PERSISTENCE OF ATRAZINE AND ALACHLOR IN GROUND WATER AQUIFERS AND SOIL

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Abstract. Degradation of atrazine and alachlor in saturated aquifer materials and soil was studied in the laboratory. A static aquifer was represented by a set of stagnant flasks and a well-mixed aquifer was simulated by recirculating columns. Water was tested at selected time intervals over six months and analyzed for herbicides and metabolites. Under all conditions, atrazine was more persistent than alachlor. Increased temperature had little effect on atrazine dissipation but did increase alachlor degradation rates, especially in the sterilized treatments. The addition of carbon and nitrogen prolonged the initial period before the onset of degradation in some of the columns. Enhanced mass transfer of the herbicides, nutrients, and oxygen in the recirculating columns dramatically increased dissipation of atrazine and alachlor. The degradation rates of atrazine and alachlor were 2 to 5 times faster in the recirculating columns than in the stagnant flasks. Atrazine was more persistent in the aquifer materials than in the soils, while alachlor dissipation was similar in the soils and recirculating aquifer columns, but was slower in the stagnant flasks. The prolonged persistence of atrazine under static, aquifer conditions ($t_{1/2}$ = 206 to 710 days) indicates that natural mechanisms are not sufficient to alleviate the risk of atrazine buildup over time; however, in a well mixed aquifer, atrazine degradation rates should be higher ($t_{1/2}$ = 66 to 106 days) and the threat of atrazine accumulation is diminished. Alachlor persistence at low concentrations ($<10\,\mu g\,L^{-1}$) in aquifers should not pose a long-term threat to ground water supplies.

Keywords: alachlor, atrazine, aquifer, degradation, environment, ground water, herbicides, leachate, soil, transport, water

Introduction

Residents in rural areas often rely on ground water for consumption and irrigation, with little or no prior treatment. However, the risk of contamination from agricultural pesticides is high in these areas (Bernard *et al.*, 2005; Fallon *et al.*, 2002). Pesticide residues are often detected in ground water supplies (Gooddy *et al.*, 2005; Karrow *et al.*, 2005; Phyu *et al.*, 2005; Reeder *et al.*, 2005). Two widely used pesticides, atrazine [6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5 triazine-2,3-diamine] and alachlor [2-chloro-2′,6′-diethyl-N-(methoxymethyl)acetanilide], have been found in ground water in the Midwest. The human and ecological risk from these herbicides is a point of concern (Gammon *et al.*, 2005; El-Sakka *et al.*, 2002).

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Atrazine is in a class of herbicides known as s-triazines and is used to control broadleaf weeds and some annual grasses in corn and grain sorghum. The herbicide is classified as moderately mobile in soil (Cohen *et al.*, 1984). Degradation of atrazine plays a major role in determining the persistence of atrazine in soil and subsurface environments. Degradation rates depend on soil type, organic matter, pH, temperature, moisture levels, oxygen levels, and microbial activity (Lesan and Bhandari, 2004; Dorado *et al.*, 2003; Smith and Walker, 1989). Warmer temperatures and moisture levels near field capacity promote the most rapid degradation.

The primary mechanism of chemical (abiotic) degradation is hydrolysis to produce hydroxyatrazine (Prosen and Zupancic-Krajl, 2005). Armstrong *et al.* (1967) showed hydrolysis to be enhanced at low and high pH and in soils with higher organic matter. Adsorption-catalyzed hyrolysis can occur as the result of hydrogen bonding between carboxyl groups in organic matter and atrazine ring nitrogen atoms (Prosen and Zupancic-Krajl, 2005; Gamble and Khan, 1985). The primary microbial degradation pathway is N-dealkylation of the side chains to produce deethylatrazine and deisopropylatrazine (Neumann *et al.*, 2004; Radosevich and Tuovinen, 2004). Increased temperature, moisture, organic matter content of soils appears to stimulate microbial degradation (Sonon and Schwab, 2004; Navarro *et al.*, 2004; Smalling and Aeolion, 2004; Johannesen and Aamand, 2003). Smith and Walker (1989) showed that as the moisture content and temperature increased for a heavy clay soil, the half-life of atrazine decreased. Blume *et al.* (2004) demonstrated that as soils become saturated and reducing conditions were induced, microbial activity was diminished and atrazine was degraded more slowly.

Considerable debate exists over whether abiotic or biotic pathways of degradation are more important in the natural environment. Most researchers conclude that both chemical and microbial dissipation of atrazine in soil is important (Johnson *et al.*, 2003; Sirons *et al.*, 1973). Half-life estimates for atrazine range from 21 to 80 days (Winkelmann and Klaine, 1991; Smith and Walker, 1989); although Jones *et al.* (1982) determined the half-life to be 330 and 385 days in two agricultural soils. Atrazine degradation is generally slower in the saturated environments (Chilton *et al.*, 2005; Pang *et al.*, 2005; Junk *et al.*, 1984). Perry (1990) estimated the half-life for atrazine in a Kansas aquifer to be 1000 days.

Alachlor is the active ingredient in the herbicide Intrro™ (and previously Lasso™) manufactured by Monsanto Company. This herbicide has been registered since 1969 as a preemergence, early postemergence, or preplant incorporated herbicide for control of most annual grasses or certain broadleaf species. Alachlor is most heavily used on corn, soybeans, and grain sorghum. Dissipation of alachlor from soil occurs because of photodecomposition, volatilization, chemical degradation, microbial degradation, and leaching (Sette *et al.*, 2004; Selim *et al.*, 2002). Significant volatilization losses occur only when moist soil

is exposed to windy conditions (Clay *et al.*, 2004). In addition, photodegradation has been shown to be significant (Ryu *et al.*, 2003). All five degradation products: (I)-[N-(2,6-diethylphenyl)-acetamide], (II)-[2-chloro-N-(2,6-diethylphenyl)-acetamide, (III)-[2,6-diethyl-N-(methoxymethyl)-aniline, (IV)-[1-chloroacetyl-2,3-dihydro-7-ethylindole], and (V)-(2,6-diethylaniline) were found to be produced by photodegradation. Hargrove and Merkle (1971) reported at 0% relative humidity and high temperature (38 to 46 °C), alachlor was degraded to (II) by chemical degradation due to acidic soil water film surfaces. The persistence of alachlor in soil is inversely proportional to temperature and moisture. Half-lives of alachlor in soil range from 4 to 50 days (Clay *et al.*, 2004).

Alachlor degradation is primarily a microbial process (Knapp *et al.*, 2003). Alachlor is degraded 50 times faster in active soil than in sterilized soil (Beestman and Deming, 1974). Tiedje and Hagedorn (1975) isolated a soil fungus, *Chaltomium globosum*, capable of metabolizing alachlor to four identifiable organic metabolites: (II), (III), (IV), and (V); however, six other soil fungi were unable to effectively degrade alachlor. They also hypothesized alachlor could be dechlorinated to form another intermediate between alachlor and (V), although metabolite (V) did not accumulate in their study.

Ensz *et al.* (2003) and Novick *et al.* (1986) reported that alachlor degradation is a cometabolic process. No single microorganism able to mineralize or use alachlor as a sole source of carbon has been found, although a mixed culture from alachlor treated soil was found to use alachlor as a soil carbon and energy source. The presence of other carbon sources, i.e., sucrose or glucose, increased the mineralizing ability of the microbes (Sun *et al.*, 1990) suggesting that the microbes utilized the side chain of alachlor as a carbon source without attacking the ring.

Alachlor is more persistent in saturated environments. In a saturated sandy loam, less than 10% of the added alachlor was degraded in 68 weeks (Junk *et al.*, 1984). Novick *et al.* (1986) reported that after 47 days incubation only 0.5% of the initial alachlor was mineralized in an aquifer material. After 126 days of incubation under either aerobic or anaerobic conditions, almost 90% of the alachlor remained in the aquifer materials (Pothuluri *et al.*, 1990). Cavalier *et al.* (1991) found that degradation of alachlor in ground water from different aquifers depended on temperature, initial concentration, and depth of the ground water. The half-life for alachlor in ground water ranged from 808 to 1518 days, while Perry (1990) provided an estimate of 400 days.

The overall objective of this research project was to determine measure rates of dissipation of atrazine and alachlor in aquifer and soil materials under various conditions. Three sites in Kansas were sampled for soils and aquifers, and treatments included stagnant versus flowing solution phase; temperatures of 15 and 25 °C; the presence and absence of added nutrients; and sterile versus non-sterilized solid substrates.

Materials and Methods

WATER AND AQUIFER MATERIAL COLLECTION AND ANALYSES

Aquifer, soil, and ground water samples were collected from three Kansas State University Agronomy Experiment Fields. One location was in south-central Kansas at the Hutchinson Experiment Field six miles south of Hutchinson. The other two locations were in northeast Kansas along the Kansas River at the Ashland Experiment Field (Manhattan, Kansas), and the Kansas River Valley Experiment Field (Topeka, Kansas), both in the floodplain of the Kansas River. Boreholes were drilled with a hydraulic rotary rig equipped with a 10 cm hollow stem auger. After the hole was compacted to reduce sloughing, aquifer sediment was brought to the surface. The material was placed in containers and transported back to the laboratory. A subsample of the aquifer material was placed in a sterilized Whirl-Pak bag (Nasco Company, Fort Atkinson, WI) for microbial enumeration.

Drilling through the aquifer continued until bedrock was located. The bottom plate of the auger was punched out, allowing the hole to fill with water. Slotted PVC pipe was inserted to form the well casing. Water was pumped out of the well for ten minutes until the water was reasonably clear. Water was collected and placed in carboys. Additional water samples were placed in glass bottles for inorganic, organic, and microbial analysis. The bottles for the organic and microbial analysis were previously sterilized by autoclaving. A portion of the inorganic water samples were acidified with nitric acid for determination of calcium and magnesium.

Soil samples were taken for comparative purposes. Duplicate soil samples were aseptically placed in sterilized plastic bags for microbial analysis. The soil samples were taken from the upper 15 cm at Topeka and Hutchinson; however, the soil sample from Ashland was obtained from a depth of 60 cm. All samples were kept in an ice chest for transport back to the lab.

At the Ashland site, the water table was located at 6.1 m and shale was struck at 15.2 m. The aquifer material, medium gravel, was obtained from a depth of 7.6 m, and the water sample was taken at the same depth. At the Topeka site, the water table was found at 5.5 m and shale was found at 23 m. The aquifer material, coarse gravel, was obtained at a depth of 7.0 m, while water was taken at a depth of 7.6 m. The water table was only 3.7 m at the Hutchinson site, and red shale was located at 7.6 m. The aquifer material, a mixture of fine sand and clay, was obtained from a depth of 4.6 m as was the water sample.

After standard analysis of temperature, pH, specific conductance, redox potential, and dissolved oxygen (Table I), the ground water samples were filtered through #42 Whatman filter paper, and the major cations, Ca, Mg, K, Na, Fe, Mn, Zn, and Cu, were analyzed by a Perkin-Elmer 460 Atomic Absorption Spectrophotometer using an air-acetylene flame and solution flow rate of 8 mL min^{-1}. Originally, the acidified samples were used to measured cation concentrations; however, the nitric acid dissolved some of the minerals in the sediment of these samples, so

TABLE I

Chemical analyses of different ground waters used in this experiment

Constitutent	Topeka Aquifer	Ashland Aquifer	Hutchinson Aquifer
Iron (Fe) ($mg\,L^{-1}$)	ND	0.71	0.20
Manganese (Mn) ($mg\,L^{-1}$)	0.27	0.14	ND
Calcium (Ca) ($mg\,L^{-1}$)	125	105.8	53.4
Magnesium (Mg) ($mg\,L^{-1}$)	14.5	17.6	9.4
Sodium (Na) ($mg\,L^{-1}$)	6.2	31.3	100.9
Potassium (K) ($mg\,L^{-1}$)	8.16	12.6	0.99
Zinc (Zn) ($mg\,L^{-1}$)	ND	ND	ND
Copper (Cu) ($mg\,L^{-1}$)	ND	ND	ND
Bicarbonate (HCO_3) ($mg\,L^{-1}$)	420.9	353.8	317.2
Sulfate (SO_4) ($mg\,L^{-1}$)	18.4	16.8	13.14
Nitrate (NO_3-N) ($mg\,L^{-1}$)	2.1	0.32	10.1
Phosphate (PO_4-P) ($mg\,L^{-1}$)	ND	ND	ND
Chloride (Cl) ($mg\,L^{-1}$)	4.7	36.8	18.6
Bromide (Br) ($mg\,L^{-1}$)	ND	0.19	NC
Fluoride (F) ($mg\,L^{-1}$)	0.3	0.57	0.99
Dissolved Solids ($mg\,L^{-1}$)	410	423	377
Hardness as $CaCO_3$ ($mg\,L^{-1}$)	373	337	173
Specific Cond. ($\mu mhos\,cm^{-1}$)	630	650	580
pH	7.26	7.65	7.63
Temperature (°C)	15.5	15.8	14.8
Eh (mV)	32.5	115.1	151.7
Dissolved Oxygen	0.53	0.87	2.76
Organic Carbon	2.49	3.76	1.04
Microorganisms ($CFU\,mL^{-1}$)	10,400	10,600	1370

ND – Not detected.

CFU – Colony forming units per milliliter of water.

the concentrations reported are from the analysis of the unacidified samples. The ground water samples were also analyzed for the anions, Cl^-, Br^-, F^-, NO_2^-, NO_3^-, $H_2PO_4^-$, and SO_4^{2-} using a Dionex System 2110i Ion Chromotograph with a Dionex AS3 anion separation column, and Dionex MPIC-NG1 and HPIC-AG3 precolumns at a flow rate of $2\,mL\,min^{-1}$. The bicarbonate, $HCO_3{}^-$, concentration was determined by titration. The dissolved organic carbon (DOC) was determined using a Dohrmann DC-180 automated carbon analyzer with an ultra-violet promoted persulfate oxidation method with a linearized non-dispersive detector. The analysis and calibration mode was non-purgeable organic carbon (NPOC) with injection loop. The pickup loop volume was 10 mL, and the injection loop volume was 1 mL with two injections per sample.

TABLE II
Physical and chemical properties of the soils and aquifer materials

Sample	% Gravel (>2 mm)	% Sand	% Silt	% Clay	pH	CEC (cmol kg^{-1})	Organic Carbon (g kg^{-1})	Microbial Population (log_{10} CFU g^{-1}[a])
Eudora silt loam (Topeka)	0	21	66	13	7.2	9.7	8.1	7.8
Eudora silt loam (Ashland)	0	37	56	8	7.5	6.4	1.4	7.5
Ost loam (Hutchison)	0	23	54	23	6.9	16.9	12.1	7.9
Topeka Aquifer (gravelly sand)	21	96	3	1	8.7	0.76	0.3	5.1
Ashland Aquifer (sand)	6	94	5	1	8.8	1.0	0.6	5.9
Hutchinson Aquifer (loamy sand)	6	82	11	7	8.6	2.9	0.9	5.9

[a]Colony forming units per gram of soil. Values are the mean of the three replicates.

Particle size was analyzed using the pipet method (Gee and Bauder, 1986) (Table II). The pH of the samples was determined in a 1:1 solution of soil and water using a Ross combination pH electrode which had been calibrated using pH 4 and 7 buffers. The cation exchange capacity (CEC) was determined using the ammonium acetate (NH_4OAc) method adapted from Chapman (1965). Analysis of total carbon was by the LECO Carbon Analyzer. A dichromate oxidation technique was used to determine organic matter (Schulte, 1980).

MICROBIAL ASSAYS

Both total plate counts and individual counts for bacteria, fungi, actinomycetes were conducted. Agar plates were prepared by adding 15.5 g of nutrient agar to 500 mL water, autoclaving the agar solution at 121 °C for 20 min, and pouring 15 mL per plate. For bacteria, 25 mL of cycloheximide solution was added through a sterile filter per 500 mL of autoclaved nutrient agar. For fungi, 25 mL of rifampicin was added through sterile filter per 500 mL of autoclaved nutrient agar. For actinomycetes, preparation of the agar included: 3 g agar, 0.2 g dextrose, 20 mg KH_2PO_4, 20 mg $NaNO_3$, 20 mg KCl, and 20 mg $MgSO_4$ dissolved in 200 mL of distilled deionized water. The pH was adjusted to 7.0 using 0.1 N HCl or 0.1NaOH.

The soil dilutions were prepared by aseptically transferring 10 g of soil, 90 mL sterile 0.85% NaCl solution and one drop of Tween 80 into a blender and mixing for 2 min at high speed for the 10^{-1} dilution. The dilutions continued until the 10^{-6}

dilution was reached. The amount of dilution depended on the microbe and sample (soil/aquifer). The plates were inoculated by adding 0.1 mL of a dilution with a sterile syringe to the prepared agar plates. Every dilution/sample combination was replicated three times. The plates were incubated at 25 °C for 4 to 7 days. The plates with 30 to 300 colonies per plates were counted.

EXPERIMENTAL DESIGN FOR DEGRADATION STUDIES

Two different experimental systems were established in the lab: a set of stagnant flasks at 15 and 25 °C simulating a static aquatic aquifer, and recirculating columns at 15 °C simulating a well-mixed aquifer. Three treatments were employed for these conditions: added nutrients, unamended, and sterilized. The entire experimental design was replicated three times for each system.

For the static system, 700 g of aquifer material from the three different aquifers was placed in 1000 mL Erlenmeyer flasks along with 700 mL of ground water from the appropriate aquifer. No soil was used in the static system. Carbon and nitrogen were added to one set of flasks for each aquifer in an attempt to stimulate microbial activiy. Cellobiose, $C_{12}H_{22}O_{11}$ (Sigma Chemical Company, St Louis, MO) was used as a carbon source. The flasks received 3.5 mL of a 10 g L^{-1} stock solution to achieve a final concentration of 50 mg L^{-1} as carbon. Ammonium nitrate, NH_4NO_3, was used as a nitrogen source. A final concentration of 10 mg L^{-1} was obtained by adding 1.4 mL of a 5 g L^{-1} stock solution.

For the sterilized treatment, the aquifer material was placed in 1000 mL flasks and autoclaved at 121 °C for 1 h and incubated for 24 h. This procedure was repeated twice. The ground water and rubber stoppers for each flask were autoclaved at 121 °C for 30 min. The ground water was then aseptically transferred to the flasks in a sterile hood. To insure no microbial activity, 0.70 g of sodium azide, NaN_3 (Sigma Chemical Company) was added as a microbial inhibitor, resulting in a final azide concentration of 1000 mg L^{-1}.

Analytical atrazine and alachlor standards were obtained from CIBA-GEIGY Corporation and Monsanto Company, respectively. A 116.6 mg L^{-1} aqueous solution of analytical atrazine and alachlor was prepared. Each flask received 120 μL of the analytical atrazine solution and 60 μL of the analytical alachlor solution, resulting in final concentrations of 20 and 10 μg L^{-1} for atrazine and alachlor, respectively. These concentrations were slightly higher than those normally found in aquifers from nonpoint sources. After the two herbicides were added, stoppers were inserted in the top of each flask. These rubber stoppers had a glass tube running through the center which had a rubber septum on the end. The glass tube with the rubber septum served as a sampling port for water samples. Once the rubber stoppers were inserted, the flasks were mixed thoroughly by inverting them three times.

The flasks were incubated at 15 or 25 °C for 182 days. These temperatures were chosen because 15 °C is a typical temperature of ground water (Table I), and 25 °C

represented a maximum temperature for the aquifer and optimum conditions for degradation. Water samples were taken with a 20 mL disposable, sterile, syringe at selected time intervals (0, 7, 21, 42, 70, 98, 126, 154, and 182 days) during the course of the experiment. The needle of the syringe was inserted into the rubber septum, and solution was drawn from the flask. The water sample was placed in a 20 mL glass scintillation vial and stored at 4 °C until analysis. After the water sample was taken, 20 mL of water from the appropriate aquifer was added to each flask to maintain a constant volume of solution. The flasks were inverted three times after each sampling, but were not shaken between samplings. The water was sampled 24 hours after addition of the herbicides. This was established as time zero for measuring concentrations. For all experimental units, adjustments in total herbicide were calculated and accounted for the small amounts of mass removed at each sampling event. The reported concentrations and mass balances reflect these changes.

For the recirculating columns, the columns were polypropylene with height of 12.6 cm and a diameter of 6.4 cm giving a column volume of 405 cm^3. The columns were fitted on both ends with threaded caps that sealed with a rubber O-ring.

The three soils were sieved at field moisture through a 2 mm sieve. The soil was added to the column in small increments and packed with a cylindrical plastic tamper. The final soil mass (500 g on a dry weight basis) was corrected for moisture content gave an approximate bulk density of 1.22 g cm^{-3}. The aquifer material was not sieved prior to packing. Each aquifer material was packed into the columns using the same procedure as for the soil. The final mass for the aquifer material was 700 g giving an approximately bulk density of 1.71 g cm^{-3}. Glass wool was inserted in the water inlet and outlet and filter paper was placed on the surface of the soil/aquifer material to prevent sedimentation in the tubing during water circulation.

The procedure used to pack the sterilized columns (soil and aquifer) was slightly different. The solid material was autoclaved in 121 °C for one hour, incubated for 24 h, and repeated twice. The ground water, rubber stoppers, and glass tubing were also autoclaved. Both ends of the columns were sealed to prevent contamination from air entry. The columns were saturated from the bottom over a 24-h period. The columns were connected from the bottom to 1000 mL Erlenmeyer flasks containing the appropriate ground water. The sterilized flasks contained 1000 mg L^{-1} sodium azide. A 0.001 M $CaSO_4$ solution was used to saturate the soil columns. The outlet at the top of the column was left open for the escape of air, except for the sterilized columns where a cotton plug was put in the outlet to prevent contamination. The flasks originally were below the bottom of the columns. The flasks were raised approximately 4 to 5 cm every 8 h until the water level reached the top of the columns allowing them to become saturated. Water was added to each flask as the sediment in the columns adsorbed more water. The final solution volume was 1100 mL for the aquifer columns and 1200 mL for the soil columns.

The columns were placed in wooden racks in a controlled environment chamber with the solution below them. The water was circulated from the flask upward

through the columns and back to the flasks by a peristaltic pump at a rate of $2\,mL\,min^{-1}$. Tygon tubing was used to connect the flasks to the columns. The peristaltic pumps were run for only ten hours per day. When the pumps were shut down, plastic clamps were used to prevent the solution from draining out of the columns.

The water was recirculated through the columns one week before nutrients or herbicides were added. The carbon and nitrogen concentrations for the nutrient treatment and the initial concentrations of atrazine and alachlor for the aquifer columns were the same as for the static system. There were two treatments for the soil columns: sterilized and non-sterilized. The soil columns received an initial concentration of $400\,\mu g\,L^{-1}$ of atrazine and alachlor. Control columns containing distilled, deionized water also were constructed. These columns had initial concentrations of 20 and $10\,\mu g\,L^{-1}$ of atrazine and alachlor, respectively. All columns were leached with recirculating water for 182 days, and water samples taken from the flasks as previously described. The columns were allowed to circulate 24 h before the first water sample was taken, establishing a time zero for measuring concentrations.

After 182 days, the degradation studies ceased. One hundred mL of solution was transferred from the stagnant flasks to glass bottles and stored at 4 °C for the herbicide analysis. For inorganic and organic analyses of the water, the samples were filtered into separate 125 mL plastic bottles and stored at 4 °C until analysis. The reminder of the water was decanted, and the solid aquifer material was placed in sterilized plastic bags and stored at 4 °C until herbicide analysis and microbial counts were conducted.

Water samples were collected from the recirculating flasks for inorganic, organic, and herbicide analysis using the procedures described above for the stagnant flasks. Before any solid material was collected, the columns were allowed to drain overnight to remove excess water. Then the caps were removed from both ends of the columns. A plastic plunger was used to push the material from the column and into a sterilized plastic bag. Each column was sectioned into two halves during collection to investigate herbicide concentrations differences within the column. The solid material from the sterilized columns was collected in a sterile hood, and stored at 4 °C.

HERBICIDE EXTRACTION AND ANALYSES

Before the degradation studies were conducted, soil, aquifer material, and ground water were tested for atrazine and alachlor. All concentrations of atrazine and alachlor were below detection limits. During the experiment, herbicides were analyzed within seven days of sampling. At the end of the experiment, the water samples were analyzed within seven days of sampling. The solid samples were analyzed within one month of sampling.

Two grams of soil or aquifer material were transferred into a 20 mL scintillation vial. Ten mL of methanol were added, the vials were sealed with foil-lined

caps, and the slurries were mixed on a reciprocal shaker for one hour. The slurries were filtered and washed with 2 mL of methanol. Each filtrate was brought to a volume of 100 mL with distilled, deionized water. The samples were then spiked with 1 μL of 1614 mg L^{-1} terbuthylazine (TBT) (terbuthylazine (TBT) (6-chloro-*N*-(1,1-dimethylethyl)-*N'*-ethyl-1,3,5-triazine-2,4-diamine, CIBA-GEIGY Corporation, Greensboro, NC) as an internal standard, and the solution was extracted with a C_{18} procedure.

C_{18} Sep-Pak catridges (Waters and Associates, Milford, MA) were attached to a 100 mL pipet and conditioned by sequentially eluting (by gravity flow) with 2 mL methanol, 6 mL ethyl acetate, 2 mL methanol, and 2 mL water. The excess water was blown from the cartridge before the samples were added. The sample was drawn into a 100 mL pipet and passed through the C_{18} cartridge by gravity flow. The loaded cartridges were then extracted with 2.5 mL ethyl acetate and eluted into a 25 mL culture tube. The residual ethyl acetate was blown from the cartridges. To remove excess water from the samples, the tubes were vigorously mixed and allowed to settle. The ethyl acetate then was transferred to a culture tube with 1 g anhydrous Na_2SO_4, and the suspensions were stirred on a vortex mixer. The supernatant liquid was transferred to 10 mL centrifuge tubes for the volume reduction procedure. Extraction from the water samples was identical, except the soil extraction step was omitted. The 20 mL or 100 mL water samples were passed through the C_{18} cartridges. The rest of the procedure was the same as described above. Because these samples contained very low concentrations of atrazine and alachlor, they were further concentrated by placing the centrifuge tubes in 55 °C water bath and reducing the volume to 100 μL with a slow stream of purified nitrogen gas. The ethyl acetate was then transferred to a 100 μL plastic vial and sealed for analysis.

The soil/aquifer sediment extraction procedure was modified for the detection of the atrazine metabolites, deethylatrazine (DEA) and deisopropylatrizine (DIA). Previous research (Schwab *et al.*, 1991) showed that in the presence of methanol (5 to 20% v/v), significant breakthrough from the C_{18} cartridges occurred for both degradation products in the aqueous solutions greater than 50 mL. To alleviate this problem, the methanol was evaporated prior to dilution of the sample to 100 mL. After shaking the slurries, the filtrate was collected in the centrifuge tubes, and TBT was injected into each sample. The centrifuge tubes were placed in a water bath at 80 °C, and the methanol was reduced to a volume of 0.5 mL under a stream of nitrogen gas. The residual methanol was transferred to a glass bottle and diluted to 100 mL with distilled, deionized water, resulting in a 0.5% (v/v) methanol water mixture. The remainder of the procedure was followed as described previously. Recovery standards showed that 0.5% methanol in water did not result in breakthrough from the cartridge.

Samples were analyzed by gas chromoatography (GC) on a Hewlett-Packard 5890 Gas Chromatograph using a split injection, J&W DB5 20 m $\times$ 0.18 mm $\times$ 0.40 μm fused silica capillary column (J&W Scientific, Folsom, CA), and

nitrogen-phosphorus detector. Helium was used as the carrier gas at a flow rate of 14.5 mL min^{-1}. A 2 μL sample was injected, and the column temperature was maintained at 50 °C for 2 min. The temperature was increased to 210 °C at a rate of 50 °C min^{-1} followed by a rate of 5 °C until a temperature of 240 °C was reached. An injector temperature of 170 °C and detector temperature of 220 °C were maintained throughout the analysis. The use of TBT as an internal standard provided excellent standard curves of peak-height ratios versus concentration ratios for atrazine, alachlor, deethylatrizine, and deisopropylatrazine (Schwab *et al.*, 1991). Injecting TBT prior to the volume reductioin alleviated the concern for an exact volume to be achieved. Two recovery standards, three spiked samples, and two blanks also were run with each set of 64 samples. The quantification limit for atrazine, alachlor, DEA, and DIA in a 20 mL water sample was 0.20 μg L^{-1}, 1.00 μg L^{-1}, 0.20 μg L^{-1}, and 0.20 μg L^{-1}, respectively. For a 100 mL sample, the quantification limit was lower by a factor of five. The quantification limit for solid samples was approximately 1 to 2 μg kg^{-1} for atrazine and 4 to 5 μg kg^{-1} for alachlor.

STATISTICAL ANALYSES

All data were subjected to analysis of variance. The expected mean squares correspond to a linear model for a multi-factor experiment arranged in a split plot - split block design in which blocks are random and factors are fixed. Fisher's least significant difference (LSD) was used to compare means. For all variables, the comparisons of interest were the interaction means in which all means were compared.

Results

PROPERTIES OF EXPERIMENTAL MATERIALS

The chemical analyses for the ground water are shown in Table I. The water from Topeka and Ashland had high levels of calcium and bicarbonate, while the Hutchinson water contained much higher sodium. The total dissolved solids (TDS) were less than 500 mg L^{-1} for all three aquifers. Most of the constituent concentrations for all three waters fell within normal ranges. The nitrate concentration for the Ashland aquifer was very low while that for the Hutchinson aquifer was near the maximum contaminant level for drinking water. The low nitrogen level in the Ashland aquifer and low phosphate levels (detection limit of 0.05 mg L^{-1}) in all three ground waters may have limited microbial activity. The pH of the ground water was close to that published in other studies (Spruill, 1985).

The temperature of the ground water was 15 °C, even though water samples were taken in early June when the air temperature was 35 °C. The Eh values and low iron concentrations indicated that all three ground waters were oxidized. The

dissolved oxygen (DO) level was low for the Topeka and Ashland aquifers, but higher for Hutchinson material. The higher DO concentration in the Hutchinson aquifer was due to this being the shallowest of the three aquifers. The organic carbon content was low for all three aquifers with the lowest concentration in the Hutchinson aquifer. The organic carbon in the water was significantly lower than in the sediments (Table II).

The physical and chemical properties of the soil and aquifer materials are given in Table II. The texture of the aquifer sediment was gravelly sand for Topeka, primarily sand and gravel for Ashland, with a high silt and clay fraction for Hutchinson. The organic carbon content of the aquifer materials and Eudora soil from Ashland was very low. The total carbon values (data not shown) were higher for the three aquifer sediments and for the Eudora soil at Ashland, indicating the presence of carbonates. The cation exchange capacity (CEC) values for the soils and aquifer materials closely correlate with the organic carbon and clay content for each solid. The low CECs for the aquifer sediments indicate low adsorption potential of the herbicides onto these coarse-textured materials.

The high pH of the aquifer materials was not expected because the pH of the water for the three aquifers was near 1 to 2 pH units lower (Table II). Sinclair *et al.*, (1990) found the pH of the aquifer sediments from northeastern Kansas to range from 8.2 to 9.6, with these high pH values attributed to the presence of carbonates. Once these aquifer sediments are drained, carbonate minerals could precipitate onto the solid material. When the solids are rewetted for the pH measurement, the carbonates could dissolve and produce a higher pH. The slightly alkaline pH for the Eudora soil at Ashland was likely caused by carbonates.

The microbial populations of the aquifers were 100-fold less than for the soils and were in agreement with values reported in the literature, 10^5 to 10^8 g^{-1} in shallow and deep aquifer sediments (Sinclair *et al.*, 1990; Balkwill *et al.*, 1989; Wilson *et al.*, 1983). The majority of the microorganisms from the three aquifers in this study were bacteria and actinomycetes. The fungal populations were 10^2 g^{-1} sediment. Fungi are inhibited at higher pH such as that found in the sediments. The large numbers of actinomycetes (10^4 g^{-1}) was not expected because other studies of aquifers had shown no actinomycetes present in aquifers (Wilson *et al.*, 1983; Sinclair *et al.*, 1990). The actinomycetes populations were 100-fold less than the soils. The values given in Table II for microbial populations are for total aerobic organisms. No experiments were conducted to enumerate for anaerobic bacteria or investigate the physiological diversity of bacteria from these aquifers.

ATRAZINE DISSIPATION

The degradation patterns for atrazine for different treatments for the various aquifer materials are shown in Figures 1, 2 and 3 . An analysis of variance on the amount of atrazine remaining in solution (C_t/C_0) was performed to determine if degradation was affected by different treatments. The disappearance of atrazine in the stagnant

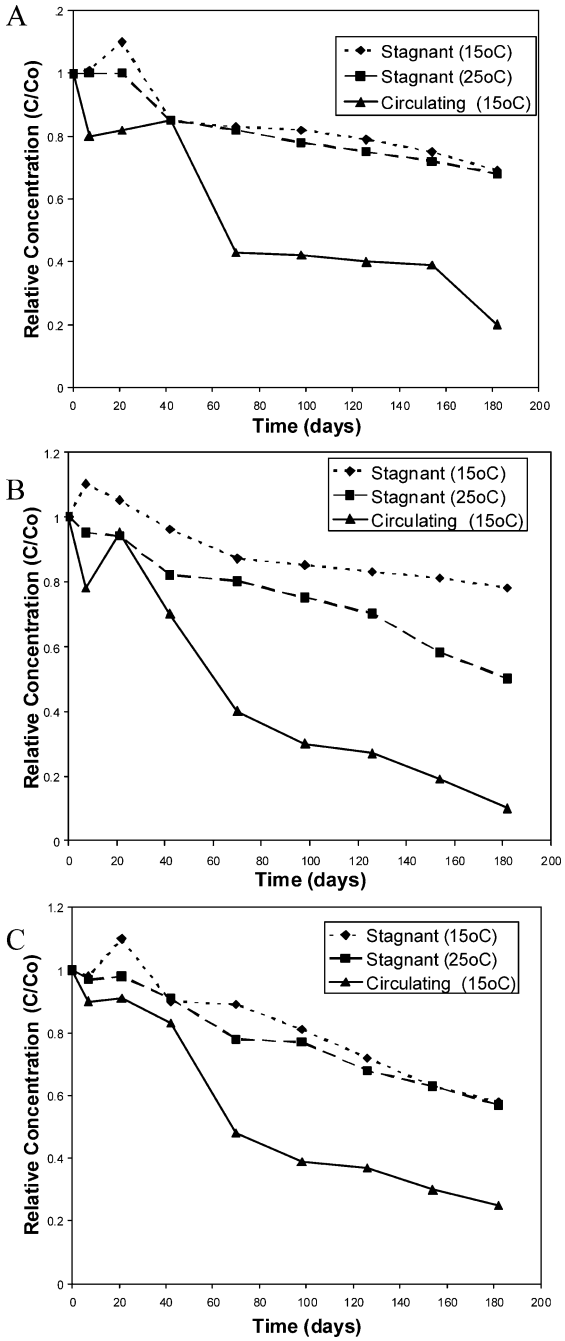

Figure 1. Atrazine degradation in the presence of additional nutrients for reactors under stagnant (15 °C), stagnant (25 °C), and recirculating (15 °C) conditions. Data points are the mean of three replications. The least significant difference between these treatments at the $p < 0.05$ level is 0.075. Figures are identified as: (A) Topeka, (B) Ashland, (C) Hutchinson.

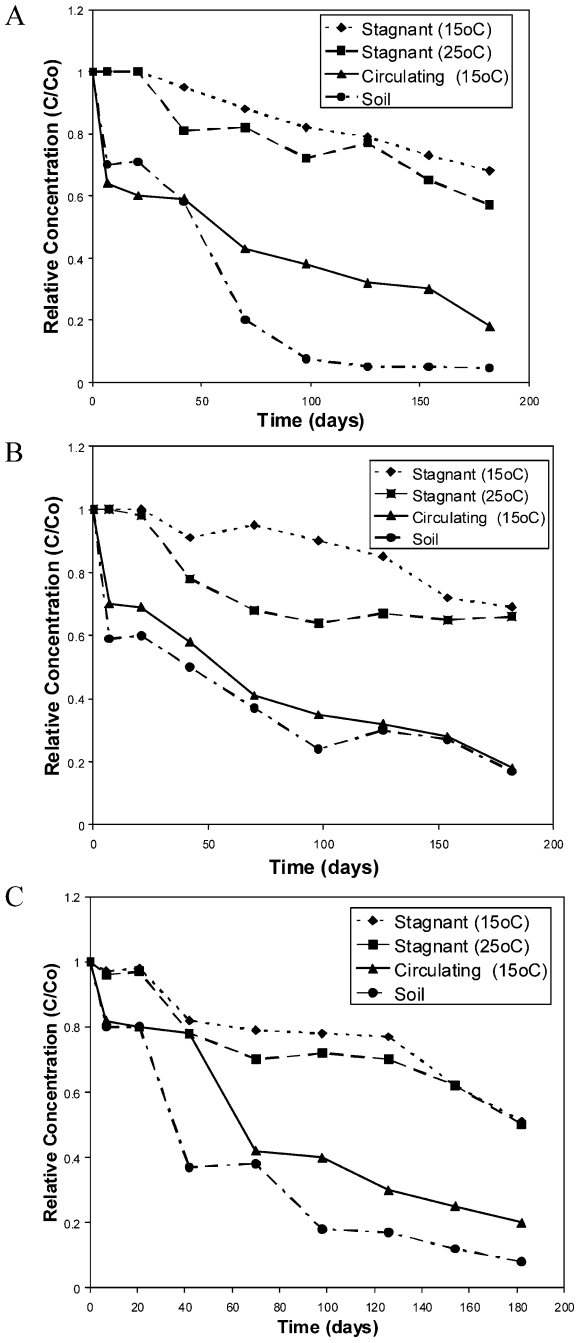

Figure 2. Atrazine degradation in unamended aquifer material and water under stagnant (15 °C), stagnant (25 °C), recirculating (15 °C) conditions, and in soil. Data points are the mean of three replications. The least significant difference between these treatments at the $p < 0.05$ level is 0.068. Figures are identified as: (A) Topeka, (B) Ashland, (C) Hutchinson.

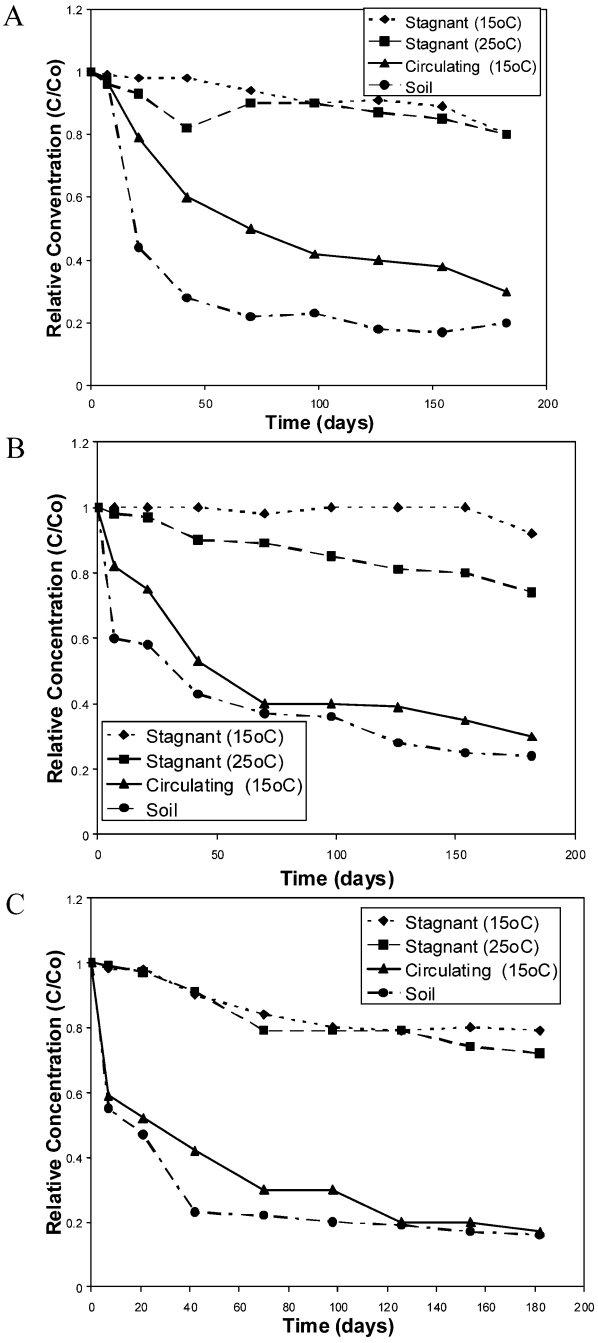

Figure 3. Atrazine degradation in sterilized aquifer material and water under stagnant (15 °C), stagnant (25 °C), recirculating (25 °C) conditions, and in soil. Data points are the mean of three replications. The least significant difference between these treatments at the $p < 0.05$ level is 0.070. Figures are identified as: (A) Topeka, (B) Ashland, (C) Hutchinson.

flasks was different for the sterilized treatments than for the unsterilized treatments. For the first 80 to 100 days, degradation in the sterilized systems was not significantly different ($P < 0.05$) from that in the nonsterilized flasks for all three aquifers with exception of the Ashland aquifer at 15 °C. After 100 days, degradation in the sterilized, stagnant flasks slowed considerably with 80% or more of the atrazine remaining at the end of the experiment. Chemical degradation was probably the primary mechanisms for removal of atrazine during the first 100 days in the stagnant flasks. During the latter half of the experiment, microbial degradation apparently became important. However, we had no means to separate chemical from biological degradation.

Even though sterilization inhibited atrazine biodegradation, chemical degradation was important, especially in the recirculating reactors. Wehtje *et al.* (1983) found that hydroxyatrizine was the only degradation product formed after 70 days under aquifer conditions. Other researchers report that atrazine hydrolysis is promoted by adsorption onto the solid material (Brouwer *et al.*, 1990; Armstrong *et al.*, 1967). In our study, between 70 and 100 days, degradation in the sterilized, stagnant flasks virtually ceased. Atrazine likely was adsorbed to "active" sites in the first 70 days but then was more slowly adsorbed to "restricted" sites and, therefore, hydrolysis slowed. The same plateau effect was observed for the sterilized, recirculating aquifer and soil columns, but the initial degradation was faster and reached a plateau sooner.

An initial period of limited microbial decomposition (lag phase) has been observed under a variety of conditions for xenobiotics in ground water (Williams *et al.*, 2003; Broholm and Arvin, 2000; Sorenson *et al.*, 1994; Swindoll *et al.*, 1988). None of the aquifers used in this study had detectable background levels of atrazine; therefore, it is likely the microorganisms had not been previously exposed to atrazine. A lag phase was observed in our reactors based on CO_2 production (data not shown); however, it is difficult to ascertain the exact cause because microbial biomass was not monitored. The additions of carbon and nitrogen failed to significantly enhance atrazine degradation under most conditions. In some cases, the nutrient additions tended to inhibit degradation, inducing a lag phase in the circulating columns for all aquifer materials. Swindoll *et al*. (1988) found that addition of alternate carbon sources inhibited the mineralization of xenobiotic substrates in aquifer materials.

Temperature had no significant affect on atrazine degradation in the Topeka and Hutchinson aquifer material and only a small effect for the Ashland aquifer sediment. McCormick and Hiltbold (1966) found that the decomposition of atrazine doubled with a 10 °C rise in temperature for surface soils. This did not hold true for atrazine degradation in this study. It appears that most of the increased atrazine degradation in the Ashland aquifer was due to enhanced chemical degradation at 25 °C

Of all factors tested, circulation of the water produced the most dramatic effects. Circulation of the water through the aquifer material resulted in the highest

disappearance of atrazine. Dissipation was just as rapid or more rapid in the sterilized treatment as in the biotic treatments for recirculating columns, indicating chemical degradation was the predominant pathway of breakdown of atrazine. The stagnant flasks were not continually mixed; therefore, the sediment sank to the bottom and the water remained on top, whereas the sediment in the recirculating columns was constantly exposed to the solution. Mass flow of atrazine within the aquifer material would have been greatly enhanced in the recirculating columns versus the stagnant flasks. The availability of atrazine to microsites within the solid matrix would enhance degradation, and the increased mixing in the recirculating columns would have insured higher nutrient and oxygen levels within the aquifer sediment.

With the solution being mixed throughout the sediment in the recirculating columns, enhanced adsorption would be favored. The pH of the sediments (Table II) was higher that the ground waters, and this condition promotes hydrolysis at the solid/solution interface versus hydrolysis in the ground water. The increased adsorption and subsequent hydrolysis in the recirculating columns would further explain the faster dissipation of atrazine. However, adsorption was not the principal cause for the disappearance of atrazine at the beginning of the experiment because the solution had been circulated through the columns 24 hours before the time-zero concentrations were determined. The aquifer material from Hutchison had the finest texture and also showed the largest difference between degradation rates in the stagnant systems and the recirculating columns. In contrast, the coarse-textured material for Topeka showed smaller differences in degradation rates between the two systems, due to the fact that adsorption to this sediment was less than the Hutchinson sediment (Splichal, 1991).

Atrazine degradation was higher in the non-sterilized soil than in the unamended aquifer material for Topeka and Hutchinson but was essentially the same for Ashland. One would expect dissipation to be faster in the soils because of increased microbial numbers and diversity, higher nutrient levels, and increased adsorption. However, the soil columns were placed under the same conditions as the aquifer material; saturated and reduced conditions, low oxygen levels, no outside nutrient sources, at 15 °C. Atrazine degradation is severely reduced in soils under saturated conditions (Chilton *et al.*, 2005; Pang *et al.*, 2005); therefore, it would be surprising to see greater degradation than in field surface soils. The soils also received 400 $\mu g\,L^{-1}$ atrazine, whereas the aquifers received only 20 $\mu g\,L^{-1}$ atrazine.

In the soil, atrazine was degraded faster than in the aquifer material with the exception of the Eudora soil from Ashland. This soil sample was taken at the depth of 60 cm and had the lowest organic carbon content and microbial numbers of the three soils. This may explain why degradation was not significantly higher in the Eudora soil compared with the Ashland aquifer material. The organic carbon concentration in the solution from the soil columns varied from 1054 mg L^{-1} to 2000 mg L^{-1} for the three soils. This was 100 to 1000 fold greater than the organic carbon concentrations in the leachate from the reactors containing aquifer materials. The

higher carbon content in the soils would provide another explanation for increased microbial and chemical degradation.

For the sterilized treatments, the Eudora soil from Topeka and Ashland has a higher degradation rate than the aquifer material. Degradation was not significantly different for the Hutchinson site between the soil and aquifer material. This difference in degradation can be attributed to greater adsorption onto the finer-textured soils. The greatest difference in degradation rates was in the Topeka columns because the soil and aquifer material had the largest difference in texture, whereas the soil and aquifer material from Hutchinson were similar in texture. Another factor contributing to higher chemical degradation in the soils was pH. The pH of the solution from the three soils was 5.5, 6.7, and 5.9 for Topeka, Ashland, and Hutchinson, respectively. The lower pH for the Topeka and Hutchinson soils would promote atrazine hydrolysis.

Differences in biodegradation between the sterilized and non-sterilized systems beginning at approximately 100 days was further confirmed by the appearance of deethylatrazine (DEA) and deiosopropylatrazine (DIA) only in the non-sterilized treatment on day 98 (Table III). Sterilized treatments did not contain detectable DEA or DIA. Because DIA and DEA are products of biodegration and not chemical degradation, the dissipation of of atrazine in the sterilized treatments must be predominantly chemical degradation. Others have reported similar results for soils (Winkelmann and Klaine, 1991). The concentration of metabolites was higher in the stagnant flasks than in the recirculating columns for all the aquifers (Table III). There were no significant differences in atrazine degradation between the different treatments for the recirculating columns, indicating the predominance of chemical dissipation. The small concentrations of DEA and DIA in the recirculating columns support this conclusion.

KINETICS OF ATRAZINE DEGRADATION

Zero-order and first-order degradation kinetics were examined using linear regression analysis. Three criteria were used to choose the best order: coefficient of determination (r^2), F-statistic, and lack-of-fit test. In many cases, zero-order kinetics resulted in an r^2 and F-statistic comparable to first-order kinetics, but the predicted values for zero-order kinetics showed lack-of-fit; therefore, first-order kinetics were chosen to describe atrazine degradation (Table IV). The rate constant, k, respresents the slope of the regression line. First-order half-lives were calculated by the equation: $t_{1/2} = 0.693/k$ where $t_{1/2}$ = half-life and k = rate constant. The time to reach the MCL of 3 μg/L from an intial concentration of 20 μg/L, can be calculated by $t_{MCL} = 1.897/k$ where $1.897 = -\ln(C_t/C_0) = -\ln(3/20)$.

Using first-order kinetics for all treatments, comparisons of rate constants between treatments and aquifer materials were feasible. There were no statistical differences in degradation rates between aquifer materials for a given treatment (Table IV). There was no effect of nutrient additions. Sterilization inhibited atrazine

TABLE III

Atrazine metabolite concentration ($\mu g\,L^{-1}$) as a function of time and treatment

			Deethylatrazine				Deiospropylatrazine		
Aquifer	Treatment	Nutrients	98 days	126 days	154 days	182 days	98 days	126 days	154 days
Topeka	Stagnant (15 °C)	Amended	0.663	0.374	0.561	0.901	0.200	0.410	0.444
Topeka		Unamended	0.792	0.673	0.846	0.780	0.200	0.562	0.663
Topeka	Stagnant (25 °C)	Amended	0.730	0.828	0.825	1.046	0.632	0.678	0.532
Topeka		Unamended	0.370	0.607	0.926	1.069	0.206	0.578	0.678
Topeka	Circulating (15 °C)	Amended	0.266	0.391	0.277	0.297	0.215	0.233	0.201
Topeka		Unamended	0.120	0.187	0.362	0.308	0.200	0.200	0.392
Ashland	Stagnant (15 °C)	Amended	0.695	0.364	0.665	1.023	0,502	0.281	0.536
Ashland		Unamended	0.838	0.756	0.772	1.102	0.716	0.695	0.598
Ashland	Stagnant (25 °C)	Amended	0.666	1.027	1.386	1.916	0.321	0.998	1.011
Ashland		Unamended	0.777	1.341	1.029	1.753	0.495	0.940	0.553
Ashland	Circulating (15 °C)	Amended	0.288	0.376	0.441	0.555	0.279	0.206	0.287
Ashland		Unamended	0.161	0.263	0.345	0.187	0.200	0.220	0.284
Hutchinson	Stagnant (15 °C)	Amended	0.398	0.670	0.665	0.855	0.583	0.708	0.540
Hutchinson		Unamended	0.801	0.793	0.939	1.243	0.269	0.579	0.615
Hutchinson	Stagnant (25 °C)	Amended	0.532	0.660	1.224	1.227	0.238	0.416	0.846
Hutchinson		Unamended	0.863	1.066	1.032	0.992	0.980	0.660	0.961
Hutchinson	Circulating (15 °C)	Amended	0.256	0.359	0.384	0.495	0.208	0.251	0.302
Hutchinson		Unamended	0.443	0.387	0.521	0.616	0.200	0.279	0.228

All values are the mean of three replications.

No values are shown for day 182 for deisopropylatrazine concentrations were below the detection limit.

TABLE IV

First-order degradation rate constants (k) and predicted half-lives ($t_{1/2}$) of atrazine

Material	Treatment	Nutrients	k (95% Confidence Interval) day^{-1}	r^2	$t_{1/2}$	t_{MCL}
Topeka Aquifer	Stagnant (15 °C)	Amended	−0.0027 (−0.0032, −0.0021)	0.80**	259	709
Topeka Aquifer		Unamended	−0.0028 (−0.0034, −0.0021)	0.74**	249	683
Topeka Aquifer		Sterilized	−0.0007 (−0.0013, −0.0002)	0.18*	953	2610
Topeka Aquifer	Stagnant (25 °C)	Unamended	−0.0032 (−0.0037, −0,0027)	0.86**	219	598
Topeka Aquifer		Amended	−0.0033 (−0.0037, −0.0028)	0.89**	212	581
Topeka Aquifer		Sterilized	−0.0015 (−0.0019, −0.0012)	0.75**	458	1254
Topeka Aquifer	Circulating (15 °C)	Amended	−0.0076 (−0.0095, −0.0057)	0.72**	92	251
Topeka Aquifer		Unamended	−0.0089 (−0.0108, −0.0069)	0.77**	78	214
Topeka Aquifer		Sterilized	−0.0071 (−0.0071, −0.0065)	0.96**	98	268
Topeka Soil	Circulating (15 °C)	Unsterilized	−0.0246 (−0.0284, −0.0208)	0.87**	28	77
Topeka Soil		Sterilized	−0.0079 (−0.0098, −0.0060)	0.74**	88	241
Ashland Aquifer	Stagnant (15 °C)	Amended	−0.0018 (−0.0027, −0.0009)	0.40**	384	1052
Ashland Aquifer		Unamended	−0.0020 (−0.0025, −0.0015)	0.72**	348	952
Ashland Aquifer		Sterilized	−0.0002 (−0.0005, +0.0002)	NS	4750	13083
Ashland Aquifer	Stagnant (25 °C)	Amended	−0.0033 (−0.0036, −0.0030)	0.95**	210	574
Ashland Aquifer		Unamended	−0.0027 (−0.0033, −0.0020)	0.71**	261	714
Ashland Aquifer		Sterilized	−0.0014 (−0.0018, −0.0010)	0.70**	492	1348
Ashland Aquifer	Circulating (15 °C)	Amended	−0.0105 (−0.0119, −0.0092)	0.91**	66	180
Ashland Aquifer		Unamended	−0.0083 (−0.0103,− 0.0063)	0.74**	84	229

(*Continued on next page*)

TABLE IV
(Continued)

Material	Treatment	Nutrients	k (95% Confidence Interval) day^{-1}	r^2	$t_{1/2}$	t_{MCL}
Ashland Aquifer		Sterilized	−0.0066 (−0.0078, −0.0053)	0.82**	106	290
Ashland Soil	Circulating (15 °C)	Nonsterilized	−0.0089 (−0.0104, −0.0074)	0.85**	78	212
Ashland Soil		Sterilized	−0.0075 (−0.0088, −0.0062)	0.85**	92	252
Hutchinson Aquifer	Stagnant (15 °C)	Amended	−0.0030 (−0.0036, −0.0025)	0.84**	227	622
Hutchinson Aquifer		Unamended	−0.0031 (−0.0036, −0.0026)	0.87**	221	606
Hutchinson Aquifer		Sterilized	−0.0010 (−0.0014, −0.0005)	0.40**	710	1943
Hutchinson Aquifer	Stagnant (25 °C)	Unamended	−0.0034 (−0.0039, −0.0028)	0.86**	206	562
Hutchinson Aquifer		Amended	−0.0033 (−0.0041, −0.0026)	0.78**	208	569
Hutchinson Aquifer		Sterilized	−0.0016 (−0.0022, −0.0010)	0.56**	431	1181
Hutchinson Aquifer	Circulating (15 °C)	Amended	−0.0090 (−0.0099, −0.0081)	0.94**	77	210
Hutchinson Aquifer		Unamended	−0.0099 (−0.0109, −0.0089)	0.94**	70	191
Hutchinson Aquifer		Sterilized	−0.0087 (−0.0109, −0.0065)	0.71**	79	218
Hutchinson Soil	Circulating (15 °C)	Nonsterilized	−0.0203 (−0.0235, −0.0170)	0.86**	34	94
Hutchinson Soil		Sterilized	−0.0080 (−0.0093, −0.0067)	0.85**	87	237

*, **Significant at the 0.05 and 0.01 probability levels, respectively. All values are the mean of three replications.
t_{MCL} – time to reach the maximum contaminant level of 3 μg L^{-1} assuming an initial concentration of 20 μg L^{-1} and first order degradation.
NS – not significant.

degradation in the stagnant flasks but not recirculating columns for all three aquifers. The regression used to yield kinetic parameters was not significant for the sterilized Ashland aquifer at 15 °C because no degradation was observed over time. Chemical degradation was significant for the first half of the experiment in the stagnant flasks, but then decreased. This resulted in the predicted half-live for the sterilized, stagnant material to be 2–4 times longer than for the biotic systems.

Degradation rates were not significantly increased in the biotic, stagnant flasks at 25 °C versus 15 °C (Table IV). On the other hand, degradation rates for the sterilized, stagnant flasks were nearly doubled with a 10-degree increase in temperature. Apparently, the increase in temperature was more effective in catalyzing chemical degradation than microbial degradation.

The most interesting values in Table IV are the rate constants and estimated half-lives for the recirculating columns. The half-lives for the recirculating columns were 2 to 10 times shorter than for the stagnant flasks, depending on the treatment and aquifer. The sterilized columns had the highest increase in degradation rates compared to the stagnant flasks. There were no differences in degradation rates between the sterilized and biotic systems for the recirculating columns. Atrazine degradation was the highest in the nonsterilized soils, with the exception of the Eudora soil from Ashland. The predicted half-life for atrazine in the non-sterilized soil are in agreement with literature values (Issa and Wood, 2005; Pang *et al.*, 2005; Blume *et al.*, 2004; Winkelmann and Klaine, 1991). The time for ground water (unamended) to reach the MCL for atrazine with an initial concentration on 20 mg L^{-1} was, in some cases, in excess of two years (Table IV).

ALACHLOR DISSIPATION

Alachlor degradation patterns are shown in Figures 4, 5 and 6. As in the case of atrazine, alachlor degradation for different treatments was similar among the aquifer materials. Many of the same trends observed for atrazine degradation also were noted for alachlor degradation. The addition of nutrients did not significantly increase or inhibit degradation for any systems except for the Topeka columns.

Unlike atrazine degradation, no lag phase was observed for alachlor. This was in contrast to a published study (Cavalier *et al.*, 1991) in which a lag phase of 6 to 8 months for alachlor in three different ground waters (without sediment) incubated at 15 °C or 22 °C was observed. Studies on alachlor degradation in the saturated zone have found evidence of significant abiotic degradation (Miller and Chin, 2005; Xu *et al.*, 2001). Results of this experiment show that chemical degradation can play a major factor in reducing alachlor concentrations in aquifers. Even in the sterilized aquifers, 50% or more of the alachlor was degraded for nearly all aquifer materials under static or circulating conditions. In fact, there were no statistical differences in dissipation for the sterilized Ashland and Hutchinson recirculating columns compared to the non-sterilized columns. Alachlor dissipation in the sterilized, stagnant flasks was significantly less than in the non-sterilized, stagnant flasks, especially at

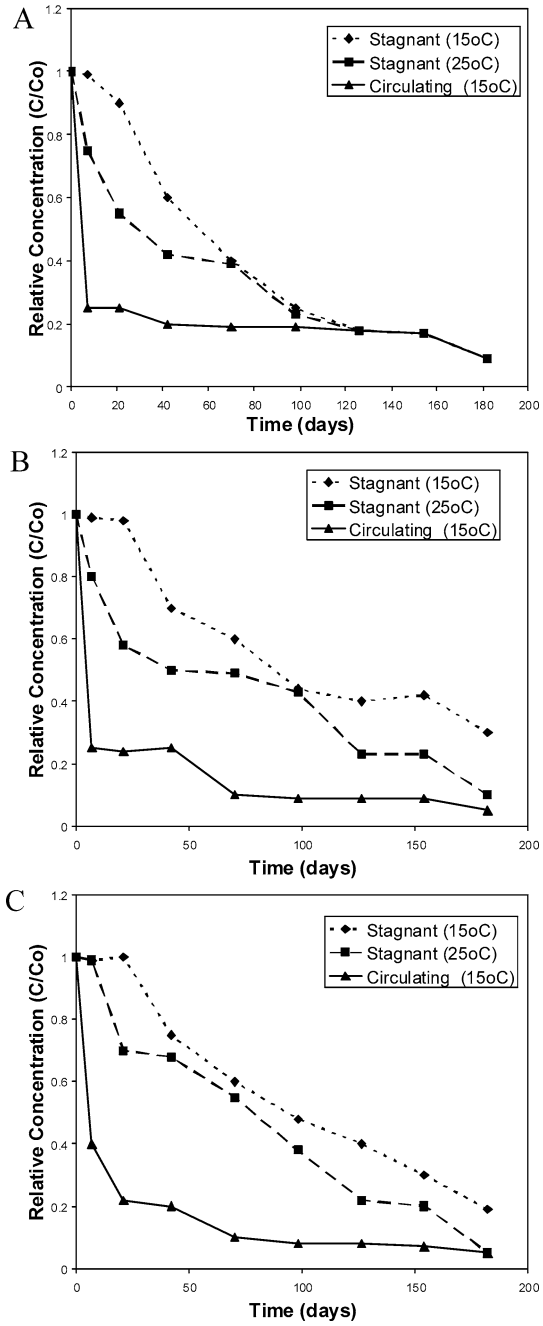

Figure 4. Alachlor degradation in the presence of additional nutrients for reactors under stagnant (15 °C), stagnant (25 °C), and recirculating (25 °C) conditions. Data points are the mean of three replications. The least significant difference between these treatments at the $p < 0.05$ level is 0.109. Figures are identified as: (A) Topeka, (B) Ashland, (C) Hutchinson.

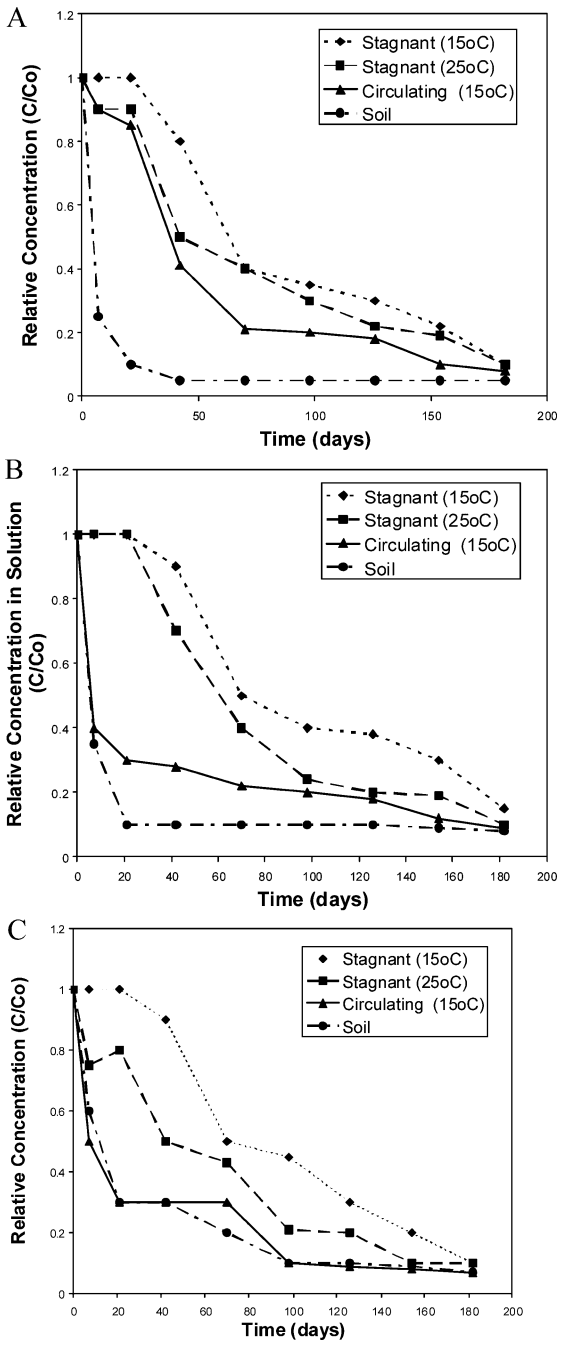

Figure 5. Alachlor degradation in unamended aquifer material and water under stagnant (15 °C), stagnant (25 °C), recirculating (25 °C) conditions, and in soil. Data points are the mean of three replications. The least significant difference between these treatments at the $p < 0.05$ level is 0.081. Figures are identified as: (A) Topeka, (B) Ashland, (C) Hutchinson.

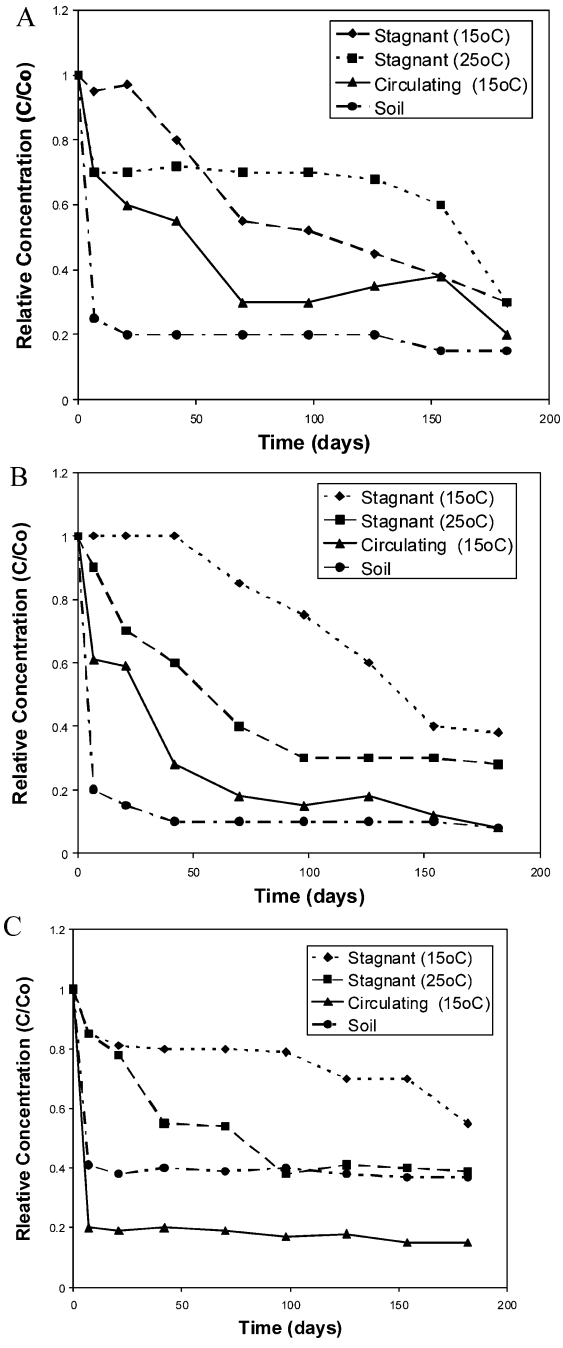

Figure 6. Alachlor degradation in sterilized aquifer material and water under stagnant (15 °C), stagnant (25 °C), recirculating (25 °C) conditions, and in soil. Data points are the mean of three replications. The least significant difference between these treatments at the $p < 0.05$ level is 0.032. Figures are identified as: (A) Topeka, (B) Ashland, (C) Hutchinson.

15 °C. Microbial degradation was the primary mechanism for alachlor removal in the non-sterile, stagnant flasks at 15 °C.

Decreased alachlor dissipation in the sterilized aquifers after 98 days was similar to that observed for atrazine. Chemical degradation in the stagnant flasks was most likely a diffusion controlled process. The stagnant flasks were not continually mixed, and eventually chemical degradation of alachlor slowed because the herbicide was not available to the entire solid-matrix within the flask. Increased temperature had a larger effect on alachlor degradation than on atrazine degradation for the unsterilized and sterilized treatment. Increased temperature enhanced alachlor in the following order: sterilized >unamended ≈ nutrients added treatment.

Alachlor degradation, as with atrazine degradation, increased in the recirculating columns. Degradation was so rapid that the detection limit (1 μg L^{-1}) was reached after 98 days for the Hutchinson aquifer and for the nutrients-amended and sterilized treatment for the Ashland aquifer. The zero-slope of the lines after 98 days may be an artifact of the analytical method; however, biodegradation has been shown to be threshold-controlled, and contaminant concentrations below 1 to 2 μg L^{-1} may not contain sufficient carbon to support bacterial growth (Cavalier *et al.*, 1991). Increased mass transfer of nutrients (carbon) and oxygen was a probable cause of increased alachlor degradation in the recirculating columns compared to the stagnant flasks.

Even though alachlor was added to the soils at a relatively high concentration of 400 μg L^{-1}, degradation was higher in the Topeka and Ashland non-sterilized soils than in the corresponding unamended aquifers. This result was not unexpected because both carbon levels and microbial activity were higher in the soil. The alachlor degradation was generally slower in the sterilized soils and reached a plateau after 21 days.

KINETICS OF ALACHLOR DEGRADATION

The criteria used to choose the reaction order was the same as for atrazine. Once again, both zero-order and first-order kinetics resulted in strong r^2 and F-statistic values for degradation in the stagnant flasks; however, the zero-order kinetics showed lack-of-fit in several cases. The half-lives predicted from zero-order kinetics also were longer than the data would suggest, while the first-order half-lives were slightly less. For the recirculating columns, a different problem was encountered. No simple kinetics (0, 1st, 2nd) described the data sufficiently. Second-order kinetics ($1/C$–$1/C_o$ versus time) reduced the half-lives to more closely match the data, but the r^2 and F-statistic were less acceptable than for first-order kinetics.

First-order kinetics were chosen to predict degradation rates and half-lives for alachlor (Table V). In the case of alachlor, $t_{MCL} = 0.916/k$. The r^2 values for alachlor were generally lower for alachlor than for atrazine but were similar to those reported in the literature for alachlor degradation in aquifers (Cavalier *et al.*, 1991; Pothuluri *et al.*, 1990;). The lower r^2 for the sterilized Eudora soil (Topeka)

TABLE V
First-order degradation rate constants (k) and predicted half-lives ($t_{1/2}$) of alachlor

Material	Treatment	Nutrients	k (95% Confidence Interval) day^{-1}	r^2	$t_{1/2}$	t_{MCL}
Topeka Aquifer	Stagnant (15 °C)	Amended	−0.0174 (−0.0195, −0.0152)	0.91**	40	53
Topeka Aquifer		Unamended	−0.0180 (−0.0216, −0.0144)	0.80**	39	51
Topeka Aquifer		Sterilized	−0.0060 (−0.0075, −0.0044)	0.71**	116	153
Topeka Aquifer	Stagnant (25 °C)	Unamended	−0.0179 (−0.0209, −0.0149)	0.85**	39	51
Topeka Aquifer		Amended	−0.0179 (−0.0203, −0.0154)	0.90**	39	51
Topeka Aquifer		Sterilized	−0.0073 (−0.0104, −0.0041)	0.45**	96	126
Topeka Aquifer	Circulating (15 °C)	Amended	−0.0130 (−0.0178, −0.0082)	0.54**	53	70
Topeka Aquifer		Unamended	−0.0085 (−0.0114, −0.0056)	0.58**	82	108
Topeka Aquifer		Sterilized	−0.0029 (−0.0047, −0.0011)	0.27**	239	315
Topeka Soil	Circulating (15 °C)	Unsterilized	−0.0221 (−0.0289, −0.0153)	0.63**	31	41
Topeka Soil		Sterilized	−0.0030 (−0.0078, +0.0019)	NS	233	308
Ashland Aquifer	Stagnant (15 °C)	Amended	−0.0090 (−0.0120, −0.0060)	0.59**	77	102
Ashland Aquifer		Unamended	−0.0128 (−0.0149, −0.0044)	0.85**	54	72
Ashland Aquifer		Sterilized	−0.0061 (−0.0078, −0.0044)	0.67**	113	150
Ashland Aquifer	Stagnant (25 °C)	Amended	−0.0113 (−0.0130, −0.0096)	0.70**	61	81
Ashland Aquifer		Unamended	−0.0144 (−0.0178, −0.0110)	0.83**	48	64
Ashland Aquifer		Sterilized	−0.0094 (−0.0138, −0.0051)	0.43**	73	97
Ashland Aquifer	Circulating (15 °C)	Amended	−0.0181 (−0.0227, −0.0135)	0.72**	38	51
Ashland Aquifer		Unamended	−0.0143 (−0.0188, −0..0097)	0.61**	49	64
Ashland Aquifer		Sterilized	−0.0140 (−0.0166, −0.0097)	0.82**	50	66

(Continued on next page)

TABLE V
(Continued)

Material	Treatment	Nutrients	k (95% Confidence Interval) day^{-1}	r^2	$t_{1/2}$	t_{MCL}
Ashland Soil	Circulating (15 °C)	Nonsterilized	−0.0169 (−0.0220, −0.0188)	0.64**	41	54
Ashland Soil		Sterilized	−0.0147 (−0.0199, −0.0095)	0.56**	47	62
Hutchinson Aquifer	Stagnant (15 °C)	Amended	−0.0135 (−0.0172, −0.0098)	0.69**	51	68
Hutchinson Aquifer		Unamended	−0.0107 (−0.0124, −0.0090)	0.87**	66	86
Hutchinson Aquifer		Sterilized	−0.0029 (−0.0044, −0.0015)	0.42**	235	311
Hutchinson Aquifer	Stagnant (25 °C)	Unamended	−0.0170 (−0.0200, −0.0139)	0.83**	41	54
Hutchinson Aquifer		Amended	−0.0158 (−0.0188, −0.0128)	0.82**	44	58
Hutchinson Aquifer		Sterilized	−0.0058 (−0.0075, −0.0040)	0.62**	120	159
Hutchinson Aquifer	Circulating (15 °C)	Amended	−0.0201 (−0.0241, −0.0161)	0.80**	34	45
Hutchinson Aquifer		Unamended	−0.0200 (−0.0247, 0.0154)	0.75**	35	46
Hutchinson Aquifer		Sterilized	−0.0155 (−0.0207, −0.0102)	0.58**	45	59
Hutchinson Soil	Circulating (15 °C)	Nonsterilized	−0.0206 (−0.0262, −0.0150)	0.68**	34	45
Hutchinson Soil		Sterilized	−0.0044 (−0.0084, −0.0005)	0.17*	157	208

*, ** Significant at the 0.05 and 0.01 probability levels, respectively. All values are the mean of three replications.
t_{MCL} – time to reach the maximum contaminant level of 4 μg L^{-1} assuming an initial concentration of 10 μg L^{-1} and first order degradation.
NS – not significant.

and Ost soil (Hutchinson) were due to the degradation curves that are not explained by first order kinetics.

No statistical differences were seen in the rate constants between the nutrient amended treatments and the unamended treatments for any of the three aquifers (Table V). Degradation was slower in the sterilized, stagnant flasks than in the biotic, stagnant flasks, but a significance difference was not observed between sterile and non-sterile treatments in the recirculating columns with the exception of the Topeka aquifer. The half-lives were 2 to 5 times longer for the sterilized, stagnant flasks as compared to the non-sterilized, stagnant flasks (Table V). Increasing temperature had a slight effect on biodegradation rates, but also significantly increased degradation in the sterilized, stagnant flasks.

Degradation for the recirculating columns was faster than for the stagnant flasks (Table V); however, the differences were much less than in the case of atrazine. The differences were less because alachlor degradation was faster than atrazine degradation in the stagnant flasks and first-order kinetics overestimated half-lives for alachlor in the recirculating columns. Visual inspection of the data for the recirculating columns would suggest that the alachlor half-life for Ashland and Hutchinson material is 7 to 21 days. Alachlor was degraded as rapidly in the aquifer columns as in the soil for Ashland and Hutchinson; however, degradation rates were slower in the stagnant flasks as compared to the soil. Degradation in the aquifer materials was not nutrient limited or slowed because of the lack of alachlor degraders, but was dependent on the mass transfer of alachlor within the solid matrix.

The predicted half-lives for the soils are in the upper range of estimated values from field experiments on surface soils (Aga and Thurman, 2001; Weed *et al.*, 1998.). The alachlor may have been more persistent in these soils because they were saturated. Alachlor half-lives in the aquifers were far shorter than those reported in similar research. Pothuluri *et al.* (1990), Perry (1990) and Cavalier *et al.* (1991) predicted half-lives of 335–1500 days for alachlor in aquifers. In the laboratory studies conducted by Pothuluri *et al.* (1990), alachlor was added at an initial concentration of 1.56 mg kg^{-1} sediment (100 to 1000 times higher than reported concentrations of alachlor found in ground water). It is possible that the microorganisms in the ground water had difficulty adapting to such a high concentration of alachlor, thus slowing degradation.

Of note is that the alachlor half-life in the aquifer material was much shorter than the atrazine half-life in all cases. Degradation of alachlor was 4 to 5 times faster than atrazine in the stagnant flasks and twice as fast in the recirculating columns. Field and laboratory studies on atrazine and alachlor have shown that atrazine is more persistent than alachlor in soil, so it is not unexpected to find that atrazine is more recalcitrant than alachlor under aquifer conditions. The time to reach the maximum contaminant levels for drinking water was lower for alachlor as compared to atrazine. The length of time was 5 to 10 times shorter for alachlor because the initial concentration was less, the maximum contaminant level was higher, and the rate constant, k, was larger.

Conclusions

The degradation patterns for atrazine and alachlor for different treatments were similar between aquifers. The additions of carbon and nitrogen did not impact herbicide degradation, indicating that these nutrients were not limiting in these specific aquifers. Degradation rates were low for atrazine and no measurable increases could be detected as temperature increased for the biological system; however, increased temperature did enhance chemical degradation in sterilized treatments. Higher temperature was effective in accelerating alachlor degradation. The importance of chemical and microbial degradation was shown for both herbicides. Chemical degradation appeared to be an important mechanism for atrazine and alachlor degradation in the recirculating columns and the predominant mechanism in all sterilized treatments. Microbial degradation was necessary to fully degrade the herbicides because chemical degradation in the stagnant system slowed substantially after 100 days. The aquifer materials had a finite capacity for chemical degradation of atrazine and alachlor when the herbicide was not dispersed well within the system.

One major conclusion from this experiment was the importance of mass transfer of the herbicide, nutrients, and oxygen. The degradation rates of atrazine and alachlor were 2 to 5 times faster in the recirculating columns than in the stagnant flasks. The supply of nutrients and oxygen to the microorganisms was vital for microbial degradation. The more reduced conditions (i.e., lack of mass transfer of $O_{2(g)}$) in the stagnant flasks versus the recirculating columns also may have inhibited microbial degradation. In addition, the herbicides were exposed to a much lower soil or sediment surface area in the stagnant flasks when compared to the recirculating columns.

Atrazine was more persistent than alachlor under all conditions, and both herbicides degraded slower in the aquifers than in the soil. However, under well-mixed conditions, atrazine degradation should occur at a moderate rate. Alachlor is much less recalcitrant and should not pose a long-term threat to ground water supplies at concentrations resulting from non-point source pollution. However, if high concentrations ($20\,\mu g\,L^{-1}$) of atrazine are present in an aquifer, the long time period estimated for atrazine degradation reach the MCL in drinking water is serious. Natural attentuation mechanisms would not adequately clean-up the water for 1 to 3 years.

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