Biodegradation **7:** 137–149, 1996.

Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm

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Accepted 8 September 1995

Key words: atrazine, bacteria, biodegradation, mineralization, sorption

Abstract

The purpose of the present study was to assess atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) mineralization by indigenous microbial communities and to investigate constraints associated with atrazine biodegradation in environmental samples collected from surface soil and subsurface zones at an agricultural site in Ohio. Atrazine mineralization in soil and sediment samples was monitored as $^{14}CO_2$ evolution in biometers which were amended with ^{14}C-labeled atrazine. Variables of interest were the position of the label ([U-^{14}C-ring]-atrazine and [2-^{14}C-ethyl]-atrazine), incubation temperature (25°C and 10°C), inoculation with a previously characterized atrazine-mineralizing bacterial isolate (M91–3), and the effect of sterilization prior to inoculation. In uninoculated biometers, mineralization rate constants declined with increasing sample depth. First-order mineralization rate constants were somewhat lower for [2-^{14}C-ethyl]-atrazine when compared to those of [U-^{14}C-ring]-atrazine. Moreover, the total amount of $^{14}CO_2$ released was less with [2-^{14}C-ethyl]-atrazine. Mineralization at 10°C was slow and linear. In inoculated biometers, less $^{14}CO_2$ was released in [2-^{14}C-ethyl]-atrazine experiments as compared with [U-^{14}C-ring]-atrazine probably as a result of assimilatory incorporation of ^{14}C into biomass. The mineralization rate constants (k) and overall extents of mineralization (P_{max}) were higher in biometers that were not sterilized prior to inoculation, suggesting that the native microbial populations in the sediments were contributing to the overall release of $^{14}CO_2$ from [U-^{14}C-ring]-atrazine and [2-^{14}C-ethyl]-atrazine. A positive correlation between k and aqueous phase atrazine concentrations (C_{eq}) in the biometers was observed at 25°C, suggesting that sorption of atrazine influenced mineralization rates. The sorption effect on atrazine mineralization was greatly diminished at 10°C. It was concluded that sorption can limit biodegradation rates of weakly-sorbing solutes at high solid-to-solution ratios and at ambient surface temperatures if an active degrading population is present. Under vadose zone and subsurface aquifer conditions, however, low temperatures and the lack of degrading organisms are likely to be primary factors limiting the biodegradation of atrazine.

Abbreviations: C_{eq}, solution phase atrazine concentration at equilibrium; C_s, amount of atrazine sorbed; CLA, [2-^{14}C-ethyl]-atrazine; k, first-order mineralization rate constant; K_d, sorption coefficient; m, slope; P_{max}, maximum amount of CO_2 released; RLA, [U-^{14}C-ring]-atrazine.

Introduction

With the advent of conservation tillage practices, the use of herbicides to control weeds on agricultural land has risen dramatically. Widely used herbicides such as atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and alachlor (2-chloro-2',6'-diethyl-N-[methoxy-methyl]acetanilide) are frequently detected in ground water and surface water (Muir and Baker, 1976; Belluck et al., 1991; Thurman et al., 1992). Despite their frequent detection, factors influencing the biodegradation of herbicides in the vadose zone and aquifers are not well understood. The dissipation of atrazine and its transforma-

tion products in surface soils has been well documented (Swain, 1981; Jones et al., 1982; Smith and Walker, 1989; Winkelmann and Klaine 1991a,b). Biodegradation is one of the most important processes governing the environmental fate of atrazine. The initial step in the biological degradation of atrazine involves the sequential removal of the alkyl side chains followed by deamination, dehalogenation, and ring cleavage. The actual sequence of the initial steps may vary in different microorganisms. The alkyl carbon has been shown to serve as a carbon and energy source for atrazine-degrading microorganisms (Cook, 1987; Erickson and Lee, 1989). The ring carbon of atrazine is fully oxidized (+IV) and does not serve catabolic or biosynthetic purposes in degradative pathways. Utilization of both the chain-C and release of amino- and ring-N have been observed (Yanze-Kontchou and Gschwind, 1994; Mandelbaum et al., 1995; Radosevich et al., 1995). Proposed biodegradative pathways have been reviewed by Cook (1987) and Erickson and Lee (1989). The biodegradation of atrazine in subsurface zones appears to be spatially variable and usually slow (Sinclair and Lee, 1992; Johnson and Furhmann, 1993).

In a prelude to the present work, atrazine was shown to persist for at least a year in subsurface sediment enrichment experiments (Radosevich et al., 1993). Samples showing degradative activity had half-lives exceeding 170 d. Slow biodegradation rates were considered to be primarily due to a low density of atrazine-degrading microorganisms in over 80 samples examined (Radosevich et al., 1993). In a similar study with subsurface samples from the same site, McMahon et al. (1992) reported laboratory-measured mineralization rate constants in the range of $<4.5\text{x}10^{-5}$ to $5.4\text{x}10^{-4}$ d^{-1} for [2-^{14}C-ethyl]-atrazine. They concluded that dealkylation of atrazine was significant with respect to the temporal scale of ground water movement at the site. These data suggest extremely slow atrazine mineralization rates in subsurface environments. The present work was initiated to investigate whether slow and variable atrazine mineralization rates are due to unfavorably low subsurface temperatures, sorption, nutrient limitation, or a lack of an active atrazine-degrading microbial population.

Materials and methods

Sample collection

The sampling sites were located at the Ohio Management Systems Evaluation Area (MSEA) on a 260 ha farm in Pike County, Ohio. The predominant soil series at the MSEA site are Huntington (fluventic hapludoll), Rossburg (fluventic hapludoll), and Nolin (fluventic eutrochrept) silt loams overlying sands that grade into gravely sand at a 2–3 m depth. The site is on the Scioto Buried Valley Aquifer which has layers of outwash consisting mostly of sand and gravel deposited to a depth of 20–26 m by drainage of melt water from the Pleistocene glaciation. The water table normally ranges from 3.5 to 7 m below the soil surface. The temperature of the ground water measured in monitoring wells at the Ohio MSEA site ranged from 9.1 to 14.6°C and the median was 12.3°C. The site has been described in greater detail by Ward et al. (1993).

Soil and sediment samples were collected in September, 1992 during the installation of a ground water monitoring well designated S15 and a bore hole designated S16. Well S15 was at a site under a conventional management system involving continuous corn production and received annual inputs of a variety of pesticides, including atrazine. Bore hole S16 was used as a reference site about 1 km down gradient of S15, and was placed near the Scioto River. The reference site had no recent history of pesticide treatment. Two surface soil samples and 12 subsurface sediment samples, ranging in depth from 0–18 m, were collected at S15 and S16. The sediment collection process has been previously described (Radosevich et al., 1993). Soil and sediment samples were stored at 4°C until analyzed.

Determination of sorption parameters

The sediment samples were sieved (2 mm sieve) without prior drying. Particle size distribution of the sediments (80–100 g dry wt., 2 mm fraction) was determined using the pipette method (Gee and Bauder, 1986). The sand (>46 μm), silt (46–2 μm), and clay (< 2 μm) fractions were normalized to the <5 mm size fraction on a weight basis which was representative of the material used in biometer experiments. The sediment samples collected below 1.8 m were predominantly composed of sand or larger size fractions. In the < 5 mm fraction sand ranged from 11.2% to 91.7%. The samples collected above 1.8 m contained

predominantly silt and clay and no material greater than 2 mm.

Atrazine sorption to sediments was determined using a batch equilibration technique. Aqueous atrazine solutions containing 0, 0.05, 0.1, 0.5, 1, 3, and 5 mg L^{-1} were prepared in 1 mM $CaCl_2$. Moist sediment samples (5 g dry wt.) were suspended in centrifuge tubes with 30 mL of atrazine solution (1:6 solid-to-solution ratio) and equilibrated on a reciprocal shaker for 48 hours at 25°C. After equilibration, the suspensions were centrifuged and the concentration of atrazine in the supernatant was determined by reverse-phase high performance liquid chromatography (HPLC) as previously described (Radosevich et al., 1995).

Sorption data were fitted to the Freundlich equation:

$$C_s = K_d C_{eq}^n$$

where C_s is the amount sorbed (mg kg^{-1}), K_d is a measure of the degree of sorption, C_{eq} is the equilibrium solution concentration of atrazine (mg L^{-1}), and n, a fitting parameter, indicates the degree of nonlinearity between solution concentration and the amount adsorbed. The sorption parameters were used to calculate equilibrium solution atrazine concentrations in biometers which were prepared at a standard solid to solution ratio (5:2, w/v) (see below).

Biometer experiments

Biometers used in this work were constructed from 50 mL serum bottles with 1.5 mL HPLC autosampler vials suspended with Cu wire from butyl rubber stoppers. The vials were filled with 1 mL of 1 M KOH trapping solution. Sediment samples (5 g) in biometers were amended with 1 μCi of an aqueous [U-^{14}C-ring]-atrazine (RLA) or 0.66 μCi [2-^{14}C-ethyl]-atrazine (CLA) solution. The initial concentration of atrazine in all biometers was 2.4 mg kg^{-1} (11 nmol g^{-1}), which was representative of a field application rate of about 2.8 kg ha^{-1}. Unless otherwise indicated, samples were processed within 48 h of collection for the uninoculated RLA biometer experiments. All treatments were prepared in duplicate. Abiotic controls were prepared by autoclaving at 120°C for 20 min prior to amendment with sterile atrazine solution. The biometers were incubated at 25±2°C and at 10±0.5°C. The biometers were periodically sampled by removing the traps and replacing them with sterilized vials containing fresh KOH solution. The entire trapping solution volume was transferred from the traps and placed in scintillation vials containing 10 mL scintillation cocktail (Scintiverse BD, Fisher Scientific) for counting the radioactivity. Complete mineralization of RLA and CLA would yield three and one equivalents of $^{14}CO_2$, respectively.

The CLA biometer experiments were initiated three weeks after sample collection. Because the samples were stored for three weeks before the CLA experiments were initiated, glucose respiration was used to assess the change in the microbial activity due to storage. For this purpose, [U-^{14}C]-glucose was used in biometer experiments within 48 h of sediment sample collection and again after 3 weeks of storage at 4°C. The mineralization of [U-^{14}C]-glucose would yield six equivalents of $^{14}CO_2$. Four S15 sediment samples were used for glucose mineralization measurements. Soil samples in biometers received 1 μCi [U-^{14}C]-glucose (4 mg kg^{-1}, 22 nmol g^{-1}) and were incubated at 25±2°C.

Mineralization rates for atrazine and glucose were determined using a first-order equation for CO_2 production (Guerin and Boyd, 1992):

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

in which the rate constant (k) was the only adjustable parameter, P is the amount of $^{14}CO_2$ released at time (t) and P_{max} is the maximum amount of $^{14}CO_2$ released.

Biometers for measurement of $^{14}CO_2$ evolution from inoculated sediments were amended with either 0.1 μCi RLA or CLA. The test bacterium, designated as M91–3, was an atrazine-mineralizing soil isolate (Radosevich et al., 1995) which was obtained from a surface soil sample collected at a pesticide mixing area at the Western Branch of the Ohio Agricultural Research and Development Center. The amount of the inoculum was standardized by optical density (660 nm). The inoculum was 70 μg dry wt. bacterium per biometer estimated from the plot of average dry wt. vs. optical density of the culture. This was equivalent to approximately 7 x 10^7 bacteria g^{-1} sediment (2 x 10^{-10} mg $cell^{-1}$). The culture was grown to early stationary phase in a mineral salts medium (Radosevich et al., 1995) which contained atrazine (0.1 mM) as the sole source of N and glucose (1.1 mM) as the C source. For inoculation, cells were harvested by centrifugation (9000 g at 4°C) and washed three times in sterile saline (0.85% NaCl) solution. The cells were resuspended in

Table 1. First-order mineralization rate constants (k) for glucose mineralization in uninoculated surface and subsurface sediments initiated after 48 h and 3 weeks of storage at 4°C. The values in parentheses indicate 95% confidence intervals.

Mean sample Depth (m)	48 h k (d^{-1})	48 h r^2	3 weeks k (d^{-1})	3 weeks r^2
0	0.10 (0.06–0.14)	0.75	0.13 (0.08–0.18)	0.78
1.7	0.05 (0.04–0.06)	0.93	0.14 (0.08–0.19)	0.77
2.3	0.17 (0.12–0.23)	0.93	0.28 (0.21–0.35)	0.94

saline solution to an O.D. (660 nm) of 0.33, and 0.5 mL of the suspension was used to inoculate the biometers.

Atrazine amendment and inoculation of the biometers resulted in a total liquid addition of 2 mL and a solid-to-solution ratio of 5:2 (w/v). An additional set of biometers was prepared in which the sediments were sterilized by autoclaving prior to atrazine amendment and inoculation. All treatments were prepared in duplicate and sterilized uninoculated sediments were included as abiotic controls.

Results

Glucose mineralization in surface and subsurface samples

Because samples were stored for three weeks before the CLA experiments were initiated, loss of microbial activity due to storage was evaluated by measuring glucose mineralization with selected samples. The recovery of $^{14}CO_2$ amounted to about 75% of added glucose in the surface soil sample and to about 45% in the subsurface samples. The glucose mineralization data were fitted to first-order rate equation and the respective rate constants are listed in Table 1. Three of the six data sets examined showed poor fits with r^2 values (coefficient of determination) <0.80, suggesting independence of glucose mineralization rate from substrate concentration. Three weeks of sample storage at 4°C resulted in a 5–30% decrease in the extent of subsequent glucose mineralization in the S15–0 m and S15–2.3 m samples. The level of respiratory activity after three weeks of sample storage was deemed sufficiently high to warrant atrazine mineralization experiments.

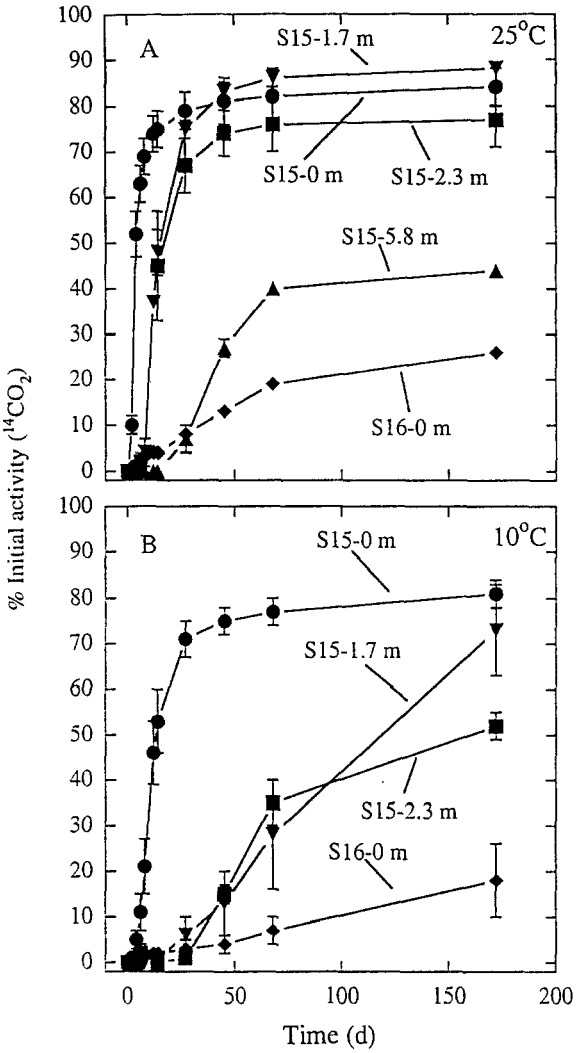

Fig. 1. Cumulative evolution of $^{14}CO_2$ from RLA samples as affected by temperature of incubation. Bars represent one standard deviation.

[U-^{14}C-ring]-atrazine mineralization

Mineralization at 25° C. Of the 14 samples collected at sites S15 and S16, five exhibited RLA mineralization activity at 25°C (Figure 1A). The rate and extent of atrazine mineralization decreased from the surface to 5.8 m depth in the S15 samples. The extent of RLA mineralization in the near-surface samples approached the theoretical maximum of 100%. The average mass balance in parallel experiments was 95±7%, comprising (i) trapped $^{14}CO_2$ and (ii) the residual ^{14}C (biomass,

residual atrazine, and metabolites) combusted at the termination of the incubation. In general, the data confirmed the 3:1 stoichiometric ratio of the theoretical maximum $^{14}CO_2$ yield from the complete mineralization of RLA. Little or no assimilation of the ^{14}C of RLA into biomass was expected since these C atoms are already fully oxidized in the atrazine ring structure. Although unusually high, given the previous atrazine treatment history at S15, extensive mineralization was expected. Only the surface sample from the reference site S16 mineralized atrazine (Fig. 1A). All other samples collected from sites S15 and S16 evolved less than 1% $^{14}CO_2$ of the added RLA in the course of the 172 d experiment. Less than 0.5% of the added radioactivity was released as $^{14}CO_2$ from the sterile controls.

Atrazine mineralization followed first-order kinetics in the S15–0 m, S15–1.7 m, S15-2.3 m, and S15–5.8 m samples, and in the S16–0 m sample. The rate-constants (k d^{-1}) for these five samples were 0.204 (r^2=0.91), 0.032 (r^2=0.89), 0.04 (r^2=0.91), 0.016 (r^2=0.87), and 0.016 (r^2=0.98), respectively. The total amount of atrazine mineralized (P_{max}) was higher in S15–0 m, S15–1.7 m, and S15–2.3 m samples when compared to the S15–5.8 m and S16–0 m samples (Fig. 1A). The S15–0 m sample had virtually no lag period preceding $^{14}CO_2$ evolution. The others were characterized by lag periods of varying length of up to four weeks. The lag periods associated with mineralization may reflect differences in initial cell densities and induction times required for gene expression of atrazine-degrading enzymes.

Effect of cold storage. Biometer experiments with RLA were repeated for the S15–0 m and the S15–1.7 m samples after three weeks of sample storage at 4°C. The results of atrazine mineralization were similar to those obtained with samples processed within 48 h. In the surface soil, atrazine mineralization began immediately after the addition of RLA, and neither the rate constant (0.19 d^{-1}, r^2=0.96) nor the P_{max} value (80%) was significantly different (p=0.05) from the previous experimental results. In the other sample (S15–1.7 m), storage did not significantly affect the rate constant (0.04 d^{-1}, r^2=0.91) but there was a 30% reduction in P_{max} of atrazine mineralization.

Mineralization at 10°C. Four of the 14 samples exhibited appreciable atrazine mineralization when incubated at 10°C (Fig. 1B). Mineralization was greatest in the surface soil from S15 and decreased with depth to 2.3 m. Atrazine mineralization was not detected with the S15–5.8 m sample incubated at 10°C. Only the surface soil sample collected at S16 mineralized atrazine at 10°C (Fig. 1B). The S15–0 m sample was the only one to exhibit first-order mineralization kinetics at 10°C (k=0.058 d^{-1}; r^2=0.94). The mineralization kinetics of all other samples could not be fitted to the first-order rate equation over the 172 d time course, suggesting that other factors in addition to substrate concentration influenced the rates. Deviation from first-order kinetics was also evident from the continuing increase in $^{14}CO_2$ evolution throughout the time course.

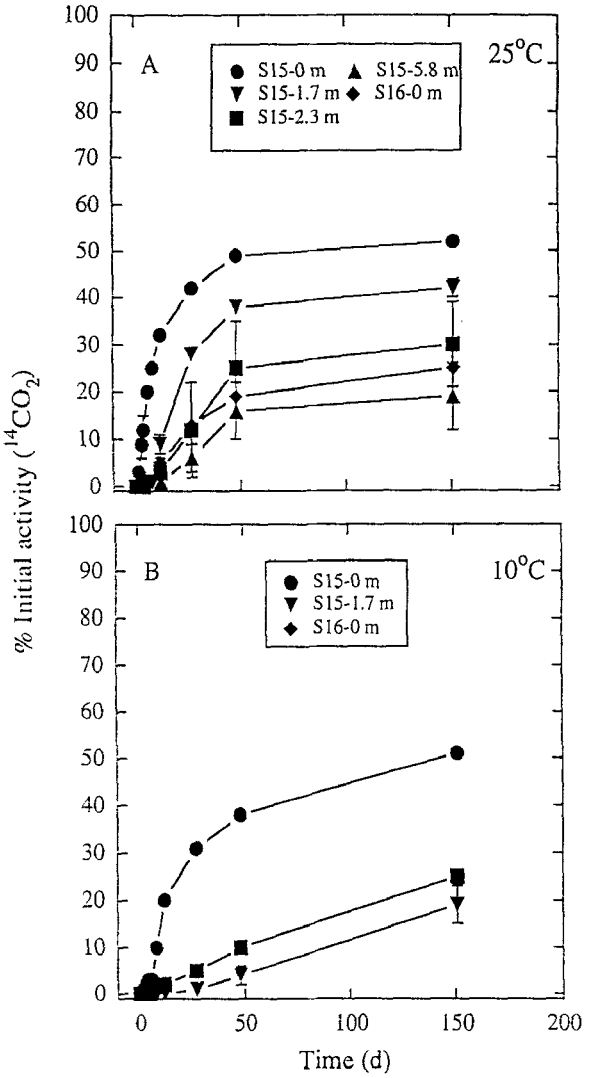

Fig. 2. Cumulative evolution of $^{14}CO_2$ from CLA samples as affected by temperature of incubation. Bars represent one standard deviation.

3.4 [2-^{14}C-ethyl]-atrazine mineralization

Mineralization at 25° C. The mineralization experiments were repeated with chain-labeled atrazine (CLA) to evaluate partial biotransformation (deethylation) of atrazine in the samples collected at S15 and S16. The mineralization of CLA at 25°C and 10°C is shown in Fig. 2 A and B. The same samples which mineralized RLA (S15–0 m, S15–1.7 m, and S15–2.3 m samples) also produced $^{14}CO_2$ from CLA. The P_{max} of $^{14}CO_2$ evolved from CLA ranged from 10% to 33% of that determined in the RLA experiments.

Caution should be taken when considering direct comparisons of P_{max} data for CLA and RLA. The decrease in $^{14}CO_2$ evolution may be attributed to the incorporation of the [2-^{14}C-ethyl]-carbon into biomass. The fraction of ^{14}C used for cellular assimilation is not recovered as $^{14}CO_2$. Therefore, it is likely that the mineralization experiments underestimated the extent of deethylation.

The first-order rate constants were 0.084 d^{-1} (r^2=0.98), 0.03 d^{-1} (r^2=0.91), and 0.0086 d^{-1} (r^2=0.98) for the S15–0 m, S15–1.7 m, and S15–2.3 m samples, respectively. The P_{max} of CLA mineralization after 151 d of incubation was highest in the S15–0 m sample (Fig. 2A). The mineralization could not be adequately described by a first-order rate equation for data sets S15–5.8 m and S16–0 m.

Mineralization at 10° C. Only three samples mineralized CLA at 10°C (Fig. 2B). None of the other samples collected at S15 and S16 mineralized more than 1% of the added CLA at this incubation temperature. The S15–0 m mineralization could be described using first-order kinetics with a k value of 0.036 d^{-1} (r^2=0.97). Mineralization in the S15–1.7 m and the S15–2.3 m sediment samples was linear, with lag periods exceeding 25 d. Evolution of $^{14}CO_2$ continued to increase throughout the 150 d time course.

Mineralization in inoculated biometer experiments

All samples collected at site (S16) except the surface soil showed negligible $^{14}CO_2$ evolution. Atrazine degradation was reevaluated in these samples in the presence of an inoculum of atrazine-degrading bacteria. Biological constraints on atrazine degradation were examined in these inoculated biometer experiments by using sediment samples with and without prior sterilization. Both RLA and CLA were used as substrates for these experiments.

All S16 samples that failed to mineralize either CLA or RLA in uninoculated experiments showed fairly rapid mineralization upon inoculation with M91–3. These results suggested that the lack of $^{14}CO_2$ evolution in the S16 samples was due to the absence of microorganisms capable of mineralizing atrazine.

Inoculation with M91–3 greatly enhanced the mineralization of RLA under all treatment conditions. RLA rate constants in the inoculated biometers increased approximately one order of magnitude and were comparable to those measured for uninoculated S15 surface soil. The rate constants exhibited no distinct variation with depth (Table 2).

RLA and CLA mineralization data for all 25°C incubations are presented in Fig. 3 and Fig. 4, respectively. The k values for RLA mineralization at both 25 and 10°C were greater in the unsterilized sediments when compared to the corresponding pre-sterilized samples (Table 2 and 3). A similar trend was observed for the maximum extent of mineralization (P_{max}) (data not shown). These differences suggested that the native microbial populations contributed to the overall $^{14}CO_2$ evolution. The products of atrazine ring-cleavage by M91–3 are biuret and urea (Radosevich et al., 1995). Both compounds would contain labeled carbon and may be readily mineralized by the native population thereby resulting in the additional $^{14}CO_2$ released in unsterilized, inoculated biometers.

When CLA was the substrate, the k values were greater in the pre-sterilized sediments (Table 3). These differences may reflect the assimilation of ^{14}C from CLA into microbial biomass. Assimilation of ^{14}C would not be expected from RLA since the ring carbon of atrazine is fully oxidized (valence +IV) and would require reduction prior to assimilation.

Atrazine sorption

Atrazine sorption was independent of sediment sample depth (Table 4). Values of K_d ranged from a high of 7.0 L kg^{-1} in the sediment collected from 9.4 m to a low of 0.4 L kg^{-1} at a depth of 6.4 m (Table 4). The degree of nonlinearity (indicated by the deviation of n from 1.0) varied from 0.97 in the 1.8 m sediments to 0.78 in the 15.5 m sediments, suggesting a combination of surface adsorption and partitioning of atrazine in soil organic matter. The range in K_d values suggested variations in the equilibrium aqueous phase atrazine concentration in biometer experiments prior to inoculation.

Table 2. Summary of mineralization rate constants from unsterilized inoculated S16 biometer experiments.

Mean sample Depth (m)	RLA, 25°C k (d^{-1})	RLA, 25°C Half-life (d)	r^2	RLA, 10°C k (d^{-1})	RLA, 10°C Half-life (d)
1.8	0.2136	3	0.99	0.0475	15
3.3	0.1034	7	0.97	0.0305	23
6.4	0.2736	3	0.95	0.07929	9
9.4	0.0936	7	0.99	0.0226	31
13.7	0.1099	6	0.96	0.0432	16
15.5	0.1152	6	0.96	0.0271	26
17.6	0.1426	5	0.97	0.0247	28
Mean sample Depth (m)	CLA, 25°C k (d^{-1})	CLA, 25°C Half-life (d)	r^2	CLA, 10°C k (d^{-1})	CLA, 10°C Half-life (d)
1.8	0.0442	16	0.99	0.0210	33
3.3	0.0331	21	0.94	0.0153	45
6.4	0.0324	21	0.97	0.0188	37
9.4	0.0282	25	0.99	0.0130	53
13.7	0.0245	28	0.98	0.0140	50
15.5	0.0252	28	0.96	0.0168	41
17.6	0.0252	28	0.99	0.0181	38

Table 3. Summary of mineralization rate constants from pre-sterilized inoculated S16 biometer experiments.

Mean sample Depth (m)	RLA, 25°C k (d^{-1})	RLA, 25°C Half-life (d)	r^2	RLA, 10°C k (d^{-1})	RLA, 10°C Half-life (d)
1.8	0.1008	7	1.00	0.0177	39
3.3	0.0648	11	0.98	0.0167	41
6.4	0.0888	8	0.97	0.0169	41
9.4	0.0499	14	0.95	0.0122	57
13.7	0.0566	12	0.98	0.0146	47
15.5	0.0576	12	0.99	0.0134	52
17.6	0.0624	11	0.97	0.0158	44
Mean sample Depth (m)	CLA, 25°C k (d^{-1})	CLA, 25°C Half-life (d)	r^2	CLA, 10°C k (d^{-1})	CLA, 10°C Half-life (d)
1.8	0.0806	9	0.96	0.0205	34
3.3	0.0713	10	0.99	0.0256	27
6.4	0.0977	7	0.94	0.0388	18
9.4	0.0384	18	0.97	0.0158	44
13.7	0.0734	9	0.98	0.0226	31
15.5	0.0631	11	0.99	0.0250	28
17.6	0.0523	13	0.99	0.0250	28

Relationship between RLA mineralization and K_d and C_{eq}

Mineralization rate constants were inversely correlated to K_d (Figures 5 A and B). This relationship suggested that mineralization rates were limited by atrazine sorption. The effect of sorption on atrazine mineralization rate was greatest for samples incubated at 25°C.

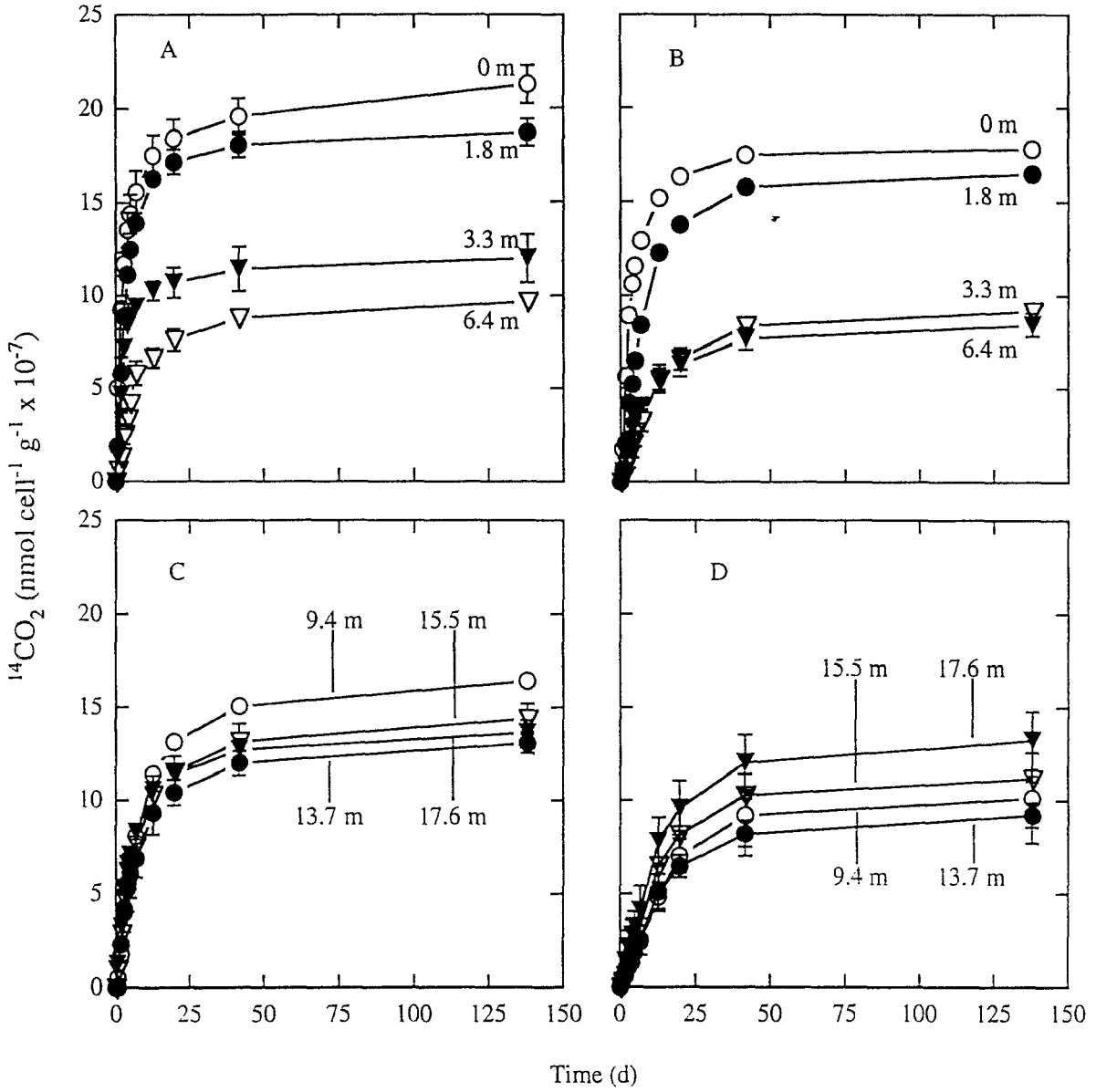

Fig. 3. Mineralization of RLA from S16 sediments ranging in depth from 0 to 17.6 m inoculated with M91–3 to an initial cell density of 7 x 10^7 cells g^{-1}. The sediments were amended without prior sterilization (A and C) and with prior sterilization (B and D) and incubated at 25°C. Bars represent one standard deviation.

The r^2 values were improved in samples that had been pre-sterilized (Fig. 5 A and B).

The effect of sorption was also examined by plotting k against the solution-phase atrazine concentration (C_{eq}) calculated from the measured K_d values (Fig. 5 C and D). These data indicated that a reduction in C_{eq} due to atrazine sorption decreased the respective k values at 25°C. The relationship was less apparent at 10°C. It can be concluded from these results that differences in sorption may partially explain the variability of k in the biometer experiments.

Relationship between CLA mineralization and K_d and C_{eq}

For CLA experiments, the inverse relationship between k and K_d was less apparent than in the corresponding RLA experiments (Fig. 6 A and B). The respective CLA slopes (m values) were lower by at least one

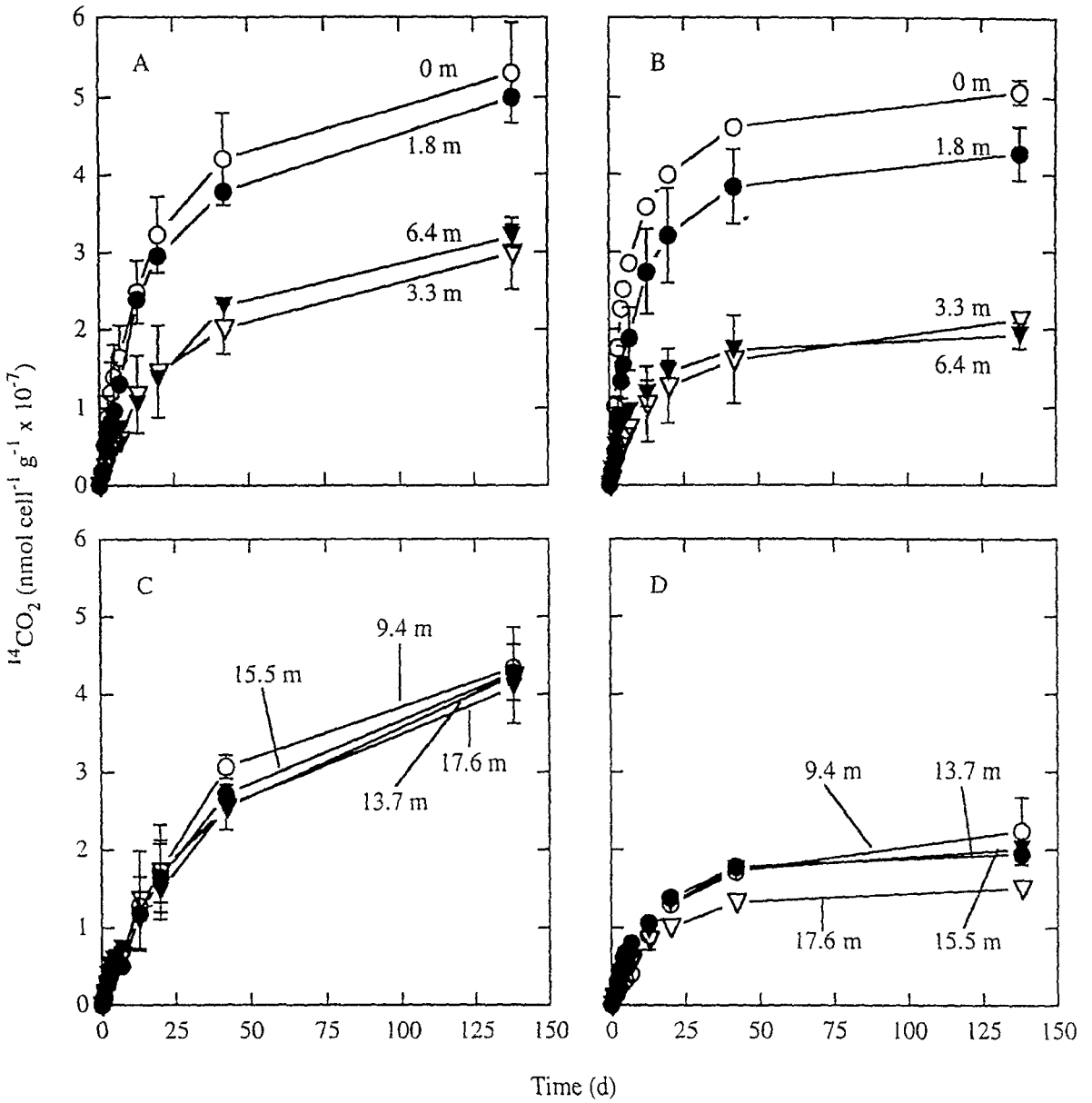

Fig. 4. Mineralization of CLA from S16 sediments ranging in depth from 0 to 17.6 m inoculated with M91–3 to an initial cell density of 7 x 10^7 cells g^{-1}. The sediments were amended without prior sterilization (A and C) and with prior sterilization (B and D) and incubated at 25°C. Bars represent one standard deviation.

order of magnitude. Similar differences were observed for the relationship between k and C_{eq} in the unsterilized experiments (Fig. 6 C and D). When the sediments were sterilized prior to inoculation and amendment with CLA, the effects of sorption were more apparent.

Discussion

In another study involving the mineralization of CLA in sediment samples, the rate constants were estimated to be in the range of $<4.5\text{x}10^{-5}$ to $5.4\text{x}10^{-4}$ d^{-1} (McMahon et al., 1992). However, the P_{max} values of CLA mineralization in these experiments were less than 1.5% of the added CLA. The rate constants reported by McMahon et al. (1992) were determined from only three experimental measurements, and no details

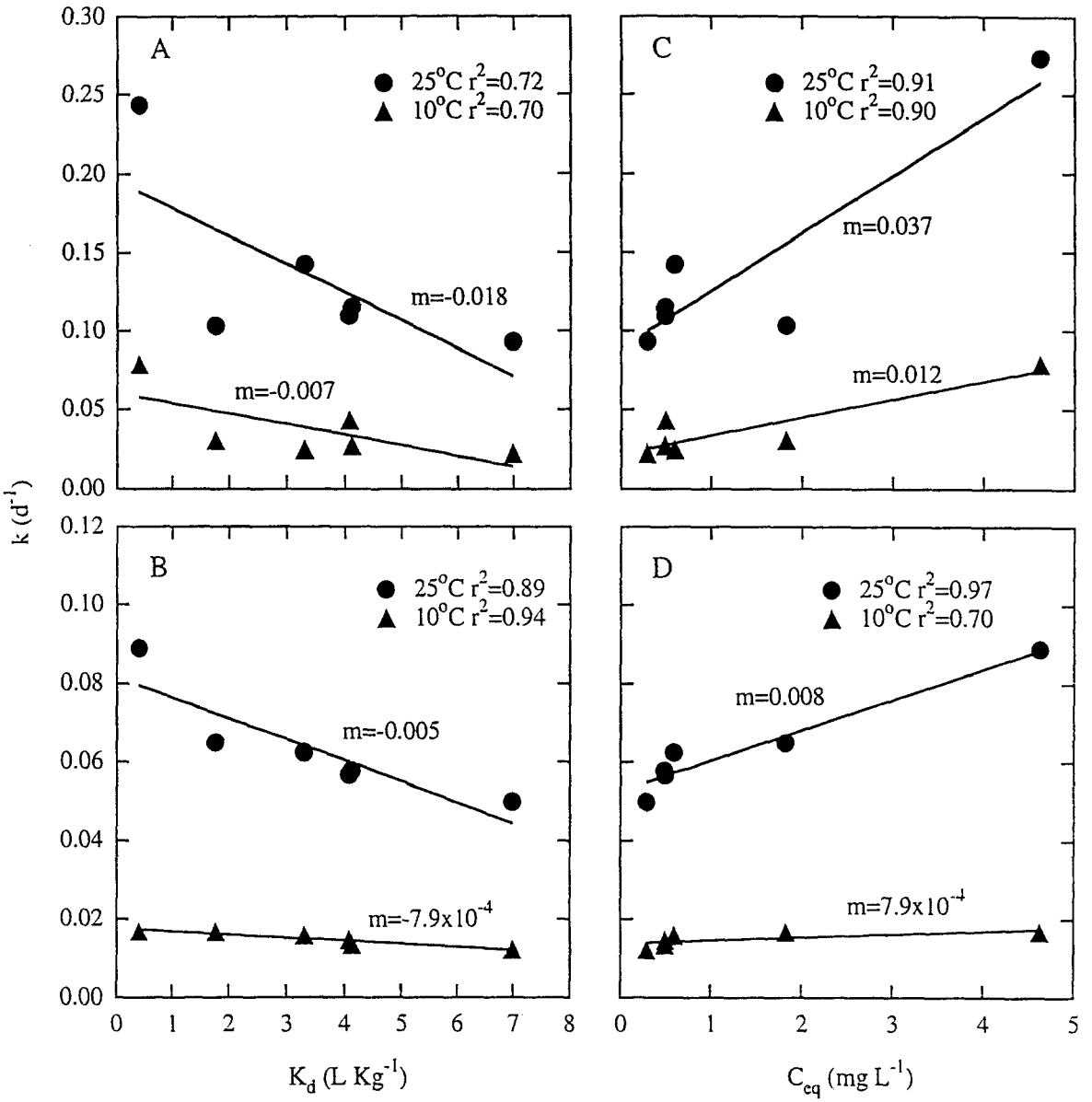

Fig. 5. Relationship between RLA mineralization rate constants (k d^{-1}) and soil sorption coefficients (K_d L kg^{-1}) and aqueous phase atrazine concentration (C_{eq} mg L^{-1}) in (A and C) unsterilized and (B and D) previously sterilized inoculated sediments ranging in depth from 3.3 m to 17.6 m and incubated at 25 and 10°C. The respective slope values (m) are also given.

were given on either the data fitting procedure or the r^2 values. The relative error in these measurements becomes increasingly more significant at these low mineralization values. The first-order rate constants determined in the present study exceeded these previously published estimates by two to three orders of magnitude.

Atrazine biodegradation in the uninoculated biometers decreased at the lower incubation temperature. Compared with the 25°C data, a four-fold reduction in the rate constant of RLA mineralization was evident at the 10°C incubation in the most active sample, S15 surface soil. With all the other samples showing activity, there was an apparent change in the order of the reaction as a result of the incubation temperature. The lack of first-order kinetics suggested that the rates were influenced by additional factors besides substrate concentration and therefore unaffected by changes in solution phase atrazine concentration due to sorption. These data illustrate that kinetic parameters measured

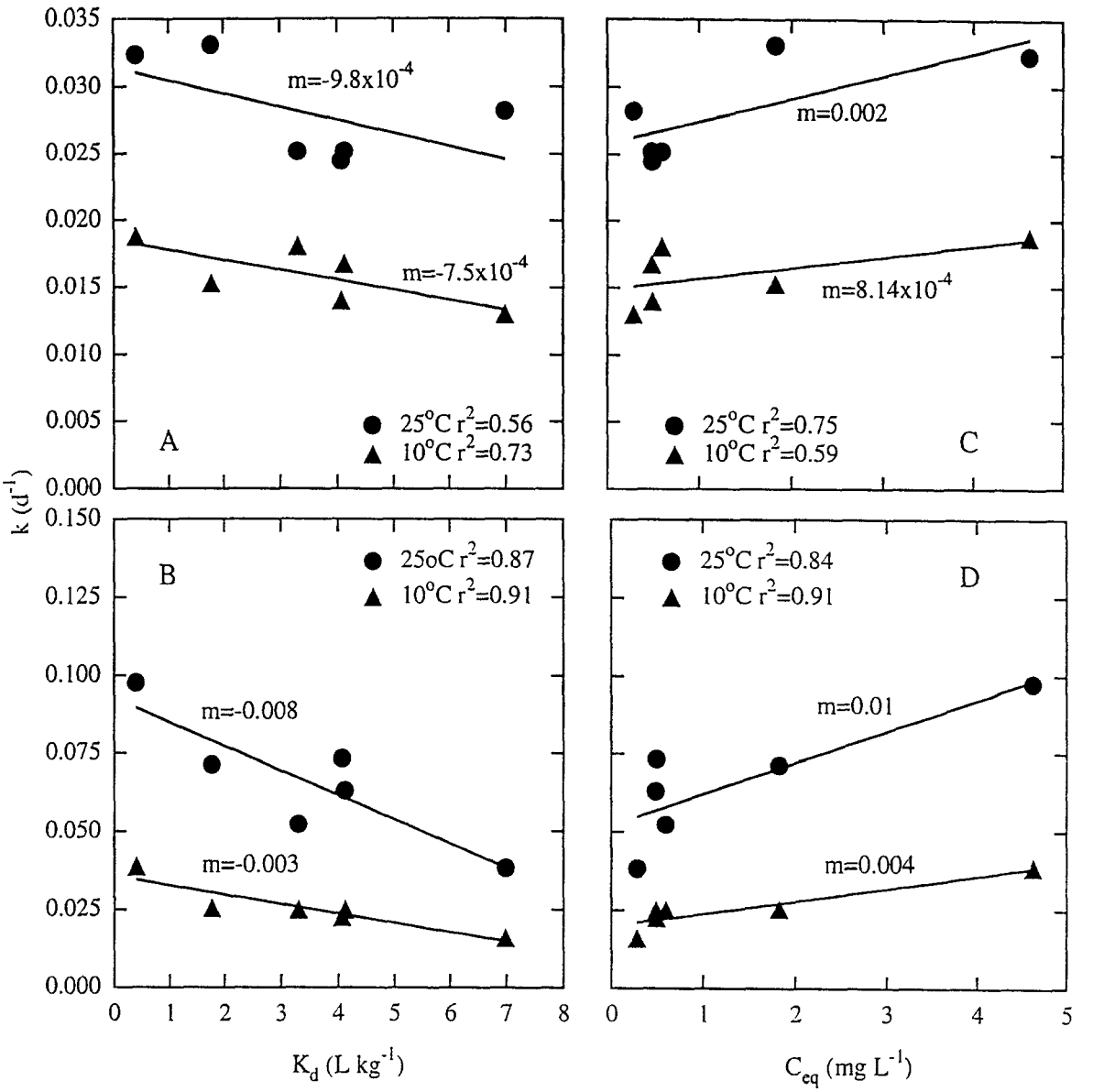

Fig. 6. Relationship between CLA mineralization rate constants (k d^{-1}) and soil sorption coefficients (K_d L kg^{-1}) and aqueous phase atrazine concentration (C_{eq} mg L^{-1}) in (A and C) unsterilized and (B and D) previously sterilized inoculated sediments ranging in depth from 3.3 m to 17.6 m and incubated at 25 and 10°C. The respective slope values (m) are also given.

at ambient surface temperatures may have little relevance in estimating dissipation rates in subsurface sediments.

The results of the present work also demonstrate that biodegradation of atrazine in the subsurface is spatially variable, in keeping with previous findings (Sinclair and Lee, 1992; Johnson and Fuhrmann, 1993). In previous attempts (Radosevich et al., 1993) to enrich for atrazine-degraders from the subsurface sediments collected from the Ohio MSEA, the parent compound persisted in over 90% of the sediment samples examined (over 80 samples from 22 bore holes). In samples showing degradative activity, atrazine half-lives exceeded 170 d. In the present study, active atrazine mineralization was particularly pronounced in sediments underlying site S15 which had a previous, long-term atrazine application history. Although the data are relatively limited with respect to the extent and number of sampling sites, they show that the reference site with no history of atrazine application exhibited slow

Table 4. Atrazine sorption parameters for the sediments collected at site S16.

Mean sample Depth (m)	K_d(L kg^{-1}) (< 2 mm)	n	r^2	K_d'(L kg^{-1}) (< 5 mm)*
0	3.34	0.92	0.996	3.34
1.8	1.44	0.97	0.999	1.44
3.3	1.84	0.88	0.996	1.76
6.4	1.15	0.96	0.999	0.40
9.4	7.1	0.79	0.997	7.0
13.7	4.18	0.84	0.997	4.07
15.5	4.38	0.78	0.992	4.12
17.6	3.73	0.79	0.996	3.30

* K_d' represents the measured K_d value corrected to the < 5 mm fraction.

mineralization of both CLA and RLA, suggesting that atrazine treatment had enhanced mineralization activity at S15. The biological basis of this enhancement has not been characterized.

Half-lives calculated from the first-order rate constants of RLA mineralization were 3.4 and 43 d for the two surface soil samples and ranged between 17 and 43 d for the active subsurface sediment samples. These values suggest considerably faster turnover rates of atrazine when compared with half-life estimates derived from enrichment cultures (Radosevich et al., 1993). However, 9 of the 14 samples examined showed no detectable mineralization. The enhanced mineralization of both RLA and CLA in the inoculated sediments from site S16 suggests that atrazine mineralization in the subsurface is limited by the absence of atrazine degraders rather than by sorption, nutrient availability or other inherent sediment properties.

Sorption was concluded to be a limiting factor for atrazine mineralization only when sediments were inoculated to a high cell density with an active atrazine-mineralizing isolate. The inverse relationship observed between the mineralization rate constants and the sorption coefficients implied that sorbed atrazine was less available for biodegradation in some samples, and that the reduction in atrazine mineralization was the result of a decrease in the solution-phase atrazine concentration. Sorption was not a significant limitation to biodegradation of atrazine at 10°C. The results of this study demonstrate that sorption can limit the biodegradation of atrazine when biodegradation rates are high (i.e., when the temperature and the number of active atrazine-degraders are not limiting). However, it seems unlikely that sorption/desorption processes pose a primary limitation to the biodegradation of atrazine in vadose zone and aquifer environments where temperatures and a lack of microbial degraders are more likely to be rate-limiting factors.

Acknowledgments

We thank A. Springer for sample collection. This research was conducted as part of the Ohio Management Systems Evaluation Area project which is a cooperative research and educational effort of the Ohio Agricultural Research and Development Center, The Ohio State University, OSU Extension, the USDA-ARS, USDA-CSRS, USDA-ES, USGS, US EPA, and other state and federal agencies. We thank the USDA National Needs Fellowship Program in Water Quality and The Ohio State University Fellowship Programs for graduate student support of the primary author. Salary and research support were provided to SJT by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

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