JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Atrazine and Terbutryn Degradation in Deposits from Groundwater Environment within the Boreal Region in Lahti, Finland

K. Marja Talja,*,† Sanna Kaukonen,† Johanna Kilpi-Koski,† Ismo Malin,‡ Timo Kairesalo,† Martin Romantschuk,† Jari Tuominen,‡ and Merja H. Kontro†

Department of Ecological and Environmental Sciences, University of Helsinki, Niemenkatu 73, 15140 Lahti, Finland; and Ramboll Analytics Ltd., Niemenkatu 73, 15140 Lahti, Finland

The degradation of pesticides atrazine and terbutryn was investigated under aerobic and anaerobic conditions in the northern boreal region subsurface deposits and sterilized controls from the depths of 6.3−21.0 m below the surface and 1.2−16.9 m below the groundwater table. During 1.3−1.7 years of laboratory incubation, atrazine degradation under aerobic conditions varied from rapid (half-live 38 days) to no degradation. Anaerobically, atrazine half-lives were 430−829 days. Organic matter, nitrogen, and lead in deposits correlated positively with the atrazine concentration in groundwater. Aerobic and anaerobic terbutryn half-lives were 193−644 and 266−400 days, respectively. Microbial aerobic atrazine and terbutryn degradation was confirmed in the deep deposits near the water table. Under aerobic conditions, the high amounts of Cr, Mn, Ni, and Zn in deposits decreased the chemical degradation of terbutryn.

KEYWORDS: Atrazine; terbutryn; degradation; groundwater; oxygen.

INTRODUCTION

The widespread use of pesticides all over the world has resulted in leaching into the groundwater in many countries. In the European Union, the maximum allowable concentration of a pesticide or degradation product in water is 0.10 μg/L, and the limit for the sum of all pesticides is 0.50 μg/L (*1*). Triazine pesticides form a wide group of compounds used for the weed control, with atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine] with a chlorine branch being among the most common herbicides used in many parts of the world. Atrazine is moderately hydrophilic with a solubility in water of 33 mg/L at 22 °C. Terbutryn [*N*-(1,1-dimethylethyl)-*N*′-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine] with a methylthio branch is another commonly used herbicide with a water solubility of 22 mg/L at 22 °C (*2*). In Finland, the sale of atrazine ended in 1992 and that of terbutryn in 2004.

Atrazine has been found in groundwater in areas where herbicide has been commonly used, like in regions with shallow groundwater tables beneath agricultural areas, gardens, dealers, railways, and roads (*3*−*5*). The concentration of atrazine in groundwater varies from site to site, depending on the chemical behavior in soil, soil type, particle size, organic matter content, weather conditions like temperature and rain, and field management practices (*5, 6*). Atrazine is degraded by both abiotic and biotic processes in soil and water (*7*). The degradation products of atrazine may comprise a significant amount in groundwater (*8*). Photodegradation, hydrolysis, and chemical reactions with metal oxides including manganese oxides are abiotic processes that degrade atrazine (*9, 10*). Indigenous microbial communities have been able to mineralize atrazine. The microbiological degradation by either a single species or a microbial consortia is regarded as the most important mechanism for atrazine mineralization, the best studied atrazine-degrading bacterium being *Pseudomonas* sp. strain ADP (*11, 12*).

The complete biodegradation of atrazine includes dehalogenation, N-dealkylation, deamination, ring cleavage, and finally mineralization to carbon dioxide and ammonia. Through dealkylation reactions, desethylatrazine (DEA), deisopropylatrazine (DIA), and desethyldeisopropylatrazine (DEDIA) are formed (*6, 11*). The factors related to the absence of biotic atrazine degradation in groundwater environment include shortage of oxygen and nutrients, like carbon and nitrogen, low atrazine concentration, and lack of selective pressure for degrading microbes. In addition, low microbial biomass and activity, little variation in conditions, low temperature, and adsorption in soil have decreased the biotic atrazine degradation (*13*−*16*). Much less information exists on the fate and degradation of terbutryn in soil and water. Muir and Yarechewski (*17*) have reported that microbial degradation is the essential mechanism of terbutryn attenuation, hydroxylterbutryn, terbutryn sulfoxide, and *N*-desethylterbutryn being the major degradation products.

* Corresponding author. Tel+358-9-19120337. Fax . +358-9-19120289. E-mail marja.talja@helsinki.fi.
† University of Helsinki.
‡ Ramboll Analytics Ltd.

10.1021/jf802528a CCC: $40.75
Published on Web 11/20/2008

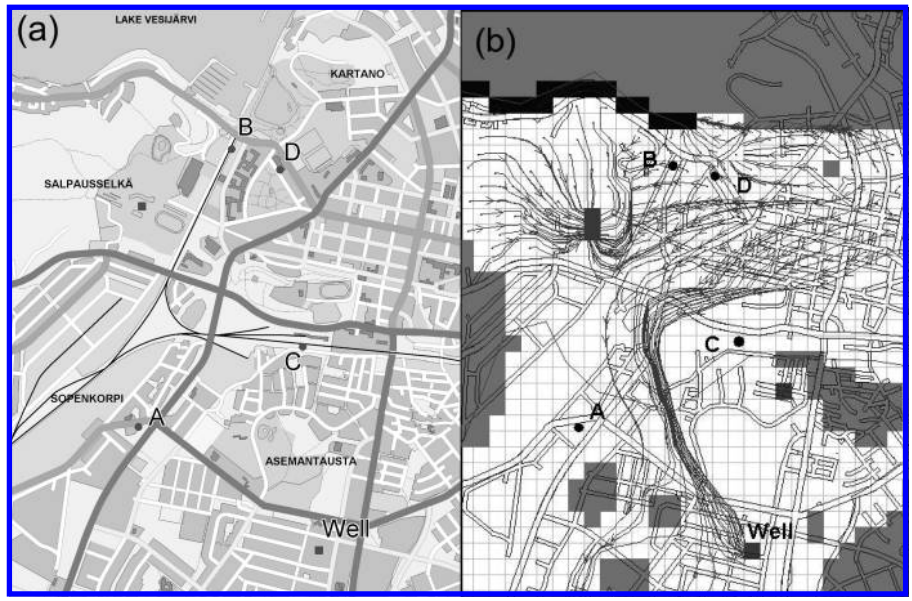

Figure 1. Sampling points in Lahti (**a**) and the groundwater flow in the study area (**b**).

The half-lives for degradation of atrazine and terbutryn and chemical composition were determined in deep (6.3−21.0 m) groundwater deposits from the northern boreal region in Finland, where aquifers are shallow with depths from 1 to 30 m. Groundwater usually contains soluble humic substances, reduced iron, and manganese. The hypothesis of the work was that the half-lives depend on microbial activity, oxygen, and the organic and inorganic composition of deposits. The atrazine degradation studies have mostly been done in the temperate region, and terbutryn behavior in groundwater has not been extensively studied.

MATERIALS AND METHODS

Sampling. The studied aquifer has atrazine and degradation products in the groundwater of a well and certain sampling pipes in an area located below the town of Lahti, Finland, which has approximately 100 000 inhabitants, industrial areas, city gardens, roads, and railway (**Figure 1**). In May 2003, the subsurface deposits, that is, sedimentary materials in the bottom of the well and pipes with atrazine in the groundwater, were taken from the well using an Ekman grab sampler (Duncan and Associates, Cumbria, United Kingdom) and from pipes B and C using a Waterra HL 21507 pump equipped with aggregate Power 2601 BV (2.5 kW, Hollola, Finland). The control deposits without atrazine in the groundwater were taken from pipe D within this same aquifer, and from pipe A situated in the adjacent aquifer close to the study area. The fine-grained fraction of subsurface deposits was accumulated to the groundwater sampling pipes through sieves with pore size of 0.3 mm, while the well bottom deposit was sandy. The groundwater flow within the study area has been modeled (**Figure 1**). The water sediment slurry was allowed to settle, and water was removed.

Chemical and Physical Analysis. The groundwater temperature and dissolved oxygen were determined on site before the deposit collection using a Ysi 650 electrode (03A010AE, Yellow Springs Instruments). The water pH was measured with a Mettler Delta 340 (Halstead, England). The dry weight of the deposits was determined from weight loss of triplicate samples of 1−3 g of deposits heated at 105 °C for 16 h (*18*). The organic matter content was determined as the loss upon ignition at 550 °C for 4 h (*19*). Total carbon and nitrogen were determined with a LECO Model 2000 CNS analyzer (St. Joseph, MI) using helium as a carrier gas (*20*). Three parallel samples of 0.321 ± 0.101 g were weighted and analyzed according to the instructions of LECO. Nitrogen oxides were reduced to N_2, which was determined using an electric conductivity detector. Carbon was burned to CO_2 under oxygen flow at 1350 °C, and CO_2 was analyzed using an infrared (IR) detector. Total carbon and nitrogen were analyzed by Ramboll Analytics Ltd. (Lahti, Finland). For the soluble NH_4^+ and NO_3^- analyses, the deposit:water (1:5, wt vol^{-1}) extraction was done for 1 h, and the solution was separated by filtration through gauze. To determine NH_4^+, the water extract was distilled using 0.3 M boric acid, bromocresol green (0.1 g), and methyl red (0.02 g) in 100 mL of methanol as an indicator. The NH_4^+ was then titrated with 0.5 mM H_2SO_4 (*21*). To determine NO_3^-, the water extract was filtered (0.45 μm) and analyzed using ion chromatography (*22, 23*). To analyze elements Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, 3 mL of deionized, distilled water, 3 mL of 37% HCl, and 1 mL of nitric acid were added to approximately 1 g of insoluble deposit (dry weight). The sample was warmed until the material was completely digested, filtrated, and brought to 50 mL using deionized, distilled water. The volume was adjusted, and the sample was analyzed by inductively coupled plasma atomic emission spectrometry. Standard reference solutions were diluted to prepare standard curves for quantification (*24, 25*). The NH_4^+, NO_3^-, and element analyses were done by Ramboll Analytics using methods accredited according to the guidelines of FINAS, Finnish Accreditation Service T039 (*26*).

Degradation Experiment. A 15.0 g portion of deposits (dry weight) and 50 mL of sterilized distilled water in 100 mL flasks were supplemented with both 30 mg L^{-1} of atrazine (100 mg kg^{-1} dry weight) and 20 mg L^{-1} of terbutryn (67 mg kg^{-1} dry weight) from stock solutions in methanol of 10 g L^{-1}. The flasks, with a hole (diameter 5 mm) in their screw cap, covered with an aluminum folio, were shaken (120 rpm) (Laboshake, Gerhardt, Königswinter, Germany) at 16 °C in the dark for 1.3−1.7 years. The 500 μL samples were taken for analyses at time points 49, 176, 296, 409, 490, and 623 days under aerobic conditions and 76, 161, 291, 396, 495 and 595 days under anaerobic conditions. The flasks were weighed at the beginning of the experiment and before samplings, and the evaporated water was replaced with sterile distilled water. All deposits and autoclave-sterilized controls were shaken in triplicate in aerobic and anaerobic conditions. Sterile controls were autoclaved (Instru, Santasalo-Sohlberg, Helsinki, Finland) for 1 h (121 °C, 101 kPa) on three successive days. Anaerobic conditions were established in the incubation jars filled with the experimental flasks using the reagent Anaerocult A (Merck, Darmstadt, Germany) and confirmed using a colorimetric anaerobic indicator (Anaerotest, Merck, Darmstadt, Germany) checked at least twice a week throughout the entire experiment.

To confirm the biotic pesticide degradation, 100 μL of liquid from the degradation experiment was spread on mineral agar 465I having 100 mg L^{-1} of atrazine or terbutryn as a nitrogen source. Five (atrazine) and 11 (terbutryn) colonies with different appearances were replated until pure cultures were obtained. The degradation of pesticides was

confirmed by the HPLC analysis after cultivation of isolated strains in mineral medium 465I (pH 7.25, German Collection of Microorganisms and Cell Cultures, DSMZ) containing $Na_2HPO_4 \cdot 2H_2O$, 3.5 g L^{-1}; KH_2PO_4, 1.0 g L^{-1}; $MgCl_2 \cdot 6H_2O$, 0.1 g L^{-1}; $CaCl_2 \cdot H_2O$, 0.03 g L^{-1}; sodium citrate, 1 g L^{-1}; in 465I agar containing atrazine or terbutryn 100 mg L^{-1}; in 465I broth containing atrazine 30 mg L^{-1} or terbutryn 20 mg L^{-1}; and 1 mL of a trace element stock solution containing EDTA, 0.5 g L^{-1}; $FeSO_4 \cdot 7H_2O$, 0.2 g L^{-1}; and 100 mL L^{-1} of the following trace element solution: $ZnSO_4 \cdot 7H_2O$, 0.10 g L^{-1}; $MnCl_2 \cdot 4H_2O$, 0.03 g L^{-1}; H_3BO_3, 0.30 g L^{-1}; $CoCl_2 \cdot 6H_2O$, 0.20 g L^{-1}; $CuCl_2 \cdot 2H_2O$, 0.01 g L^{-1}; $NiCl_2 \cdot 6H_2O$, 0.02 g L^{-1}; $Na_2MoO_4 \cdot 2H_2O$, 0.03 g L^{-1}. The medium was solidified with 1.5% agar.

Analysis of Pesticides. Standards in methanol:water (3:1 v/v) contained atrazine and terbutryn at five concentrations ranging from 2.3 to 129.9 μM with 70.7 μM simazine as an internal standard. The responses were linear with the correlation coefficients ($R^2 \pm$ standard deviation) of 0.997 $\pm$ 0.003 for atrazine and 0.994 $\pm$ 0.009 for terbutryn. The detection limit was approximately 2.6 μM for atrazine and 2.8 μM for terbutryn. The internal standard, 70.7 μM simazine, was added to a 100 μL sample, which then was brought to 600 μL with methanol:water (3:1 v/v). After filtration through a 0.45 μm GHP membrane (Acrodisc, Gelman, Pall Corp., Ltd.), 20 μL was analyzed using an HPLC equipped with a sample processor (Waters 712 WISP, Milford, MA), two chromatography pumps (model 6000A, Waters Associates Inc., Milford, MA), a Symmetry C_{18} column (3.9 $\times$ 150 mm, C-18, Waters, MA), and a UV detector (Hewlett-Packard HP 1050) set at 225 nm. The system was controlled with a Maxima 820 chromatography workstation (Millipore, Ventura, CA), which also performed the data collection from the UV detector. The flow rate of the mobile phase was 1 mL min^{-1}. The chromatographic separation was carried out using a linear gradient profile of a 10 mM phosphate buffer (pH 7.0), with acetonitrile increasing from 30% to 70% in 12 min, held at 70% for 1 min, and then decreased in 5 min back to 30%, which was maintained for 5 min.

Concentrations of atrazine and the degradation products DEA and DIA in water from the sampling pipes and groundwater well were from the drinking water quality analyses done twice a year by waterworks in Ramboll Analytics Ltd. (Lahti, Finland) with the accredited method based on the solid-phase extraction of pesticides, followed by the high resolution gas−liquid chromatography−mass spectrometry.

Calculations. The half-lives of atrazine and terbutryn were calculated using the first-order rate equation $\ln C(t) = -kt + \ln (C_0)$, where C_0 is the initial pesticide concentration, $C(t)$ is the concentration at the time t (days), and k is the rate constant. In fitting data to the regression curve, the statistical significance $p < 0.001$ and $r < -0.697$ were obtained for half-lives of $n = 18-21$ (aerobic pipe A deposit, $n = 9$). The data were also fitted to the zero-order kinetic equation, resulting in poor statistical significance. Pearson two-tailed correlation and analysis of variance were performed with SPSS-statistical package for Windows (SPSS Inc., Chicago, IL). The two (atrazine) or three (terbutryn) factor nonparametric Kruskal−Wallis test (K−W) (*27*) was used for the analysis of variance, due to the significance of $p < 0.05$ in Levene's test of equality of error variances. The significant interactions were obtained between samples, oxygen (aerobic, anaerobic), and sterilization (deposit, sterilized control) for terbutryn (**Table 1**). The samples were then analyzed separately using the K−W test, and finally the interaction between oxygen and sterilization was determined using the Mann−Whitney test (M−W) (*28*).

RESULTS AND DISCUSSION

Atrazine. In the pipe A deposit atrazine level was below the detection limit under aerobic conditions in 176 days (half-life 38 d), while no degradation occurred in the sterilized deposit control, and anaerobically the half-life was 672−719 d (**Tables 2** and **3**, **Figure 2**). For atrazine biodegradation, a half-life of 67.7 d has been measured in New Zealand from 1.0−1.2 m below the surface (mbs) (*29*). To confirm the biotic atrazine degradation in the pipe A deposit, five pure cultures with different appearances were isolated. The microbes were cultivated aerobically in a mineral medium, and atrazine degradation as a nitrogen source was analyzed by the HPLC. Despite of clear microbial degradation in the deposit, no atrazine was detected in groundwater from pipe A. This adjacent aquifer could have an atrazine exposure history and an adapted microbial population degrading atrazine (**Figure 1**).

Table 1. Three (terbutryn: sample, oxygen, sterilization) and Two (atrazine: sample, sterilization) Factor Kruskal−Wallis Test Results of Half-Lives

	H (Kruskal−Wallis test)	
	atrazine	terbutyn
oxygen (aerobic/anaerobic)		0.02
sterilization (deposit/sterilized control)	4.39[c]	0.41
sample	4.48	1.78
oxygen−sterilization		0.04
sample−oxygen		12.00[c]
sample−sterilization	3.80	18.80[a]
sample−oxygen−sterilization		18.61[a]
Pipe A		
oxygen		180.92[a]
sterilization		33.23[a]
oxygen−sterilization		19.39[a]
Pipe B		
oxygen		54.26[a]
sterilization		4.01[c]
oxygen−sterilization		40.01[a]
Pipe C		
oxygen		6.98[b]
sterilization		101.77[a]
oxygen−sterilization		106.67[a]
Pipe D		
oxygen		11.85[a]
sterilization		122.08[a]
oxygen−sterilization		158.01[a]
Well		
oxygen		27.92[a]
sterilization		189.64[a]
oxygen−sterilization		113.39[a]

[a] $p < 0.001$. [b] $0.001 < p < 0.01$. [c] $0.01 < p < 0.05$.

No aerobic microbial atrazine degradation was detected in the main study aquifer well and pipe B, C, and D deposits compared to the sterilized controls, although groundwater from the well and pipes B and C contained atrazine (**Tables 2** and **3**, **Figure 2**). In anaerobic deposits, the atrazine half-lives of 715−754 d were equal or even longer than in sterilized controls of 430−829 d (K−W, $p < 0.05$), indicating the absence of biotic degradation. For comparison, the half-live for chemical atrazine degradation has been 74.8 days at the depth of 1.0−1.2 mbs in New Zealand (*29*), 0.14 years to no degradation at 1.5−3.5 mbs and 0.8−1.1 m below the water table (mbt) in The Netherlands (*30*), 1.2−36 years (mean 8.6 years) at 1.5−11.65 mbt in simulations in United States (Wisconsin) (*31*), and no degradation at 0.3−9.5 mbs from above the water table to 4 mbt in Denmark (*32, 33*). The autoclave sterilization disturbed the composition of deposits and may have affected the chemical degradation of atrazine. Although no biotic degradation occurred in well and pipe B, C, and D deposits, this aquifer had the typical microbial atrazine degradation products DEA and DIA in groundwater (**Table 2**). Pesticides and degradation products are small molecules and easily elute with groundwater from distant sources.

In the studied deposits from below the water table, the anaerobic abiotic atrazine degradation with half-lives of 715−754 d was faster than aerobic degradation with half-lives of 1081 d

Table 2. Depths of Groundwater Well and Sampling Pipes, Water Table, and Physicochemical Characteristics of Groundwater and Subsurface Deposits

	well	pipe A	pipe B	pipe C	pipe D
depth (m)	6.3	18.5	19.9	11.4	21.0
water table (m)	5.1	17.7	11.9	9.6	4.1
depth from water table (m)	1.2	0.8	8.0	1.8	16.9
		Groundwater			
atrazine (μg L^{-1})	0.10 ± 0.03	0	0.36 ± 0.09	0.03 ± 0.01	0
desethylatrazine (DEA) (μg L^{-1})	0.11 ± 0.03	0	0.20 ± 0.06	0.09 ± 0.03	0
deisopropylatrazine (DIA) (μg L^{-1})	0.02[b]	0	0.04 ± 0.01	0	0
oxygen (mg L^{-1})	4.5	5.7	1.5	7.3	0.16
pH	7.9	6.8	6.8	6.3	6.9
temperature (°C)	6.1	7.6	11.4	9.2	9.3
		Subsurface Deposits			
organic matter (mg g^{-1} dry wt)	13.8 ± 1.6	7.5 ± 0.3	25.1 ± 0.4	12.3 ± 0.1	5.0 ± 0.2
carbon (mg g^{-1} dry wt)	1.34 ± 0.78	1.42 ± 1.25	1.40 ± 0.85	1.11 ± 0.79	0.538 ± 0.147
nitrogen (μg g^{-1} dry wt)	123.1 ± 109.4	88.4 ± 102.2	316.0 ± 79.1	122.9 ± 106.4	90.9 ± 65.8
NH_4 (μg g dry wt)	2.0 ± 0.6	6.0 ± 1.8	11.0 ± 3.3	6.5 ± 2.0	1.8 ± 0.5
NO_3 (μg g^{-1} dry wt)	<2	<2[a]	<2[a]	<2	<2
Fe (mg g^{-1})	31.0 ± 7.8	15.0 ± 3.8	12.0 ± 3.0	26.5 ± 6.6	15.0 ± 3.8
Mn (μg g^{-1})	185 ± 46	170 ± 43	245 ± 61	275 ± 69	165 ± 41.3
Zn (μg g^{-1})	41.0 ± 14.4	44.5 ± 15.6	56.5 ± 19.8	64.5 ± 22.6	46.0 ± 16.1
Cu (μg g^{-1})	38.0 ± 11.4	19.0 ± 5.7	30.5 ± 9.2	30.0 ± 9.0	20.0 ± 6.0
Cr (μg g^{-1})	15.5 ± 4.7	24.5 ± 7.4	34.0 ± 10.2	40.5 ± 12.2	20.5 ± 6.2
Ni (μg g^{-1})	15.0 ± 4.5	17.5 ± 5.3	17.5 ± 5.3	21.0 ± 6.3	12.0 ± 3.6
Pb (μg g^{-1})	16.5 ± 5.8	11.5 ± 4.0	19.5 ± 6.8	13.0 ± 4.6	10.0 ± 3.5
Co (μg g^{-1})	7.0 ± 2.5	7.5 ± 2.6	8.0 ± 2.8	12.0 ± 4.2	7.5 ± 2.6
Cd (μg g^{-1})	<2	<2[a]	<2[a]	<2	<2

[a] Below the detection limit. [b] Below the quantification limit.

Table 3. Atrazine Half-Life under Aerobic and Anaerobic Conditions in Deposits and Sterilized Controls from Groundwater Well and Sampling Pipes A, B, C, and D

	aerobic conditions/days (years)		anaerobic conditions/days (years)	
	deposit	sterilized deposit control	deposit	sterilized deposit control
pipe A	38 ± 1 (0.1 ± 0.01)	no degradation	719 ± 44 (2.0 ± 0.1)	672 ± 36 (1.8 ± 0.1)
pipe B	1081 ± 272 (3.0 ± 0.8)	no degradation	754 ± 213 (2.1 ± 0.6)	441 ± 68 (1.2 ± 0.2)
pipe C	no degradation	no degradation	744 ± 44 (2.0 ± 0.1)	638 ± 305 (1.7 ± 0.8)
pipe D	no degradation	no degradation	715 ± 306 (2.0 ± 0.8)	430 ± 56 (1.2 ± 0.2)
well	no degradation	no degradation	754 ± 213 (2.1 ± 0.6)	829 ± 183 (2.3 ± 0.5)

to no degradation (**Table 3**, **Figure 2**). The opposite relation has been measured in surface soil and subsurface soil above the water table of 0.80−1.0 mbs (*13*). In deep deposits below the groundwater table, the soil composition could enhance the anaerobic chemical atrazine degradation better than aerobic degradation (**Tables 2** and **3**). In the deposits, iron content decreased with the increase in the depth ($r = -0.976$, $p < 0.01$). Zinc correlated positively with chromium ($r = 0.974$, $p < 0.01$), cobalt ($r = 0.890$, $p < 0.05$), and manganese ($r = 0.940$, $p < 0.05$), while manganese had a positive correlation with chromium ($r = 0.903$, $p < 0.05$). The groundwater oxygen content was the lowest in the deepest deposits from the water table ($r = -0.888$, $p < 0.05$), that is, in pipe B and D deposits from 8.0 to 16.9 mbt, while in pipe A deposit from 0.8 mbt biodegradation was measured despite of great depth of 18 mbs. The oxygen limitation has often been preventing biotic atrazine degradation (*13, 32−35*). Microbes in the pipe A deposit from groundwater temperature of 7.6 °C could be better adapted to cold than those isolated from the temperate region, where microbial atrazine degradation has been detected at 15 °C in surface soil (*36*) and sediments from 1.0 to 1.2 mbs (*29*) and at 10 °C in vadose sediments from 1.7 to 2.3 mbs (*16*).

The atrazine and DEA concentrations in groundwater were high, when nitrogen (atrazine, $r = 0.986$, $p < 0.01$; DEA, $r = 0.891$, $p < 0.05$), organic matter (atrazine, $r = 0.961$, $p < 0.01$; DEA, $r = 0.977$, $p < 0.01$), and lead (atrazine, $r = 0.915$, $p < 0.05$; DEA, r=0.960, $p < 0.01$) contents were high in subsurface deposits (**Table 2**). The organic matter occurred in deposits with nitrogen ($r = 0.951$, $p < 0.05$) and lead ($r = 0.959$, $p < 0.01$). The interactions among atrazine, organic matter, nitrogen, and lead could decrease atrazine availability for the chemical and microbial degradation (*14, 37−39*), especially in the deposit from pipe B with the highest amount of these compounds and half-lives of 754−1081 d. The highest organic matter and nitrogen contents of pipe B deposit likely resulted from the bank filtration of water from Lake Vesijärvi to the groundwater (**Figure 2**). When released from the organic matter, nitrogen could prevent biotic atrazine degradation as a nitrogen source (*40, 41*), and lead could be toxic to microbes (*42, 43*). However, the groundwater lead concentration (5 μg L^{-1}, 0.024 μM) likely did not affect microbial growth, the minimum inhibitory concentration being 0.125−6.04 mM for *Pseudomonas* spp (*44, 45*). Although the high organic matter content has increased microbial atrazine degradation and vice versa (*39−41,46,47*), in the boreal region the natural microbial activity in the groundwater environment is low. In pipe A deposit with complete aerobic biotic atrazine degradation, the organic matter content was 3.3-fold lower than in pipe B deposit (**Tables 2** and **3**, **Figure 2**). Interestingly, in the anaerobic pipe B deposit, the autoclave disturbance of the chemical structure decreased the half-life to the shortest of the deposits, 441 d (K−W, $p < 0.05$).

Terbutryn. The significant three-way interactions between sample, oxygen, and sterilization were obtained for terbutryn

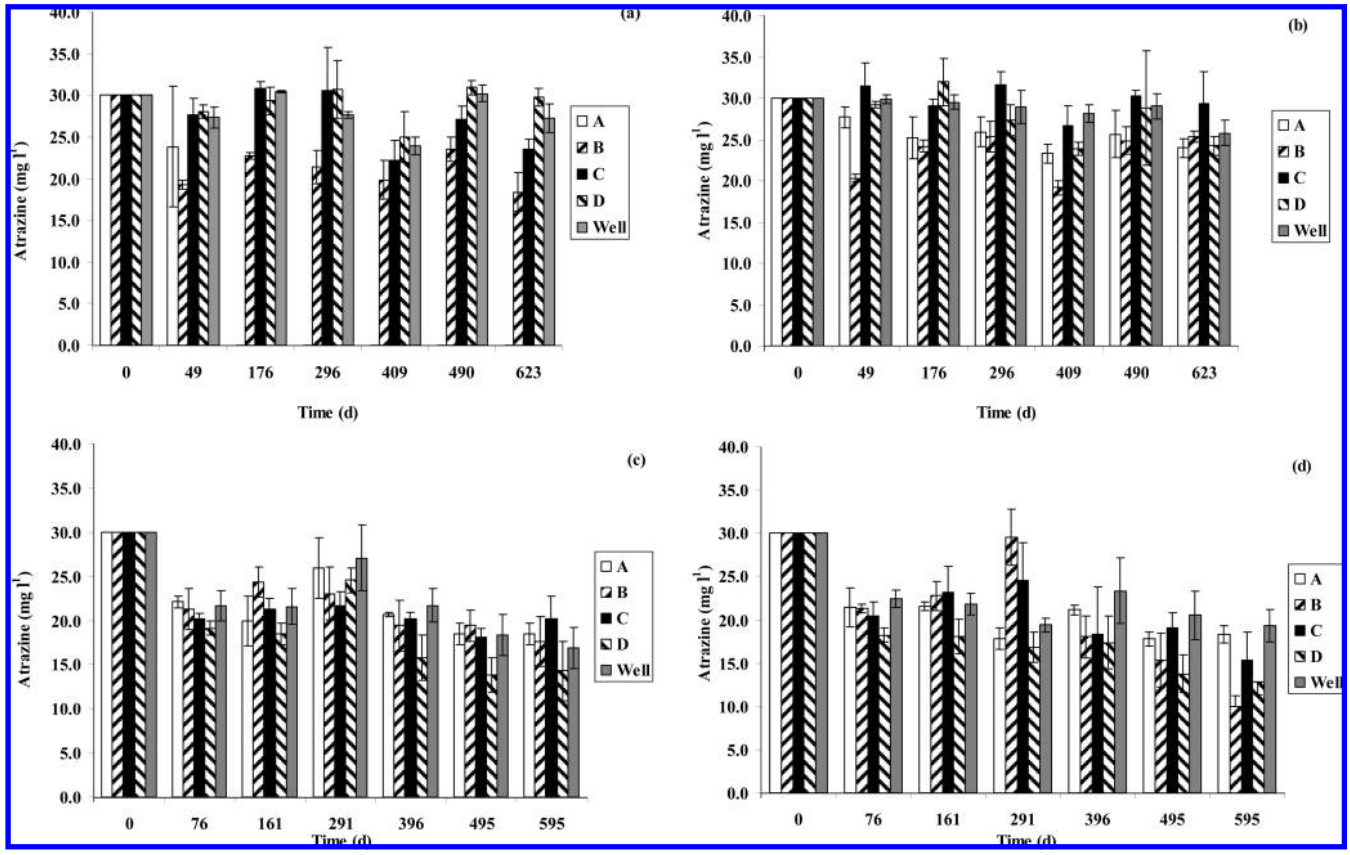

Figure 2. Degradation of atrazine in deposits from sampling points A, B, C, and D and well under aerobic conditions (a) with sterilized controls (b) and anaerobically (c) with sterilized controls (d).

Table 4. Terbutryn Half-Life under Aerobic and Anaerobic Conditions in Deposits and Sterilized Controls from Groundwater Well and Sampling Pipes A, B, C, and D

	aerobic degradation/days (years)		anaerobic degradation/days (years)	
	deposit	sterilized deposit control	deposit	sterilized deposit control
A	177 ± 48[a,b] (0.5 ± 0.1)	304 ± 74 (0.8 ± 0.2)	387 ± 74 (1.1 ± 0.2)	400 ± 13 (1.1 ± 0.1)
B	349 ± 62[a](1.0 ± 0.2)	443 ± 17[c] (1.2 ± 0.1)	320 ± 9 (0.9 ± 0.1)	286 ± 25 (0.8 ± 0.1)
C	229 ± 4[a,b] (0.6 ± 0.1)	483 ± 87[c] (1.3 ± 0.2)	299 ± 28 (0.8 ± 0.1)	302 ± 45 (0.8 ± 0.1)
D	499 ± 114[a,b] (1.4 ± 0.3)	181 ± 7[c] (0.5 ± 0.1)	338 ± 66 (0.9 ± 0.2)	362 ± 40 (1.0 ± 0.1)
Well	644 ± 192[a,b] (1.8 ± 0.5)	205 ± 16[c] (0.6 ± 0.1)	294 ± 25 (0.8 ± 0.1)	266 ± 32 (0.7 ± 0.1)

[a] Differed from aerobic sterilized deposit control (Mann−Whitney, $p < 0.05$). [b] Differed from anaerobic deposit (Mann−Whitney, $p < 0.05$). [c] Differed from anaerobic sterilized deposit control (Mann−Whitney, $p < 0.05$).

degradation (**Table 1**). The terbutryn half-lives of 177−229 d under aerobic conditions in deposits from pipes A and C with the lowest water table of 0.8−1.8 mbt were significantly shorter than in sterilized controls (M−W, $p < 0.05$) or anaerobic conditions (M−W, $p < 0.05$) (**Tables 1** and **4**, **Figure 3**). The aerobic microbial terbutryn degradation in pipe A and C deposits was confirmed after isolation of four and seven different strains, respectively, similarly as was presented for atrazine. No terbutryn was detected in groundwater from pipes A and C; however, an adapted microbial population able to degrade terbutryn may have developed, since terbutryn partly replaced atrazine after the 1992 ban.

In deposits from pipe D and well, the half-lives of 499−644 d under aerobic conditions were longer than in the sterilized controls of 181−205 d (M−W, $p < 0.05$) or under anaerobic conditions of 294−338 d (M−W, $p < 0.05$), the latter not differing significantly from sterilized control half-lives of 266−362 d (**Tables 1** and **4**, **Figure 3**). The chemical degradation seemed to occur in pipe D and well deposits, aerobically being slower than anaerobically. The half-lives in anaerobic deposits (294−387 d) and sterilized controls (266−400 d) with no significant differences could be indicative of chemical degradation instead of biotic degradation.

The aerobic terbutryn half-lives of 177−644 d varied more than under anaerobic conditions of 266−400 d (**Table 4**, **Figure 3**). In water above river and pond sediments, the terbutryn half-life has been 180−240 d and in sediments approximately 380 d (*17*), which is within the range of variation determined in this study. In aerobic deposits, the variation likely resulted from the above-presented differences in biotic and abiotic terbutryn degradation. In sterilized controls under aerobic conditions, the fast chemical terbutryn degradation was related to the low amounts of Cr ($r = 0.962$, $p < 0.01$), Mn ($r = 0.920$, $p < 0.05$), Zn ($r = 0.911$, $p < 0.05$), and Ni ($r = 0.897$, $p < 0.05$) (**Tables 2** and **4**, **Figure 3**). This is in agreement with the results of Lyapchenko et al. (*48*), who have reported that terbutryn metal complexes were stable and inhibited degradation.

Comparison of Atrazine and Terbutryn Degradation. The half-lives of atrazine and terbutryn within the northern boreal region generally were of the same order of magnitude as has

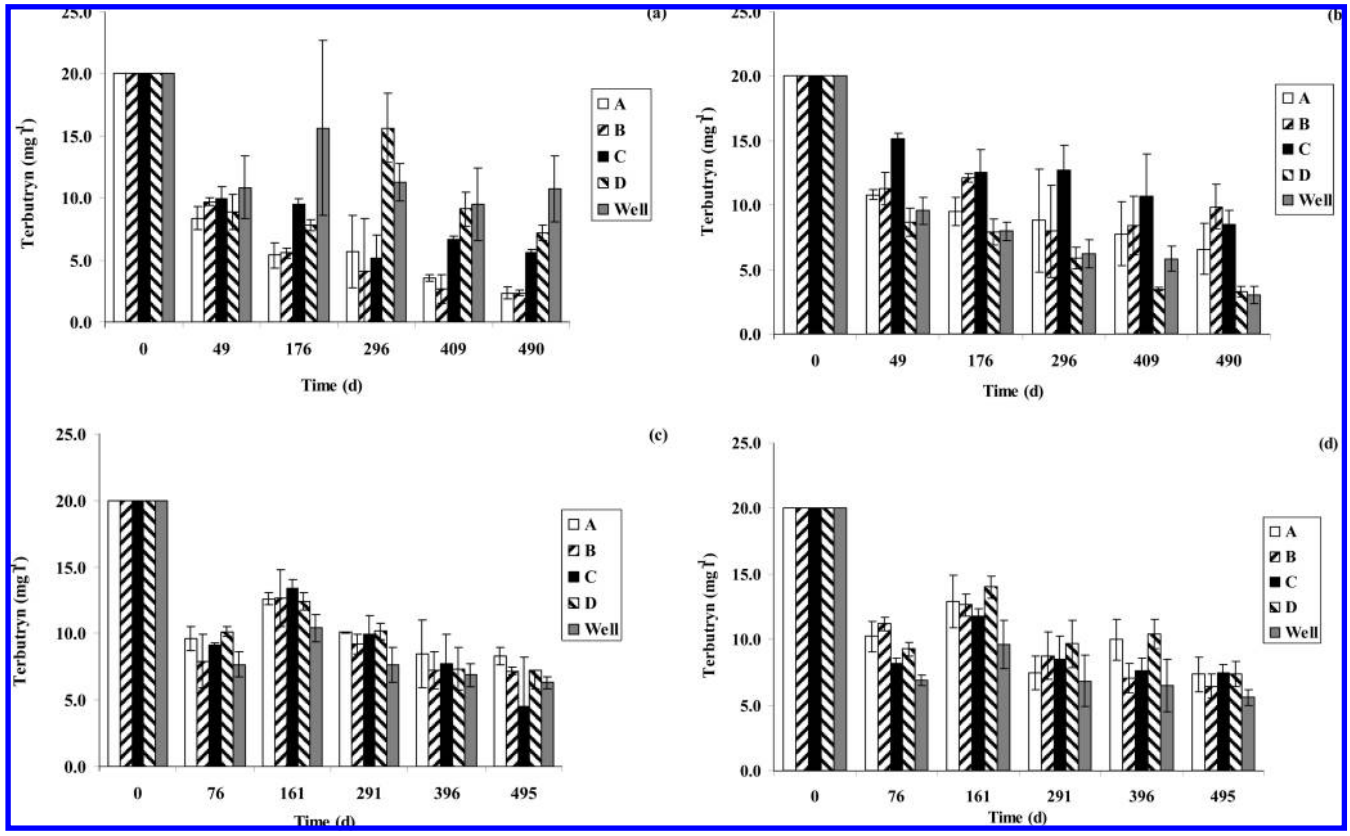

Figure 3. Degradation of terbutryn in deposits from sampling points A, B, C, and D and well under aerobic conditions (a) with sterilized controls (b) and anaerobic conditions (c) with sterilized controls (d).

been reported in the temperate region (*17, 29*−*33*). The biotic atrazine and terbutryn degradation was found in deposits closest to the water table. The abiotic degradation of atrazine was faster anaerobically than aerobically, while an opposite relation was measured for terbutryn. The abiotic terbutryn half-lives of 181−644 d generally were shorter than atrazine half-lives of 430 d to no degradation. Photodegradation of terbutryn has also been faster than that of atrazine (*9*). The C−S bond of terbutryn may break more easily than the C−Cl bond of atrazine. Cl-triazines like atrazine have formed bonds with organic matter stronger than S-triazines like terbutryn, indicating that sorption may inhibit atrazine degradation more than terbutryn degradation (*49*). The complex, poorly degradable humic substances, typical for the northern boreal region, may form interactions with pesticides and prevent both chemical and microbial degradation.

LITERATURE CITED

(1) European Union, Council Directive 98/83/EC on the quality of water intended for human consumption. Official Journal of the European Communities, 3 November, 1998.

(2) Tomlin, C. D. S. *The Pesticide Manual*, 12 ed.; The British Crop Protection Council: Surrey, UK. 2000.

(3) Boyd, R. A. Herbicides and herbicide degradates in shallow groundwater and the Cedar River near a municipal well field, Cedar Rapids, Iowa. *Sci. Total Environ.* **2000**, *248*, 241–253.

(4) Carabias-Martínez, R.; Rodríguez-Gonzalo, E.; Fernández-Laespada, M. E.; Calvo-Seronero, L.; Sánchez-San Roman, F. J. Evolution over time of the agricultural pollution of waters in an area of Salamanca and Zamora (Spain). *Water Res.* **2003**, *37*, 928–938.

(5) Worrall, F.; Kolpin, D. W. Aquifer vulnerability to pesticide pollution—Combining soil, land-use and aquifer properties with molecular descriptors. *J. Hydrol.* **2004**, *293*, 191–204.

(6) Thurman, E. M.; Meyer, M. T.; Mills, M. S.; Zimmermann, L. R.; Perry, C. A. Formation and transport of deethylatrazine and deisopropylatrazine in surface water. *Environ. Sci. Technol.* **1994**, *28*, 2267–2277.

(7) Kruger, E.; Rice, P.; Anhalt, J.; Anderson, T.; Coats, J. Comparative fates of atrazine and deethylatrazine in sterile and nonsterile soils. *J. Environ. Qual.* **1997**, *26*, 95–101.

(8) Kolpin, D. W.; Thurman, E. M.; Linhart, S. M. Finding minimal herbicide concentrations in ground water? Try looking for their degradates. *Sci. Total Environ.* **2000**, *248*, 115–122.

(9) Lányi, K.; Dinya, Z. Photodegradation study of some triazine-type herbicides. *Microchem. J.* **2004**, *75*, 1–13.

(10) Shin, J. Y.; Cheney, M. A. Abiotic transformation of atrazine in aqueous suspension of four synthetic manganese oxides. *Colloids Surf. A* **2004**, *242*, 85–92.

(11) Wackett, L. P.; Sadowsky, M. J.; Martinez, B.; Shapir, N. Biodegradation of atrazine and related s-triazine compounds: From enzymes to field studies. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 39–45.

(12) Smith, D.; Alvey, S.; Crowley, D. E. Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. *FEMS Microbiol. Ecol.* **2005**, *53*, 265–273.

(13) Accinelli, C.; Dinelli, G.; Vicari, A.; Catizone, P. Atrazine and metolachlor degradation in subsoils. *Biol. Fertil. Soils* **2001**, *33*, 495–500.

(14) Blume, E.; Bischoff, M.; Moorman, T. B.; Turco, R. F. Degradation and binding of atrazine in surface and subsurface soils. *J. Agric. Food Chem.* **2004**, *52*, 7382–7388.

(15) Leistra, M.; Smelt, J. H. An appraisal of methods for measurements of pesticide transformation in the groundwater zone. *Pest Man. Sci.* **2001**, *57*, 333–340.

(16) Radosevich, M.; Traina, S. J.; Tuovinen, O. H. Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm. *Biodegradation* **1996**, *7*, 137–149.

(17) Muir, D. C.; Yarechewski, A. L. Degradation of terbutryn in sediments and water under various redox conditions. *J. Environ. Sci. Health B* **1982**, *17*, 363–380.

(18) SFS-EN 13040. Soil improvers and growing media. Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density. Finnish Standards Association SFS, Helsinki, Finland, 2000.
(19) SFS-EN 13039. Soil improvers and growing media. Determination of organic matter content and ash. Finnish Standards Association SFS, Helsinki, Finland, 2000.
(20) LECO Corp. Total organic carbon and nitrogen in soils, Organic Application Note 203−821−165, LECO Corp., St. Joseph, MO, 2003.
(21) SFS 5505. Determination of inorganic and organic nitrogen in waste water. Modified Kjeldahl method. Finnish Standards Association SFS, Helsinki, Finland,1988 (in Finnish).
(22) SFS-EN ISO 10304−1. Water quality. Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulphate ions, using liquid chromatography of ions. Part 1: Method for water with low contamination. Finnish Standards Association SFS, Helsinki, Finland, 1995.
(23) SFS-EN ISO 10304−2. Water quality. Determination of dissolved anions by liquid chromatography of ions. Part 2: Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulphate in waste water. Finnish Standards Association SFS, Helsinki, Finland,1997.
(24) ISO 11885. Water quality. Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy. Finnish Standard Association, Helsinki, Finland, 1996.
(25) ISO/CD 2 2036. Soil quality. Determination of trace elements in extracts of soil by inductively coupled plasma atomic emission spectrometry (ICP AES). International Standard Organisation, 2005.
(26) SFS-EN ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories. Finnish Standards Association SFS, Helsinki, Finland, 2005.
(27) Kruskal, W. H.; Wallis, W. A. Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* **1952**, *47*, 583–621.
(28) Mann, H. B.; Whitney, D. R. On the test of whether one or two random variables is stochastically larger than the other. *Ann. Mathem. Stat.* **1947**, *18*, 50–60.
(29) Pang, L.; Close, M.; Flintoft, M. Degradation and sorption of atrazine, hexazinone and procymidone in coastal sand aquifer media. *Pest Man. Sci.* **2005**, *61*, 133–143.
(30) Van der Pas, L. J. T.; Leistra, M.; Boesten, J.J.T.I. Rate of transformation of atrazine and bentazone in water-saturated sandy subsoils. *Pestic. Sci.* **1998**, *53*, 223–323.
(31) Levy, J.; Chesters, G. Simulation of atrazine and metabolite transport and fate in a sandy-till aquifer. *J. Contam. Hydrol.* **1995**, *20*, 67–88.
(32) Larsen, L.; Aamand, J. Degradation of herbicides in two sandy aquifers under different redox conditions. *Chemosphere* **2001**, *44*, 231–236.
(33) Rügge, K.; Bjerg, P. L.; MosbÆk, H.; Christensen, T. H. Fate of MCPP and atrazine in an anaerobic landfill leachate plume (Grindsted, Denmark). *Water Res.* **1999**, *33*, 2455–2458.
(34) Papiernik, S. K.; Spalding, R. F. Atrazine, deethylatrazine and deisopropylatrazine persistence measured in groundwater in-situ under low-oxygen conditions. *J. Agric. Food Chem.* **1998**, *46*, 749–754.
(35) Patterson, B. M.; Franzmann, P. D.; Davis, G. B.; Elbers, J.; Zappin, L. R. Using polymer mats to biograde atrazine in groundwater laboratory column experiments. *J. Contam. Hydrol.* **2002**, *54*, 195–213.
(36) Mandelbaum, R. T.; Wackett, L. P.; Allen, D. L. Mineralization of the s-triazine ring of atrazine by stable bacterial mixed cultures. *Appl. Environ. Microbiol.* **1993**, *59*, 1695–1701.
(37) Scow, K. M.; Fan, S.; Johnson, C.; Ma, G. M. Biodegradation of sorbed chemicals in soil. *Environ. Health Perspect.* **1995**, *103*, 94–95.
(38) Lucio, M.; Schmitt-Kopplin, P. Modelling the binding of triazine herbicides to humic substances using capillary electrophoresis. *Environ. Chem. Lett.* **2006**, *4*, 15–21.
(39) Smalling, K. L.; Aelion, C. M. Distribution of atrazine into three chemical fractions: impact of sediment depth and organic carbon content. *Environ. Toxicol. Chem.* **2004**, *23*, 1164–1171.
(40) Abdelhafid, R.; Houot, S.; Barriuso, E. Dependence of atrazine degradation on C and N availability in adapted and non-adapted soils. *Soil Biol. Biochem.* **2000**, *32*, 389–401.
(41) Rhine, E. D.; Fuhrmann, J. J.; Radosevich, M. Microbial community responses to atrazine exposure and nutrient availability: linking degradation capacity to community structure. *Microb. Ecol.* **2003**, *46*, 145–160.
(42) Roane, T. M. Lead resistance in two bacterial isolates from heavy metal- contaminated soils. *Microb. Ecol.* **1998**, *37*, 218–224.
(43) Suhadolc, M.; Schroll, R.; Gattinger, A.; Schloter, M.; Munch, J. C.; Lestan, D. Effects of modified Pb-, Zn-, and Cd- availability on the microbial communities and on the degradation of isoproturon in a heavy metal contaminated soil. *Soil Biol. Biochem.* **2004**, *36*, 1943–1954.
(44) Teitzel, G. M.; Parsek, M. R. Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. *Appl. Environ. Microbiol.* **2003**, *69*, 2313–2320.
(45) Workentine, M. L.; Harrison, J. J.; Stenroos, P. U.; Ceri, H.; Turner, R. J. *Pseudomonas fluorescens'* view of the periodic table. *Environ. Microbiol., Society for Applied Microbiology and Blackwell Publishing Ltd.* **2007**,
(46) Moorman, T. B.; Cowan, J. K.; Arthur, E. L.; Coats, J. R. Organic amendments to enhance herbicide biodegradation in contaminated soils. *Biol. Fertil. Soils* **2001**, *33*, 541–545.
(47) Shapir, N.; Mandelbaum, R. T.; Fine, P. Atrazine mineralization by indigenous and introduced *Pseudomonas* sp. strain ADP in sand irrigated with municipal wastewater and amended with composted sludge. *Soil Biol. Biochem.* **2000**, *32*, 887–897.
(48) Lyapchenko, N.; Eitner, K.; Schroeder, G.; Brzezinski, B. The complexes of some s-triazine herbicides with metal cations studied by ESI mass spectrometry and theoretical calculations. *J. Mol. Struct.* **2003**, *690*, 45–51.
(49) Chefetz, B.; Bilkis, Y. I.; Polubesova, T. Sorption-desorption behaviour of triazine and phenylurea herbicides in Kishon river sediments. *Water Res.* **2004**, *38*, 4383–4394.

Received for review August 16, 2008. Revised manuscript received October 16, 2008. Accepted October 17, 2008. We thank Ph.D. Juha Mikola for comments on statistics and Mrs.Minna Korhonen for the technical assistance. The work was supported by Maj and Tor Nessling Foundation, the Ministry of Agriculture and Forestry of Finland, Maaja Vesitekniikan Tuki Ry, Lahti Aqua Ltd., and the Onni and Hilja Tuovinen Foundation.

JF802528A