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Effects of Sorption on Biological Degradation Rates of (2,4-Dichlorophenoxy)acetic Acid in Soils†

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Three mathematical models were proposed to describe the effects of sorption of both bacteria and the herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D) on the biological degradation rates of 2,4-D in soils. Model 1 assumed that sorbed 2,4-D is not degraded, that only bacteria in solution are capable of degrading 2,4-D in solution, and that sorbed bacteria are not capable of degrading either sorbed or solution 2,4-D. Model 2 stated that only bacteria in the solution phase degrade 2,4-D in solution and that only sorbed bacteria degrade sorbed 2,4-D. Model 3 proposed that sorbed 2,4-D is completely protected from degradation and that both sorbed and solution bacteria are capable of degrading 2,4-D in solution. These models were tested by a series of controlled laboratory experiments. Models 1 and 2 did not describe the data satisfactorily and were rejected. Model 3 described the experimental results quite well, indicating that sorbed 2,4-D was completely protected from biological degradation and that sorbed- and solution-phase bacteria degraded solution-phase 2,4-D with almost equal efficiencies.

Most research to date concerning pesticide degradation has focused on describing the metabolic pathways by which these chemicals are degraded and on empirically describing the kinetics of degradation (1–3). Little work, however, has been done to describe mechanistically the processes which govern the degradation of pesticides (4, 8).

Some factors which influence intracellular degradation rates may be related to availability of the pesticide to the degrading organisms. When pesticides such as paraquat and diquat are intercalated into clays (2, 13) or are irreversibly bound to soil organic matter as is dichloroaniline (6), they are isolated from the degrading organisms and are thereby protected from intracellular degradation. These cases are somewhat unusual, however, because most pesticides reversibly partition between the soil solution and the soil organic matter (7, 11). Although sorption may increase the amount of chemical degradation as in the case of surface-catalyzed hydrolysis of triazine herbicides (1, 12), it is not known whether sorption per se renders a pesticide unavailable for uptake by microbes. Since bacteria themselves may be sorbed, it is conceivable that bacteria and pesticide may be sorbed on adjacent locations on the soil surface, thereby facilitating scavenging of the chemical by the sorbed bacteria. Thus, pesticide sorption might either enhance or decrease microbial degradation rates in soils, depending upon whether the sorbed pesticide is available.

The major objective of the research reported here was to determine whether an intracellularly metabolized pesticide, (2,4-dichlorophenoxy)acetic acid (2,4-D), may be degraded while it is sorbed to soil and, if so, to compare 2,4-D degradation rates in the sorbed and the solution phases. The relative contributions to 2,4-D degradation by sorbed- versus solution-phase (free) bacteria were also investigated. Because variables in the experimental design were closely controlled, the results may vary from those one would expect from an in situ field study.

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THEORY

Three different mathematical models were evaluated to study the effects of sorption on the biological degradation of 2,4-D. Relationships between degradation and sorption of both bacteria and 2,4-D were incorporated into these models.

A number of assumptions were made to simplify the formulation of the models. First, it was assumed that the mineralization of 2,4-D (i.e., degradation to CO_2) could be described in terms of first-order kinetics. Bacterial growth was not accounted for, so experimental conditions had to be controlled to negate any growth during the course of the experiment. If growth had been allowed, the number of bacteria would have changed, and the microbes could have grown more quickly in one phase than in another. Also inherent in these models was the assumption that no intermediate metabolites left the cell. The metabolites could have had different adsorption characteristics than the parent compound and would have complicated considerably the formulation of these models.

The sorption of bacteria and 2,4-D was assumed to be characterized by linear isotherms described by the following equations. For 2,4-D,

$$S = K_D C \tag{1a}$$

For bacteria,

$$N_s = K_B N_w \tag{1b}$$

where S (micrograms gram^{-1}) and N_s (cells gram^{-1}) are the amounts of 2,4-D and bacteria, respectively, sorbed on soil; K_D and K_B are the respective sorption coefficients (milliliters gram^{-1}); and C (micrograms milliliter^{-1}) and N_w (cells milliliter^{-1}) are the solution-phase concentrations of 2,4-D and bacteria, respectively. Linear sorption isotherms were assumed so that the models could be solved analytically.

The herbicide 2,4-D was chosen for these experiments because (i) a pure colony of 2,4-D-degrading bacteria could easily be isolated, (ii) 2,4-D is intracellularly degraded, and (iii) 2,4-D mineralization can be described by first-order kinetics. It was also believed that 2,4-D is metabolized

rapidly enough that no by-products are likely to leave the cell before complete mineralization to CO_2. The validity of these assumptions was explored and will be discussed below.

The first model (model 1) stated that only 2,4-D in solution is degraded and that it is degraded only by bacteria in solution. This model implicitly assumed that sorbed 2,4-D is completely protected from degradation and that sorbed bacteria are unable to degrade any 2,4-D in solution. This model may be written as follows:

$$dT/dt = -k_w C N_w W \quad (2)$$

where dT/dt is the change in mass of the pesticide with time (micrograms minute^{-1}), k_w is the degradation rate coefficient (milliliters cell^{-1} minute^{-1}), W is the volume of water (milliliters) in the system, and C and N_w are as defined for equation 1. k_w includes the rate at which the bacteria in solution encounter 2,4-D in solution, the rate of 2,4-D uptake by the cells, and the kinetics of the biochemical pathways involved.

Model 2 stated that bacteria in a given phase degrade only the 2,4-D in that phase; i.e., only sorbed bacteria degrade sorbed 2,4-D, and only bacteria in solution will degrade solution-phase 2,4-D. The total amount of 2,4-D degraded would then equal the amount degraded from the solution phase ($k_w C N_w W$) plus that degraded in the sorbed phase ($k_{ss} S N_s M$), as shown by the following equation:

$$dT/dt = -(k_w C N_w W + k_{ss} S N_s M) \quad (3)$$

where k_{ss} (grams cell^{-1} minute^{-1}) is the rate coefficient for degradation in sorbed phase, M is the mass of soil (grams), and other terms are as defined earlier. In this model, k_{ss} is composed of the rate at which cells are likely to come into contact with sorbed 2,4-D, the rate of uptake of the 2,4-D, and the kinetics of the biochemical pathways.

The third model (model 3) explored the possibility that only 2,4-D in solution is available for degradation but that bacteria in both adsorbed and solution phases would be capable of degrading 2,4-D. The total amount of 2,4-D degraded would be equal to that degraded in solution by bacteria in solution ($k_w C N_w W$) plus the amount degraded in solution by sorbed bacteria ($k_{sw} C N_s M$), as follows:

$$dT/dt = -(k_w C N_w W + k_{sw} C N_s M) \quad (4)$$

In this case, k_{sw} (milliliters cell^{-1} minute^{-1}) represents the combined effects of the rates at which sorbed bacteria encounter 2,4-D in solution, take it up, and then mineralize it to CO_2. Solving these equations analytically for the production of CO_2 (refer to the Appendix for a more detailed derivation), we find

$$P = P_{max}[1 - \exp(-k_i^* t)] \quad (5)$$

where P is the amount (micrograms) of 2,4-D mineralized over time, P_{max} is the maximum amount (micrograms) of potentially mineralizable 2,4-D, and k_i^* is the first-order degradation rate coefficient for each model. Values of k_i^* may be estimated by fitting equation 5 to the experimental data for CO_2 evolution (mineralization).

As can be seen from the definitions of k_i^* (see the Appendix), if W, M, K_D, and N_s and N_w are known, the only unknowns are the values of the degradation coefficients (k_w, k_{sw}, and k_{ss}) in the solution and sorbed phases. By varying the soil/solution ratios (M/W), the number of bacteria and the amount of 2,4-D in each phase may be controlled, thereby varying k_i^*. By rearranging the equations defining k_i^*, it can be seen that a linear regression can be performed on the data, with k_{ss} and k_{sw} describing the slope of the line (equal to zero for model 1) and k_w as the y intercept. These forms of the equations are listed below. For model 1,

$$k_1^*[(W + MK_D)/(N_w W)] = k_w \quad (6)$$

For model 2,

$$k_2^*[(W + MK_D)/(N_w W)] = k_{ss}[(N_s MK_D)/(N_w W)] + k_w \quad (7)$$

For model 3,

$$k_3^*[(W + MK_D)/(N_w W)] = k_{sw}[(N_s M)/(N_w W)] + k_w \quad (8)$$

If model 1 satisfactorily described the data, we expected the calculated values of k_w to be constant across different soils and different M/Ws. If either model 2 or 3 described the data, we expected the k_{ss} of K_{sw} and k_w terms from the correct model to be independent of soil type.

MATERIALS AND METHODS

Soils. Three soils and one clay were chosen for this study. Soils with a wide range of properties (Table 1) were selected so that the three models could be tested under a broad range of conditions.

Determination of optimum bacterial concentration. A pure culture of 2,4-D-degrading bacteria isolated by L. T. Ou (University of Florida) was used in this study. These bacteria were tentatively characterized as being similar to the genus *Flavobacterium*. The optimum concentration of bacteria for the rapid degradation of 2,4-D via first-order kinetics was found by first growing the cells at 25°C in 2 liters of 2,4-D mineral medium (9), which was continually shaken, and then harvesting during late log phase (4 to 5 days). These cells were then centrifuged, washed with 100 ml of pH 7 phosphate buffer (4.8 g of K_2HPO_4 and 1.2 g of KH_2PO_4 per liter), centrifuged again, and suspended in phosphate buffer.

A Petroff-Hauser counting chamber was then used to determine bacterial concentrations, serial dilutions made to yield final concentrations of 10^{10}, 10^9, 10^8, and 10^7 cells ml of phosphate buffer^{-1}. Of each concentration, 1 ml was then injected through the top of a 125-ml Erlenmyer flask containing 102.5 μg of 2,4-D and 1 μCi of [^{14}C]2,4-D dissolved in 10 ml of pH 7 phosphate buffer. These flasks were shaken on a rotary shaker and maintained at 25°C.

Samples (0.5 ml) were taken from each flask at 15, 25, 35, 45, 60, 90, 180, 240, and 330 min, and the samples were immediately added to scintillation vials along with 10 ml of scintillation cocktail (6 g of PPO [2,5-diphenyloxazole], 0.75 g of POPOP [1,4-bis-(5-phenyloxazolyl)benzene], 400 ml of 2-methoxyethanol, 600 ml of toluene). Previous tests had

TABLE 1. Selected properties of soils

Soil or clay	Texture	Major clay minerals	% Organic carbon
Webster	Silty clay loam	Smectite, mica, vermiculite, kaolinite	3.59
Eustis[a]	Silt and clay	Kaolinite, gibbsite	7.10
Cecil	Loamy sand	Kaolinite, gibbsite, vermiculite, iron oxides	1.02
Montmorillonite	Clay	Smectite	0

[a] Only the silt and clay fraction of this fine sandy soil was used.

confirmed that significant amounts of $^{14}CO_2$ were not retained in the aqueous sample as HCO_3^-. The scintillation cocktail stopped the mineralization of 2,4-D. Each sample was then analyzed for ^{14}C by using a liquid scintillation counter, and the amount of ^{14}C was related to the amount of 2,4-D mineralized. Data for the loss of 2,4-D from solution were fitted to first-order degradation kinetics to estimate k_i* values in equation 5. All experiments were duplicated.

2,4-D sorption isotherms. Soil samples were first sterilized by autoclaving and then ground to pass through a 60-mesh (nominal diameter, 250 μm) sieve. The absence of 2,4-D-degrading organisms in these soils was checked by plating a 1/10 dilution onto 2,4-D mineral medium and 2% agar and incubating for 2 days at 25°C. The amount of pesticide sorption by the three soils and the clay was determined by a batch slurry method as described by Green et al. (5). Uniformly ring-labeled [^{14}C]2,4-D with a specific activity of 2.38 mCi mmol^{-1} used in this study was purified by preparative thin-layer chromatography as described by Ou et al. (9). The sorption data were fitted to equation 1a, and the value of 2,4-D sorption coefficient K_D was estimated.

Montmorillonite is an expanding 2:1 clay which therefore intercalates water. This intercalation excludes 2,4-D from some of the water, and a correction should be made to account for this. The amount of water absorbed by the clay was measured by mixing 10.2 ml of phosphate buffer with 0.5, 1.0, and 2.0 g of clay, shaking for 30 min, and then filtering. The volume of the residual buffer was measured, and the amount lost was assumed to have been absorbed by the clay. A linear regression was performed on these data, indicating that 3.57 ml of buffer was absorbed per g of clay. This correction factor was used for all data analysis involving montmorillonite.

Bacterial sorption isotherms. The amounts of bacteria sorbed by the soils were determined similarly to those for 2,4-D sorption. The bacteria were labeled with ^{14}C by growing a culture in 50 ml of 2,4-D mineral medium (1,000 μg of 2,4-D ml^{-1}) containing 30 μCi of [^{14}C]2,4-D for 4 days, until the culture had reached the top of the log-growth phase. These cells were then harvested by centrifugation, washed in phosphate buffer, centrifuged again, and suspended in pH 7 phosphate buffer. A 0.5-ml sample of this bacterial suspension was counted by liquid scintillation, with the bacterial concentration being determined with a Petroff-Hauser counting chamber. Dilutions of 10^9 and 10^8 cells ml^{-1} were made from this stock suspension. Of each dilution, 2 ml was added to 1 g of soil and shaken on a tumbling shaker for 5 h at 25°C. Due to absorption of the buffer solution by montmorillonite, only 0.5 g of clay was used. The suspension was allowed to clear by settling, which ranged from ca. 30 min for the Eustis soil to 12 h for the Webster soil. After the supernatant had cleared, 0.5 ml was sampled and ^{14}C activity was measured by liquid scintillation counting. Equilibrium amounts of bacteria in the sorbed and solution phases were calculated, and the data were fitted to equation 1b, yielding the value for bacterial sorption coefficient K_B.

The above technique for bacterial sorption assumed that all of the ^{14}C remained within the cell and was not evolved as $^{14}CO_2$. This assumption was checked by collecting $^{14}CO_2$ above a sample of labeled bacteria and soil for the duration of the experiment. No $^{14}CO_2$ was collected, indicating that the ^{14}C stayed incorporated in the biomass. Good agreement was found between bacterial counts determined by the radioisotope method and by using the Petroff-Hauser counting chamber.

2,4-D mineralization studies. The 2,4-D mineralization experiments were set up so that time course experiments could be conducted with several soil/solution ratios. All experiments were duplicated.

Pure cultures of 2,4-D-degrading bacteria were grown in a mineral medium (9) containing 1,000 μg of 2,4-D ml^{-1}. Cells were harvested by centrifugation during the late log growth phase and were then washed with sterile phosphate buffer, centrifuged, and suspended in sterile phosphate buffer to make a final bacterial concentration of ca. 10^9 cells ml^{-1} as determined with a Petroff-Hauser counting chamber.

[^{14}C]2,4-D (0.5 μCi) in methanol was added to each reaction vessel, and the methanol was evaporated off. A sterile phosphate buffer containing 102.5 μg of 2,4-D was added to each reaction vessel containing soil. All soils were sterilized by autoclaving and ground to pass a 60-mesh sieve. To each reaction vessel, 10 ml of sterile phosphate buffer was added. KOH pellets (four to five) were added to stainless-steel CO_2 traps suspended from the top of each reaction vessel, and the vessels were stoppered.

Of the bacterial suspension, 1 ml was injected by hypodermic syringe through a hole in the stopper. This hole was immediately sealed with putty, and the vessels were placed on a rotary shaker and maintained at 25°C. The reaction was stopped in individual vessels at either 30, 60, 120, 190, or 300 min by injection of either 0.1% acidified $HgCl_2$ or 37% Formalin. Each of these solutions was found to stop the mineralization of 2,4-D immediately. The vessels were allowed to sit overnight to allow enough time for the trapping of $^{14}CO_2$ by the KOH. The traps were then dropped into tubes containing 20 ml of water and mixed thoroughly, and 0.5 ml was sampled and counted by liquid scintillation. This method was found to yield an average recovery of 95%. These data were used to calculate the amount of 2,4-D mineralized (P) as a function of time. These data were fitted to equation 5, and the first-order degradation rate coefficient, k_i*, was determined.

RESULTS AND DISCUSSION

Preliminary experiments. Certain assumptions made in the development of the three models (equations 2, 3, and 4) were checked by a series of preliminary experiments.

A suspension of *Flavobacterium*-like cells was desired which could be easily obtained and could rapidly degrade 10 μg of 2,4-D ml^{-1} by first-order kinetics. At 10^7 cells ml^{-1}, 2,4-D degradation followed zero-order kinetics. Concentrations of 10^9 and 10^{10} cells ml^{-1} produced first-order kinetics, but it was difficult to concentrate the bacterial suspension to this degree. A suspension of 10^8 cells ml^{-1} was easily obtainable, and the mineralization of 2,4-D followed first-order kinetics. For this suspension, the value of degradation rate coefficient k_w was 6.8×10^{-11} ml cell^{-1} min^{-1}. Data for the mineralization of 2,4-D by these different bacterial suspensions are presented in Fig. 1. It was found that not all of the 2,4-D present in the system was mineralized; typically, only 60 to 70% of the 2,4-D was mineralized. This may have been due in part to the incorporation of carbon into microbial biomass and in part to possible inhibition of degrading enzymes.

The assumption of instantaneous sorption was checked by measuring the amount of 2,4-D sorbed after contact times varying from 5 to 60 min. It was found that the sorption kinetics were indeed rapid, with ≥98% of 2,4-D sorbed at equilibrium having been sorbed within the first 5 min. Equilibrium sorption of both 2,4-D and bacteria was described well ($r \geq 0.99$) by a linear isotherm (equation 1). The

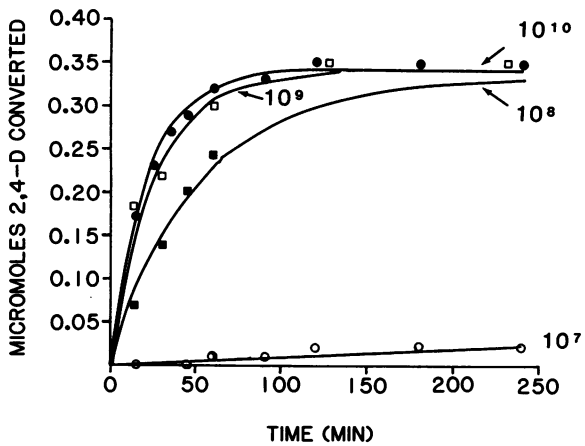

FIG. 1. Mineralization of 2,4-D by suspensions of a pure bacterial culture. Numbers by the curves indicate bacterial concentrations (cells milliliter^{-1}).

measured values of the sorption coefficients K_D and K_B are listed in Table 2. Sorption of both 2,4-D and bacteria was largely controlled by the soil organic-carbon content, with more sorption occurring as the organic-carbon content increased. This result was consistent with the trend reported for sorption of a number of pesticides on soils and sediments (7, 11). 2,4-D was not sorbed on the negatively charged montmorillonite, and there was actually some repulsion seen as a result of a similar charge on 2,4-D. The intercalated water in montmorillonite excluded 2,4-D, resulting in a higher 2,4-D concentration being found in the supernatant than that added in the batch-slurry sorption experiments. In all experiments with montmorillonite, a correction was made for the intercalated water. No adsorption of bacteria was noted for montmorillonite, although one might expect some sorption in the presence of high electrolyte concentrations (3).

Model verification. Model 1 stated that only 2,4-D in solution was available for degradation and that only bacteria in solution would be capable of degrading it. As is evident from equation 6, if this model were correct, the calculated values of k_w (the degradation rate coefficient for bacteria in solution degrading solution 2,4-D) should have been constant with varying soil/solution ratios and for different soils. Results from an analysis of the experimental data by using model 1 are presented in Table 3. It is evident that the k_w values were not constant, with the exception of those obtained for montmorillonite. For a given soil, k_w values increased with increasing soil/solution ratios. Between soils, k_w values also increased with increasing organic-carbon content, bacterial sorption, and 2,4-D sorption. Montmorillonite was the exception, with k_w values remaining essentially constant with increasing soil/solution ratios. By a rearrangement of equation A3 in the appendix, it seems evident that $MS = T/[1 + (W/MK_D)]$ and that for a given soil (fixed K_D), MS increases as (W/M) decreases. Thus, as the soil/solution ratio increases, sorption of both bacteria and 2,4-D should increase, except for montmorillonite ($K_D = 0$; $K_B = 0$), in which neither 2,4-D nor bacteria were sorbed. These results indicate that model 1 did not accurately describe the data. They suggest either that sorbed 2,4-D was being degraded or that sorbed bacteria may have been degrading the 2,4-D in solution.

Model 2 stated that the 2,4-D in solution was degraded only by those bacteria in solution and that the sorbed 2,4-D was degraded only by those bacteria that were sorbed. Fitting the data to equation 7, as discussed above, indicates that the intercept of the lines should have been equal to k_w, the degradation rate coefficient in solution, and the slopes of the lines should have yielded k_{ss}, the degradation rate coefficient for the sorbed phase. If this model were correct, k_{ss} values should have been the same for all soils. The data are presented graphically in Fig. 2, with the k_w, k_{ss}, and r values being listed in Table 4.

There was a good agreement (within a factor of 3) between all k_w values calculated with this model, and a good correlation for k_{ss} values within soil type was found when the data were regressed (Fig. 2). The agreement of k_{ss} values across soils, however, was not good, with the calculated values ranging from 6.96×10^{-12} g cell^{-1} min^{-1} for Eustis soil to 5.8×10^{-10} g cell^{-1} min^{-1} for Cecil soil, nearly a 100-fold difference. This wide range of values for k_{ss} indicates that model 2 did not accurately describe the data. As sorption of both 2,4-D and bacteria increased, the calculated values of k_{ss} decreased, suggesting that there may have been at least a partial protection of 2,4-D from degradation when it was sorbed.

Model 3 (equation 8) tested for the possibility that 2,4-D in solution could be degraded by bacteria in both the solution and sorbed phases and that sorbed 2,4-D was completely protected from degradation. Results from analyzing the experimental data by this model are presented in Table 5 and

TABLE 2. Sorption coefficients (K_D and K_B) for 2,4-D and bacteria

Soil or clay	Sorption coefficient (ml g^{-1})	
	K_D	K_B
Webster	1.15	66.0
Eustis (silt and clay)	9.05	85.1
Cecil	0.09	8.1
Montmorillonite	0	0

TABLE 3. Degradation rate coefficients (k_w) for model 1

Soil or clay	M/W (g ml^{-1})	k_w ($\times 10^{11}$) (ml cell^{-1} min^{-1})
No soil	0	6.28
Webster	0.00889	8.23
	0.0889	28.4
	0.175	52.2
	0.349	101.0
	0.437	126.0
	0.524	118.7
	0.699	218.0
	0.889	278.0
Cecil	0.0429	9.42
	0.0858	11.5
	0.258	19.24
	0.343	17.9
	0.515	26.0
	0.687	38.0
	0.858	42.1
Eustis (silt and clay)	0.0429	3.44
	0.172	15.4
Montmorillonite	0.051	8.81
	0.123	7.50

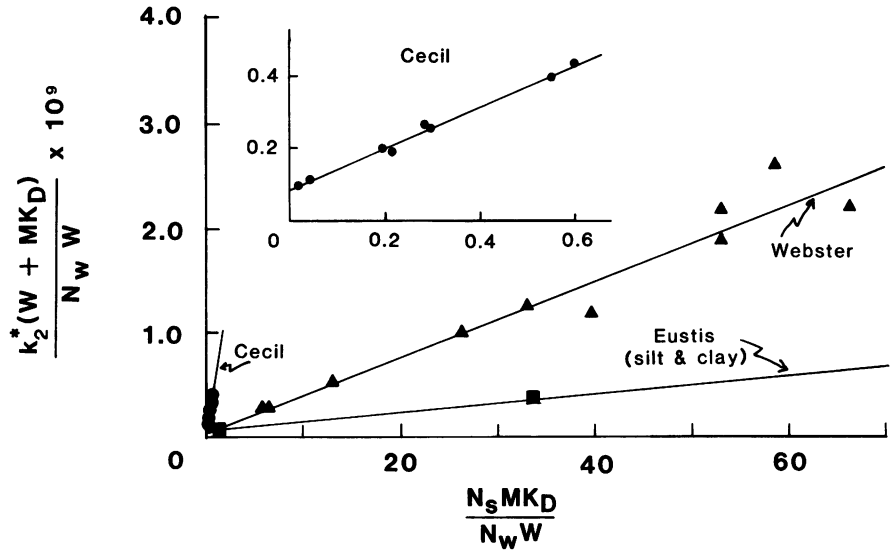

FIG. 2. Regression analysis of the 2,4-D mineralization data by using model 2 (equation 6).

in Fig. 3. The slope of the line in Fig. 3 represents k_{sw}, the degradation rate coefficient for sorbed bacteria degrading solution 2,4-D. The intercept value is k_w, the degradation rate coefficient for solution bacteria degrading solution 2,4-D. A very good correlation ($r \geq 0.99$) was found between all soils, with $k_{sw} = 4.1 \times 10^{-11}$ ml cell^{-1} min^{-1} ($\pm 3.0 \times 10^{-12}$ ml cell^{-1} min^{-1}) and $k_w = 8.5 \times 10^{-11}$ ml cell^{-1} min^{-1} ($\pm 7.41 \times 10^{-11}$ ml cell^{-1} min^{-1}). The ranges noted on these coefficients express the 95% confidence intervals. The high correlation with varying soil types and soil/solution ratios indicates that this model adequately described the data and that 2,4-D was protected when it was sorbed.

The results obtained for montmorillonite were not analyzed by either model 2 or 3 because there was no sorption of either the bacteria or 2,4-D. The results obtained from the montmorillonite experiment, however, were in accordance with the results one would expect with model 3 (Table 3). Since there was no sorption, one would expect mineralization to take place at the same rate as when no clay was present. After corrections had been made for the intercalated water, these results were all similar to the case where no clay or soil was present. Only the corrected values are listed in Table 3.

Values found for degradation coefficients k_w and k_{sw} were similar, but, given the error in determining k_w, it was not possible to conclude whether the two rate coefficients were equivalent or significantly different. Their values were of the same order of magnitude, but k_{sw} could have been as much as four times smaller or larger than k_w.

It was not possible to distinguish between a case in which bacteria were sorbed to soils and one in which small clay particles adsorbed to the bacteria. Since clay particles are smaller than 2 μm, it is possible that both situations occurred in the experiments. If clay particles covered the cell, 2,4-D in solution could have been inhibited from diffusing past the clay barrier and into the cell. This would have resulted in k_{sw} being lower than k_w. If bacteria were sorbed to larger soil particles, however, more surface area could have been available for the uptake of 2,4-D, and a higher k_{sw} would then have been seen than if clay were sorbed to bacteria. This k_{sw} value should still have been lower than the value for k_w. Since soils used in the experiments covered a range of clay contents and since no real difference was observed for the values of k_{sw} calculated for different soils, it can be assumed that these effects, if present, were minimal.

The protection of sorbed 2,4-D from biodegradation may be a result of two factors, exclusion and the inability to scavenge. Sorbed 2,4-D may have been located sufficiently deep within the soil-organic matter matrix that the bacteria were unable to attack it. Bacteria may also have been simply unable to take up 2,4-D from the sorbed phase. It was not possible to distinguish between these two cases. More research is needed to resolve this problem.

The results presented here were for a single strain of bacteria degrading 2,4-D in a rigorously controlled environment and may not be applicable to conditions one would expect to find in situ, where mixed populations may be degrading the pesticide for a much longer time than the 5-h incubation period used here. The model presented here may not be applicable to pesticides that are degraded extracellularly or that require long contact times to reach sorption equilibrium. The next logical step in describing the effects of sorption on degradation rates is to perform laboratory experiments which take these factors into account and to see how well model 3 performs under more natural conditions.

TABLE 4. Degradation rate coefficients (k_w and k_{ss}) for model 2

Soil	k_w ($\times 10^{11}$) (ml cell^{-1} min^{-1})	k_{ss} ($\times 10^{11}$) (g cell^{-1} min^{-1})	r
Webster	4.13	3.65	0.974
Cecil	7.03	57.6	0.993
Eustis (silt and clay)	2.13	0.696	0.998

TABLE 5. Degradation rate coefficients (k_w and k_{sw}) for model 3

Soil	k_w ($\times 10^{11}$) (ml cell^{-1} min^{-1})	k_{sw} ($\times 10^{11}$) (ml cell^{-1} min^{-1})	r
Webster	4.13	4.75	0.984
Cecil	7.03	5.07	0.986
Eustis (silt and clay)	2.13	6.13	0.998
All soils	8.46	4.10	0.986

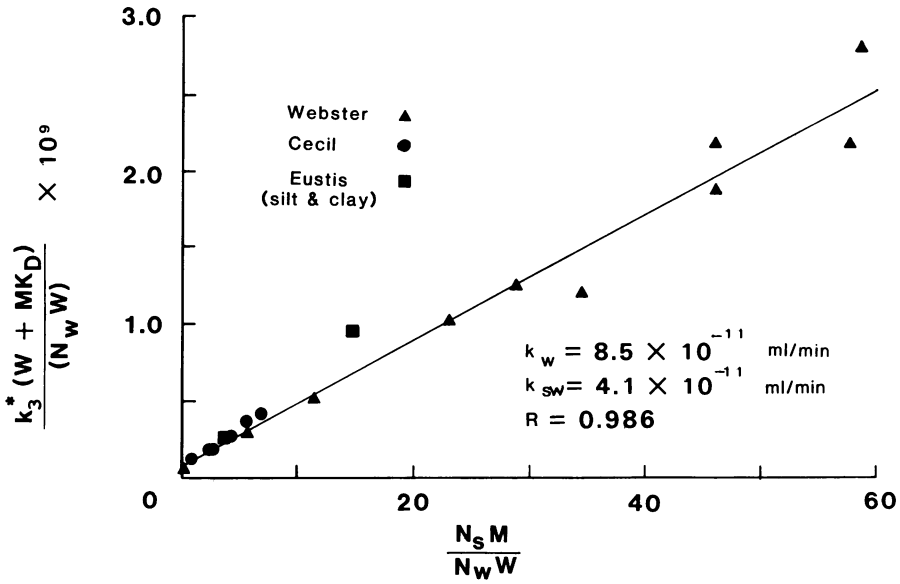

FIG. 3. Regression analysis of the 2,4-D mineralization data by using model 3 (equation 8).

APPENDIX

The total amount (T [micrograms]) of 2,4-D present in the system is equal to the amount in solution (WC) plus that sorbed to soil (MS), as follows:

$$T = WC + MS \quad \text{(A1)}$$

Since $S = K_D C$, equation A1 may be rewritten in either of the following ways:

$$T = (W + K_D M)C, \text{ or } T = \left(\frac{W}{K_D} + M\right)S \quad \text{(A2)}$$

Therefore,

$$C = T/(W + K_D M), \text{ and } S = T\Big/\left(\frac{W}{K_D} + M\right) \quad \text{(A3)}$$

Each of the models may be rewritten by substituting the expressions in equation A3 for C and S in equations 2, 3, and 4 and rearranging to yield the following for models 1, 2, and 3, respectively:

$$dT/dt = -[(k_w N_w W)/(W + MK_D)]T \quad \text{(A4)}$$

$$dT/dt = -[(k_w W N_w + k_{ss} N_s M K_D)/(W + MK_D)]T \quad \text{(A5)}$$

$$dT/dt = -[k_w W N_w + k_{sw} N_s M)/(W + MK_D)]T \quad \text{(A6)}$$

The terms inside the brackets will be redefined as k_i^*, where i = 1, 2, or 3, with the generalized form of each of these equations being written as

$$dT/dt = -k_i^* T \quad \text{(A7)}$$

The integral form of equation A7 is given as equation 5. The value of k_i^* for each model may be found by fitting the amount of 2,4-D mineralized with time to equation 5.

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