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Degradation of Alachlor in Natural and Sludge-Amended Soils, Studied by Gas and Liquid Chromatography Coupled to Mass Spectrometry (GC–MS and HPLC–MS)

Sonia Rodríguez-Cruz[†] and Silvia Lacorte*

Department of Environmental Chemistry, IIQAB-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

Alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide] is an herbicide used worldwide. The relative rates of disappearance of alachlor, the formation kinetics of alachlor ethane sulfonic acid (ESA), and the formation of other degradation products in two different soils (a soil with natural organic matter and a sludge-amended soil) has been studied. For such a purpose, soil samples were spiked with alachlor at 2.5 mg kg⁻¹, concentration generally applied in agricultural soils, and were submitted to sunlight, simulating natural field conditions. Extracts were analyzed by GC-MS and HPLC-MS in scan mode. A good correlation was observed between both techniques, and HPLC-MS allowed the determination of two eluting peaks corresponding to the two stereoisomeric forms of alachlor ESA. Degradation of alachlor in the two soils followed first-order kinetics. Half-life in the natural soil was 4.2 \pm 0.1 days, and half-life in the sludge-amended soil was 5.8 \pm 0.8 days. The higher half-life observed in the sludge-amended soil was attributed to the higher sorption of alachlor to this soil compared to the natural soil. The degradation of alachlor in both soils gave rise to the production of alachlor ESA. Its concentration increased during the incubation period, and after 27 days, its concentration was about 0.59 mg kg⁻¹ in the natural soil and 0.37 mg kg⁻¹ in the sludgeamended soil. The other two alachlor transformation products were identified using GC-MS, and the abundance of these degradation products increased while alachlor was degraded.

KEYWORDS: Alachlor; alachlor ESA; GC-MS; HPLC-MS; soil; degradation

INTRODUCTION

Alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide] is a herbicide used in pre-emergence to control annual grasses and many broad-leaved weeds in corn and other crops. It has been classified by the World Health Organization (1993) as a human carcinogen. Because of its widespread use, alachlor has been found in both surface water and groundwater from different countries (1, 2). In Spain, alachlor has been detected in soil and water as a result of its usage in corn cultivation (3–5).

Alachlor is highly to moderately mobile in soil, and the mobilization decreases with an increase in organic carbon (OC) and clay content in soil. Soil organic matter is the major factor for alachlor adsorption, and it is sorbed in a lesser extent by clay colloids (6). The magnitude and adsorption mechanism of this herbicide are affected not only by the amount but also by the nature and properties of soil organic matter. The application of organic amendments such as municipal sewage sludge to soil may affect the status of soil organic matter and influence its

properties (7), increasing pesticide sorption (6). It has been observed that, when the amount of humic acids increased, alachlor degradation was significantly slowed and the degradation kinetics were shifted to a first-order model (8).

In soil, alachlor is transformed to its metabolites primarily by biodegradation. The half-life of its disappearance from soil has been reported to be between 4 and 8 days (9-11), although very little mineralization has been observed (12). This indicates that biotransformation proceeds via cometabolism (13). Rates of the loss of alachlor in soil can be increased as the temperature and soil moisture increase (14, 15).

Because of biological dechlorination, alachlor is transformed into its corresponding ethane sulfonic acid (ESA) metabolite in soil (9, 16). In a field-disappearance study, the observed disappearance half-life was 8 days and alachlor ESA was formed at a fast rate. Alachlor ESA is a more polar compound than alachlor and has been found more frequently and at higher concentrations than the parent herbicide in groundwater because of leaching and in surface water because of agricultural runoff (16–19). The presence of alachlor ESA in waters can create toxicological effects toward soil and aquatic organisms (20), and it is necessary to survey both the parent compound and metabolite to understand the environmental fate of this herbicide in soil and prevent groundwater contamination.

^{*} To whom correspondence should be addressed. Telephone: +34-934-006169. Fax: +34-932-045904. E-mail: slbqam@cid.csic.es.

[†]Current address: Department of Environmental Chemistry and Geochemistry, IRNA-CSIC, Cordel de Merinas 40-52, 37008 Salamanca, Spain. Telephone: +34-923-219606. Fax: +34-923-219609. E-mail: sorocruz@ usal.es.

The objectives of this study were (i) to determine the relative rates of disappearance of alachlor in soils, (ii) to investigate the formation kinetics of alachlor ESA, and (iii) to identify other degradation products formed. For such purposes, gas chromatography coupled to mass spectrometry (GC–MS) and highperformance liquid chromatography coupled to mass spectrometry (HPLC–MS) both in scan mode were used. Samples were analyzed using these two techniques for comparison reasons and to detect potential degradation products formed during the exposition time. Two different soils were used, a soil with natural organic matter and a sludge-amended soil. Both soils were from agricultural fields from Catalonia (NE Spain).

MATERIALS AND METHODS

Reagents and Standards. All solvents (methanol, ethyl acetate, and water) were HPLC-grade and were supplied by Merck (Darmstadt, Germany). Alachlor was supplied by Riedel-de Haën (Seelze, Germany), and alachlor metabolite, ESA, was supplied by Monsanto Chemical Co. (St. Louis, MO). Alachlor d_{13} was purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Soil Collection. Two different agricultural soils, a natural soil and a sludge-amended soil, were taken from the 0–15 cm depth (topsoil) at two different agricultural fields in Catalonia. Natural soil characteristics were pH 7.5, 24.8% clay, 26.2% silt, 49.0% sand, 0.176% nitrogen, 1.4% carbonates, and 2.1% total OC. Sludge-amended soil corresponded to a sludge addition of 20 t ha⁻¹, and its characteristics were pH 7.5, 0.122% nitrogen, 54% carbonates, and 2.5% total OC. The clay, silt, and sand composition of sludge-amended soil have not been included because their contents cannot be determined accurately owing to the high percentage of carbonates in this soil. According to the official protocols of soil analysis (21), when the percentage of carbonates is >25%, the texture of the soil is not included because it cannot be given accurately. The original sludge contained 46.1% organic carbon.

Soil samples were individually homogenized by hand to give uniformly mixed samples free of stones and plant residues. The soil was air-dried overnight and sieved (<2 mm). Sieved soil was placed into a plastic bag and stored at 4 °C for less than 2 weeks prior to the start of the experiments. Subsamples (10 g) of each soil were dried in an oven at 110 °C overnight to determine soil moisture content.

Soil Preparation and Incubation. A solution of the analytical-grade alachlor in water was added to 300 g of each soil to give a concentration of 2.5 mg kg⁻¹ (this represents the recommended dose of pesticide application) with soil moisture at 50% water-holding capacity. The herbicide was uniformly mixed into each sample with a spatula. Each soil was transferred to one sterile glass container, which were loosely capped and incubated outdoors at ambient temperature and exposed to sunlight, simulating natural field conditions. The experiment was conducted in March 2005. During the experiment, the temperature was recorded at a meteorological station located close to the site where the glass containers were maintained. The average total daily short-wave radiation of this period was 350 W m⁻², and the mean daily temperature was 15 °C (http://www.infomet.cfr.es/metdata/). Soil was exposed to normal daylight for up to 27 days. An aliquot (about 5 g) of each soil sample was weighed and dried to correct for the percent moisture content of the soil. Moisture contents, maintained by the addition of HPLC water as necessary, were 17.0 and 14.3% for natural and sludgeamended soil, respectively.

Alachlor Extraction Procedure for Soil. A modified version of the extraction method used by Aga and Thurman (9) was used. A total of 1000 ng of surrogate standard (alachlor d_{13}) was added to the soil prior to extraction. A total of 10 g of soil was extracted in duplicate with 20 mL of a 75:25 (v/v) methanol/water mixture in a Teflon-lined, screw-capped glass tube. The soil mixture was extracted by sonication for 10 min. Then, each sample was centrifuged for 10 min, and the clear supernatant was collected into a 40 mL vial. The extraction procedure was repeated on the same soil sample, and the second supernatant was combined with the first. The combined extracts were evaporated at 30 °C using a Reacti-VapIII (Pierce, Rockford, IL) until

only 10 mL of water remained. To eliminate the water present in the extraction medium and to remove coextracted matrix impurities, the extract was transferred to a vial for automated solid-phase extraction (SPE) using 60 mg Oasis cartridges (Waters, Milford, MA). The Oasis cartridges were preconditioned sequentially with methanol (1 mL), ethyl acetate (1 mL), methanol (1 mL), and water (3 mL). Then, the soil extracts were passed through the cartridge and eluted first with ethyl acetate (15 mL) followed by methanol (15 mL). Alachlor was eluted in ethyl acetate, whereas its more polar ESA metabolite was eluted in methanol. The recoveries of the method were determined by spiking 10 g of soil with a standard mixture containing alachlor and alachlor ESA to a final concentration of 0.2 mg kg⁻¹ and performing the extraction procedure as depicted above. This spiking level was chosen assuming that degradation of alachlor would occur during the incubation to yield 10% or less of the initial concentration. Unspiked soil samples did not contain any traces of alachlor nor alachlor ESA.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. The extracts were analyzed by gas chromatography equipped with a mass spectrometric detector (GC/MS) using a Fisons 8000 Series GC (Finnigan, Sunnyvale, CA) operated in electron ionization mode. A fused-silica capillary column (30 m, 0.25 mm i.d., 0.25 μ m film thickness) containing 5% phenyl and 95% methyl polysiloxane (HP 5MS) was used. Helium was the carrier gas at a flow rate of 1 mL min⁻¹. A total of 2 μ L of extract was injected in splitless mode (spitless time of 0.80 min). The oven was set at 60 °C (1 min), and the temperature was raised to 180 °C at 8 °C min⁻¹ and to 250 °C at 8 °C min⁻¹. The final temperature was held for 1 min, and the total run time was 25 min. Acquisition was performed in scan mode from 75 to 350 amu.

Alachlor and ESA were quantified by internal standard calibration with alachlor d_{13} as an internal standard for both compounds. Quantification was performed using the base peak at m/z 146 for alachlor and alachlor ESA and m/z 156 for alachlor d_{13} . Calibration was performed from 0.01 to 25 μ g ml⁻¹. Data were acquired and processed using the Xcalibur software.

Liquid Chromatography-Mass Spectrometry Analysis. Samples were also analyzed by HPLC-MS for comparison reasons and to explore whether other degradation products might be formed. The HPLC system consisted of an HP 1100 autosampler with a 100-µL loop and HP 1090 A LC binary pump, both from Hewlett-Packard (Palo Alto, CA). Compounds were separated on a $250 \times 4 \text{ mm i.d.}, 5$ μ m particle C₁₈ reversed-phase column (Lichrospher 100 RP-18) preceded by a guard column (4 \times 4 mm, 5 μ m) of the same packing material (Merck, Darmstadt, Germany). The injection volume was 40 μ L, and the flow rate was 1 mL min⁻¹. The mobile phase used was solvent A (0.3% acetic acid in water) and solvent B [0.3% acetic acid in methanol/acetonitrile (1:2)] (22). The gradient consisted of 10% B, which increased linearly to 40% B in 12 min and then to 100% B in 13 min. The final percentage of solvent B was maintained for 1 min, and the total run time was 26 min. Detection was carried out using a HP 1040M diode-array UV-vis detector coupled in series with an LC-MSD HP 1100 mass-selective detector, equipped with an electrospray (ESI) interface. DAD detection was performed at 196 nm for alachlor and 205 nm for alachlor ESA. The extraction voltage used for alachlor was 65 V in positive ionization mode, and for ESA, the extraction voltage was 85 V in negative ionization mode.

Operating conditions of the MS system were optimized in the scan mode (scan range, m/z 103-400) in the positive and negative ionization mode. The drying gas flow was set at 12 L min⁻¹; the nebulizer pressure was 55 psi; the drying gas temperature was 250 and 350 °C; and the capillary voltage was 3500 and 4500 V in the positive and negative ionization mode, respectively. Diagnostic ions used for quantification were m/z 238 [M - OCH₃]⁺ for alachlor in PI mode and m/z 314 [M - H]⁻ for alachlor ESA under NI mode.

Calibration curves were generated from 0.01 to 25 μ g mL⁻¹. Quantitative analysis was performed using the base peak of each compound obtained from the total ion chromatogram (TIC). For alachlor (PI mode), internal standard calibration was performed using alachlor d₁₃. No internal standard was available to quantify ESA in negative ionization mode, and therefore, external standard quantification was used. Data were acquired and processed by use of the ChemStation

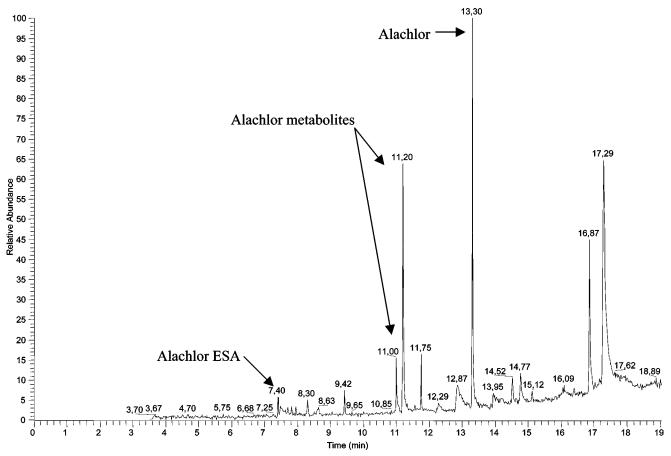


Figure 1. GC–MS chromatogram in scan mode corresponding to a soil extract from sludge-amended soil after 27 days of incubation. Peaks correspond to alachlor and different alachlor metabolites.

software (Agilent Technologies). Each analytical data reported represents the mean value of two GC-MS or LC-MS determinations.

RESULTS AND DISCUSSION

Analytical Determination of Alachlor and Alachlor ESA. There were no background interferences in either GC–MS nor HPLC–MS analysis of soil extracts; therefore, the use of Oasis SPE cartridges was efficient in eliminating coextracted matrix compounds. The few matrix peaks appearing at the end of the chromatogram did not interfere with the identification and quantification of alachlor nor alachlor ESA. With GC–MS, target analytes were well-resolved with the chromatographic run used. Calibration curves were linear in the 0.01–25 μ g mL⁻¹ range, with correlation coefficients being 0.998 for alachlor and 0.999 for alachlor ESA. Retention times for alachlor ESA, alachlor d₁₃, and alachlor were 7.40, 13.20, and 13.30 min, respectively, when they were analyzed by GC–MS (**Figure 1**).

Main ions m/z for alachlor ESA, alachlor d₁₃, and alachlor are indicated in **Table 1**. Two other alachlor transformation products were found using GC–MS. The abundance of these degradation products increased when alachlor was degraded, but they could not be quantified because of the lack of a standard. The retention times of these alachlor metabolites were 11.00 and 11.20 min (**Figure 1**). These two metabolites were detected even at the end of the incubation period (27 days). The first metabolite presented characteristic m/z ions at 161 (100) $[M - C_3H_6O_2]^+$, m/z 146 (87) $[M - C_4H_8O_2]^+$, m/z 178 (61) $[M - C_3H_6O_2]^+$, and m/z 235 (19) $[M]^+$. The second metabolite presented ions at m/z 160 (100) $[M - C_2H_5O_2]^+$, m/z 146 (58) $[M - C_3H_6O_2]^+$, m/z 189 (38) $[M - CH_3OH]^+$, and m/z 221 (29) $[M]^+$ as main ions. These two metabolites were tentatively identified as *N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide and alachlor metabolite number 14 according to Mangiapan et al. (23). *N*-(2,6-Diethylphenyl)-*N*-(methoxymethyl)acetamide is formed from dechlorination of alachlor (24).

With HPLC-MS, alachlor was detected at its protonated form or with sodium adduct, and the loss of a methoxy group was observed (**Table 1**). The internal standard, alachlor d_{13} presented the same fragmentation as the native compound (**Table 1**). For alachlor ESA, the only ion formed was at m/z 314 [M - H]⁻.

The mean recovery values of alachlor from soils spiked at 0.2 mg kg⁻¹ were of 103 and 116% for both natural and sludgeamended soils, respectively, when samples were analyzed by GC-MS. The slight higher recovery of alachlor in sludgeamended soil can be explained by the phenomena known as "matrix-induced chromatographic response enhancement" that can occur for particular pesticides, matrix types, and depending upon the status of the capillary columns (25). The influence of matrix co-extractives on the response of analytes is a well-known phenomenon in pesticide residue analysis, which can result in either a decreased detection response or an increased analytical signal (25). The mean recovery values of alachlor obtained by GC-MS were slightly higher than the respective recoveries obtained by HPLC-MS. HPLC-MS can cause ion suppression when analyzing complex matrixes such as sludge-amended soils, leading to lower recoveries, as observed for a wide range or organic compounds (26). The recoveries of this extraction procedure calculated when the samples were analyzed by HPLC-MS ranged from 93 and 95% for alachlor in natural and sludge-amended soil, respectively, and from 31 to 41% for ESA in the same soils, at a spiking concentrations of 0.2 mg

Table 1. Mass Spectral Characterization of Alachlor and Alachlor Metabolites (Ions, Chemical Structure, and Abundance), Obtained by GC-MS and HPLC-MS^a

compounds	MW	Rt (min)	HPLC-MS	Rt (min)	GC-MS
alachlor	269	24.48	292 [M + Na] ⁺ (63) 270 [M + H] ⁺ (50) 238 [M – OCH ₃] ⁺ (100)	13.30	269 [M] ⁺ (6) 188 [M - C_2H_5OCI] ⁺ (89) 160 [M - $C_3H_5O_2CI$] ⁺ (100) 146 [M - $C_4H_7O_2CI$] ⁺ (39) 118 [M - $C_5H_{10}O_7NCI$] ⁺ (21)
alachlor ESA	315	10.44–10.91	314 [M – H] [–] (100)	7.40	$\begin{array}{c} 161 \left[M - C_{3}H_{5}O_{5}S \right]^{+} (62) \\ 146 \left[M - C_{4}H_{7}O_{5}S \right]^{+} (100) \\ 118 \left[M - C_{5}H_{10}O_{5}NS \right]^{+} (84) \end{array}$
alalchlor d_{13}	282	24.50	305 [M + Na] ⁺ (95) 283 [M + H] ⁺ (90) 251 [M - OCH ₃] ⁺ (100)	13.20	207 [M - C ₂ H ₂ OCl] ⁺ (65) 174 [M - C ₃ H ₅ O ₂ Cl] ⁺ (51) 156 [M - C ₄ H ₇ O ₂ Cl] ⁺ (100) 124 [M - C ₅ H ₁₀ O ₂ NCl] ⁺ (62)
metabolite 1	235	nd ^b	nd ^b	11.00	235 [M]+ (19) 178 [M - C ₃ H ₆ O]+ (61) 161 [M - C ₃ H ₆ O ₂]+ (100) 146 [M - C ₄ H ₈ O ₂]+ (87)
metabolite 2	221	nd ^b	nd ^b	11.20	221 [M] ⁺ (29) 189 [M - CH ₃ OH] ⁺ (38) 160 [M - C ₂ H ₅ O ₂] ⁺ (100) 146 [M - C ₃ H ₆ O ₂] ⁺ (58)

^a Molecular weight (MW) and retention time (Rt, in minutes) are also given. ^b nd = not detected.

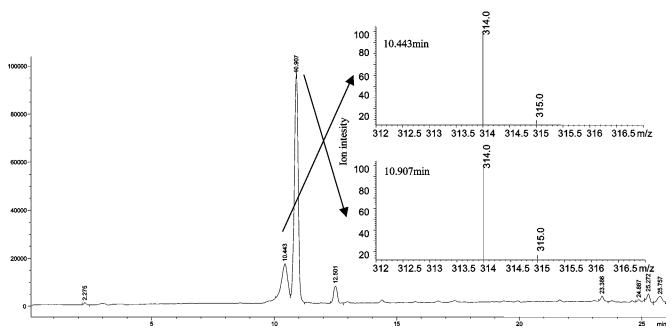


Figure 2. HPLC-MS chromatogram in negative ionization mode and under SIM conditions corresponding to a soil extract from sludge-amended soil after 15 days of incubation and mass spectrum for two stereoisomeric forms of alachlor ESA.

kg⁻¹. The concentrations of alachlor and ESA were corrected for recovery values.

Biological degradation of alachlor in soil results in the formation of the ESA derivative. This molecule exists in two stereoisomeric forms as a result of hindered rotation of the amide bond with respect to the substituted aromatic ring (27). At elevated temperatures, rapid conversion of the isomers occurs, where separation is not possible in the GC–MS analysis (28). However, by HPLC–MS, there were two eluting peaks corresponding to the *S*-cis and *S*-trans diastereomers. In the HPLC–MS chromatogram, the first eluting peak has a minor area in comparison with the second eluting peak but both peaks have the same main ion at m/z 314 corresponding to alachlor ESA (**Figure 2**). HPLC–MS was carried out at room temperature, and it has been observed that alachlor ESA exhibits split peak shapes at room temperature because of stereoisomerism (29).

Figure 3 shows the cis/trans relationship over the course of time. Within our work, we cannot determine whether the S-cis or S-trans isomer was eluting first. Cardoza et al. (27) pointed that elution of the S-trans or S-cis isomer depends upon the conditions of the separation applied, mainly on the solvent used. Alachlor ESA could present a changing isomeric ratio within a day (28), and probably, this can explain that in some samples there was only one visible isomer in the chromatogram. It has been proposed that there are changing amide S-cis/S-trans ratios when the analytes are dissolved from polar to nonpolar solvents (28). This changing preference of amide configurations may be important in the differential distribution of these compounds in soil constituents and bacteria and might influence the degradation rate and pathways of these compounds in the environment. The amide S-cis/S-trans isomerization can explain the difficulty in separating acetanilide herbicide metabolites

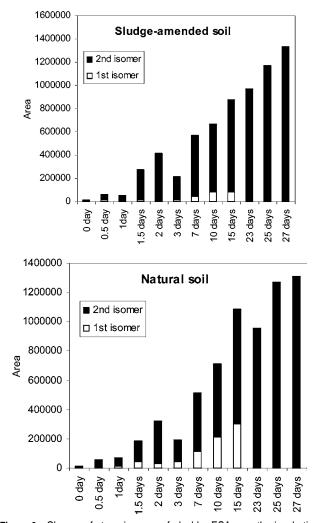


Figure 3. Change of stereoisomers of alachlor ESA over the incubation period (first and second isomers refer to the order of elution in the chromatogram).

using HPLC because broad and overlapping signals have been observed at room temperature (28). Alachlor transformation to alachlor ESA has an intermediate step, which involves a conjugation, mediated by glutathione-S-transferase (GST), between the chiral tripeptide glutathione and the chloroacetamide moiety of alachlor-forming alachlor—glutathione conjugates. After subsequent degradation of the conjugate moiety and oxidation of the SH group to sulfonic acid, this pathway may lead to the formation of alachlor ESA (13) and this degradation could be stereospecific (only a stereoisomer is produced rather than a mixture) (28).

Degradation of Alachlor in Soils. Degradation of alachlor was evaluated in a natural soil and an agricultural soil artificially enriched in organic matter by the addition of sewage sludge (20 t ha⁻¹). The degradation kinetics of alachlor and the formation of alachlor ESA derived from the analysis of soil extracts by GC–MS and HPLC–MS are showed in **Figure 4**. The data are plotted as residual concentrations of alachlor (μ g g⁻¹) or the amount of alachlor ESA produced (μ g g⁻¹) against the time of incubation for each soil. The alachlor residue levels in samples analyzed by GC–MS were found to be slightly higher than those analyzed by HPLC–MS (**Figure 4**). However, a good correlation was observed between the levels of alachlor and alachlor ESA in both natural and sludge-amended soils. The coefficent of correlation (R^2) was 0.9731 and 0.9907 for

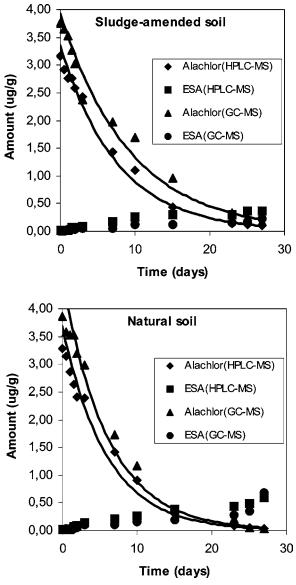


Figure 4. Degradation kinetic of alachlor and production of alachlor ESA derived from the analysis of soil extracts by GC–MS and HPLC–MS. Points are experimental data, and lines are fitting results using the first kinetic model.

alachlor in sludge-amended and natural soils, respectively, and R^2 of 0.9370 and 0.8439 for alachlor ESA.

The degradation of alachlor in the two soils followed a firstorder kinetic. Half-life was 4.1 days in the natural soil and 5.3 days in the sludge-amended soil when samples were analyzed by HPLC-MS. When samples were analyzed by GC-MS, values of half-life were not very different to those found with HPLC-MS, being 4.2 days in the natural soil and 6.4 days in the sludge-amended soil. The average half-life in the natural soil was 4.2 \pm 0.1 days, and in the sludge-amended soil, the average half-life was 5.8 \pm 0.8 days. Half-lives of alachlor in soils are similar to those reported in the literature (4-8 days)by different authors (9-11). In the present study, the higher half-life observed in the sludge-amended soil could be due to the higher organic matter content of this soil compared to the natural soil. Given that alachlor is mainly adsorbed onto the soil organic matter, it was expected that the degradation of alachlor would depend upon the OC content of the extracted soil. Sorption to clays and soil organic matter is likely to play an important role in influencing the bioavailability and retarding the migration of these compounds in subsurface environments (13). Several authors have suggested that organic matter could play an important role in enhancing alachlor sorption by amended soil (6, 7, 30). Sewage sludge may enhance herbicide sorption, decreasing the bioavailability and increasing the half-life of alachlor in agricultural soils. The properties and molecular structures of organic matter influence sorption in very different ways (31). In addition, the nature of the organic matter as polarity and degree of aromaticity influence sorption of pesticides on soils (32).

On the other hand, biodegradation is the single most significant mechanism in controlling the dissipation of alachlor in agricultural soils (13). Acetamide herbicides are relatively resistant to photolytic decomposition (33), although photodecomposition has been shown to occur in water (34). Because microbial degradation is the most important factor in determining alachlor fate in the environment, abiotic degradation was not investigated separately in this study but it was considered as a part of the degradation process.

During the incubation period, the concentration of alachlor ESA increased, and after 27 days, it was about 0.59 mg kg⁻¹ in the natural soil and 0.37 mg kg⁻¹ in the sludge-amended soil. The concentration of alachlor was about 0.03 mg kg⁻¹ in the natural soil and 0.10 mg kg⁻¹ in the sludge-amended soil after the incubation period.

On day 27, alachlor ESA represented 18.0 and 11.7% of the parent compound in natural and sludge-amended soil, respectively. At the end of the experiment, the concentration of alachlor expressed as percentage of the initial applied amount was 0.9% in natural soil and 3.2% in sludge-amended soil. The faster degradation of alachlor in the natural soil gave rise to a higher production of alachlor ESA after 27 days of incubation in comparison with the sludge-amended soil.

Although alachlor is degraded in agricultural soils, the use of sludge as an organic fertilizer affects its degradability rate, as demonstrated in the present study. The formation of alachlor ESA and other degradation products in agricultural soils produce contamination of groundwater because of the leaching potential of these compounds (35), thus affecting the quality of such when it is used for irrigation purposes or for human consumption. This study illustrates that sludge application as an organic fertilizer affects the degradation kinetics of alachlor. A greater comprehension of the role of amendments to influence the degradation of herbicides in soil will provide a better understanding of the bioavailability and potential toxicity of these contaminants and their metabolites. Furthermore, continued research is needed to identify major pesticide metabolites and to develop analytical methods to identify them in soil and other environmental media.

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