



ENVIRONMENTAL RESEARCH BRIEF

Biodegradation of Atrazine in Subsurface Environments

James L. Sinclair* and Tony R. Lee*

Abstract

The pesticide atrazine is frequently detected in ground water, including ground water used as drinking water. Little information is available on the fate of atrazine in the subsurface, including its biodegradability. The objectives of this study were to evaluate the biodegradability of atrazine under differing conditions (oxygen status, prior exposure to atrazine, subsurface sediment texture, unsaturated and saturated zone sediments) that would be commonly encountered in the subsurface. Samples of soil and sediment were taken from a borehole drilled at a location beside a highway near Stratford, Oklahoma which had received applications of atrazine annually for 12 years. A second borehole was drilled 66 feet away in a field that had not received atrazine applications. Samples were taken from different depths with respect to the water table and sediment types. Core material from the boreholes was used to make microcosms to study atrazine biodegradation. Microcosms from Stratford, OK sediment were incubated aerobically. A sample from the Norman, OK landfill was used to make microcosms that were incubated anaerobically. Identical microcosms that had been sterilized by autoclaving were used as controls to differentiate biodegradation from abiotic processes. Most or all of the atrazine spiked into active and sterile microcosms made of surface soil from both Stratford locations had disappeared by 105 days of incubation as determined by HPLC analysis. No disappearance of atrazine was observed in either the active or sterile treatment of any of the subsurface samples from either Stratford borehole. A small amount (3.8%) of ^{14}C ring labeled atrazine was mineralized to $^{14}\text{CO}_2$ by 161 days

in the active treatment of the surface soil from the Stratford roadside. Little or no atrazine was mineralized to CO_2 in microcosms of the surface soil from the Stratford field or the subsurface samples from either borehole. A slow decline of atrazine was noted in the active treatment of the Norman landfill sediments that were incubated anaerobically. Therefore, some decline of atrazine concentration was noted in anaerobic subsurface microcosms of Norman landfill sediments, but no decline was observed in the aerobic Stratford subsurface sediment microcosms. Factors responsible for the lack of atrazine degradation in the Stratford microcosms may have included the usually small bacterial populations in these samples and the resistant nature of atrazine.

Introduction

Within the last decade, there have been many reports of the occurrence of pesticides in ground water (Younos and Weigmann, 1988). One of the pesticides which occurs most commonly in ground water is atrazine. Because of health concerns, there is interest in factors which would affect the occurrence and fate of atrazine in ground water. Typically, when atrazine is found in the subsurface it is present in concentrations of 0.3 to 3 ppb (Younos and Weigmann, 1988), which is at least an order of magnitude lower than levels commonly found in surface runoff waters (Thurman et al., 1991). The amounts of atrazine which occur in the subsurface are controlled by factors which are discussed in Cheng and Koskinen (1986) and Helling and Gish (1986).

Predicting the fate of atrazine in the subsurface is difficult because little work on the behavior of atrazine has been done in the subsurface or with samples of subsurface material. Most work on atrazine degradation was done in the 1960s and 1970s and was

* *ManTech Environmental Technology, Inc., Robert S. Kerr Environmental Research Laboratory, Ada, OK.*



done either with surface soil, water or with pure cultures of microorganisms. These studies indicated that atrazine was moderately resistant to degradation in surface soils although degradation rates varied considerably in different soils (Skipper and Volk, 1972; Roeth et al., 1969). Enough atrazine often survived into the next growing season so that inhibition of sensitive plants occurred. Mineralization of atrazine to CO₂ was found to proceed very slowly with only a few percent of added atrazine being mineralized in several months time (Skipper and Volk, 1972, and Dao et al, 1979).

A limited number of studies have been done on atrazine biodegradation in the subsurface. Roeth et al. (1969) reported that atrazine was degraded 2 to 3 times faster in surface soil than in subsurface soil from 14 to 24 inches and 36 to 48 inches deep. Lavy et al. (1973) found that phytotoxic amounts of atrazine were gone after 5 months at 15 cm, 17 months at 40 cm, and were still present after 41 months at 90 cm after atrazine amended soil from these depths was reburied at these same depths in a soil pit in Nebraska. Obenhuber (1988) reported that atrazine had a half-life of 556 days in aerobic subsurface microcosms, and 2632 days in anaerobic microcosms based on ¹⁴CO₂ data. This report also demonstrated that alternating aerobic-anaerobic treatments speeded up the rate of degradation. These studies suggest that atrazine degradation proceeds at a slower rate in the subsurface than in surface soil. Recent work on microbial populations and activities in the subsurface has shown that microbial abundances (Sinclair and Ghiorse, 1989, Sinclair et al. 1990) and activities (Hicks and Fredrickson, 1989) differ between different sediment types. Thus, it is likely that pesticide biodegradation will proceed at different rates in different types of subsurface sediment.

This study was designed to provide more information on the biodegradation of atrazine in the subsurface. The specific objectives were to determine if atrazine biodegradation occurs in the subsurface, and if so, at what rates in different parts of the profile and in different sediment types.

Materials and Methods

Sites and Sampling

A search was conducted for a site where atrazine had been regularly applied and which had other desired characteristics. These features were that the site was reasonably level, had permeable sediments which would permit rapid downward movement of water, and had a nearby location with similar geology but had not received applications of atrazine, and was near Kerr Lab. The only site that met these selection criteria was the roadside by highway 177 which had been sprayed with atrazine, glyphosate and sulfometuron methyl once a year for weed control. Atrazine had been applied annually for approximately 12 years. The location was 4.2 miles north of Stratford, OK on the west side of Highway 177. Surface soil collected January 2, 1989 from the roadside was found to contain atrazine. A drilling was conducted June 9, 1989 at the roadside to evaluate whether this site would be suitable for this study. The subsurface strata were determined to be permeable to water; the water table level was found to be somewhat deep but still at an acceptable level for the migration of atrazine to ground water (10.59 m); and samples were taken for atrazine analysis. A drilling to obtain samples for experiments was conducted on July 25, 1989 in the roadside and July 26, 1989, 66 feet west of the roadside borehole in a field where atrazine had not been applied. Samples were taken from

a number of depths where sediments having different textures were found. Samples from each borehole that will be discussed in this paper are the following:

<i>Description</i>	<i>Roadside Depth (m)</i>	<i>Field Depth (m)</i>
Surface	0.0- 0.1	0.0- 0.1
Unsaturated	4.2- 4.5	3.8- 4.1
Saturated	11.8- 12.0	12.0- 12.3

The subsurface samples used in the anaerobic treatment were from the Norman, OK landfill (Beeman and Suffita, 1990). A hole was dug with a backhoe to below the water table (1.5 to 2.0 M deep). Samples were scooped into jars from at or slightly below the water table. Sediments were anaerobic at this site, and rapidly reduced resazurin.

The average annual temperature in central Oklahoma (including Stratford and Norman) is 16 to 17 °C. The temperature of shallow sediments varies with the seasons, however the deeper sediments are the average annual temperature. Seasonal variations were noted in the temperature of the Norman landfill sediments due to their shallow depth (S.A. Gibson, personal communication).

Microcosms

Microcosms were set up by aseptically weighing out 10 g of sediment and transferring this to a sterile 60 ml serum bottle. The bottles were stoppered with sterile 1 cm thick butyl rubber stoppers. Sediments were adjusted to 30% water content with sterile Milli-Q water. Sterile microcosms were made by autoclaving for 8 hours at 122 °C, after which, all manipulations of the microcosms were done in a laminar flow hood using aseptic technique. Sterile and active (nonsterile) microcosms were made from all samples. Three replicate microcosms were made for each treatment. The anaerobic Norman landfill sediments were weighed out for microcosms in an anaerobic glovebox. One ml of a 1/100,000 dilution of resazurin and a 0.1 ml of a 100 μm solution of Na₂S·9H₂O were added to each microcosm. The microcosms were incubated at 22°C in the dark. All microcosms were amended with 100 ppb of atrazine.

Sample Characterization

The following properties of samples were determined: water content, particle size distribution, total organic carbon (TOC), pH, total nitrogen, ammonium nitrogen, total phosphorus, phosphate phosphorus, and 26 metals.

Bacteria in samples were enumerated by plate counts using dilute peptone, trypticase, yeast extract, glucose (PTYG) medium (Balkwill and Ghiorse, 1985). Direct counts of bacteria were made using acridine orange direct counting (AODC) procedures described in Balkwill and Ghiorse (1985).

Extraction and Analyses

Atrazine standards were obtained from EPA and ManTech standards repositories in Cincinnati, OH and Research Triangle Park, NC, respectively. Standards were also made up from pure atrazine obtained from Chem Service (West Chester, PA). Little difference was noted between these standards when they were compared.

The atrazine extraction method of Muir and Baker (1978) was tested to determine its efficiency for atrazine extraction from soil. Soil was spiked with 1 ppm of atrazine which was allowed to remain in the soil for 1 week before extraction. Twenty ml of absolute methanol were added to 10 g of soil and were shaken for one hour. The methanol was poured into a Teflon centrifuge tube and centrifuged on a Sorvall RC2B centrifuge at 10,000 rpm using an SS34 rotor for 10 minutes. The supernatant was analyzed by high pressure liquid chromatography (HPLC) analysis and showed a 95% recovery of atrazine. This method was adopted for atrazine extraction.

Bond Elute C18 cartridges (Analytichem International, Harbor City, CA) were investigated as a method to concentrate atrazine and remove soil organic matter from methanol extracts of soil. To increase the polarity of the extract so that atrazine would be retained by the C18 material, the extract was diluted 10:1 with Milli-Q water. Two tests were run to determine how efficient the cartridges were at retaining the atrazine while the extract was being passed through the cartridge. The first test was to run a 100 parts per billion (ppb) solution of 10% methanol: 90% water through a C18 cartridge. The second method was to stack two C18 cartridges and measure the amount of atrazine which passed through the first cartridge into the second cartridge. After the extract was passed through the cartridges, 2.0 ml of absolute methanol was used to elute the atrazine from the C18 material. Analysis of the eluate indicated that for the first method of checking the extraction efficiency of the C18 cartridges, essentially all of the atrazine in the initial solution was recovered in the methanol eluate. For the second method, it was found that 1.7% of the total amount of atrazine had passed through the first cartridge into the second cartridge. Therefore, the C18 cartridge method of concentrating the extracts was found to be very efficient and was selected as the method of concentration to be used.

HPLC analysis was done using a Scientific Systems Inc. (SSI) isocratic HPLC system. Samples dissolved in methanol were diluted 50:50 with Milli-Q water; and if any turbidity was noted, they were centrifuged in a Microfuge (Fotodyne, Gosheim, F.R. Germany) for 10 minutes. Samples were injected into a 50 μ l sample loop. A mobile phase of 28% acetonitrile, 68% water, 4% methanol and 0.0002% acetic acid (v/v) at pH 4.6 gave the best results for separating atrazine from interfering peaks. Some samples were analyzed by increasing the methanol concentration stepwise up to 7% with a corresponding decrease in the percentage of water. An Alltech C8 150 X 4.6 mm column and an Alltech C8 guard column, both with 5 μ m packing material were used. A flow rate of 2.0 ml per minute was used initially and then reduced to 1.5 ml per minute later to reduce pressure and stress on the column. A UV-VIS detector was used at 222 nm. The extraction method used in this study mobilized a considerable amount of soil organic matter. To prevent this organic matter from degrading the performance of the column and other parts of the system, regular flushes and cleanings of the column and other parts were necessary including a 10 minute methanol flush between each sample analysis followed by a 20 minute baseline re-equilibration flush with the mobile phase.

Mineralization of the atrazine ring was determined by measuring $^{14}\text{CO}_2$ production from ^{14}C ring-labeled atrazine added to the microcosms. The amount of ^{14}C labeled atrazine added to the microcosms was 50,000 DPM with enough nonlabeled atrazine added to make up a total of 100 ppb. CO_2 was trapped in 0.25 ml of 1 N NaOH in a centerwell apparatus (Kontes Glass, Vineland,

NJ) suspended from the stopper of the bottle. One day before the bottles were sampled 1% H_2SO_4 was added to the bottles to drive off carbonate- CO_2 . A pilot study using this method indicated that CO_2 inside the bottles was almost completely removed by the CO_2 trap after 1 hour and therefore this method is satisfactory for determining CO_2 evolution in this type of microcosm (Ghiorse et al., 1989 and Madsen et al., 1991). A search was conducted for a suitable scintillation cocktail which would give a high counting efficiency in the presence of the 1 N NaOH CO_2 absorbent. A 6:1 ratio of Beckman Ready-Solv EP cocktail (Beckman Instruments Inc., Fullerton, CA) to 1 N NaOH was found to give equal counts to a 6:1 ratio of the same cocktail with water even when a precipitate formed in the NaOH and cocktail mixture. Therefore this cocktail was used in the experiments.

Results

The physical, chemical and biological properties of the Stratford samples recovered from the boreholes are listed in Table 1. The bacterial populations in all of the samples used in this experiment including those from the sandy strata were lower than usual (Sinclair and Ghiorse, 1989) with even the highest plate counts from these samples being less than 10^5 per gram of sediment. The 12.0 m deep sample from the field had <50 plateable bacteria per g. The other characteristics showed differences in the samples as would be expected. Nitrogen and phosphorus showed a general trend toward lower values with depth. Samples used for microcosms were analyzed for 26 metals (data not shown). There was little relation between the metals content of the samples and biological populations.

Atrazine assays of the samples taken January 2, 1989 from the roadside indicated that there was about 1 part per million (ppm) of atrazine in the surface soil. Another surface soil sample taken from the roadside March 13, 1989 had 9.3 ppb indicating that there was a loss of 99% of the atrazine over a period of 2 months and 11 days. The temperatures during this time were cold so biological activity would have been minimal. There had been substantial rain and snow melt during this period and there was evidence of the flow of water in the ditch. Therefore, it appears that most of the atrazine loss occurred as a result of surface runoff. At the time of the July, 1989 drilling 26 ppb atrazine was found in the surface soil and 0.46 ppb was found 4.2 m deep in the unsaturated zone (Table 1).

The results of the atrazine disappearance experiment are shown in Figures 1 and 2. Atrazine loss occurred at a steady rate in microcosms of both the active and the sterile treatments of the surface soil of the roadside. By the end of the experiment at 161 days, more than 80% of the atrazine originally spiked into the microcosms had been degraded or bound in both the active and sterile treatments in the roadside surface soil. In the surface soil of the field where atrazine had not been applied there was a sharp decline in concentration up to 77 days, after which atrazine had declined to below the limit of detection in both active and sterile treatments. Data shown for microcosms of subsurface material (Figure 1) indicate that there was no atrazine loss from any of the samples. No differences were noted between the active and sterile treatments of the subsurface sample microcosms, and not even abiotic hydrolysis was observed in the other subsurface samples regardless of the differing characteristics of the samples.

By contrast, in subsurface samples taken from the Norman landfill (Figure 2), a steady decline in the concentration of atrazine was

Table 1. Physical, chemical and biological properties of core samples taken July 25 and 26, 1989 and used for microcosm experiments. Diversity of bacterial colony types is the number of distinctly different bacterial colony types appearing on the PTYG plates.

Site/ Depth (m)	Description	% H ₂ O	% Sand/ Silt/Clay	pH	%TOC	NO ₂ -N+NO ₃ -N Mg/Kg	NH ₄ ⁺ -N Mg/Kg	Total N Mg/Kg	PO ₄ ⁻³ -P Mg/Kg	Total P Mg/Kg	Atrazine ug/kg	AODC /gdw	Plate Counts PTYG/gdw	Diversity Colony Types
Roadside														
4	0	Surface	16.0	79/9/12	7.6	0.76	36.2	277	1.61	116	26	8.8 X 10 ⁸	3.5 X 10 ⁷	29
	4.2	Unsaturated	17.5	76/14/10	5.6	0.03	10.0	105	0.4	50	0.46	8.6 X 10 ⁸	3.6 X 10 ²	5
	11.8	Saturated	20.1	89/4/7	6.5	0.01	1.4	32.6	0.03	18.4	<0.2	6.7 X 10 ⁸	8.4 X 10 ⁴	8
Field														
	0	Surface	0.4	85/3/12	6.3	0.41	11.3	415	5.04	105	<0.2	5.6 X 10 ⁸	1.1 X 10 ⁷	38
	3.8	Unsaturated	7.8	90/4/6	5.3	0.03	7.5	50	<0.02	34.4	<0.2	3.8 X 10 ⁸	2.6 X 10 ⁴	7
	12.0	Saturated	16.0	92/2/6	6.3	0.01	2.5	28.6	0.04	20.4	<0.2	5.6 X 10 ⁸	<10 ¹	0

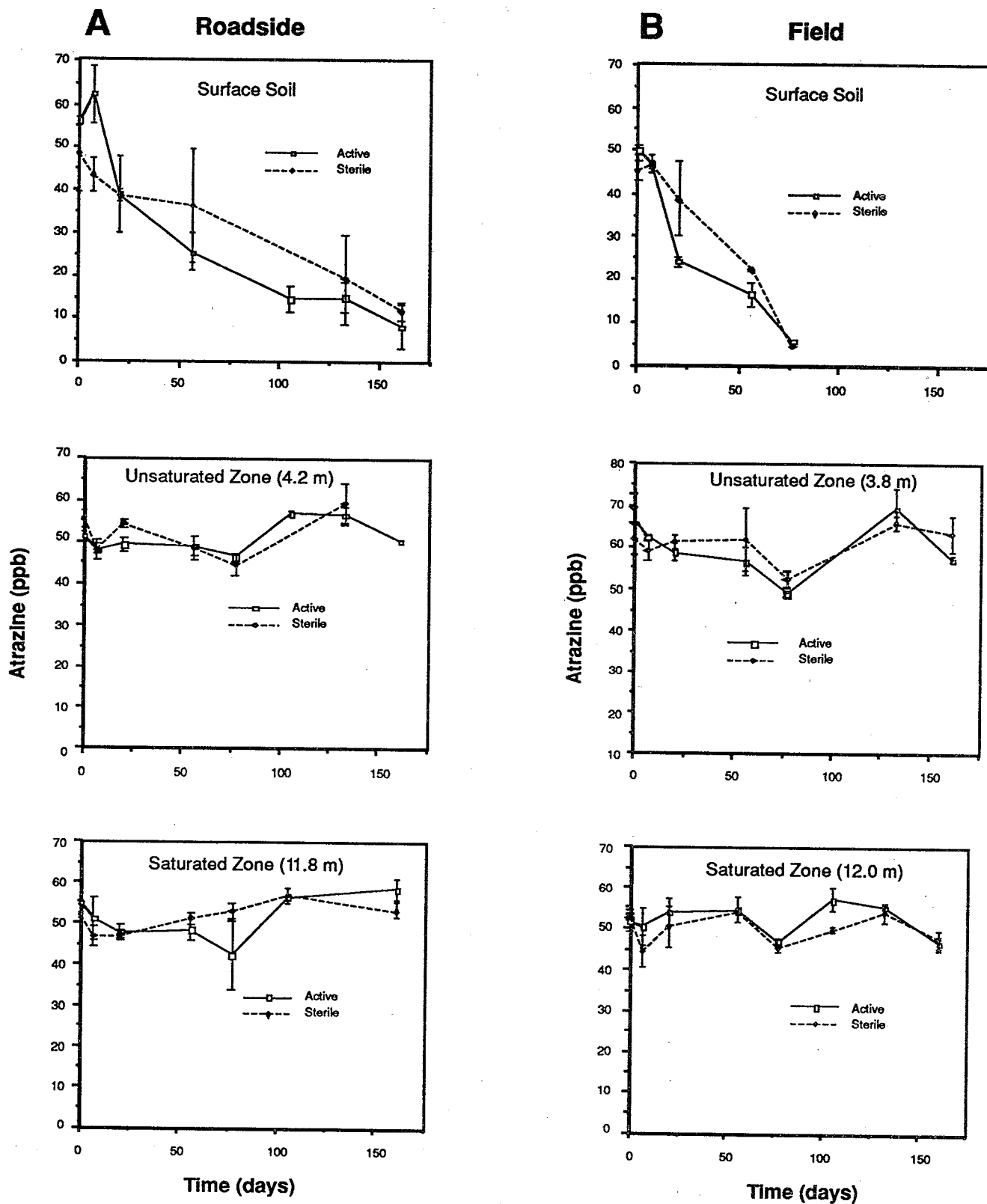


Figure 1. Atrazine levels as determined by HPLC analysis are shown for microcosms of soil and sediments from the Stratford, OK site. Panel A shows microcosms from a profile of depths from the roadside where atrazine had been sprayed. Panel B shows microcosms from a profile from the field 66 ft. from the roadside where atrazine had not been sprayed. The error bars are ± 1 S.D.

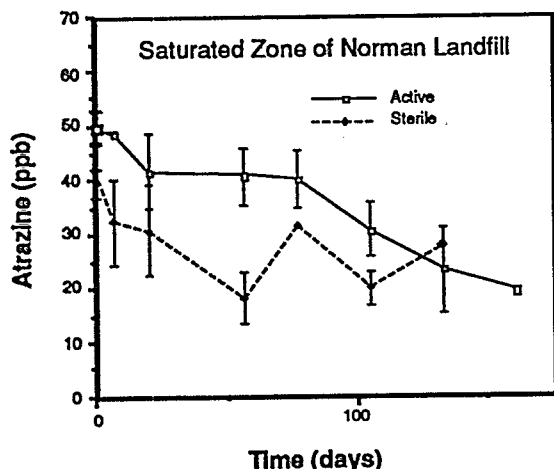


Figure 2. Atrazine levels as determined by HPLC analysis are shown for microcosms from the saturated zone of the Norman Landfill. The error bars are ± 1 S.D.

noted in the active treatment throughout the experiment. By the end of the experiment very little of the added atrazine was detectable. In the sterile treatment, it was not possible to determine whether there was any degradation of atrazine because of interferences in the samples.

The results of the atrazine mineralization experiment (Figure 3) indicated that there was a slow release of ^{14}C labeled CO_2 from labeled atrazine added to the surface soil of the roadside. By 161 days of incubation, 3.8% of the activity originally added as labeled atrazine was released as $^{14}\text{CO}_2$. A very small amount of $^{14}\text{CO}_2$ was released from the surface soil of the field in the nonsterile treatment as compared to the sterile treatment. By 161 days, this amounted to 0.4% of the total amount of activity added. Very little activity appeared in the CO_2 traps of the microcosms of the subsurface samples. Most of the activity which was trapped appeared at 133 or 161 days and often appeared equally in the nonsterile and the sterile microcosms. Examples of nearly equal amounts of activity found in nonsterile and sterile treatments can be seen in the roadside sample from 11.8 m deep and the field samples from 3.8 and 12.0 m deep. No clear example was observed where microcosms of the nonsterile treatment of a subsurface sample had more activity in the CO_2 trap than could be seen in the sterile treatment from the same subsurface sample.

Discussion

Most of the atrazine applied to the roadside appeared to be lost to surface runoff, however there was some penetration of atrazine into the upper part of the unsaturated zone. The amount of atrazine which penetrated into the 4.2 m deep sample (0.46 ppb) was below the EPA's health advisory limit of 3 ppb of atrazine in ground water (USEPA, 1989) and detectable levels of atrazine were not found in deeper samples. Therefore, the indigenous microbial populations were exposed to atrazine in the surface soil and upper parts of the unsaturated zone of the roadside. Despite this prior exposure in at least part of the profile, there was no evidence of microbial acclimation to atrazine in the surface or subsurface of the roadside in the atrazine disappearance experiment. In the roadside and the field, abiotic hydrolysis

seemed to be responsible for almost all of the atrazine breakdown since nearly equal amounts of loss were observed in the active and sterile treatments. There seemed to be a more rapid rate of hydrolysis in the surface soil of the field than in the roadside although the reason for this is not clear. The lack of biodegradation of atrazine in the subsurface samples may not have been unexpected, however the lack of abiotic hydrolysis was unusual and the reason for this is not evident.

An objective of this work was to determine how the rate of biodegradation of atrazine differed in sediments having different characteristics, such as texture or position with respect to the water table. Regardless of differing characteristics, no atrazine degradation was observed in any of the subsurface samples. A similar observation about atrazine biodegradation was made by Konopka and Turco (1991). Atrazine was apparently resistant enough to degradation to mask any differences in biodegradation which might be observed in sediments which had different microbial population sizes or types during the 161-day time course of the experiment. Degradation of atrazine was observed in the Norman landfill sediments which were incubated anaerobically. This result illustrates that atrazine may degrade in the subsurface under some conditions.

Very little complete mineralization of atrazine to CO_2 was observed in any sample. These results are similar to those of Skipper and Volk (1972) and others who also observed little atrazine mineralization in surface or subsurface soils. There did appear to be a microbial acclimation effect in the roadside surface soil as compared to the field surface soil for atrazine mineralization. Atrazine mineralization to CO_2 appeared to be a biological process because it was only observed in the active treatment. About 10 times as much atrazine mineralization occurred in the roadside surface soil microcosms as occurred in the field surface soil microcosms. No evidence could be seen for atrazine mineralization in any of the subsurface sediment samples. It is not clear why ^{14}C activity was found in the CO_2 trap cups of the sterile treatments of several of the subsurface sediment microcosms but not in the sterile treatment surface soil microcosms. It is unlikely that the activity was due to $^{14}\text{CO}_2$ formed by microbial contaminants in the sterile subsurface sediment microcosms because this activity was noted in all of the replicates of these microcosms.

It is difficult to judge how representative the results of these experiments are of atrazine biodegradation at other subsurface sites because of the lower than usual bacterial populations at the Stratford site (compared to Sinclair and Ghiorse, 1989 or Sinclair *et al.*, 1990). Nonetheless, the microbial populations did have the ability to degrade alachlor even though alachlor had not been applied to this site. Dr. T.B. Moorman of the USDA in Stoneville, MS (1991 Abstr. Amer. Soc. Microbiol. Ann. Meeting Q94, p. 292) has reported that several percent of alachlor added to microcosms of some subsurface sediments from the Stratford site was mineralized. Alachlor did degrade differently in samples having different textural characteristics or position with respect to the water table. The alachlor degradation in the Stratford samples was reported to be more rapid than that found in subsurface samples from Plains, GA (T.B. Moorman, personal communication, and Pothuluri *et al.*, 1990). Therefore the slow rate of atrazine biodegradation in the subsurface of this site may be a reflection of the resistance of atrazine to degradation and its decreasing rate of degradation with depth in soil (Lavy *et al.*, 1973). Nonetheless, because of the sparse bacterial populations and the lack of abiotic hydrolysis in the subsurface samples from this site, other sites

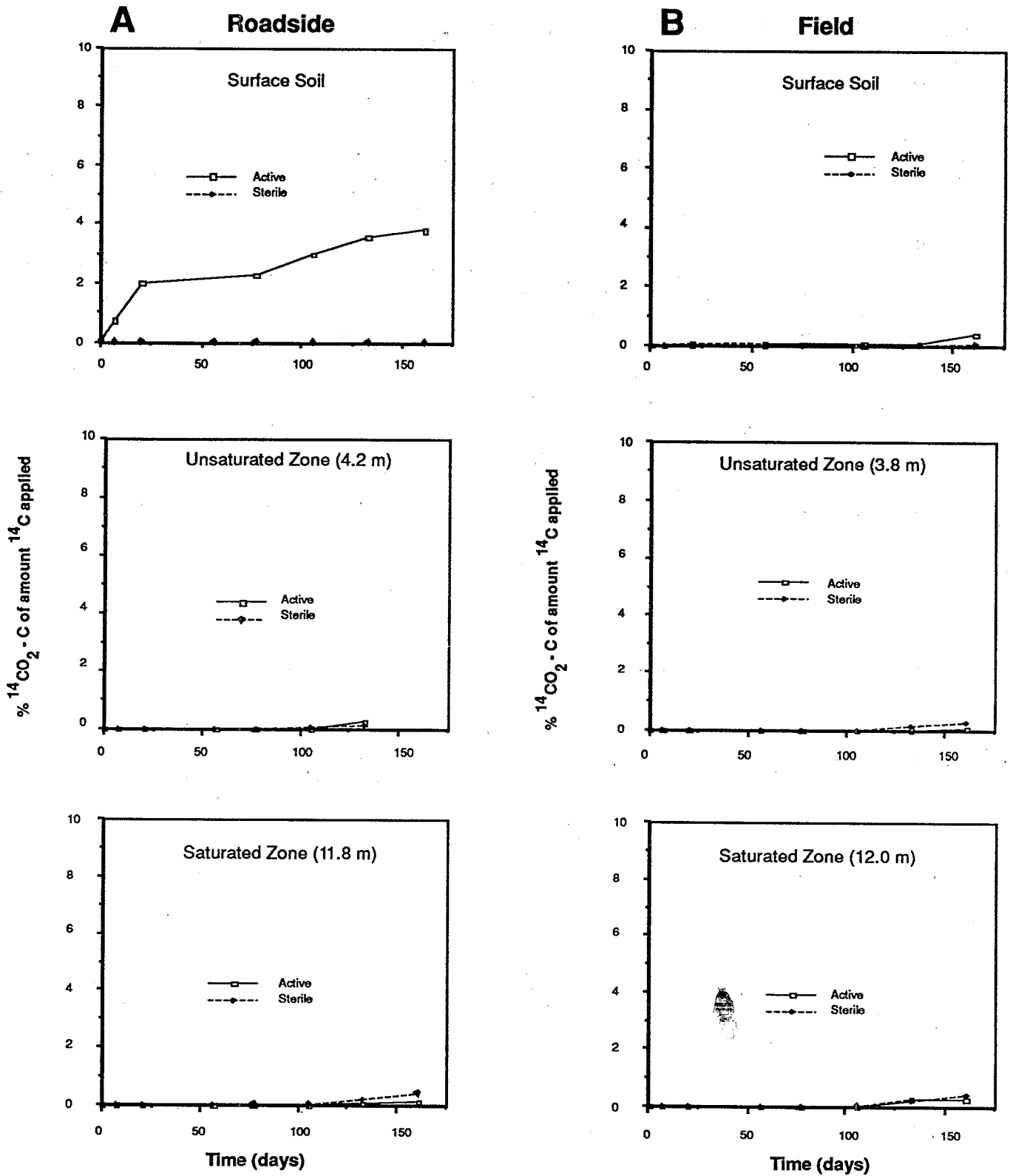


Figure 3. The percent of ^{14}C evolved as $^{14}\text{CO}_2\text{-C}$ from added ^{14}C ring-labeled atrazine is shown. Panel A shows the results from microcosms of the roadside where atrazine had been sprayed and panel B shows the results from the field 66 ft. from the roadside where atrazine had not been sprayed.

should be studied to determine if atrazine degrades more readily in the subsurface at other locations.

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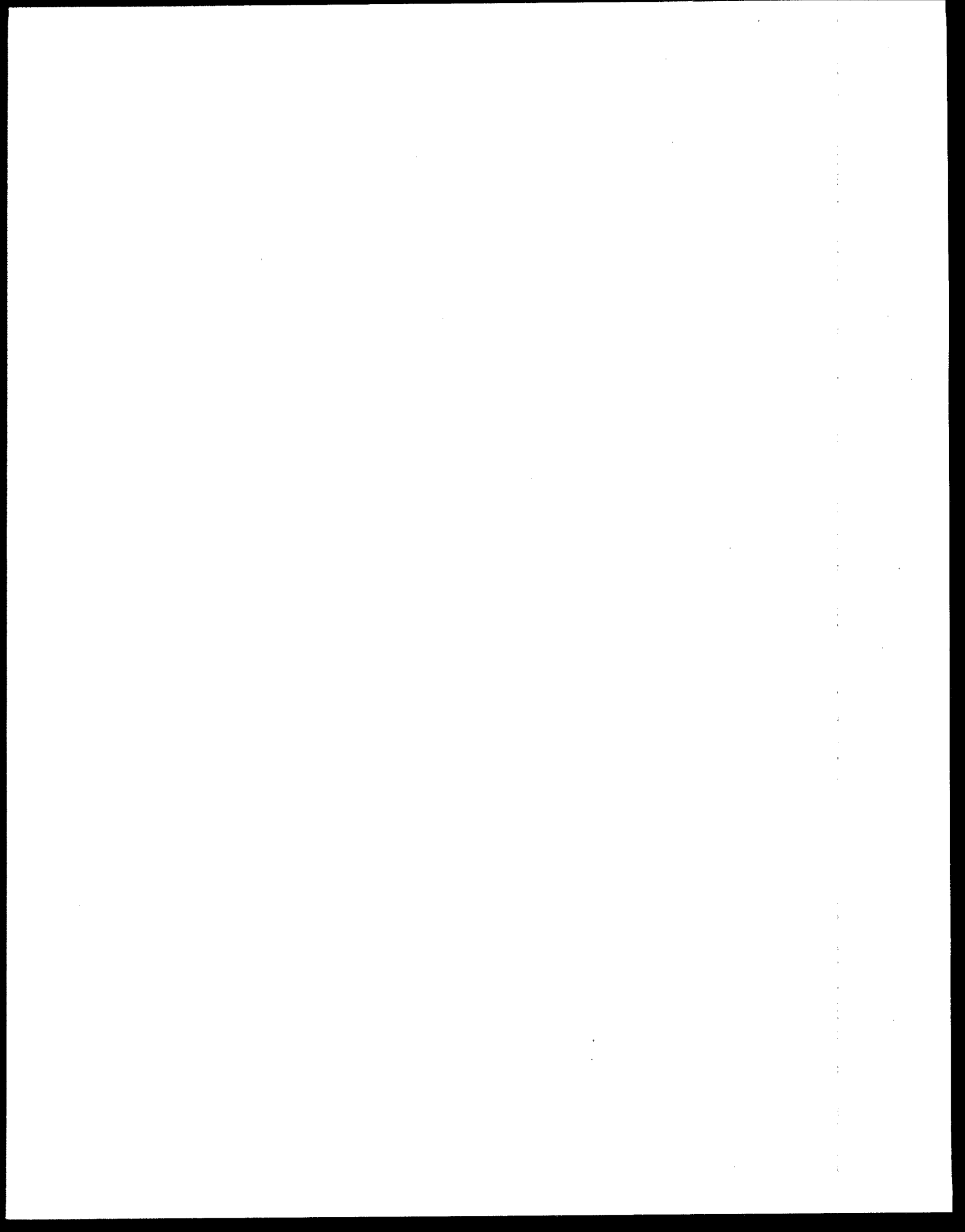
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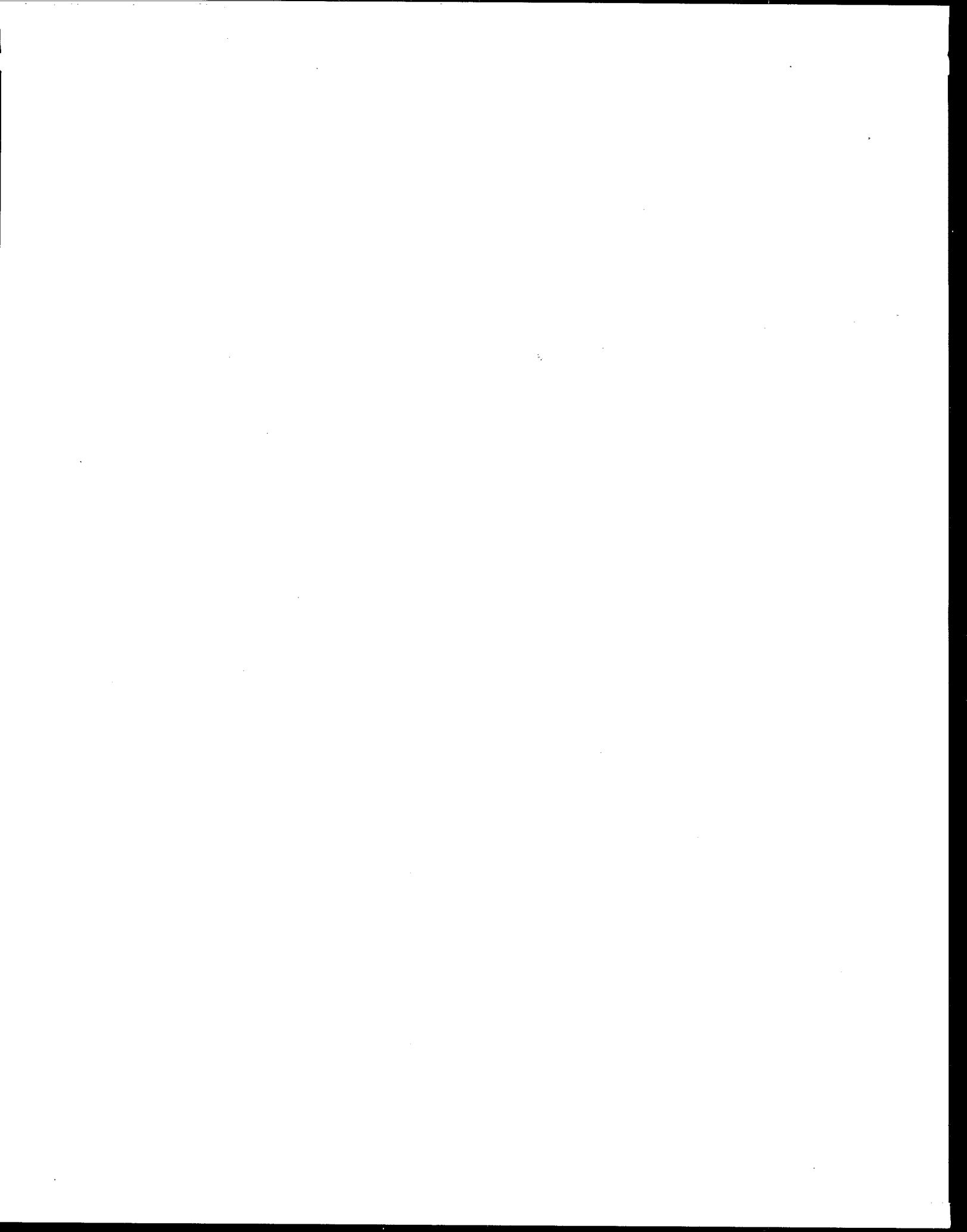
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