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Atrazine and metolachlor degradation in subsoils

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Abstract Degradation of atrazine [2-chloro-4-etylamino-6-isopropylamino-1,3,5-triazine] and metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide] in sterile and non-sterile soil samples collected at two different soil depths (0–20 and 80–110 cm) and incubated under aerobic and anaerobic conditions was studied. Under aerobic conditions, the half-life of atrazine in non-sterile surface soil was 49 days. In non-sterile subsoil, the half-life of atrazine (119 days) was increased by 2.5 times compared in surface soils and was not statistically different from half-lives in sterile soils (115 and 110 days in surface soil and subsoil, respectively). Metolachlor degradation occurred only in non-sterile surface soil, with a half-life of 37 days. Under anaerobic conditions, atrazine degradation was markedly slower than under aerobic conditions, with a half-life of 124 and 407 days in non-sterile surface soil and non-sterile subsoil, respectively. No significant difference was found in atrazine degradation in both sterile surface soil (693 days) and subsoil (770 days). Under anaerobic conditions, degradation of metolachlor was observed only in non-sterile surface soil. Results suggest that atrazine degraded both chemically and biologically, while metolachlor degraded only biologically. In addition, observed Eh values of soil samples incubated under anaerobic conditions suggest a significant involvement of soil microorganisms in the overall degradation process of atrazine under anaerobic conditions.

Keywords Atrazine · Metolachlor · Surface soil · Subsurface soil · Degradation

Introduction

Investigations dealing with herbicide degradation in soil are mainly carried out using surface soil (0–20 cm) (Brejda et al. 1988; Winkleman and Klaine 1991; Topp et al. 1994). However, results obtained from surface soil are not directly comparable with results that can be achieved in subsurface soil. In fact, herbicides that leach into subsurface soil encounter a different set of environmental conditions. Microbial biomass and microbial activity as well as available nutrients in subsurface soil are different from those found in surface soil (Federle et al. 1986; Brockman et al. 1992). Degradation of herbicides may proceed at rates that are considerably lower in subsoil than in surface soil (Moorman and Harper 1989; Kruger et al. 1997).

The detection of pesticides in groundwater continues to concern the general public. In USA and most of the European countries, pesticides, including atrazine and metolachlor, have been found in groundwater (Friesel et al. 1986; Lock 1987; Roux et al. 1991; Klint et al. 1993). Groundwater does not have the natural cleansing mechanisms common in surface water (McCarty et al. 1981), thus determination of the fate of herbicides in subsurface soil is essential to assess their potential to contaminate groundwater (Issa and Wood 1999).

Atrazine and metolachlor are two commonly used pesticides. Atrazine degradation in soil is either a microbial and chemical process (Blumhorst and Weber 1994; Assaf and Turco 1994; Mandelbaum et al. 1995). Several researchers have isolated bacteria or mixed cultures that could use atrazine as the sole C source (Behki and Khan 1986; Yanze-Kontchou and Gschwind 1994) or as the sole N source (Assaf and Turco 1994; Alvey and Crowley 1995; Mandelbaum et al. 1995; Radosevich et al. 1997).

Metolachlor degradation in soil is a biological rather than a chemical process (Bouchard et al. 1982; Saxena et al. 1987; Miller et al. 1997) and it is considered to be a cometabolic process (Krause et al. 1985; Saxena et al. 1987).

In the literature, information on the transformation rate of atrazine and metolachlor in subsoil materials is limited. While atrazine degradation in subsoil materials was reported by some authors (Kruger et al. 1997; Issa and Wood 1999), metolachor was found to be persistent

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in subsurface soil (Miller et al. 1997). Despite several investigations of atrazine and metolachlor degradation, little is known about the relationship between microbial biomass and activity and herbicide transformation rates.

The objectives of this study were to determine degradation rates of atrazine and metolachlor in soil collected at two different depths (0–20 and 80–100 cm). Considering that anaerobic conditions may exist in some aquifers and are, at least transiently, present in subsoils (Pothuluri et al. 1990), rates of degradation of atrazine and metolachlor were determined under aerobic and anaerobic conditions. Differences in soil biomass along the soil profile were also determined, by means of the fumigation-incubation method. To evaluate soil oxidation/reduction status under anaerobic condition, soil Eh was measured during soil incubation.

Materials and methods

Soils

Soil samples were taken from a field with no previous herbicide history at the Experimental Farm of the University of Bologna at Ozzano, near Bologna (Italy). The soil is classified as a Udertic Ustochrepts, fine, mixed, mesic and has a loamy texture. Soil profile properties are described in Table 1.

Samples from 0–20, 40–60 and 80–100 cm were taken using a soil probe. The soil probe was washed with water and acetone prior to each sampling to avoid contamination. Collected samples were stored in sterilized plastic bags and transported to the laboratory. Soil samples were air-dried and passed through a 2-mm sieve. All these operations were done under a laminar-flow hood to prevent significant contamination by microorganisms. A portion of soil collected from 0–20 and 80–100 cm depths was sterilized by autoclaving twice for 1 h at 121°C and 103 kPa.

Atrazine and metolachlor degradation

A water-dispersible, granular, commercial formulation of atrazine [2-chloro-4-etylamino-6-isopropylamino-1,3,5-triazine; 90% of active ingredient (a.i.)] and an emulsifiable concentrate formulation of metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide; 80% of a.i.] were used. Atrazine and metolachlor were provided by Novartis (Basel, Switzerland). Analytical grade atrazine (99.1%) and metolachlor (96.1%) were provided by Dr Ehrenstorfer (Augsburg, Germany) and employed as analytical standards.

Twenty-five grams (air-dry basis) of the microbial active and sterile soil from 0–20 and 80–100 cm depths were weighed in sterilized Petri dishes and treated with appropriate sterile water solutions of atrazine and metolachlor to provide a final concentration of 5 mg a.i. kg^{-1} soil (air-dry basis).

Soil moisture of the 144 samples was adjusted to the field capacity by adding sterile ultrapure water. The moisture content of surface and subsurface soil at field capacity (–33 kPa) was determined by using a porous pressure-plate apparatus. Soil samples were kept in the dark in a climatic chamber at 15°C. Aerobic conditions were maintained by keeping the Petri dishes uncovered. The moisture level of the soil samples incubated in aerobic conditions was checked daily by weighing and adjusted using sterile ultrapure water. Soil samples incubated in anaerobic conditions were kept in the same chamber but Petri dishes were arranged in anaerobic jars (AnaeroJar AG 25). Anaerobic jars were hermetically sealed and anaerobic conditions were established by using reagent AnaeroGen AN 25, which is able to consume O_2 and release CO_2. A colorimetric indicator (Anaerobic Indicator) allowed us to check if anaerobic conditions were maintained. AnaeroJar AG 25, AnaeroGen AN 25 and Anaerobic Indicator were provided by Oxoid (Basingstoke, Hampshire, UK). No significative water losses were observed during the incubation period.

Different incubation intervals were employed for aerobic and anaerobic incubation conditions. Incubation intervals were 0, 5, 10, 20, 40 and 60 days for the aerobic conditions and 0, 20, 40, 60, 120 and 140 days for the anaerobic conditions.

The whole experiment was designed as a 2×2×2 factorial, replicated 3 times, with one variable (half-life) and with sterility (active and sterile soil), O_2 level (aerobic and anaerobic conditions) and soil depth (surface and subsurface soil) as factors. Least significant differences were determined by standard *t*-test at the 0.05% level of significance after ANOVA.

HPLC analysis of herbicides

Soil contained in Petri dishes was removed and placed in glass vials. Atrazine and metolachlor were extracted from the soil by adding 10 ml acetonitrile (gradient grade, Merck) to each vial and shaking vigorously for 2 h in a horizontal shaker at room temperature. The supernatant was filtered through a 0.2-µm filter and directly analysed on a Beckman (Palo Alto, Calif.) System Gold 126 liquid chromatograph with a 250×4.6-mm Beckman Ultrasphere C_{18} 5-µm particle size column. The compounds were detected at 222 nm and 210 nm using a Beckman Model 168 dual-wavelength UV detector. A Spark Holland Basic Marathon (Emmen, The Netherlands) autosampler was employed. Atrazine and metolachlor analyses were done in gradient mode at the flow rate of 1 ml min^{-1}. The gradient separation of atrazine was performed by main-

Table 1 Soil properties. *n.d.* Not determined

Soil depth (cm)	Particle size[a]			Moisture content[b] (–33 kPa) (%)	pH[c] (1:2.5)	Eh[d] (mV)	Total N[e]	Organic C[f]	Biomass C[g] (µg C g^{-1} air-dried soil)
	Sand	Silt	Clay				(%)		
	(%)								
0–20	40	29	29	28.2	8.1	474	0.6	0.7	63.2
20–40	38	31	31	30.1	8.1	n.d.	0.5	0.6	n.d.
40–60	40	30	30	29.8	8	n.d.	0.5	0.6	31.1
60–80	40	30	30	26.1	8	n.d.	0.3	0.4	n.d.
80–100	38	31	31	27.8	8	305	0.2	0.3	8.4

[a] Determined by hydrometer method
[b] Determined by porous plate/pressure apparatus
[c] Determined in 1:2.5 soil:water suspension
[d] Determined in fresh soil by Pt electrode
[e] Determined by Kjeldahl digestion
[f] Determined by dichromate oxidation
[g] Determined by chloroform fumigation-incubation method

taining initial conditions at water-acetonitrile (70:30) for 1 min, then increasing the acetonitrile content linearly for 10 min to reach a final water-acetonitrile ratio of 40:60. Metolachlor separation was done by maintaining the initial water-acetonitrile (45:55) ratio for 1 min, then increasing the acetonitrile content linearly for 10 min to reach a final water-acetonitrile ratio of 40:60. The injection volume was 50 µl for each compound. Retention time and diode array scan of analytical grade atrazine and metolachlor were employed for identification of active ingredient in soil extracts. Extraction efficiencies of atrazine and metolachlor were determined in soil samples at different times of the experiment and were always above 92%. Peak area was used for residue quantification. Degradation half-lives were calculated by linear regression of the natural logarithm of the percentage of herbicide remaining against the time and the slope of each line compared with ANOVA.

Microbial biomass measurements

Soil microbial C was measured by a slight modification of the original method proposed by Jenkinson and Powlson (1976). Soil of three different depths (0–20, 40–60 and 80–100 cm) was employed. Five replicates of each treatment (fumigated and unfumigated soil) and soil depth were adopted. Twenty-five grams of soil (air-dry basis) was weighed in culture tubes (3 cm internal diameter×10 cm). For the fumigation treatment, the moisture content of soil samples was adjusted to 50% of the field capacity and fumigated with alcohol-free $CHCl_3$ for 24 h. After the removal of $CHCl_3$ vapour, the soil moisture was adjusted to the field capacity by adding an equivalent volume of distilled water plus a small volume (0.3 ml) of liquid obtained by filtration of a peat sludge through a 0.4-µm filter. The filtered peat sludge was added to obtain a suspension rich in microorganisms and to assure a homogeneous distribution in the fumigated soil mass. Unfumigated soil samples were moistened to the field capacity using ultrapure sterile water. Fumigated and unfumigated soil samples were incubated in 500-ml sealed glass cylinders containing 15 ml of 0.5 M NaOH in separate vials. Five cylinders without soil samples were used as the control. Soil samples were kept in the dark in a climatic chamber at 25°C. Cylinders were opened 18, 42, 66, 90 and 120 hours after the incubation started and NaOH solutions removed. CO_2-C recovered in each NaOH solution was measured by titration following addition of $BaCl_2$. Biomass C (B_c) was calculated from a modification of the expression proposed by Jenkinson and Powlson (1976): $B_c=F_c/k_c$ where F_c=(CO_2-C evolved by fumigated soil during 0–5 days) minus (CO_2-C evolved by non-fumigated soil during 0–5 days). A k_c factor of 0.45 was used for converting the CO_2-C flush to biomass C content (Jenkinson and Ladd 1981). As originally proposed by Jenkinson and Powlson (1976), biomass C is calculated considering an incubation time of 10 days. Preliminary tests with soil used for the experiment showed that incubation of soil samples for 5 and 10 days did not cause differences in calculated soil biomass C values. Consequently, a 5-day incubation was adopted to estimate biomass C.

Soil redox potential measurements

Changes in oxidation-reduction status (redox potential, Eh) in soil samples incubated in anaerobic conditions were estimated. Twenty-five grams of soil from 0–20 and 80–100 cm depths was weighed in culture tubes (3 cm i.d.×10 cm). Autoclaved soil samples of the two soil depths were included. Soil samples were separately treated with appropriate sterile water solutions of atrazine and metolachlor to provide a final concentration of 5 mg a.i. kg^{-1} soil (air-dry basis). Soil moisture was adjusted to field capacity by adding sterile ultrapure water. Soil samples were incubated at 15°C using the same anaerobic system previously described. Soil Eh was tested at 0, 20, 40, 60 and 140 days after incubation. For each soil depth, sterilization treatment, herbicide and sampling interval, three replicates were adopted.

Soil Eh was measured using a combined platinum and Ag/AgCl as reference electrode attached to a pH/mV meter (model GLP 22; Crison Instruments, Alelle, Spain). The electrode was tested in a 468-mV standard solution provided by Crison Instruments.

For each sample interval, anaerobic jars were opened and test tubes were immediately sealed to prevent changes in oxidation-reduction status. Soil Eh was measured after the electrode, introduced 2.5 cm below the soil surface, had equilibrated in soil (Wang and Bettany 1994). According to McGeehan and Naylor (1994), the head space of each sample was purged with O_2-free N_2 gas during Eh measurements. To achieve this, test tubes were introduced into a plastic cylinder saturated with O_2-free N_2 gas. The reported Eh values are weighted averages of the potentials of all the redox couples present (Bohn 1968).

Results and discussion

Atrazine and metolachlor soil degradation

Atrazine degradation in surface (0–20 cm) and subsurface (80–100 cm) soil followed first-order kinetics both under aerobic and anaerobic conditions. The coefficients of regression of the natural logarithms of concentration against time ranged from 0.92 to 0.97 and were significant ($P<0.05$), thus indicating that the assumption of first-order kinetics was acceptable.

The three main effects, namely soil sterility, soil depth and incubation condition, were statistically significant (Table 2). In microbially active soil, the mean half-life of atrazine (175 days) was 2.4 times lower than that in sterile soil (422 days). In surface soil, atrazine showed a faster degradation with a mean half-life (245 days), which was 1.4 times lower than that in subsurface soil (352 days). In addition, the aerobic incubation of soil

Table 2 Atrazine half-life (days) (±SE) under aerobic and anaerobic conditions as a function of soil depth and sterilization treatment. Mean values followed by the *same letter* are not significantly different as determined by ANOVA and a standard *t*-test of the 0.05% level of significance

	Aerobic incubation		Anaerobic incubation		Mean
	Active	Sterile	Active	Sterile	
Surface soil (0–20 cm)	49.9±5.5 (a)	113.6±3.9 (b)	123.8±2.2 (c)	693.2±5.8 (e)	245.1 (A')
Subsurface soil (80–100 cm)	119.5±5.6 (b)	110.0±3.7 (b)	407.7±2.1 (d)	770.2±1.1 (e)	351.8 (B')
Mean	84.7 (a')	111.8 (b')	265.7 (c')	731.7 (d')	
Mean	98.2 (A)		498.7 (B)		

Table 3 Metolachlor half-life (days) (±SE) under aerobic and anaerobic conditions as a function of soil depth and sterilization treatment. *degr.* Degradation

	Aerobic incubation		Anaerobic incubation	
	Active	Sterile	Active	Sterile
Surface soil (0–20 cm)	37.5±3.2	No degr.	No degr.	No degr.
Subsurface soil (80–100 cm)	No degr.	No degr.	No degr.	No degr.

samples caused a 5.1 times increase in atrazine degradation (98 days) with respect to the soil incubated under anaerobic conditions (499 days). These results confirm the primary role of biological processes under aerobic conditions in soil degradation of atrazine (Assaf and Turco 1994; De Laune et al. 1997; Miller et al. 1997; Issa and Wood 1999).

The statistically significant interaction of soil sterility×soil depth×aeration allowed us to hypothesize the involvement of different degradation processes in soil transformation of atrazine (Table 2). Under aerobic conditions, similar half-lives of atrazine were observed in active subsurface soil (120 days) and in sterile soil from both surface (114 days) and subsurface soil (110 days). In contrast, in non-sterile surface soil atrazine showed a faster degradation with a half-life (50 days) which was approximately 2.3 times less than that observed in the other aerobic soils. Atrazine dissipation in sterile soil was postulated to be mainly due to chemical hydrolysis (Assaf and Turco 1994). Results from the present study confirmed this and showed that both biological and chemical degradation are involved in atrazine degradation in surface and subsurface soils. This is in agreement with the findings of Miller et al. (1997) and Rodriguez and Harkin (1997).

Under anaerobic conditions, a modest transformation of atrazine was observed, as also reported by Chung et al. (1995) and De Laune et al. (1997). In active soil, atrazine was less persistent in surface (124 days) than in subsurface soil (408 days). In addition, in sterile soil, atrazine showed a slower degradation with a half-life (732 days) 2.7 times higher than that in active soil (266 days). No significant difference was found in atrazine degradation from sterile surface and subsurface soil. Furthermore, under anaerobic conditions, the abiotic transformation of atrazine (733 days) was markedly slower than under an aerobic environment (112 days). Evidence that atrazine shows similar behavior in subsurface environments was provided by Druliner (1989), who observed an inverse correlation between atrazine and dissolved O_2 concentrations in a Nebraska aquifer.

Metolachlor degradation was observed only under aerobic conditions in active surface soil (Table 3), with first-order kinetics (r^2=0.94; P<0.05), and an estimated half-life of 38 days. No metolachlor transformation was recorded under aerobic and anaerobic incubation conditions in active subsurface soil or sterile surface and subsurface soil. These findings are in agreement with those of Bouchard et al. (1982) and Miller et al. (1997), who reported microbial degradation under aerobic conditions as the prevalent transformation pathway of metolachlor in soil.

Soil properties

The respiration trends of fumigated and unfumigated soil samples collected at three soil depths (0–20; 40–60 and 80–100 cm) are shown in Fig. 1. In the 120-h incubation, the cumulative respiration of unfumigated soil from 0–20 cm depth was approximately doubled with respect to those of unfumigated soil from 40–60 and 80–100 cm depths. Soil fumigation caused a significant increase in CO_2 evolution. In particular, the cumulative CO_2 emission of fumigated surface soil was 2.4 and 4.3 times higher than that of soil from 40–60 and 80–100 cm depths, respectively. During incubation, different respiration trends of fumigated and unfumigated soil were observed as a function of soil depth. In surface soil, after 10-h incubations, the respiration rate of fumigated samples was statistically different from basal respiration (i.e. unfumigated samples). In contrast, for both 40–60 and 80–100 cm soil depths a relevant lag-phase was observed. In soil from 40–60 and 80–100 cm depths, the CO_2 emission of fumigated soil was statistically different from that of unfumigated soil after a 40- and 60-h incubation, respectively. The absolute values of CO_2 evolution and the trends of soil respiration are linked to the decline of microbial biomass throughout the soil profile. In the 0–100 cm soil depth, no relevant changes in texture or pH were found as a function of soil depth (Table 1). Conversely, relevant decreases in total N, organic C and microbial biomass were observed. In particular, the microbial biomass C of surface soil (0–20 cm) was 2.0 and 7.5 times higher than that found in soil from 40–60 and 80–100 cm depths, respectively. A similar decrease in soil biomass throughout the soil profile is reported by Speir et al. (1984) and Willems et al. (1996). The observed decline of soil biomass may explain the slow degradation of atrazine and the metolachlor persistence in subsurface soil (80–100 cm) under aerobic conditions.

According to Bohn (1971) and Erhardt-Zabik and Wolt (1996), Eh values recorded in active 0–20 (Eh= 462 mV) and 80–100 cm (Eh=330 mV) soil correspond respectively to oxidizing and moderately reducing conditions (Table 1). As a consequence, the incubation of soil from the 80–100 cm depth under aerobic conditions presumably represented an alteration with respect to the original environment for optimal growth of subsurface microorganisms. In agreement with Tratnyek and Wolfe (1990), a significant modification of soil redox potential due to heat sterilization was observed (data not shown). In particular, the sterilization treatment of samples from 80–100 cm soil depth caused a shift in the redox poten-

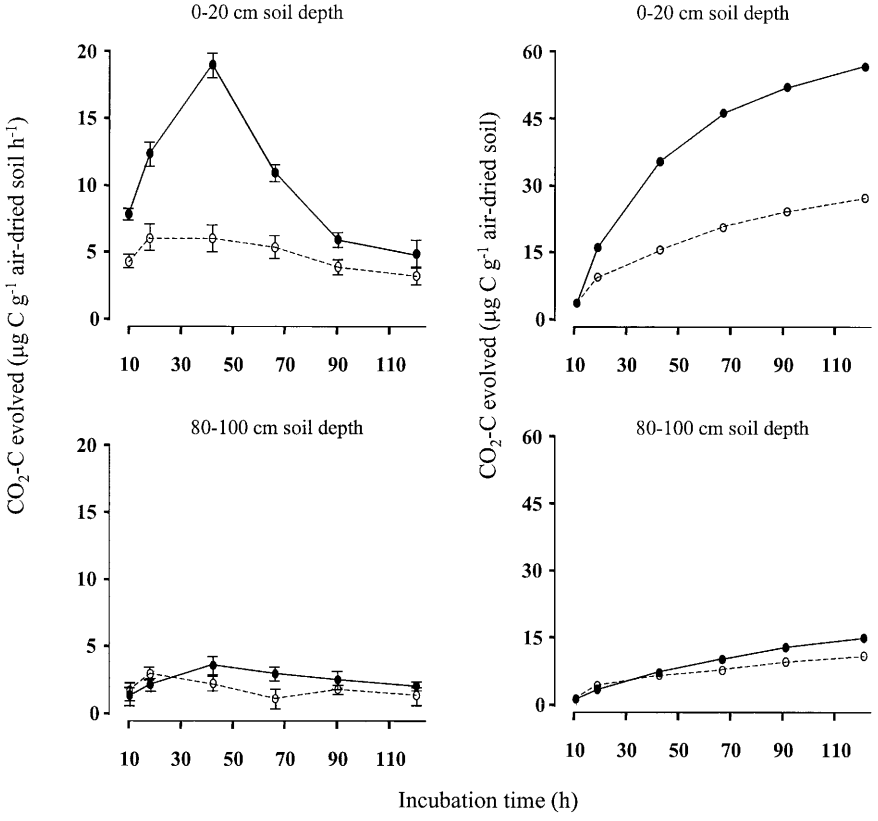

Fig. 1 Respiration rate (*left*) and cumulative respiration (*right*) of fumigated (●) and unfumigated (○) soil samples of two different soil depths (*above* 0–20 cm; *below* 80–100 cm). *Bars* represent SEs (n=5)

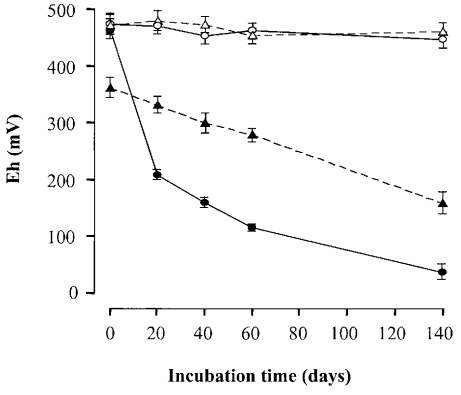

Fig. 2 Measured Eh values of surface soil (● non-sterile, ○ sterile) and subsoil samples (▲ non-sterile, △ sterile) as a function of incubation time. Since pesticide treatment did not influence Eh values, data shown represent the mean of treated samples with atrazine and metolachlor. *Bars* represent SEs (n=6)

tial from moderately reducing to oxidizing conditions. In addition, the incubation under anaerobic conditions produced different effects in sterile and active soil. No variation of Eh values was observed for sterile surface and subsurface soil (Fig. 2). On the contrary, a relevant decrease in redox potential was found in both active surface and subsurface soil. After a 140-days incubation of soil from both 0–20 and 80–100 cm depths, reducing conditions, corresponding respectively to Eh values of 45 and 190 mV, were recorded.

The different trends of Eh values in sterile and active soil support the hypothesis that decreasing Eh in soil is mainly due to biological activities under anaerobic conditions (Wolfe and Maclady 1992). On the basis of this finding, under anaerobic conditions the involvement of anaerobic biological degradation processes in the degredation of atrazine in active soil could be postulated (Adrian and Suflita 1994). The redox potentials in both surface and subsurface soils were not sufficiently low to support reductive dechlorination of atrazine (Boesten and Van Der Pas 1993). In order to identify the mechanisms of observed anaerobic biological degradation, further investigations based on the identification of metabolites are needed.

Overall, this study showed that atrazine and metolachlor degradation was influenced by both soil depth and redox levels as a function of aerobic and anaerobic incubation conditions. It is suggested that atrazine entering a soil profile characterized by reducing conditions (i.e. subsurface soil and aquifer) and low microbial activities is highly persistent. Except for surface soil, no biotic or abiotic degradation of metolachlor was observed in any of the incubations with subsurface soil. For these reasons, atrazine and especially metolachlor that leach into subsoil could represent a relevant risk for groundwater quality.

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