sandy loam	46.6	44.1	81.7	54.3	88.5	21.8	61.9	19.9
8	Yolo silt loam	66.4	36.0	69.6	29.3	70.0	41.6	58.2	21.2
9	Madrid loam	62.8	45.6	74.9	58.1	77.9	31.0	81.8	28.1
10	Ross silt loam	51.6	27.7	56.5	25.9	78.3	35.2	66.8	21.4
11	Adjidaumo clay	43.9	27.7	63.1	41.8	79.3	41.2	62.0	21.6
12	Angola silty clay loam	55.7	34.1	65.6	48.8	77.8	32.8	69.2	24.0
13	Langford silt loam	61.7	41.9	69.4	57.1	80.2	34.9	74.8	25.2
14	Madalin clay	50.0	33.7	65.4	46.7	81.2	30.5	61.6	14.0
15	Lima loam	55.9	27.9	52.9	22.7	89.8	23.1	66.5	15.2
16	Quillayute silt loam	65.2	30.2	67.9	13.6	48.4	23.3	66.1	11.1

Table 2. Properties of soils.

Soil	Organic C (g · kg ⁻¹)	Particle size distribution (%)			CEC (cmol · kg ⁻¹)	Surface area (m ² · g ⁻¹)
		Sand	Silt	Clay		
1	0.70	77.1	1.80	20.5	2.15	29.4
2	3.00	1.50	33.9	64.6	14.7	115
3	3.40	58.7	10.7	30.6	5.75	67.3
4	3.40	10.1	69.5	20.4	20.6	132
5	4.10	6.50	47.7	45.8	24.8	145
6	6.20	8.80	55.7	35.5	20.0	136
7	9.90	66.2	26.2	7.60	20.9	124
8	13.4	19.5	56.0	24.5	22.1	132
9	35.8	9.30	37.2	13.5	13.7	39.1
10	37.1	19.3	61.2	19.5	20.7	46.9
11	44.9	3.60	35.9	60.5	36.3	122
12	46.4	12.1	57.2	30.5	25.5	64.4
13	47.3	3.00	55.3	21.7	22.0	47.6
14	69.5	30.0	22.0	48.0	37.8	126
15	71.7	34.2	44.3	21.5	32.7	53.9
16	110	21.0	57.0	22.0	54.1	87.6

Table 3. Simple correlation coefficients (*r*) between phenanthrene sequestration and soil properties.^a

Soil property	% Mineralized		% Extracted	
	0 day	200 days	0 day	200 days
Organic C	0.072	-0.554*	-0.722**	-0.740***
Log (organic C)	-0.002	-0.494*	-0.845***	-0.703**
Sand	-0.280	0.044	0.348	0.111
Log (sand)	-0.115	-0.139	0.112	-0.178
Silt	0.579*	0.018	-0.401	-0.359
Log (silt)	0.366	0.007	-0.496*	-0.350
Clay	-0.278	-0.080	-0.027	0.263
Log (clay)	-0.154	-0.191	-0.047	0.180
CEC	0.066	-0.481*	-0.607*	-0.679**
Log (CEC)	0.104	-0.282	-0.652**	-0.546*
Surface area	0.002	0.204	0.216	0.149
Log (surface area)	-0.021	0.151	0.130	0.068

^aSignificance level was $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***). If not marked, significance level was $p > 0.05$.

Table 4. Simple correlation coefficients (*r*) between atrazine sequestration and soil properties.^a

Soil property	% Mineralized		% Extracted	
	0 day	200 days	0 day	200 days
Organic C	-0.112	-0.740**	-0.216	-0.730**
Log (organic C)	0.016	-0.863***	-0.339	-0.889***
Sand	0.588*	-0.107	0.275	0.227
Log (sand)	0.575*	-0.356	0.238	0.025
Silt	-0.434	-0.194	-0.193	-0.310
Log (silt)	-0.428	-0.401	-0.307	-0.539*
Clay	-0.334	0.381	-0.167	0.039
Log (clay)	-0.371	0.434	-0.122	0.114
CEC	-0.338	-0.594*	-0.163	-0.679**
Log (CEC)	-0.321	-0.615*	-0.294	-0.749***
Surface area	-0.403	0.191	0.045	0.016
Log (surface area)	-0.448	0.091	-0.016	-0.089

^aSignificance level was $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***). If not marked, significance level was $p > 0.05$.

correlation and linear or multiple linear-regression analyses were performed using the SAS system (version 6.12, SAS Institute, Cary, NC, USA). For linear or multiple linear regression, a default value of $p = 0.15$ was employed. Stepwise procedures were used to identify sets of independent variables that could best estimate the response variables. The multicollinearity of independent variables was assessed using COLLIN and VIF option in the model statement. When resulting models showed problems with multicollinearity, sets of independent variables were selected manually.

Results

Previous studies have shown that the extent of sequestration declines with time.^{4,9)} Chung and Alexander⁵⁾ showed that the extent of bioavailability, as measured based on mineralization of phenanthrene or atrazine, decreased rapidly until 60 days and then rather slowly until 200 days. They⁵⁾ also showed that the extent of extractability, as measured through mild extrac-

tion of phenanthrene or atrazine, decreased with a similar trend. In general, the decrease in the extent of availability followed hockey-stick shape.¹⁻³⁾ Table 1 shows the values measured for the extents of sequestration at 0 day and 200 days. The values used to indicate extent of sequestration were the percentage mineralized (% mineralized) or the percentage extracted (% extracted) at 0 or 200 day. The data indicated that some of the added chemicals were not bioavailable or extracted even at 0 day and that the degree of sequestration varied among 16 soils. It was thus difficult to estimate the initial concentration of a contaminating chemical, which, however, may be possible to be estimated using the extents of sequestration at 200 days and the soil properties. Soil properties of 16 soils are listed in increasing order of organic C content (Table 2). The soil properties including organic C, particle size distribution, CEC, and surface area, varied greatly.

Relationship between the extents of sequestration and properties of 16 soils or the logarithm of properties were determined by obtaining simple correlation coefficients (*r*). In case

Table 5. Multiple linear regression analyses between soil properties and percentages of initial bioavailability or extractability.

Compound	Measure of sequestration	Regression model ^a	R ²
Phenanthrene	% Mineralized	$y = 41.31 - 0.128 \text{ Organic C}$	0.307
	% Extracted	$y = 61.08 - 0.396 \text{ Organic C}$	0.548
Atrazine	% Mineralized	$y = 37.56 - 7.781 \text{ Log (organic C)} + 8.172 \text{ Log (clay)} + 0.951 \text{ Log (sand)}$	0.894
	% Extracted	$y = 68.07 - 12.53 \text{ Log (organic C)}$	0.790

^aIndependent variables, which were significantly correlated ($p < 0.15$) with response variables, were tested in regression models for their ability to predict the response variables.

of phenanthrene, the r values for % mineralized at 0 day vs. silt content and at 200 day vs. organic C content were >0.50 , while those for % mineralized vs. other properties were not significant except for CEC (Table 3). The r values for % extracted vs. organic C content was >0.70 and those for % mineralized vs. CEC was >0.60 . In case of atrazine, the r values for % mineralized and % extracted at 200 days vs. organic C content was >0.72 . Sand content had r values of >0.57 with % mineralized at 0 day, and clay content had r values of >0.38 with % mineralized at 200 days. The high r values with organic C content concurred with CEC. This view is supported by the finding that organic C content and CEC of 16 soils were highly correlated ($r = 0.853$). The logarithmic val-

ues of soil properties sometimes gave better correlation coefficients than the nonlogarithmic ones (Tables 3 and 4), especially in the case of organic C.

To assess whether the initial concentration could be estimated, a measure of sequestration (i.e., % mineralized or extracted) should be first predicted based on various soil properties. Thus, the multiple linear-regression analyses were performed for the measures of sequestration at 200 day since the actual concentration of chemical in soil, a necessary component to calculate the initial concentration, is not available at 0 day in a real situation (see below for details). The multiple-regression equation had the following form:

$$Y = b_0 + b_1X_1 + \dots + b_kX_k$$

where Y is the predicted value (in percent) of sequestration measure, b_0 is the Y intercept, b_1 is the regression coefficient of the first independent variable X_1 , b_k is the regression coefficient of the k th variable X_k , and X_1 and X_k are soil properties. Coefficients of determination (R^2) for the predicted value were also calculated. The values of R^2 varied appreciably, and the combination of soil properties that gave the highest values depended not only on the measure of sequestration but also on the compound (Table 5). For % mineralized, three soil properties (log organic C, log clay, and log sand) have been chosen, while for the others, one property (log organic C or organic C) was selected. However, neither CEC nor surface area was selected for the multiple regression analysis. For all the measures of sequestration, better correlations were obtained for the sequestration of atrazine than for the that of phenanthrene.

Equations 2 ($R^2 = 0.548$) and 3 ($R^2 = 0.894$) from Table 5 were employed to calculate the initial concentrations of phenanthrene and atrazine, respectively. The extracted or mineralized amounts of each chemical at 200 day were obtained for each soil from original data set of Table 1. Subsequently, the amounts were divided by the percentage/100, where the percentages were obtained from the selected equations and soil properties (Fig. 1). As expected from R^2 value, some of the calculated values of phenanthrene appreciably deviated from the actual added amount of $1 \text{ mg} \cdot \text{kg}^{-1}$. Notably, the calculated values deviated considerably with soil of high organic C (Soils 8-16), but not much with the soils of low organic C (Soils 1-7). However, in most cases, the calculated value of atrazine stayed close to the actual treatment of $6 \text{ mg} \cdot \text{kg}^{-1}$.

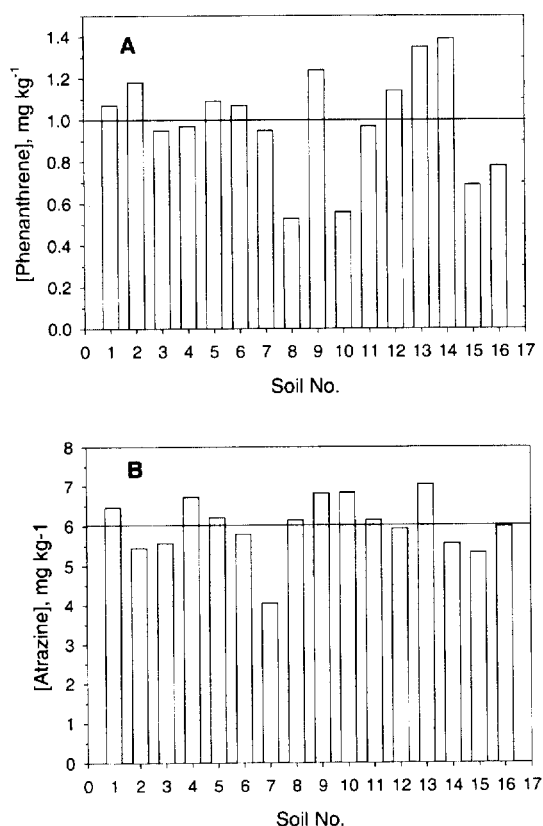


Fig. 1. Initial and predicted concentrations of phenanthrene (A) and atrazine (B). Lines indicate the initial concentrations at which phenanthrene ($1 \text{ mg} \cdot \text{kg}^{-1}$) or atrazine ($6 \text{ mg} \cdot \text{kg}^{-1}$) were added to soils.

Discussion

Several lines of evidence point to the sequestration of the chemicals that persist in soil.¹⁻⁹⁾ Many recent studies showed that increased contact time between chemicals and soil decreased the availability of the chemicals toward the microbes responsible for biodegradation, making them more difficult to be bioremediated. In addition, the increased contact time also decreased the extractibility of the chemicals from the soil.⁴⁻⁹⁾ Sorption and desorption may be important mechanisms for chemical fates in soil. Alexander¹⁻³⁾ summarizes hocky-stick pattern of sorption and desorption mechanisms, where each one follows a two-phase pattern, a rapid phase followed by a much slower phase. The toxicity of many pesticides to plants and insects declines with increasing residence time in soil. All the evidences mentioned above indicate that there are underlining scientific basis for the sequestration and bioavailability of organic chemicals in soil. The basis may be critical for us to determine an environmentally acceptable endpoint and develop an economically viable remediation technology.

Most polluted soils were contaminated for at least many years. Thus, sequestration is particularly significant, considering its relation to bioremediation and the toxicology of poorly degradable chemicals. Although many studies have revealed factors affecting the sequestration and aging, more remains that are not understood well.¹⁴⁾ However, we can use known facts to enhance the bioavailability of organic compounds in soil to meet the level permitted by the regulatory agencies or to reduce the toxicity of the compounds in soil. Before the known technologies are applied, it is of utmost importance to get information on the type and initial concentration of contaminating chemicals. The information could help us understand how much chemicals were degraded or sequestered. The information on the initial concentration of the chemicals would help to establish the environmentally acceptable endpoint as well as the economically viable bioremediation strategies. This is because vigorous extraction removes both the bioavailable and the sequestered fraction of the target compounds so that it overestimates the concentration accessible to living organisms.^{1-3,14)} Bioremediation often does not reduce the level of target chemicals below regulatory standard based on the vigorous extraction methods. Thus, the remaining amount would pose little risk. Therefore, although the regulatory procedure suggests a failure, the result of the remediation may have met its actual objectives.^{1-3,14)}

Regardless of the usefulness of the present study, more points should be considered before the estimation technique for the initial concentration can be applied to the field. For example, soils are often contaminated with a mixture of chemicals. Thus, we need to gather further information on how each chemical behaves and interacts with each other in a vari-

ety of soils to affect the sequestration and, thus, the estimation of the initial concentrations.

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