

## Metolachlor Photodegradation Study in Aqueous Media under Natural and Simulated Solar Irradiation

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To elucidate the photochemical behavior of pesticide metolachlor, degradation was carried out in aqueous media of different compositions such as sea, river, lake, and distilled water under natural and simulated solar irradiation. In addition, the effect of important constituents of natural water such as dissolved organic matter (DOM, isolated from Pamvotis Lake) and nitrate ions was also examined. It was found that photodegradation proceeds via a pseudo-first-order reaction in all cases. The presence of DOM inhibits the photolysis reaction with half-lives ranging from 87 to 693 h whereas the degradation rate was accelerated up to 11 times in the presence of  $\text{NO}_3^-$ . In addition, the toxicity of the degradation products formed (generally through hydroxylation, dealkylation, and cyclization reactions) was also performed using the marine luminescent bacterium *Vibrio fischeri*. Our results indicated a toxicity increase of the irradiated solution showing that photoproducts of higher acute toxic effects were formed.

**KEYWORDS:** Photodegradation; metolachlor; dissolved organic matter; fate

### INTRODUCTION

Current agricultural practices rely heavily on pesticides for crop production. The use of these anthropogenic chemicals is an integral part of world food production as illustrated by the fact that million of tons of pesticides are applied to soil and foliage each year. Recent findings that cite the presence of pesticides in drinking water supplies illustrate the fact that some fraction of the pesticides applied to a particular location can be transported off site and into surface waters. Given the potential human and wildlife health risks associated with toxic pesticides in surface waters, it is important to determine the probability that a certain chemical will persist in the environment by examining the various reactions of the molecule.

Metolachlor, first synthesized in 1976 by Ciba-Geigy, is a germination inhibitor used mainly for control of grassy weeds (1). It is heavily applied on crops such as soybeans, corn, and beans and is the most intensively used herbicide belonging to the chemical class of chloroacetamides. In the Mediterranean region, metolachlor is one of the most widely applied herbicides with annual usage exceeding 5740 kg (2). It is structurally similar (Figure 1) to several other widely used chloroacetamide herbicides such as alachlor, butachlor, and so forth. The structure, as the name implies, has been developed around the acetanilide moiety; however, the difference is manifested mainly

in the methoxyalkyl side chain. This moiety regulates selectivity, water solubility, and other such herbicidal properties.

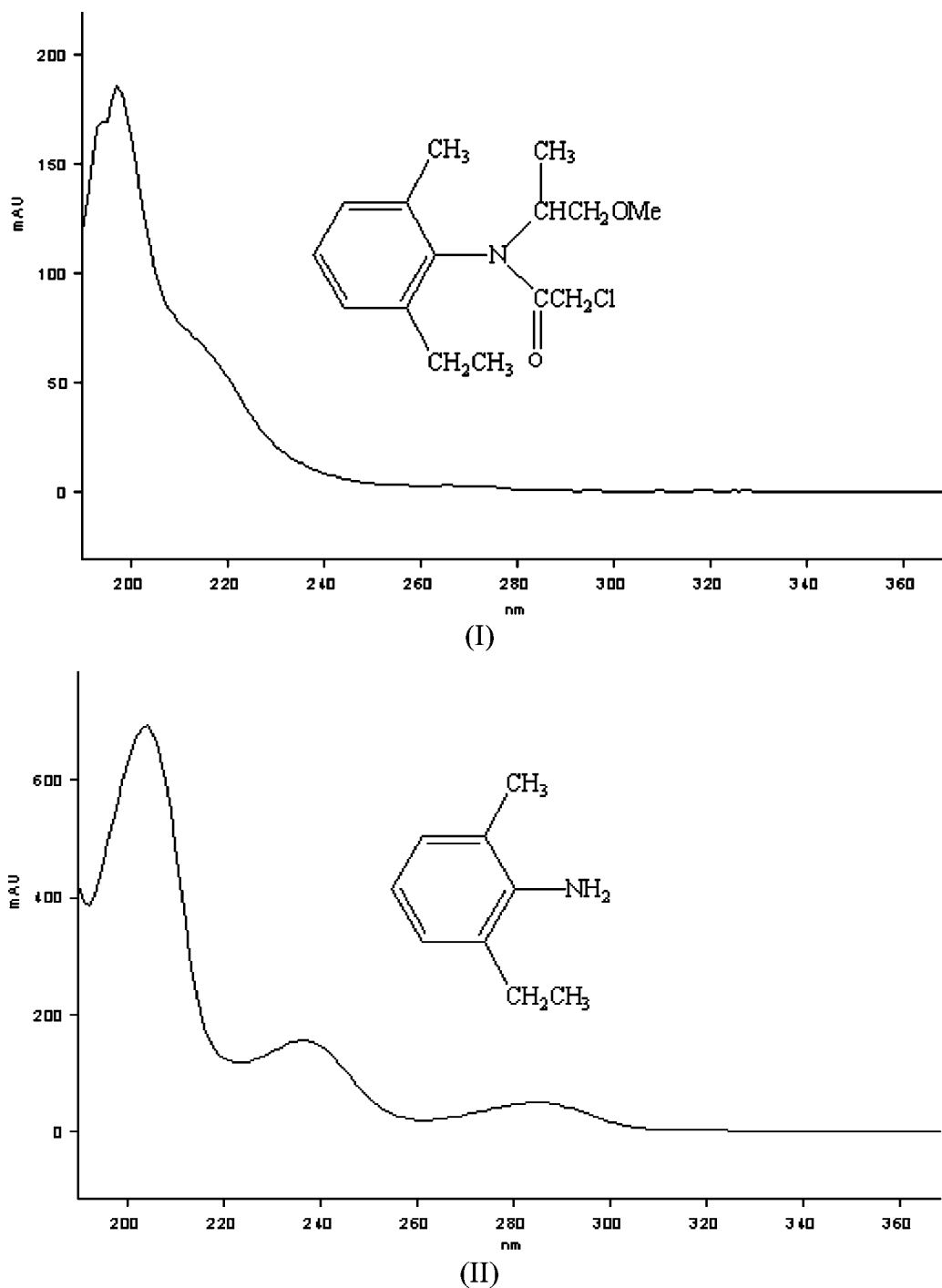
Although metolachlor is not registered as an aquatic herbicide, its residues carried from terrestrial applications do infiltrate ground and surface waters at concentrations ranging from 0.01 to 0.40  $\mu\text{g/L}$  (3–6). It has the potential to leach into groundwater because of its relatively high water solubility (550 mg/L at 20 °C) and low sorption to soil particles.

Several studies have examined the aquatic fate and persistence of metolachlor (7–10) indicating that it is fairly resistant to hydrolysis at a wide pH range (2–10). Less than 5% loss of the compound was observed after 50 days, except at pH 10, where a 20% loss took place. As far as the half-life of metolachlor in agricultural soil under field conditions is concerned, it has been reported to be between 15 and 50 days (11). Several studies have confirmed that microbial transformations are the essential mechanisms responsible for the degradation of metolachlor (12–15). Microorganisms capable of metabolizing it have been isolated to elucidate degradation using mixed and pure cultures. However, although dissipation of metolachlor in soil is caused by microorganisms, mineralization proceeds very slowly. During the past two decades, many authors have studied the biodegradation of metolachlor and other acylanilide herbicides by using selected microorganisms (16–19). After 6 weeks, less than 5% of ring [ $^{14}\text{C}$ ]-metolachlor has been recovered as  $^{14}\text{CO}_2$  (20). Among the various transformation products formed during metolachlor biotic and abiotic degradation, 2-ethyl-6-methylaniline is thought to be an environmentally stable aniline degradation product (21). The levels of this

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**Figure 1.** Metolachlor (I) and 2-ethyl-6-methylaniline (II) structures and UV/vis spectra.

metabolite are generally higher than metolachlor in water and sediment samples while toxicity tests have shown that it is more toxic than the parent compound (21). Therefore, a potential risk of toxic exposure on the environment exists, despite the absence or low levels of metolachlor, which is conventionally monitored.

Photolysis is one of the most important abiotic degradation pathways of organic micropollutants under environmental conditions. A few studies have dealt with metolachlor photodegradation mainly in lake waters and soils under various conditions (22–24). It is well known that natural waters contain a variety of dissolved, colloidal, and suspended organic and mineral constituents from soil or plant origin that might influence the photolysis of chemicals in water in different ways: either by reducing photolysis (quenching the excited states of organic molecules), shielding them from incident

radiation (25), or by acting as photosensitizers to promote photodegradation of organic micropollutants (26).

Moreover, naturally occurring nitrate ions in surface waters are a primary source of hydroxyl radicals, one of the principal intermediates in natural water photochemistry that are responsible for the degradation of organic contaminants (27). Therefore, sunlight-induced hydroxyl radical production by  $\text{NO}_3^-$  plays a significant role in the phototransformation of organic micropollutants. Hence, because of metolachlor's large application in agriculture and its persistence in the environment, it would be of interest to elucidate the major factors influencing the photochemical behavior of this micropollutant in the aquatic ecosystem. The latter becomes more interesting when degradation studies are carried out in natural field conditions, taking into consideration factors such as temperature variation and daily

**Table 1.** Characteristics of Selected Environmental Waters

water type	pH	conductivity ( $\mu\text{S}/\text{cm}$ )	TDS <sup>a</sup> (mg/L)	TOC <sup>b</sup> (mg/L)	salinity (‰)	NO <sub>3</sub> <sup>-</sup> (mg/L)
distilled	5.35	0.9	nd <sup>c</sup>	bdl <sup>d</sup>	nd	nd
Ionian Sea	7.62	50411	165	2.10	33.4	3.31
Louros River	7.81	382	208	2.93	0.40	2.28
Pamvotis Lake	7.87	516	361	11.03	0.44	10.30

<sup>a</sup> Total dissolved solids. <sup>b</sup> TOC = total organic carbon. <sup>c</sup> nd = not determined. <sup>d</sup> bdl = below detection limit.

and seasonal variation of natural sunlight intensity. Moreover, the study of the photochemical behavior of a contaminant is a key issue in terms of the formation of toxic transformation products. Therefore, toxicity assessment becomes necessary. Although several methods and organisms for direct acute toxicity assessment are available (28), the Microtox assay using the marine bacteria *Vibrio fischeri* is especially rapid and reliable (29).

To meet these requirements, we examined the aquatic photochemical degradation of metolachlor under field and laboratory conditions. The goals of this research were to (i) determine the photodecomposition kinetics of metolachlor in different natural waters under natural and simulated solar irradiation, (ii) account for the effect of isolated humic and fulvic substances (dissolved organic matter) as well as the nitrate ions on the reaction rate, and (iii) evaluate the toxicity of the irradiated solutions. An effort was also made to investigate the influence of irradiated solutions on the degradation products formed through direct and indirect photolytic reactions.

## MATERIALS AND METHODS

**Chemicals.** Metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-2-methoxy-1-methylethyl-acetamide] and 2-ethyl-6-methyl-aniline (metabolite) standards of high purity were purchased from Riedel-de Haen (Seelze, Germany) and Dr. Ehrenstorfer GmbH (Germany), respectively. Humic and fulvic acids were isolated from Pamvotis Lake using the IHSS isolation method preestablished by Thurman and Malcolm in 1981 by the so-called XAD technique (30). Pesticide analysis grade solvents (acetone, methanol, dichloromethane, and ethyl acetate) were purchased from Pestiscan (Labsacan Ltd., Dublin, Ireland). HPLC analysis grade solvents (methanol, acetonitrile, and water) were purchased from Merck (Darmstadt, Germany). Sodium sulfate (pro-analysis) and NaNO<sub>3</sub> were obtained from Merck and Riedel de Haen, respectively. SDB (styrene-divinylbenzene copolymer) extraction disks (47 mm) were purchased from 3M Empore (St. Paul, MN), and a conventional filtration apparatus was supplied from Supelco (Bellefonte, PA). Synthetic field water (SFW) solution consisted of distilled water at which appropriate amounts of isolated HA/FA and nitrate ions were added at concentrations close to environmental levels.

**Water Sampling.** Natural waters used in the experiments were collected from the Epirus region of northwestern Greece and were as follows: seawater (Ionian Sea), lake water (Pamvotis Lake), and river water (Louros River). The natural water samples were collected from the top meter of each water body in 2.5-L pre-cleaned amber glass bottles and stored at 4 °C prior to use. All water samples were used without previous treatment, filtration, or sterilization. Their physicochemical characteristics are given in Table 1. Concentration of total organic carbon (TOC) in the water samples was measured with a Shimadzu Total Carbon 5000 Analyzer (TOC-5000 Shimadzu) using the high-temperature catalytic oxidation method (HTCO).

**Chromatographic Conditions.** The LC system comprised a Shimadzu online DGU-14A degassing system coupled to an FCV-10AL controller unit and an LC-10AD high-pressure solvent delivery pump, with a 20- $\mu\text{L}$  sample loop injector and a Shimadzu SPD-M10A UV/diode-array detector (used at 220 nm). The column material was a Discovery C<sub>18</sub> (Supelco), with 5- $\mu\text{m}$  particles (25 cm  $\times$  4.6 mm i.d.)

with a guard column of the same material (8 mm  $\times$  3 mm). Isocratic elution with a mobile phase composed of water/acetonitrile (20/80% v/v) was used for the elution of the analytes from the column. The flow rate was 0.8 mL/min and the volume injected was 20  $\mu\text{L}$ . The oven temperature was set to 40 °C.

**Photolysis in Environmental Waters under Natural Solar Irradiation (Outdoors Experiment).** The natural waters as well as distilled water were spiked with an appropriate volume of metolachlor solution in methanol to give a concentration level of 5 mg/L. Then, the solutions were put into capped quartz glass reservoirs of 1000 mL and placed on the terrace of the Chemistry Department for the duration of June–July 2003. A dark control experiment was also performed by exposing aluminum foil covered reservoirs filled with the same pesticide solutions to the same environmental conditions. The average total daily short-wave radiation for this period was 578 W/m<sup>2</sup>, with 10 h mean sunshine duration from sunrise to sunset. The mean daily air temperature was 24 °C, while maximum and minimum air temperatures were 33 and 16 °C, respectively.

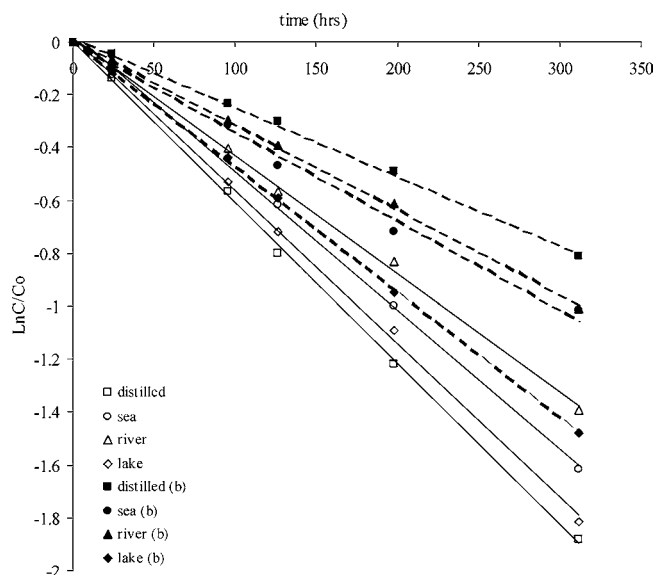
**Photolysis in Natural Waters under Simulated Solar Irradiation.** The photochemical stability of metolachlor was also tested using a Suntest CPS+ Photosimulator from Heraeus (Hanau, Germany), equipped with a xenon arc lamp (1500 W) and special glass filters restricting the transmission of wavelength below 290 nm. The light source was on the top of the reactor and an average irradiation intensity of 750 W/m<sup>2</sup> was maintained throughout the experiments measured by an internal radiometer. Chamber and black panel temperatures were regulated by pressurized air-cooling circuit and monitored using thermocouples supplied by the manufacturer. The temperature of samples did not exceed 25 °C using tap water cooling circuit for the UV-reactor. Irradiation experiments were carried out on a 10-cm-diameter cylindrical quartz glass UV-reactor by exposing 50 mL of the four different aqueous solutions (distilled, sea, river, and lake water) of metolachlor (5 mg/L) under artificial solar irradiation. A dark control experiment was also conducted in this series of experiments.

**Photolysis in the Presence of Isolated Humic/Fulvic Acids and Nitrate Ions.** To examine the effect of dissolved organic matter (DOM), aqueous solutions of metolachlor (5 mg/L) in distilled water (50 mL) were exposed to simulate solar irradiation using the Suntest apparatus and the same UV-reactor, in the presence of humic (HA) and fulvic (FA) acids isolated from Pamvotis lake. Concentrations of the acids were 4, 8, 16, and 24 mg/L. The influence of NO<sub>3</sub><sup>-</sup> ions on the reaction rate at concentrations 1, 2.5, 5, and 10 mg/L were also examined. The above concentrations of humic/fulvic acids and nitrate ions were chosen to resemble those found in natural waters.

**Photolysis in the Presence of an SFW (Synthetic Field Water) Solution.** The purpose of using an SFW solution was to provide a matrix containing the major constituents that affect a micropollutant's fate in natural waters such as humic materials (humic and fulvic acids) and nitrate ions. We were particularly interested in comparing metolachlor degradation rates in SFW with lake water at which metolachlor's persistence was the highest compared to other natural waters. The concentrations of the chosen constituents of the SFW were similar to those occurring in Pamvotis lake water: 3.6 mg/L HA, 7.3 mg/L FA, and 10.3 mg/L NO<sub>3</sub><sup>-</sup>.

**Quantification and Extraction Procedure.** Aliquots of 1.0 mL were taken out from the reservoirs (outdoor experiment) and the reactor (laboratory experiment) at predetermined times of irradiation and were injected to the liquid chromatograph to follow the reaction kinetics. Quantification was performed with external calibration curves constructed under the same experimental procedure.

The determination of 2-ethyl-6-methylaniline (an environmentally stable degradation product of metolachlor) was carried out using solid-phase extraction (SPE) disks and LC analysis with a modification of the method proposed by Hostetler and Thurman (31). The same extraction method was also applied for the isolation of other photo-products formed, which were identified by GC/MS techniques as already reported by our group (32). Fifty milliliters of the irradiation solutions were extracted separately at different time intervals by means of SPE as follows: SDB extraction disks were conditioned with 10 mL of acetone for 2 h. To allow better extraction, 0.25 mL of methanol modifier was added to the residues. The disks were placed on the



**Figure 2.** Photodegradation of metolachlor in (a) distilled, sea, river, and lake water and (b) dark controls under natural solar irradiation.

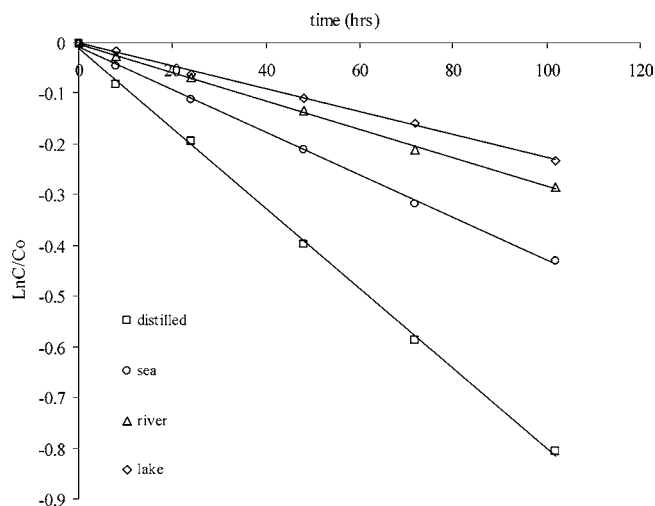
filtration apparatus and washed with 5 mL of solvent mixture dichloromethane:ethyl acetate (1:1, v/v) under vacuum followed with 5 mL of methanol for 3 min, with no vacuum applied. The disks were not allowed to dry and the samples were allowed to percolate through the disks under vacuum. Then, the compounds bound to the disks were collected by using 5 mL of the elution mixture dichloromethane/ethyl acetate (1:1, v/v) twice. The fractions were finally evaporated to 0.1 mL in a gentle stream of nitrogen. Then, 20.0  $\mu$ L and 1.5  $\mu$ L were injected into the LC-UV/DAD and GC/MS instrument, respectively. Before proceeding with the illumination of metolachlor, the recovery offered by the proposed SPE/LC-DAD method was investigated to assess and verify the validity of our observations. The recoveries ranged from 73.6% to 81.2% in all natural waters analyzed.

**Calculation of Half-Life.** Pseudo-first-order rate constants for metolachlor were observed for all experiments, obtained by linear regression of logarithmic concentration values ( $\ln[C_t]/[C_0]$ ) determined as a function of time. As far as the photodegradation constants ( $k_{\text{phot}}$ ) are concerned, they were calculated by subtracting the exponents of different degradation curves representing the apparent degradation ( $k_{\text{app}}$ ) and the degradation as a result of hydrolysis, volatilization, and biodegradation (dark experiment,  $k_{\text{dark}}$ ). In this way, the considered rate constants ( $k_{\text{phot}} = k_{\text{app}} - k_{\text{dark}}$ ) and the half-lives ( $t_{1/2}$ ) refer to the actual photochemical reaction with the exclusion of the contribution of other factors. The half-life was determined using the equation  $t_{1/2} = \ln 2/k$ .

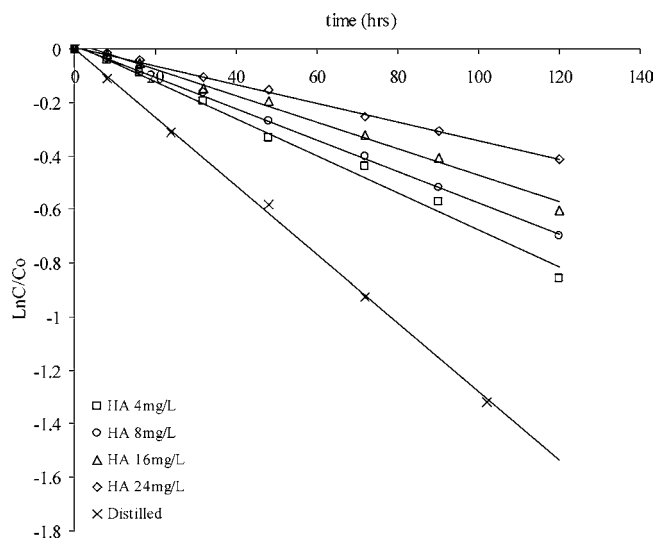
**Toxicity Evaluation.** Microtox, a bacterial assay using the bioluminescence bacteria *Vibrio fischeri* (Azur, model 500 Toxicity Analyzer), was used in this study for the toxicity evaluation. Freeze-dried bacteria, reconstitution solution, diluent (2% NaCl), and an adjustment solution (nontoxic 22% sodium chloride) were all obtained from Azur. On the basis of the reduction in bioluminescence of the marine bacterium by toxicants, the toxicity of metolachlor solution before and after irradiation collected after different time intervals was measured. The inhibition of the luminescence, compared with a toxic-free control to give the percentage of inhibition, was calculated following the established protocol and using the Microtox calculation program after contact times of 5 and 15 min.

## RESULTS AND DISCUSSION

**Photodegradation Kinetics of Metolachlor.** Metolachlor was irradiated in different types of natural waters as well as in the presence of DOM and nitrate ions. Linear pseudo-first-order plots were observed for all experiments allowing for the calculation of the rate constants (Figures 2–5 and Table 2). Experimental data indicated that the behavior of our photosimulator shows similar degradation trend to natural solar



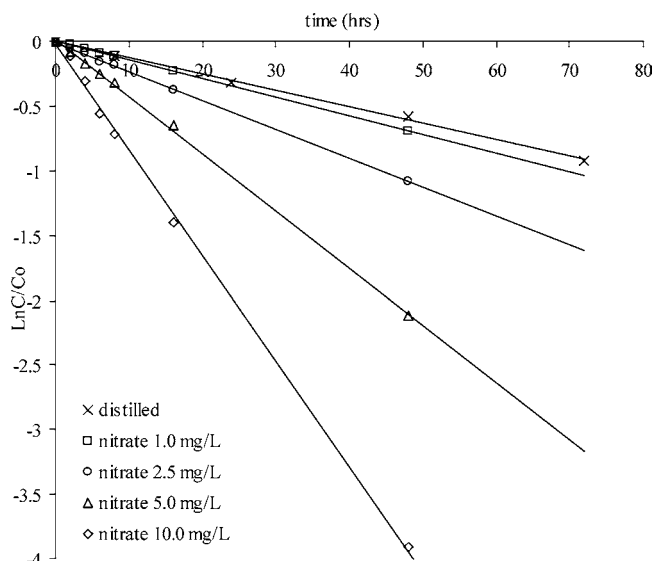
**Figure 3.** Photodegradation of metolachlor in distilled, sea, river, and lake water under simulated solar irradiation.



**Figure 4.** Photodegradation of metolachlor at various concentrations of isolated humic acids in distilled water under simulated solar irradiation.

irradiation (outdoor experiment) in the local area of Ioannina at northwestern Greece with a photolysis rate decreasing in the following order: distilled > sea > river > lake water illustrating a strong dependence on the composition of the irradiated media and especially on the concentration of dissolved organic matter. In all cases, the presence of DOM slowed the rate of photolysis. Our data clearly demonstrate that photolysis of metolachlor was slower in environmental waters compared to distilled water under both experimental conditions; in other words, as the DOM in natural waters increases the photolysis rate decreases. The diminished rate of metolachlor indicates that organic matter absorbed most of the photons emitted thereby slowing down direct photochemical reaction of metolachlor. The above statement is known as the optical filter effect (quenching) since organic matter could act as one of the most important sunlight-absorbing components of the aquatic environment (33, 34).

Moreover, particulate matter such as sediment particles and microorganisms suspended in a water column may scatter incident light, greatly reducing penetration of light beneath the surface. The slower rate of seawater is also consistent with  $\bullet\text{OH}$  scavenging by chloride ions (35). However, a sensitization effect cannot be excluded in natural waters (see byproduct evaluation). Indirect photolytic reactions are thought to proceed also because of the presence of other chemical transients (besides DOM and



**Figure 5.** Photodegradation of metolachlor at various concentrations of  $\text{NO}_3^-$  ions under simulated solar irradiation.

**Table 2.** Kinetics of the Photolysis of Metolachlor in Different Aqueous Media under Solar and Simulated Solar Irradiation

photolysis	$t_{1/2}$ (h)	$k_{\text{phot}}$ ( $\text{h}^{-1}$ )	RSD (%)	$R^2$
Solar Irradiation (Outdoor)				
distilled water	193	0.0036	11.72	0.9993
seawater	408	0.0017	12.83	0.9973
river water	578	0.0012	10.80	0.9976
lake water	693	0.0010	12.03	0.9985
Simulated Solar Irradiation (Suntest)				
distilled water	87	0.0080	7.74	0.9985
seawater	161	0.0043	6.79	0.9944
river water	248	0.0028	8.01	0.9958
lake water	301	0.0023	8.58	0.9982
$\text{NO}_3^-$ 1.0 mg/L <sup>a</sup>	48	0.0141	5.44	0.9977
$\text{NO}_3^-$ 2.5 mg/L	29	0.0239	5.27	0.9965
$\text{NO}_3^-$ 5.0 mg/L	17	0.0407	4.98	0.9994
$\text{NO}_3^-$ 10.0 mg/L	8	0.0876	5.05	0.9949
HA 4 mg/L <sup>a</sup>	103	0.0067	7.25	0.9892
HA 8 mg/L	122	0.0057	6.55	0.9980
HA 16 mg/L	147	0.0047	6.29	0.9898
HA 24 mg/L	204	0.0034	7.89	0.9968
FA 4 mg/L <sup>a</sup>	165	0.0042	8.11	0.9970
FA 8 mg/L	289	0.0024	7.47	0.9963
FA 16 mg/L	347	0.0020	6.99	0.9802
FA 24 mg/L	408	0.0017	8.11	0.9597
SFW <sup>b</sup>	277	0.0025	6.91	0.9962

<sup>a</sup> In distilled water. <sup>b</sup> SFW: synthetic field water.

nitrate) present in natural waters such as alkyl peroxy and carbonate radicals, singlet oxygen, and aqueous electrons. Carbonate radicals generated from the reaction of  $\bullet\text{OH}$  with either carbonate or bicarbonate ions play a significant role in limiting the persistence of various pesticides (36). Alkyl peroxy radicals ( $\bullet\text{OOR}$ ) can be produced through the reaction of ground-state oxygen with excited DOM chromophores, and singlet oxygen ( $^1\text{O}_2$ ) may be formed upon the absorption of sunlight by DOM and subsequent energy transfer to ground-state oxygen ( $^3\text{O}_2$ ).

To verify the inhibition effect of DOM, experiments with the same initial concentration of metolachlor and various concentrations of isolated HA (Figure 4) and FA were conducted allowing for the calculation of rate constants. It was postulated that the decrease in the rate of photodegradation could be either due to DOM competing with metolachlor for the available photons or to binding between DOM and metolachlor.

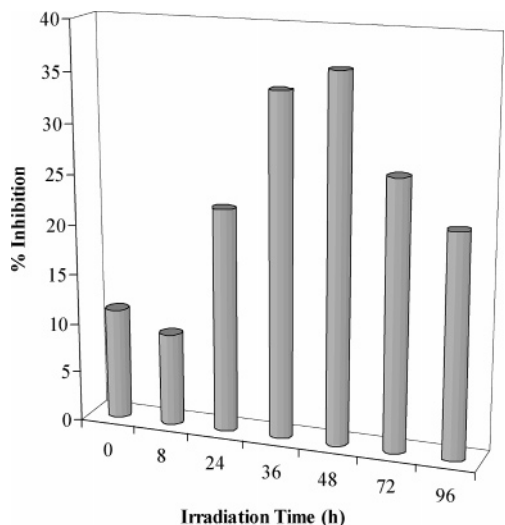
For example, experiments conducted at concentrations 4, 8, 16, and 24 mg/L of the isolated HA produced rate constants of  $0.0067 \pm 0.0005$ ,  $0.0057 \pm 0.0004$ ,  $0.0047 \pm 0.0003$ , and  $0.0034 \pm 0.0003 \text{ h}^{-1}$ . The same trend was also observed in FA. The rate constants decreased as the concentration of the isolated FA in solution increased:  $0.0042 \pm 0.0003$ ,  $0.0024 \pm 0.0002$ ,  $0.0020 \pm 0.0001$ , and  $0.0017 \pm 0.0001 \text{ h}^{-1}$  at concentrations of 4, 8, 16, and 24 mg/L, respectively. Metolachlor absorbs some irradiation in the wavelength region emitted by the lamp source, and this wavelength range overlaps with the absorbance region of DOM (HA and FA); thus, screening by DOM is significant. When metolachlor and HA/FA are present in solution together, a small fraction of the pesticide may be bound to DOM ( $K_{\text{ow}} = 3.85$ ). Since DOM commonly contains carboxylic, phenolic OH, alcoholic OH, keto, and alkoxy groups, it is likely that metolachlor interacts with DOM through hydrogen bonding, with additional interactions through charge-transfer bonds between the aromatic nucleus of the herbicide and aromatic rings of the DOM.

The magnitude of rate inhibition is different for each of the DOM samples. Because of the lack of structural characteristic data of HA and FA, it was not possible to extend our findings. Photochemical properties of humic and fulvic substances result from complex phenomena and depend on many factors, the origin of each substance being the most important.

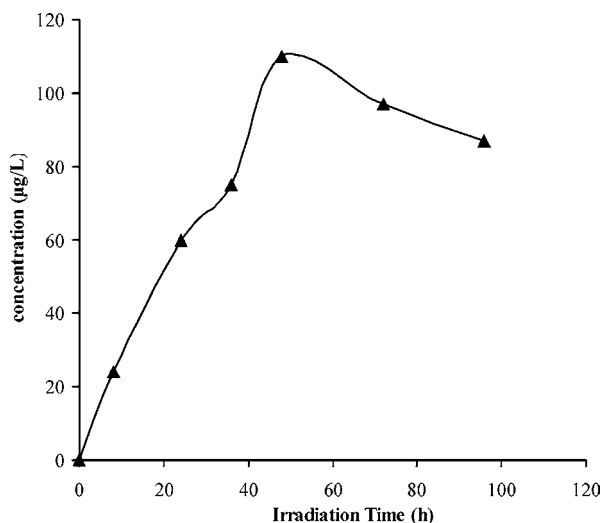
Several authors showed that nitrite/nitrate photolysis, which is a potential source of  $\bullet\text{OH}$  in natural waters, can initiate rapid reactions leading to the degradation of organic micropollutants. The exact mechanism of nitrate photolysis has been the subject of detailed investigation (37). In this study, an acceleration in the reaction rate was observed with increasing nitrate concentrations as illustrated in Figure 5. Increasing nitrate concentrations decreased the half-life of metolachlor to a large extent and accelerated the degradation rate by a factor of 11 compared to distilled water. Our group (32) has already shown that metolachlor photocatalytic degradation occurs mainly through reaction with hydroxyl radical and results suggest this reaction is predominant in the aqueous media examined.

The fate of a pollutant could vary as a function of water composition (36). Therefore, it is important to study the simultaneous interaction between organic contaminants and natural water constituents, such as dissolved organic matter and nitrate, which can simultaneously, respectively, decrease and increase photochemical reactions. Our findings demonstrate that the metolachlor degradation rate constant in SFW ( $0.0025 \pm 0.0002 \text{ h}^{-1}$ ) is close to that of lake water ( $0.0023 \pm 0.0002 \text{ h}^{-1}$ ). It appears that DOM present in SFW acts predominantly as an "inner" filter as well as in the scavenging of the hydroxyl radicals produced during nitrate photolysis causing inhibition of metolachlor phototransformation compared to distilled water. The fact that similar degradation rates have also been observed in lake water shows that these constituents are of major importance when considering indirect photodegradation processes and the fate of organic micropollutants.

**Toxicity Evaluation.** Seven samples irradiated at different time intervals were analyzed to evaluate the bioluminescence inhibition percentage of each sample. The initial toxicity of metolachlor solution (0 h of irradiation-distilled water) showed an inhibition of 11% (15 min of incubation) that is slightly decreased to 9% after 8 h of irradiation. After that time, the toxicity of the solution increases and reaches a maximum of 36% inhibition at 48 h (Figure 6). In other words, compounds more toxic than metolachlor are formed. The greater toxicity observed was initially attributed to the formation of 2-ethyl-6-



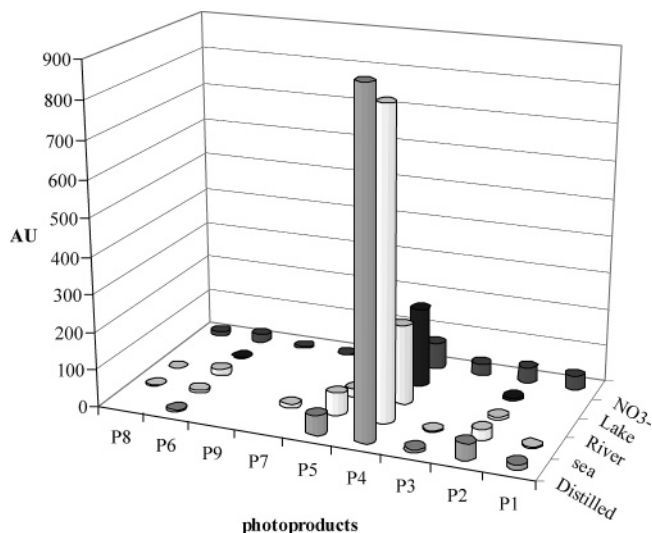
**Figure 6.** Inhibition of the luminescence of bacteria *Vibrio fischeri* as a function of the irradiation time.



**Figure 7.** Formation of 2-ethyl-6-methylaniline in distilled water under simulated solar irradiation.

methylaniline since Osano et al. (38) found that this metabolite was approximately 6 times more acutely toxic than its parent compound (metolachlor). The formation of this degradation product during metolachlor photolysis (**Figure 7**) shows that at 48 h of irradiation the concentration of 2-ethyl-6-methylaniline reached its highest value while at prolonged irradiation times a decrease was observed in accordance with the toxicity profile (**Figure 6**). However, the low concentration values detected cannot explain the high toxicity of the solution since no inhibition of the microtox assay was observed when exposed at low concentration levels of a standard 2-ethyl-6-methylaniline solution. However, a significant portion ( $\approx 44\%$ ) of products still remains unidentified according to Wilson and Mabury (22) including monochloroacetic acid (MCA), a polar product that accounts for between 5 and 30% of the photoproducts of the parent compound. Past investigations have shown that MCA is quite toxic (22). Therefore, the high toxicity of the solution observed can be attributed to other degradation products as well, while synergistic effects among the other transformation products are also considered.

**Byproduct Evaluation.** Investigations regarding photodegradation of pesticides have focused on identifying products using GC/MS or LC/MS techniques. Since in our previous work an



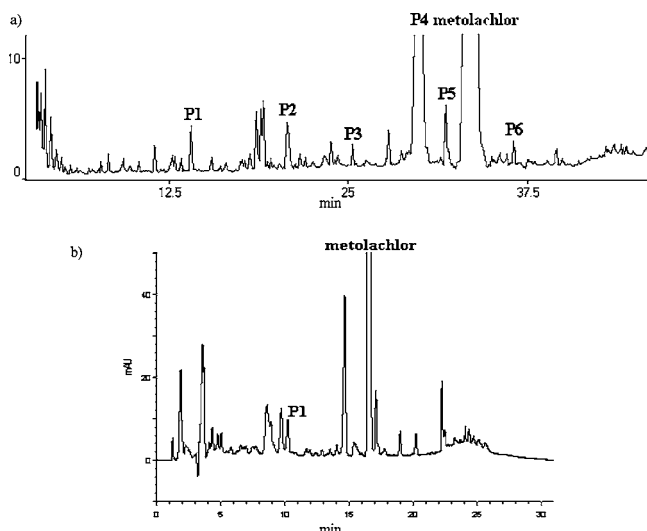
**Figure 8.** Transformation products formed (arbitrary units, AU) during metolachlor photolysis in aqueous solutions under simulated solar irradiation.

exhaustive degradation pathway (32) has been given regarding metolachlor photocatalytic degradation, an attempt was made in this study to provide any information regarding the influence of irradiated media on the mechanism and extent of the production of the byproducts. Up to nine compounds have been identified as degradation intermediates during metolachlor photodegradation: 2-ethyl-6-methyl aniline [P1]; *N*-(2-methoxy-1-methylethyl)-*N*-(2-vinyl-6-methyl) aniline [P2]; 2-chloro-*N*-(2-ethyl-6-methylphenyl) acetamide [P3]; [4-(2-ethyl-6-methylphenyl)-5-methyl-3-morpholinone] [P4]; [hydroxy-metolachlor/2-hydroxy-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide] [P5]; [2-chloro-*N*-[2-ethyl-6-(hydroxymethyl)phenyl]-*N*-(2-methoxy-1-methylethyl) acetamide] [P6]; 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-hydroxy-1-methylethyl) acetamide [P7]; [2-chloro-*N*-(2-vinyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide] [P8]; and [2-chloro-*N*-(2-hydroxyethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide] [P9] (**Figure 8**).

In all the treatments, the major degradation product identified was [P4]. For example, after 102 h of photolysis in natural waters and 48 h of artificial nitrate solution, [P4] constituted about 89, 87, 82, 87, and 30% of the photodegradation products formed in distilled, sea, river, lake, and nitrate aqueous solutions, respectively. The main transformation processes in regard to metolachlor degradation were hydroxylation (compounds [P6], [P8], [P9], [P7], and [P5]) and dealkylation (compounds [P1], [P2], and [P3]) with subsequent morpholine ring formation (compound [P4]).

Direct photolysis (distilled water) of metolachlor gave rise to six degradation intermediates (**Figure 9**). Besides the most abundant product ([P4]), *N*-alkyl dealkylation products were also formed (compounds [P1], [P2], and [P3]). It has been shown in previous works that a photoinduced base-catalyzed anilide hydrolysis mechanism occurs, leading also to the production of MCA (22). In distilled water, [P6] (phenyl-alkyl hydroxylation) and [P5] (*N*-alkyl hydroxylation) have been also formed showing that within an environmentally relevant aqueous media both hydroxylated and dealkylated products can be formed as a result of direct photolysis of metolachlor.

The product profile in seawater was similar. In addition to the transformation products formed in distilled water, two additional intermediates were detected. These were the ring hydroxylation ([P8]) and *N*-alkyl hydroxylation ([P7]) interme-



**Figure 9.** (a) GC-MS-EI total ion chromatogram and (b) LC/UV-DAD chromatogram obtained from an SPE extract of metolachlor solution (distilled water) after 102 h of irradiation with simulated solar light.

diates, showing that indirect photochemical (photosensitized) reactions initiated by naturally occurring species cannot be excluded even if an inhibition effect on metolachlor degradation was observed as previously discussed.

Regarding river and lake water in which the filter effect was more pronounced (compared to seawater) because of higher organic matter content, we can observe that *N*-alkyl dealkylation reactions attributed to direct photolysis were reduced since [P1] and [P3] were not detected. Compound [P4] was formed in much lower quantities showing that metolachlor photodegradation occurs less rapidly compared to distilled water. However, ring hydroxylation ([P8]) and phenyl-alkyl hydroxylation ([P6]) still occur in such matrixes.

In nitrate artificial solution, nine compounds have been identified. *N*-Dealkylated products [P1–P3] were formed in higher quantities compared to other aqueous solutions in this study. Besides direct photolysis, it is possible that carbonate radical, hydroxyl radicals, and alkyl peroxy radicals may be capable of initiating dealkylation products through an electron-transfer reaction (22). In addition to the dealkylated products, the formation of [P8], and [P6], [P9], as well as [P7], [P5] was expected since they refer to ring, and phenyl-alkyl, *N*-alkyl hydroxylation reactions, respectively. These reactions are likely to be involved since nitrate photolysis is well known to give rise to nonselective hydroxyl radicals that can react with and transform the target compound.

It seems that in natural waters the major degradation pathway of metolachlor degradation involves mainly the morpholinone formation ([P4]) while *N*-alkyl-dealkylation ([P2] and [P1]), *N*-alkyl-hydroxylation ([P5]), and phenyl-alkyl hydroxylation ([P6]) seem to be also formed, however, to much lower quantities. The matrix in which photolysis occurs greatly influences the mechanism and the ratio of hydroxylated or photoinduced cleavage products formed.

**Conclusion.** This study was undertaken primarily to determine the photochemical behavior of metolachlor under solar and simulated solar irradiation. For this purpose, the pollutant was irradiated in a variety of surface waters of different composition. Factors affecting the photolytic fate of the pesticide in the aqueous environment were also studied under laboratory conditions. In general, photodegradation was overall a slow process with dissolved organic matter exhibiting a strong

inhibition effect while nitrate photolysis resulted in the production of hydroxyl radicals which increased the efficiency of degradation. Generally, it is postulated that hydroxylation (occurring during metolachlor photodegradation) is associated with detoxification since hydroxylated compounds have high water solubility and are more easily degraded. This was not the case in the present study at which toxicity evaluation using the marine bacteria *Vibrio fischeri* demonstrated that metolachlor photolysis produced more toxic compounds.

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