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BIOAVAILABILITY OF ORGANIC CONTAMINANTS DURING TRANSPORT: IMPACT OF INITIAL SOIL ENVIRONMENT CONDITIONS

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ABSTRACT

Bioavailability of organic contaminants may be confounded by several processes operating simultaneously in soil subsurface. Model predictions of fate and transport of organic contaminants in soils and groundwater are sensitive to assumptions concerning rates of microbial degradation. In the current study, the herbicide 2,4-D was chosen as a model compound, since it is an aromatic chlorinated molecule, which is structurally similar to numerous other compounds of current interest. The objective was to illustrate the impact of the initial soil environment conditions on 2,4-D bioavailability under batch and column transport conditions. These conditions include: moisture content, initial concentration, stirred reactor versus stationary batch, biomass, aerobic versus anaerobic conditions, and pore water velocity (i.e., residence time). Modeling 2,4-D degradation with first-order kinetics yielded poor predictions of degradation behavior and half-life in soil. Accounting for bioavailability through the use of the modified first-order and logistic models presented more accurate predictions of both the rate and extent of 2,4-D degradation. In the transport environment, it is not clear whether microbial biomass concentration responsible for 2,4-D degradation remain constant under the tested range of pore water velocities. Apparent degradation rate constants may decrease with increasing pore water velocity due to decreases in residence time per unit length (thought of as *local opportunity time*). In summary, the effects of residence time on contaminant bioavailability may be confounded by several processes operating simultaneously. These findings emphasize the difficulty in accurately predicting the degradation and transport of organic contaminants in soils across a range of flow conditions using independently determined rate parameters.
Keywords: Biodegradation, residence time, rate-limited, 2,4-D, kinetics.

INTRODUCTION

The availability of organic contaminants in the subsurface environment is affected by a series of ill-defined, often uncharacterized processes. In some of these processes, the compound is readily evident, and it can be easily removed from the soil by conventional extraction procedures; the evidence of reduced bioavailability of these compounds is the marked decline in the rate of biodegradation (Alexander and Scow, 1989). Reduced bioavailability due to contaminant sorption does not necessarily preclude biodegradation, but does severely limit the rate and extent of contaminant transformation (Doughten, 1997). Several factors may be responsible for reduced bioavailability of sorbed contaminants: 1) desorption kinetics are slow enough that mass transfer essentially limits degradation; 2) sorbed contaminants cannot enter the cell and be acted on by intracellular enzymes; 3) extracellular enzymes are subject to sorption and lose catalytic activity; 4) sorbed contaminants may be less available and accessible to extracellular enzymatic attack; 5) additional growth factors and nutrients may be sorbed and less available for microbial growth and activity; 6) near surface conditions

may be depleted in nutrients or have a lower pH, making it unfavorable for microbial growth and survival; and 7) the microbes themselves are attached and consequently have limited access to the contaminant molecules (Alexander, 1994). A contaminant may become less available or totally unavailable for biodegradation if it enters or is deposited in a micropore that is inaccessible to microorganisms (Langner *et al.*, 1998). A contaminant may be move out of a micrpore by diffusion to a site containing a bacterium where it will be readily bioavailable for biodegradation. Several models have been developed to describe the effect of diffusion on bioavailability (Scow and Alexander, 1992; Scow and Hutson, 1992). The interest and discussion on the bioavailability of sorbed contaminants has been centered on two hypotheses. The first hypothesis is that organisms use only compounds that are in solution; consequently, degradation is rate-limited by desorption kinetics (Guerin and Boyd, 1992). The second hypothesis suggests that bacteria attached to surfaces degrade sorbed substratesand that the proximity between organism and substrate is responsible for degradation (Harms and Zehnder, 1995).

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been widely used to control the growth of broadleaf weeds in cereal grains at rate of 0.28–0.56 kg ha^{-1} (Herbicide Handbook, 1994). In this study, the 2,4-D was chosen as a model compound since this compound is an aromatic chlorinated molecule, which is structurally similar to numerous other compounds of current interest. Because acidic pesticides, such as 2,4-D, can ionize in aqueous solutions forming anion species, they are mainly adsorbed by soil organic matter (Grover, 1971) and not significantly adsorbed by clay minerals (Weber *et al.*, 1965). The preparation method of 2,4-D leads to more impurities among which are highly toxic dioxins (WHO, 1984). Biodegradation kinetics is one of the most important processes affecting the environmental fate of 2,4-D in the soil subsurface environment (Simkins and Alexander, 1984; Ogram *et al.*, 1985; Angley et a., 1992; Donnelly *et al.*, 1993; Estrella *et al.*, 1993). Like most processes occurring in nature, biodegradation is governed by numerous initial soil environment conditions (e.g., soil type, pH, moisture content, initial concentration, indigenous microbial community; Greer *et al.*, 1990; Veeh *et al.*, 1996). Under conditions favorable for degradation, 2,4-D disappears from soil within one to three weeks following application (Audus, 1960). It has been documented that disappearance of 2,4-D is primarily microbially mediated (Loss, 1975, Yadav and Reddy, 1993) by a number of microorganisms mainly *Arthrobacter sp.* and *Pseudomonas* (Loss, 1971). Degradation pathway has been elucidated by several researchers (Tiedje *et al.*, 1969; Gamar and Gaunt, 1971; Gaunt and Evans, 1971) and involves the removal of the acetic acid side chain to yield dichlorophenol followed by several suggested pathways (Evans *et al.*, 1971) to produce muconic acid which will transform into 3 and 4 carbon acids (e.g., succinic acid) that can readily be used in the Kreb's cycle (Loss, 1975). Several researchers have been studied the impact of initial concentration of 2,4-D on biodegradation, whether at low concentration (de Lipthay *et al.*, 2003) or high concentration (Ou *et al.*, 1978, Estrella *et al.*, 1993) or different range of concentrations (Langner *et al.*, 1998).

Accurate model predictions of the fate of organic contaminant in soil environment require appropriate rate equations describing biodegradation (Estrella *et al.*, 1993; Langner *et al.*, 1998) and appropriate estimates of degradation parameters (e.g., rate constants). Uncertainties in rate expressions and/or rate parameters can result in a significant variation in model predictions of contaminant fate and transport (Gaber, 2004). It was documented in the literatures by several researchers (Estrella *et al.*1993; Langner *et al.*, 1998; Gaber, 2004) that degradation rate parameters determined under batch conditions were not suitable for predicting 2,4-D breakthrough curves (BTCs). For example for a weakly sorbing compound such as 2,4-D, an increase in the half-life from 10 to 20 d could result in an increase in the predicted fraction of leached compound by approximately 2 orders of magnitude (Boesten and van der Linden, 1991). Independent of the model used to describe microbial degradation during transport, there is additional uncertainty regarding the appropriate choice of rate parameters necessary for predicting degradation.) In addition, the results obtained by Kelsey and Alexander (1985) suggest a strong dependence of *p*-nitrophenol degradation rate parameters on the solute flow regime (path length and flow rate). If these findings are generally true for organic contaminants subject to biodegradation, then our ability to predict degradation across a wide range of transport conditions using a single set of batch-determined parameters is reduced significantly. However, there is little published information concerning the impact of initial soil environment conditions on biodegradation rate. Therefore, this study was aimed at investigating some initial soil environment conditions and its influence on the bioavailability of 2,4-D under batch and column transport conditions.

MATERIALS AND METHODS

Chemicals

^{14}C-Carboxyl-labeled 2,4-D were obtained from Sigma Chemical (St. Louis, MO). Radiopurity was verified by high-pressure liquid chromatography (HPLC) on a C-18 reverse phase column at 254 nm using acetonitrile-0.2% phosphoric acid (25:75) with a flow rate of 1.5 mL min^{-1} (R_t =6.5 min.).

Soil

The soil used in this study is an Amsterdam silt loam (*fine-silty, mixed Typic Haploboroll*) sampled from the A.H. Post Ag. Exp. Station, Bozeman, MT. (Table I). The soil was air-dried (5.32% moisture) and sieved (< 2mm) before use.

Table 1: Characteristics of Amsterdam silt loam soil used in batch and transport experiments.[†]

Soil pH (1:1 H_2O)	Organic Matter	Sand	Silt	Clay	$CaCO_3$	CEC	Soil water content	
							-33kPa	-1500kPa
		---------- % ----------				Cmol Kg^{-1}	--- Kg Kg^{-1} ---	
7.8	1.7	14.5	52.5	33.0	2.0	22.9	0.273	0.128

[†] Values represents means of two replicates.

Batch sorption-desorption isotherms

The batch sorption-desorption isotherms were conducted as described in detailed by Gaber (2004). 10-g samples of soil and 10 mL of 3 *mM* $CaCl_2$ solution containing various concentrations of 2,4-D were shaken in centrifuge tubes for 24 h at room temperature. Initial aqueous phase of a mixture of carboxyl-^{14}C-labeled 2,4-D and unlabeled 2,4-D in a background solution of 3 *mM* $CaCl_2$. 2,4-D concentrations were 0.15, 0.3, 0.6, 1.0, 3.0, and 10 mg L^{-1} in triplicates. After shaking, the tubes were centrifuged and the supernatant solution decanted and filtered (0.45 μm). The amount of radioactivity in the liquid phase was determined using a Packard 2200CA liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL). Sequential desorption isotherms were obtained to evaluate the existence of nonsingularity (Green *et al.*, 1980). Tubes were weighed and refilled with 3 *mM* $CaCl_2$ solution to 10 mL of liquid phase and shaken again then centrifuged. The removal and refilling procedures were repeated several times to obtain desorption isotherms with five desorption steps.

Batch biodegradation experiments

Several sets of batch biodegradation experiments were conducted to investigate the impact of initial soil environment conditions on biodegradability and bioavailability of 2,4-D. These initial conditions included moisture contents, initial 2,4-D concentration, initial microbial population, and existence of aerobic or anaerobic condition.

Batch degradation studies were conducted using 10-g samples of soil in gas-tight Erlenmeyer flasks containing two ports for continuous gas exchange. Preliminary experiments showed that initial degradation rates were sensitive to the duration of prewetting the soil prior to 2,4-D application. Therefore, soils were brought to a water content of 0.15 L (kg soil)$^{-1}$ three days prior to initiation of degradation experiments, in attempt to simulate the conditions of the transport experiments. All liquids added to the batch reactors contained a background solution of 3 *mM* $CaCl_2$. The head space was continuously purged with humidified, CO_2-free air at a flow rate of approximately 100 mL h^{-1}, passed through 10 mL of 0.5 *M* NaOH and analyzed for $^{14}CO_2$ evolved using liquid scintillation. The individual soils were analyzed for soil residual ^{14}C using biological oxidation (R.J. Harvey Instrument Corp., Hillsdale, NJ) and liquid scintillation.

A series of degradation experiments was conducted to evaluate the effect of variable soil water content on 2,4-D degradation. Soil water contents of 0.20, 0.28, 0.36, 0.44, and 0.55 L (kg soil)$^{-1}$ were evaluated (in triplicate) using an initial aqueous phase 2,4-D concentration of 1.0 mg L^{-1}. For each water content, one control experiment was conducted under sterile conditions with autoclaved soil (two consecutive treatments of 40 min at 100 kPa and 120°C). To study the impact of acclimation on the degrader population and to further investigate the lag period, a biodegradation experiment with soil prespiked twice with the field application rate of 2,4-D (three weeks and six weeks earlier) was conducted. Additional degradation experiments were conducted with slurries (shaken on a rotary shaker at 130 rpm) to reduce potential effects of rate limited transport of 2,4-D to sites of microbial

degradation. These experiments were conducted using a soil : water ratio of 1 : 3 (g : g) and two initial 2,4-D concentrations (1.0 and 0.1 mg L^{-1}): 1.0 mg L^{-1} was chosen to expose the microorganisms to the same initial 2,4-D concentration as in the nonstirred experiments, and 0.1 mg L^{-1} represented a similar absolute mass of 2,4-D per g of soil as in the nonstirred experiments. Sodium Azide (NaN_3), a biocide agent and microbial inhibitor, was used at different concentrations to study the impact of initial microbial community impact on 2,4-D biodegradation.

Analytical Methods

Three models were tested for their abilily to describe the degradation of 2,4-D and were chosen for their simplicity and possible applicability to degradation subroutines in transport models (Characklis, 1990).
The first order kinetics:

$$\frac{C}{C_o} = 1 - e^{kt}$$

where C/C_0 in the fraction CO_2 evolved, k is the first order rate constant, and t is time.
The modified first order kinetics:

$$P = P_{max}(1 - e^{kt})$$

where P is the fraction CO_2 evolved, P_{max} is the maximum extent of mineralization, k is the rate constant, and t is time.
The logistic equation

$$X = \frac{X_o e^{kt}}{1 - (\frac{X_o}{X_m}(1 - e^{kt}))}$$

where X is the fraction CO_2 evolved, Xo is the inital fraction CO_2 evolved, X_m is the maximum extent of mineralization, k is the rate constant, and t is time.

Column Transport Experiments

A series of soil column experiments with varying solute pore water velocity (i.e., residence time) was performed to investigate the impact of 2,4-D bioavailability during transport (Table 2). Disturbed soil columns were prepared by uniformly packing air dry soil into PVC tubes with an inner diameter of 5.1 cm and 30 cm length. The ends of the columns were secured with polycarbonate caps containing rubber O-rings. The bottom end cap supported a porous plastic plate with an air entry pressure of 100 kPa (Soil Measurement Systems, Tucson, AZ). Columns were equipped with an air entry port near the bottom and an air outlet in the top end cap. Valves quick disconnect couplings (Cole Parmer Instrument Co., Niles, IL) and Luer Lock-valve combinations enabled the frequent detaching and weighing of the columns for monitoring soil water contents. Eluent was supplied with a precision syringe pump (Soil Measurements Systems) at variable rates depending on the desired pore water velocity (v). A fraction collector

(Retriever II, ISCO Inc., Lincoln, NE) housed in a vacuum chamber (Soil Measurements Systems) was used to collect effluent (up to 13 mL per sample) while maintaining a constant potential at the bottom plate of the soil column (van Genuchten and Wierenga, 1986). A peristaltic pump was used to provide a continuous air stream of approximately 15 to 40 mL h^{-1} to the air inlet port (the higher flow rates were used in the longer soil columns). The slow upward stream of air through the column was intended to minimize the potential occurrence of anaerobic conditions within the column.

Soil columns were wetted from the bottom within 8 to 48 h depending on the column length, after which the bottom plate pressure was set to -30 kPa and 3 m*M* $CaCl_2$ was supplied to the top of the column. Soil water content was monitored daily by detaching and weighing the columns throughout individual experiments.After steady state flow conditions were established (constant water content and v), the eluent solution was switched from 3 m*M* $CaCl_2$ to a continuous pulse of 1.0 mg L^{-1} 2,4-D containing ^{14}C-labeled 2,4-D (specific activity of 1.2 x 10^5 to 5.7 x 10^5 Bq L^{-1} depending on the anticipated amount of degradation), ^3H_2O as a tracer (specific activity between 3.3 x 10^4 and 1.7 x 10^5 Bq L^{-1}), and 3 m*M* $CaCl_2$. Column effluents were analyzed for ^{14}C and ^3H using liquid scintillation. The evolution of $^{14}CO_2(g)$ was periodically determined by sampling CO_2 traps at the air outlet port and within the vacuum chamber. Selected effluent samples were analyzed before and after acidification and purging with $N_2(g)$ to determine the contribution of dissolved $^{14}CO_2$ to total ^{14}C in the effluent. Effluent samples from representative experiments were also analyzed using HPLC-radioisotope detection to determine the fraction of total ^{14}C present as 2,4-D.

Table 2. Column transport experimental conditions

v	θ_v	T_o	ρ	P	R_f	C_o
cm d^{-1}	$cm^3\ cm^{-3}$	PV	g cm^{-3}			m*M*
5.5	0.365	0.92	1.16	11.77	1.782	7.56E-03
5.72	0.370	0.58	1.17	49.59	1.778	5.15E-04
13.64	0.442	0.74	1.09	19.85	1.606	7.56E-03
40.55	0.445	0.82	1.14	8.45	1.630	7.56E-03
(Sterile) 65.73	0.385	0.64	1.10	22.25	1.703	7.56E-03
84.68	0.454	0.82	1.08	36.55	1.585	5.15E-04

v = pore water velocity, θ_v = volumetric water content, T_o = pulse size, PV = pore volume, ρ= bulk density, P (Peclet number) = vL/D, D = dispersion coefficient, R_f (retardation factor) = 1 + ρ/θ_v kd, C_o = initial concentration.

Experiments were continued until approximate steady-state concentrations of 2,4-D were obtained in the effluent, at which time the eluent was switched back to 3 m*M* $CaCl_2$. Column experiments were terminated when the effluent ^{14}C concentrations approached zero. Soil columns were frozen until they were sectioned into 3.5 cm segments. The individual segments were analyzed for soil residual ^{14}C using biological oxidation (R. J. Harvey Instr. Corp., Hillsdale, NJ) and liquid scintillation.

A sterile column experiment was performed to separate biological and abiological sources of 2,4-D-soil interactions. We premixed 250 g of soil

(preincubated for 2 d at a water content of 0.2 L [kg soil]$^{-1}$) with crystalline mercuric chloride at a rate of 1.0 g (kg soil)$^{-1}$ (Wolf *et al.*, 1989). All column parts, tubing and background solutions were either autoclaved twice or stored in 70 % ethanol for several days before assembling the column. The soil was packed uniformly into the column and treated as the other columns.

RESULTS AND DISCUSSION

Batch Experiments Data

Sorption-Desorption

Data of 2,4-D sorption over the study concentration range was described by the nonlinear form of Freundlich equation:

$$C_s = K_d C_L^n$$

where C_s is the quantity of herbicide sorbed per gram of adsorbent (g kg^{-1}), C_L is the equilibrium herbicide concentration in solution (g L^{-1}), K_d is the herbicide distribution coefficient (L kg^{-1}), and n is the empirical "order" of the sorption reaction, which is usually close to 1.0. Under our batch experiment conditions, we found using nonlinear least square fit, that K_d = 0.246±0.03 cm^3 g^{-1} and n = 0.784 (r^2=0.99) suggesting a slight nonlinearity (Fig. 1).

The sequential desorption "nonsingularity" data show different paths from the sorptive and desorptive directions. This observation indicated the existence of hysteresis or nonsingularity, hence; the existence of sorption-related nonequilibrium (see the inserted figure in Fig. 1).

Biodegradation

The degradation of 2,4-D has been studied extensively since its introduction as a herbicide 50 years ago. However, most individual studies have examined degradation rates in only a few soils. The novelty of the current study rests on the observations made on impact of soil environment initial condition on the bioavailability of 2,4-D confirming or disproving trends commonly reported. In the current study, degradation curves of 2,4-D under batch conditions show the effects of soil wetness, pretreatment, mixing environment, and initial substrate concentration.

Typical degradation curves were observed under different initial soil moisture contents with short lag phases compared with many previous studies. The length of the lag phase has been positively correlated with initial substrate concentration; the low initial substrate concentrations used in the current investigation supports this finding. The rate and extent of 2,4-D mineralization was relatively insensitive to moisture variations ranging from 20-40% (Fig. 2) although slight decreases in the extent of 2,4-D mineralization were observed at θ_m =0.20, where lower moisture content may have reduced 2,4-D mass transfer rates to sites of microbial activity. Soil exhibited slower rates of degradation at θ_m =0.55 (hyper-saturated conditions) than under unsaturated conditions. However, the extent of 2,4-D mineralization under saturated conditions was not significantly affected after 45 d of incubation. Table 3 shows the fitted parameters driven from the biodegradation curves.

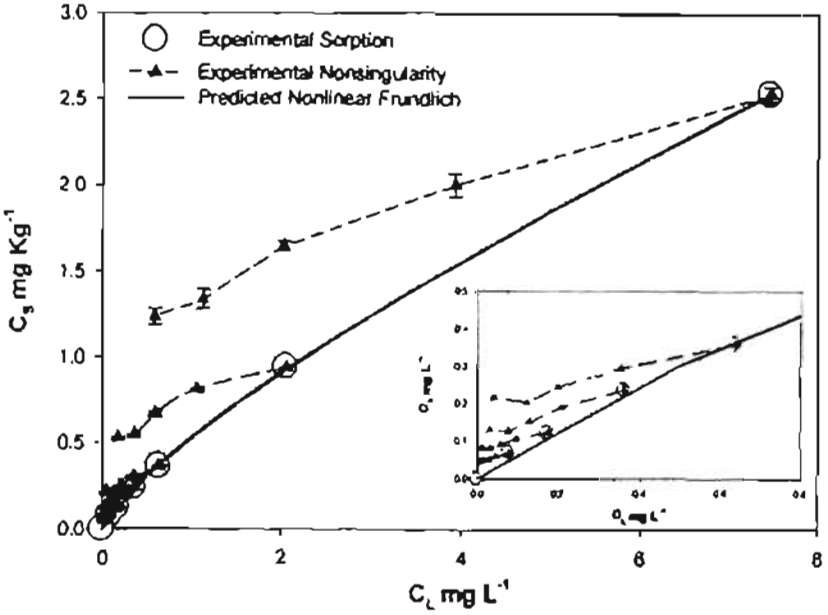

Figure 1. Experimental and Predicted Sorption-Desorption Nonsingularity of 2,4-D

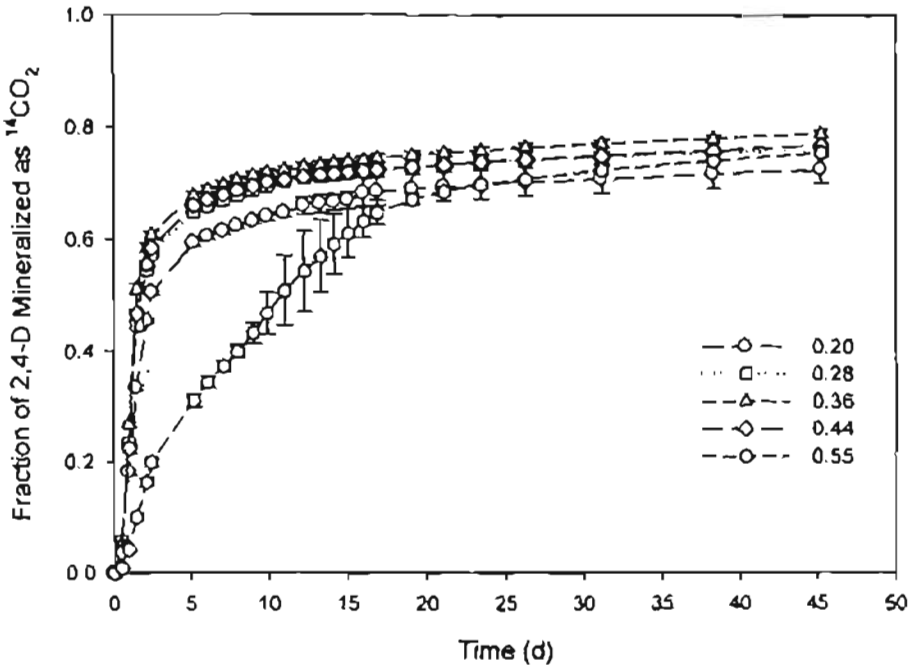

Fig. 2 Impact of Soil Water Content on 2,4-D Mineralization

Table 3. Fitted mineralization parameters affected by soil moisture contents

Water Content	r^2 (fitted curve)	K (h^{-1})	t_1 (h)[1]	% Recovery
0.20	0.9662	0.06918	39.04	94.43
0.28	0.9694	0.08162	33.20	91.86
0.36	0.9669	0.09447	30.28	93.42
0.44	0.9701	0.08894	32.60	90.01

[1] t_1 is the time required to reach one-half of the maximum mineralization rate as determined by the fitted curve.

On the other hand, soils prewetted for 3 d prior to 2,4-D degradation as compared to soil not prewetted results in difference in the rate and extent of the 2,4-D mineralization as well as the length of lag period (data no shown). This result is in agreement with Langner *et al.*, (1998) where they found a 10-fold increase in model estimates of X_0 due to prewetting treatment. It is postulated that the rate of degradation is strongly affected by the connectedness of water films along grain boundaries. As the continuity of these films decreases, mass transfer rates of the chemical decreases and degradation rates decrease as well.

To further investigate the lag period, the soil was prespiked with 2,4-D twice a month earlier before using the soil in the degradation batch, the data show the absence of lag period for the spiked soil compared with the non-spiked soil (Fig. 3) suggesting the increase in the degrader population. The disappearance of the lag period suggesting the buildup of the degrader population rather than enzyme induction or random mutation due to the earlier treatment.

Changing from stationary soil environment to well-stirred soil slurry generally resulted in lower estimates of X_0. A 10-fold decrease in initial 2,4-D concentration (to 0.1 mg L^{-1}) resulted in a shorter period of lower degradation rate preceding the rapid linear rate (Fig. 4). Similar impact of initial 2,4-D concentration on biodegradation kinetics was reported by Parker and Doxtader (1982).

Degradation rates have been shown to correspond to variations in biological activity or number of 2,4-D degraders in soil. Fig. 5 shows that treating the soil with sodium azide as a microbial inhibitor has an impact on the extent and rate of 2,4-D mineralization. The 2 m*M* treatment of sodium azide increases the lag period and decrease the extent and rate of 2,4-D mineralization from the untreated soil till 6 d then it was similar after that. This may be due to the acclimation of the survived microbial degrader at that point. On the other hand, the 5 m*M* sodium azide treatment decreases the rate and extent of 2,4-D mineralization significantly during the experiment. The bacterial plate counts of the used soils for estimating the colony forming units (CFUs) indicating the same trend.

Aerobic or anaerobic conditions play another important factor. Reductive dechlorination of halogenated pesticides such as 2,4-D has been reported to occur under anaerobic conditions (Kuhan and Suflita, 1989; Doughten, 1997). Batch degradation experiments using air or nitrogen as the flushing gas in the flasks showed substantially lower rates of mineralization under anaerobic conditions (Fig. 6).

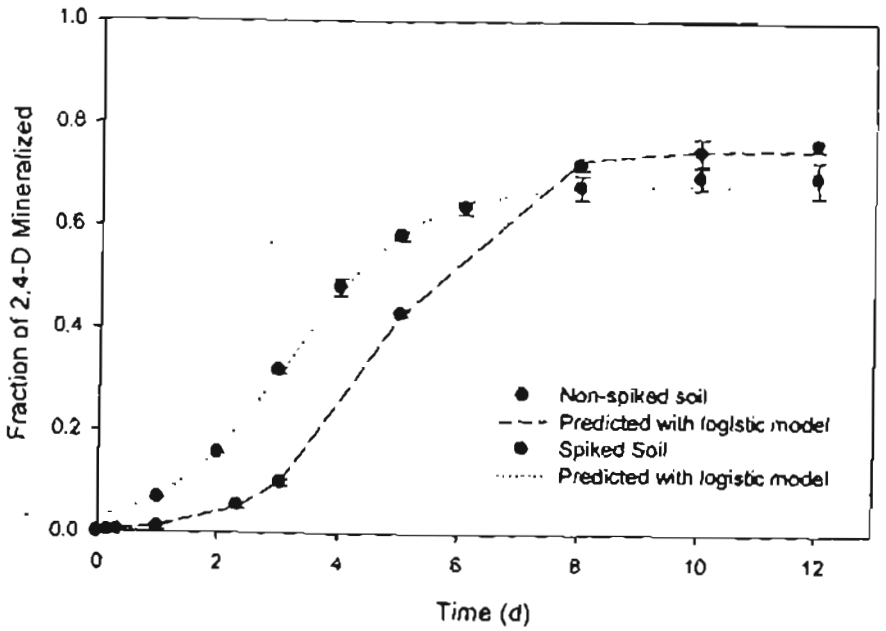

Fig. 3 Impact of Pre-spiking Treatment on 2,4-D Mineralization

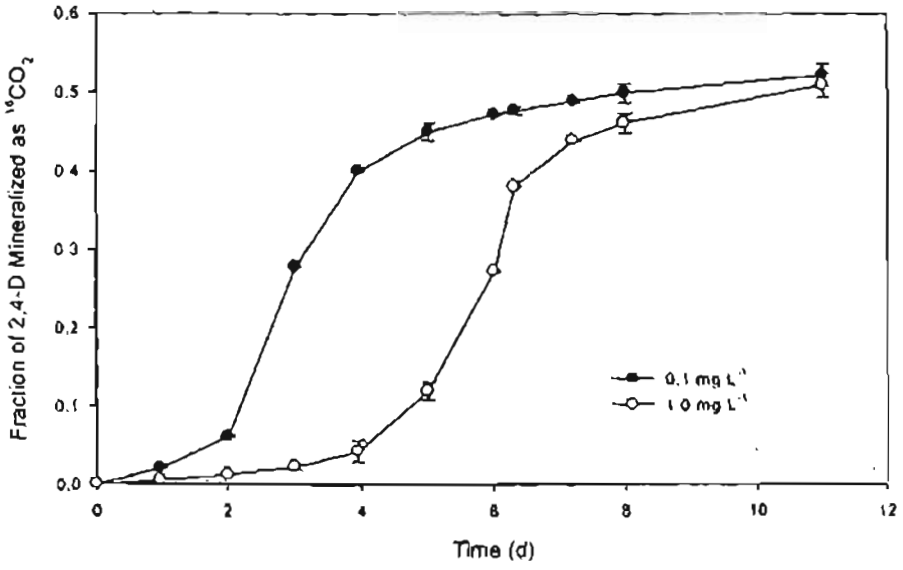

Fig. 4 Impact of 2,4-D Initial Concentration on Biodegradation Lag Period

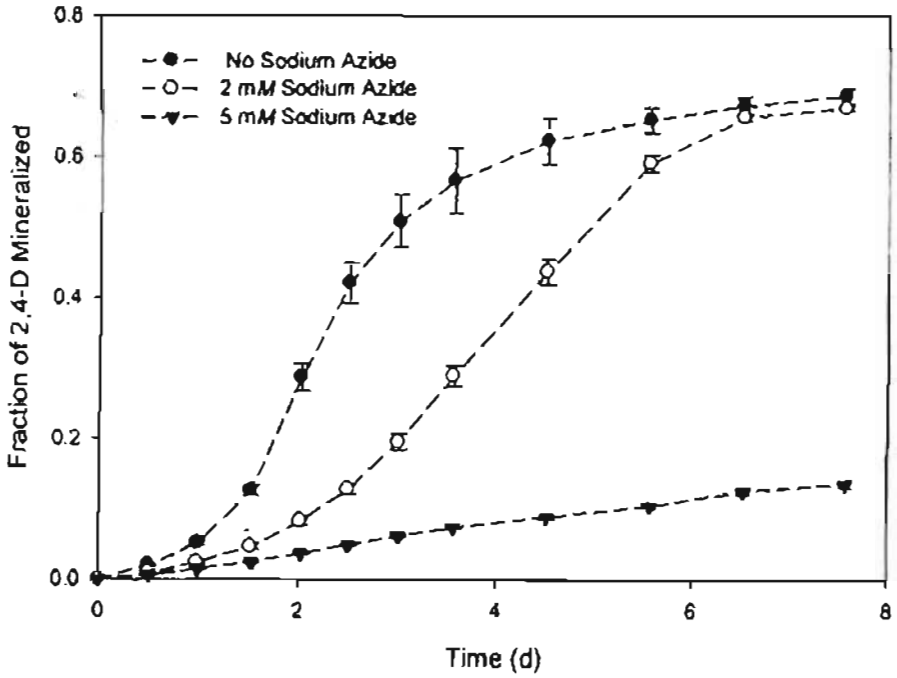

Fig. 5 Impact of Biocidal Agent (NaN_3) on 2,4-D Mineralization

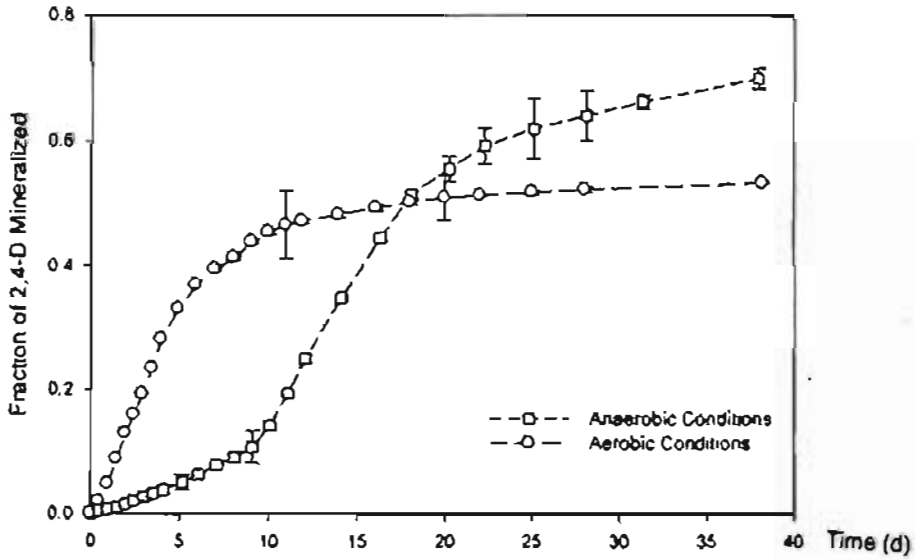

Fig. 6 Impact of Aerobic versus Anaerobic Conditions on 2,4-D Mineralization

Comparisons between the three degradation models showed that first-order kinetics describe mineralization poorly. First order kinetics are based upon the concept that rate is a function of substrate concentration and do not account for the sorption of substrate and the subsequent decrease in effective concentration. The modified first order model accounts for the lower effective concentrations of 2,4-D in the soil and describes the observed mineralization curves more effectively. A further complication is that the rate of sorption controls the length of time a substrate could potentially be available for degradation. Fast sorption rates give slow rates and low extents of mineralization; conversely, soils with slow sorption should exhibit high rates and high extents of mineralization.

The use of the logistic model was in response to observations that mineralization follows a lag time in which mineralization occurs very slowly (sigmoidal shape curves). This equation also described the observed extents of mineralization well and accounted for the short lag phase observed. However, the logistic model cannot be mathematically solved for the half-life of 2,4-D in soil and is a less attractive alternative to describing mineralization. The logistic and the modified first-order models comparably described rates of mineralization and had similar rate constants. Both of these models exceeded the ability of simple first-order kinetics to describe the observed production of $^{14}CO_2$. All three models failed to model rates of mineralization effectively or to account for slow mineralization that occurred after rapid degradation. Table 4 compares the three model parameters used to describe 2,4-D degradation. In general, predictions from both models overestimated the amount of time that 2,4-D remains in the soil with first-order kinetic predictions higher than those of the modified equation.

Table 4. Comparison among the three models used to describe the 2,4-D degradation

Model	K (h^{-1})	r^2	$t_{1/2}$ (h)	P_{max}	X_0	X_m
First-order	0.005	0.32	142	-	-	-
Modified first-order	0.03	0.96	38	0.74	-	-
Logistic	0.163	0.96	25	-	0.04	0.96

Column Transport Data

Tritiated water (^3H_2O) breakthrough curves (BTCs) were analyzed to obtain column dispersion coefficient and to test for the presence of physical nonequilibrium conditions during transport using CXTFIT 2.0 model (Toride *et al.*, 1995). This analysis suggested that physical nonequilibrium processes were not significant under our experimental conditions. A typical observed and fitted ^3H_2O BTC is presented (Fig. 7a) while the rest BTCs were similar. Our data show lack of detectable $^{14}CO_2$ evolution in the gas phase under sterile column conditions which suggested that abiotic pathways of 2,4-D degradation were insignificant under these conditions. About 97% of the applied ^{14}C-2,4-D was recovered in the effluent (Table 5), indicating only a small fraction (<1%) of the applied 2,4-D was sorbed irreversibly to the soil. This provides important evidence that residual soil ^{14}C measured in nonsterile columns was present as biomass ^{14}C rather than as irreversibly bound 2,4-D. These results are in agreement and similar to what have been found by Langner *et al.* (1998).

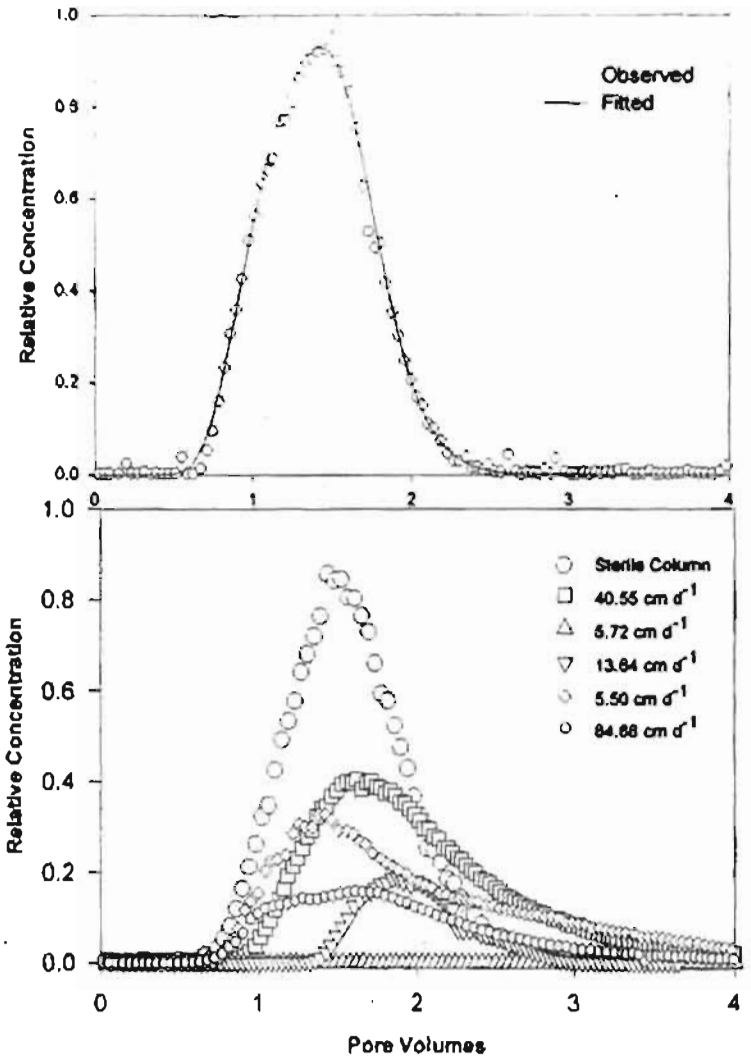

Fig. 7 Column Transport Experiments Data: a) Observed and Fitted ^3H_2O BTCs; b) Impact of Residence Time on 2,4-D BTCs

Under nonsterile column transport conditions, acidification of the effluent samples with HCL and subsequent purging with N_2 did not reduce ^{14}C concentrations indicating that carbonate-^{14}C in the column effluents were insignificant. Moreover, the HPLC radioisotope detection analysis of selected effluent samples suggested, unlike the results shown by Estrella *et al.* (1993) but similar to Langner *et al.* (1998), that the BTCs of the actual ^{14}C-2,4-D always followed the shape of the ^{14}C-BTCs obtained by scintillation analysis. This observation coupled with the nearly complete 2,4-D recovery in the effluent of the sterile control column lead us to the simplifying assumptions that (i) ^{14}C found in the column effluent was representative of ^{14}C-2,4-D, and (ii) $^{14}CO_2$ in the gas phase as well as residual ^{14}C in the soil represented microbiologically altered or degraded compound.

Qualitative ^{14}C-2,4-D fate under varying column transport conditions was obtained from the fractions of applied ^{14}C-2,4-D that were recovered as nondegraded compound in the column effluents throughout individual experiments, the collected $^{14}CO_2$ in the trap, and the soil residual fraction (Table 5).

The impact of pore water velocity (v) or residence time (RT) on degradation can be understood qualitatively by comparing effluent 2,4-D recoveries across columns and through the 2,4-D BTCs (Fig. 7b). The first two experiments are similar in v and RT (Table 5) but the initial concentration was different (Table 2) where the initial concentration in the second experiment was a 10-fold less than the first experiment. This has an influence on the amount recovered in the effluent due to bioavailability of 2,4-D. The setting of the transport experiments in the current study (pulse input) show different trend than other experimental setting (continuous input) due to the limitation of the substrate concentration (2,4-D) assuming same microbial population in both columns. Similar impact was also observed in the case of last experiment where RT was short (0.35 d) and the ^{14}C-2,4-D recovered in the effluent was low (14.3%). As the RTs increase the amount recovered in $^{14}CO_2$ trap increase. This indicates an increase in 2,4-D degradation rate with decreasing v, because the time available for degradation was approximately constant for each column. Similar results for the dependency of degradation on both the residence time and pore water velocity were obtained by Kelsey and Alexander (1995) and Langner *et al.* (1998).

In the current study, lower effluent 2,4-D recoveries always corresponded to higher amounts of ^{14}C recovered as $^{14}CO_2$(g) and soil residual ^{14}C (Table 4). However, total recoveries of applied ^{14}C decreased from 98 % to 75 % as values of ^{14}C recovered in effluent decreased. The accuracies of the ^{14}C analyses in column effluent (scintillation), soil (biological oxidation and scintillation), and CO_2 traps (scintillation) were found to be high; therefore, it is likely that in columns with significant degradation, the remaining portion of ^{14}C was lost as $^{14}CO_2$ through gas leaks.

Table 5. Fate of 2,4-D during transport through Amsterdam soil

v	RT	% ^{14}C-2,4-D-Recoved			Total
cm d^{-1}	d	Soil content	$^{14}CO_2$ trap	Effluent	%
5.50	5.45	11.92	38.17	25.90	75.99
5.72	5.42	38.02	36.12	0.96	75.10
13.64	2.20	25.70	21.61	18.73	77.04
40.55	0.74	13.75	9.09	59.11	81.85
(Sterile) 65.73	0.46	0.85	0.63	96.64	98.12
84.68	0.35	45.40	16.43	14.30	76.13

CONCLUSION

The bioavailability of 2,4-D as a model organic contaminant showed dependence on initial soil environment conditions. Some of these conditions include: moisture content, initial concentration, stirred reactor versus stationary batch, microbial population, aerobic versus anaerobic conditions, and pore water velocity (i.e., residence time). Modeling 2,4-D degradation with first-order kinetics yielded poor predictions of degradation behavior and half-life in soil. Accounting for bioavailability through the use of the modified first-order and/or logistic models presented more accurate predictions of both the rate and extent of 2,4-D degradation. The sorption-desorption process appear to has dominant impact on bioavailability and degradation, as sorbed 2,4-D becomes less available for degradation. It has been suggested that soluble 2,4-D is most easily degraded; however, differences in apparent degradation rate and extent under batch and column conditions are further complicated by the effect of residence time and mass transfer rates. It has been shown that degradation rate of 2,4-D may be constrained by mass transfer rates (i.e., desorption limited degradation) to site of microbial activity. In the transport environment, it is not clear whether biomass concentration responsible for 2,4-D degradation remain constant as a function of pore water velocity. Higher pore water velocity may result in a greater biomass transport; hence reduce the accumulation of column biomass (Stott *et al.*, 1983). It is also suggested that increases in pore water velocity may result in increase leaching of inorganic nutrients or organic substrates important for cometabolism of 2,4-D. Apparent degradation rate constants may decrease with increasing pore water velocity due to decreases in residence time per unite length (thought of as *local opportunity time*). In summary, the effects of pore water velocity (i.e., residence time) on contaminant bioavailability may be confounded by several processes operating simultaneously. These findings emphasize the difficulty in accurately predicting the degradation and transport of organic contaminants in soils across a range of flow conditions using independently determined rate parameters.

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الإتاحة الحيوية للملوثات العضوية أثناء الحركة: تأثير الظروف الابتدائية لبيئة التربة

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تتحدد عملية الإتاحة الحيوية للملوثات العضوية بعدة عمليات تحدث فى ذات الوقت فى ظروف التربة التحت سطحية. ويعتبر التنبؤ بمصير وحركة الملوثات العضوية فى التربة والمياه الجوفية باستخدام النماذج الرياضية حساسة لافتراضات معدلات التحلل الميكروبى. وقد تم اختيار مبيد الحشائش 2,4-D فى هذه الدراسة كمركب نموذجى للملوثات العضوية لتوضيح تأثير الظروف الابتدائية لبيئة التربة على مدى إتاحة المبيد تحت ظروف أنابيب الاختبار واعمدة الحركة. وتشمل هذه الظروف ما يلى : محتوى الرطوبة والتركيز الابتدائى والتفاعل مع التقليب المستمر مقابل التفاعل الساكن وتركيز الكتلة الحيوية الميكروبية والظروف الهوائية والاهوائية بالإضافة الى سرعة حركة المحلول الاراضى فى المسام، او بعبارة أخرى زمن بقاء المبيد فى التربة.

وقد استخدم النموذج الكينانيكى من الرتبة الاولى لوصف تحلل مبيد 2,4-D فى التربة وقد اعطى تنبئ غير دقيق لسلوك وتحلل المبيد فى التربة وكذا زمن نصف العمر. وعلى الجانب الاخر فان استخدام نموذج الرتبة الاولى المعدل والنموذج المنطقى قد ادى الى تنبؤ اكثر دقة لكل من معدل وكمية وتحلل المبيد نتيجة اخذهما الاتاحة الحيوية فى الاعتبار. وتحت ظروف الحركة فى اعمدة التربة فمن غير الواضح ما اذا كان تركيز الكتلة الحيوية الميكروبية المسئولة عن تحلل مبيد 2,4-D ضلت ثابتة مع اختلاف سرعة الماء فى المسام. وقد وجد ان ثوابت معدل التحلل الظاهرية يمكن ان تقل بزيادة سرعة الماء فى المسام ويرجع ذلك الى نقص زمن بقاء المبيد فى كل وحدة طولية والتى تم تصورها كزمن للفرصة المحلية.

وكملخص فان تاثيرات زمن البقاء على الاتاحة الحيوية للملوث تتحدد بعدة عمليات تحدث فى ذات الوقت وهذه النتائج تلقى الضوء على مدى صعوبة التنبؤ الدقيق لتحلل وحركة الملوثات العضوية فى التربة خلال مدى من ظروف التدفق باستخدام معدلات التحلل المقدرة فى ظروف مختلفة وبمعزل عن ظروف الحركة.