DEGRADATION OF ALACHLOR [2-CHLORO-2',6'-DIETHYL-N-(METHOXYMETHYL) ACETANILIDE] IN SOILS AND BY MICROORGANISMS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
ROBERT W. TAYLOR
1972

ABSTRACT

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Alachlor was not used as a sole source of carbon and energy for growth by soil or river water microorganisms as judged by the absence of growth in enrichment cultures.

Metabolism of 100 ppm alachlor by growing and resting (replacement) fungal mats was studied. Replacement mats of *Aspergillus niger* metabolized only 12.7% of the herbicide in a one week period. However, growing mats of *Chaetomium sp.* metabolized as much as 97.2% of the alachlor during a 17 day growth period while growing mats of *A. niger* metabolized only 18%. The gas-liquid chromatographic analysis of the *Chaetomium* medium also showed two additional peaks that could be alachlor metabolites. Analysis of the fate of the ^{14}C-ring labeled alachlor in the experiment using *Chaetomium* showed that 85.7% of the label was extracted in ethyl acetate and that, of this, only 43% could be attributed to alachlor. A minor quantity (8.9%) of the label remained in the aqueous phase and no label was trapped

as CO_2. Thus a large portion of the label (42.7%) appeared to be contained in non-polar metabolites.

When 100 ppm (w/w) of ^{14}C-alachlor was incubated in a Barry loam soil, only 16% of the original alachlor, as determined by gas chromatographic analysis, was recovered by acetone-water extraction after 21 days in both non-sterilized and gamma sterilized soils. In contrast 44-50% of the label was extracted by the same solvent; however, in the non-sterilized soil one-quarter of this label was in polar components while in the sterilized soil only one-twelfth remained in the aqueous fraction. None of the label was found as $^{14}CO_2$ after 21 days.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop and Soil Sciences

1972

Dedication

To my parents, brother and sister whose memory,
encouragement, and never ending sacrifice
gave me the inspiration to
finish this work

ACKNOWLEDGEMENTS

To Dr. J. M. Tiedje, my heartfelt gratitude is due for his encouragement, patience and indispensable guidance.

To Dr. W. G. Fields, the author is indebted for his cooperation in supplying pure cultures of the fungi used in this study.

To Dr. D. Penner and Dr. M. J. Zabik, special thanks is also due for their constructive criticisms of the manuscript.

The financial assistance from the Environmental Protection Agency, Grant no. 8RDIEP00801-06, is acknowledged.

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INTRODUCTION

Herbicides have become almost indispensable to large-sacle agriculture. They are easy to apply, are less damaging to economic plants than most mechanical weed control methods and their use is economically favorable. However, a concern of herbicide use is the possibility of environment pollution. Therefore, the biodegradability and non-toxicity of the degradation products of any herbicide should be known before it can be considered safe for use in the environment.

Anilide herbicides are relatively new and show promise for weed control in turf and a variety of economically important crops including rice, cotton, soybeans, corn, and tomatoes (Bartha, 1968). Among the attractive features of this class of herbicides are their effectiveness, selectivity, biodegradability and low mammalian toxicity (Bartha, 1968). Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide] (commercially sold as Lasso) is an anilide herbicide which recently has become popular as a weed control agent.

Little information has been published on the detection and degradation of alachlor with no information available on microbial degradation. Hargrove and Merkle (1971) found that chemical degradation of alachlor in the soil, under

conditions of low relative humidity and high temperature, resulted in the formation of 2-chloro-2',6-'diethyl acetanilide. Since the same product resulted from acid catalysis in 5 N aqueous HCl, the degradative mechanism in soil was attributed to acidic soil water films on mineral surfaces. In this case cleavage resulted in the loss of the N-substituted ether group with the aromatic system and amide linkage preserved. Beestman and Deming (Agronomy Abstract, 1972) suggest that microbial decomposition was the major route of alachlor degradation in the soil with a half-life of between 2 to 14 days. In addition, information from the manufacturer states that 40% and possibly as much as 90% of alachlor degradation in soil is carried out by the microflora.

In studies on alachlor metabolism in plants Armstrong (1972) concluded that alachlor was metabolized rapidly by yellow nutsedge to at least one water-soluble metabolite. Lamaureux, Stafford, and Tanaka (1971) studying plant metabolism of a related herbicide, propachlor, (2-chloro-N-isopropylacetanilide) found at least three water-soluble metabolites, one of which was identified as the glutathione conjugate of propachlor. Based on these few reports, metabolism of alachlor could be expected to produce water-soluble metabolites.

Bartha and Pramer (1967) demonstrated that the microbial degradation of propanil (3',4'-dichloropropionanilide) produced 3,4-dichloroaniline and propionic acid as the products, which showed that enzymatic cleavage of the parent

molecule occurred at the amide linkage. The same site of enzymatic attack could be postulated for the microbial degradation of alachlor.

Isolation of an organism capable of using alachlor as a sole source of carbon and energy for growth has not been reported. This suggests that if there is bio-degradation in nature it might be accomplished by a cometabolic mechanism. Evidence is available indicating that cometabolism may be an important phenomenon in the breakdown of pesticides (Horvath and Alexander, 1970).

A need for further studies on the degradation of alachlor is evident and this study was initiated to find out if alachlor is microbially degraded and to detect any degradation product(s).

MATERIALS AND METHODS

Fungi

Aspergillus niger and *Chaetomium sp.*, two soil fungi, were used in the study. Pure cultures of these fungi were obtained from Dr. William G. Fields, Department of Botany and Plant Pathology, Michigan State University. The cultures were maintained by aseptic transfers on potato dextrose agar slants.

Enrichment on acylanilides

The enrichment media were made from a basal salts solution consisting of 1.6 g K_2HPO_4; 0.4 g KH_2PO_4, 0.2 g NH_4NO_3, 0.2 g $MgSO_4$, and 0.017 g $CaCO_3$; 0.0014 g $FeSO_4$, made up to 1 liter with deionized water. The following chemicals were added to 100 ml portions of the salts solution to give a final concentration of 0.05%: 1) propachlor; 2) CDAA; 3) alachlor; 4) propanil; 5) 3'-chloro-acetanilide; 6) propionanilide; 7) N-methyl acetanilide; 8) acetanilide; 9) oxanilic acid. Fungal enrichment media were prepared similarly except that K_2HPO_4 was omitted and the pH was adjusted to 4.6 with 1 N H_3PO_4. The inoculum was 1 ml of water from the Red Cedar River for aquatic organisms and 1 ml of a soil suspension from a turf covered Conover loam from the Michigan State University campus for soil organisms.

Growth was recorded from visual observations, showing the appearance of turbidity or an increase in biomass, after two weeks.

Replacement cultures

One liter of malt extract broth was made by dissolving the dried broth in the basal salts solution; 100 ml portions were autoclaved in 300 ml Erlenmeyer flasks, then inoculated with pieces of *A. niger* mycelium. Fungal mats developed on this medium during a 14 day incubation period. Five of the fungal mats were freed of the malt extract broth by aseptically pouring out the spent medium. The mats were washed twice with sterilized deionized water to remove the excess fungal debris and to dilute the chloride which remained from the malt extract. During the second wash, the mats were allowed to sit on the deionized water for 16 hr. This wash was used to establish the level of background chloride. Inorganic chloride released from alachlor was determined colorimetrically according to the method of Bergmann and Sanik (1957). The other five mats remained on the malt extract broth and were used as controls for substances secreted by the mats. After the second wash the fungal mat was resuspended on 100 ml of a solution containing 10 mg alachlor (100 ppm) and the basal salts solution previously described.

Both the alachlor media and the malt extract broth in the controls were separated from the mats and extracted with two 50 ml portions of redistilled ethyl acetate at 0 time,

5 hr, 10 hr, 24 hr and 168 hr following incubation at room temperature. Simultaneously, the sterile controls which consisted of five 100 ml portions of the same 100 ppm alachlor solutions, were extracted. Each of the fungal mats was homogenized in 100 ml of redistilled ethyl acetate, the homogenate filtered, and the cell debris discarded. The ethyl acetate extracts were dried by addition of anhydrous Na_2SO_4 prior to gas chromatographic analysis. Inorganic chloride was determined on all the alachlor containing aqueous solutions after extraction with ethyl acetate.

Growing fungal mats

Two liters of 100 ppm alachlor malt extract broth solution were made up by adding 0.2 g of alachlor and 30 g of malt extract broth to the basal salts solution. From the above solution nine 100 ml portions were filter-sterilized through a 0.20 micron Millipore filter into presterile 300 ml Erlenmeyer flasks. Two flasks were inoculated with mycelial pieces of each of the following fungi: *A. niger; Chaetomium sp.; A. niger* plus acetanilide (0.01 g per flask). Three flasks which were not inoculated served as zero time controls. Two flasks of 100 ml containing only malt extract broth media were made up separately, autoclaved and inoculated with *A. niger* and *Chaetomium sp.*; these flasks served as the controls for substances secreted by the mats. Fungal mats were allowed to grow for 17 days at room temperature, after which the media and mats were extracted as described in the

replacement mat experiment above. The ethyl acetate extracts were assayed for alachlor by gas chromatography.

Growing fungal mats---^{14}C-alachlor added

A 100 ppm alachlor malt extract solution was made up as described above. Two-tenths milliliter of uniformly ring-labelled ^{14}C-alachlor (specific activity--1.02 μ Ci/μmole) in 95% ethanol was added to 500 ml of this solution (110 dps per 1 ml of media). As described above 100 ml portions were filter sterilized and placed into four sterilized biometer flasks. Twenty milliliters of sterilized 1 N NaOH was aseptically transferred to the side arm of each biometer flask. Two of the flasks were inoculated with *Chaetomium sp.* The flasks were sealed and the fungi allowed to grow for 21 days at room temperature. One of the other two inoculated flasks was extracted with redistilled ethyl acetate at zero time while the other was extracted after 21 days. After termination of the experiment the fungal mats were extracted by immersing in ethyl acetate for 24 hr; a dry weight of the residual was determined after the extraction. The aqueous solutions were extracted as described in the replacement mat experiment. Radioactivity of the aqueous residue and the ethyl acetate extract was determined by liquid scintillation counting. Following extraction, the aqueous solutions were acidified to pH 2 and re-extracted with ethyl acetate. The quantity of radioactivity in this second ethyl acetate extract was also determined. The aqueous solutions were then readjusted to approximately pH 6

before the radioactivity assay. The ethyl acetate extracts were assayed for alachlor by gas chromatography.

Extraction of alachlor from aqueous media

Alachlor was extracted from the aqueous media with two 50 ml portions of redistilled ethyl acetate. The aqueous and organic phases were shaken for one minute in a 250 ml separatory funnel and the two phases were separated after 12 hr. The two ethyl acetate fractions were combined and dried over anhydrous Na_2SO_4 prior to gas chromatographic analysis.

Soil experiment

The metabolism of alachlor was studied in a Barry loam obtained from a plot at the Michigan State University Soils Experimental Farm which had never been treated with alachlor. Five grams of soil was placed in small glass bottles (4 inches high x 1 3/4 inches in internal diameter). A 2 ml plastic vial containing 1 ml of 1 N NaOH to trap $^{14}CO_2$ was suspended from the plastic cap. Fifty µl of an alachlor-^{14}C-alachlor solution (2915 dps and a final concentration of 100 ppm) was distributed dropwise from a 28 gauge needle on the soil in each bottle. The soils were moistened with 1.5 ml of tap water and the bottles sealed. The sterile control was obtained by subjecting one of the bottles to 3 M rads of ^{60}Co-radiation (McLaren and Peterson, 1967). The soils were extracted with two 20 ml portions of an acetone-water solution (80:20) at zero time and after 2, 7, and 21 days incubation. The radioactivity of the

acetone-water extract (0.1 ml) was assayed prior to evaporation of the acetone in a water bath at 60 C under a stream of nitrogen. The remaining aqueous portion was extracted with 20 ml of ethyl acetate. The ethyl acetate phase was removed, assayed for radioactivity (1 ml) and analyzed for alachlor by gas-liquid chromatography. The radioactivity remaining in the aqueous phase was also assayed.

Gas-liquid chromatographic (GLC) analysis

Quantitation of alachlor was by GLC employing a flame ionization detector. Triphenylmethane, which was well separated from alachlor, was added as an internal standard; 1 ml of a 100 ppm solution (in ethyl acetate) was added to 10 ml of the ethyl acetate extract. The same procedure was followed for preparation of the standard curve, which included values from 5 to 100 ppm alachlor. The injection volume of all samples was 2 µl. Gas chromatographic conditions were as follows:

Perkin-Elmer Model 900 gas chromatograph
Carrier gas (Helium) flow-40 ml/min
Injector temperature -210 C
Detector temperature -250 C
Column over temperature -198 C
Column - 182 cm x 3 mm O. D. stainless steel
packed with 4% SE-30/6% QF-1 (Thompson, Walker and Moseman, 1969) on 60/80 gas Chrom Q

Radioactivity assay

The ^{14}C-label was followed by liquid scintillation counting using a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 8310. All aqueous samples were counted in Bray's solution (Bray, 1960) while samples in ethyl acetate were counted in a toluene based liquid scintillator of POP and POPOP. The CO_2 trap of 1 N NaOH was counted in Bray's solution plus 4% Cab-O-Sil. All counts were corrected for quenching by external standardization and for machine efficiency.

Chemicals

Analytical grade alachlor, propachlor, CDAA (2-chloro-N,N-diallylacetamide) and propanil were supplied by the Monsanto Company, St. Louis, Missouri. The purity of alachlor was reported to be 99.8%. 3'-Chloroacetanilide, propionanilide, N-methylacetanilide, acetanilide and oxanilic acid were obtained from Eastman Organic Chemicals, Rochester, New York. The malt extract broth was purchased from Difco Laboratories, Detroit, Michigan. Uniformly ^{14}C-ring labelled alachlor was a gift of the Monsanto Company. The radioactive purity was 99.7% as determined by thin-layer chromatography.

RESULTS AND DISCUSSION

Enrichment media containing either propachlor, CDAA or alachlor as the sole source of carbon and energy showed no visible growth of microorganisms (Table 1). Growth was observed on all the other acylanilides used in this study. There appeared to be no difference between the inoculum source with regard to range of substrate degraded. The media at neutral pH were dominated by bacteria while those at the acidic pH contained mycelial and single cell (yeast) fungi. The results of the enrichment experiment explain why an organism capable of using alachlor as the sole source of carbon and energy has not been reported. Rather, cometabolism is a more likely mechanism for alachlor biodegradation in nature.

Aspergillus was used in this study because this genus is capable of utilizing an enormous variety of substances for food owing to the large number of enzymes produced (Alexopoulos, 1962). The Chaetomiaceae are referred to as cellulose-destroying fungi which grow on paper, fabrics, dung and straw (Alexopoulous, 1952).

As shown in Table 2, alachlor was not metabolized by a replacement mat of *A. niger* until 168 hr of incubation at which time 12.7% of the alachlor was lost. This quantity of degradation compares with the value 18% inorganic chloride

Table 1 - Growth in enrichment cultures containing acylanilides as the sole source of carbon and energy

Substrate	Media at pH 7.1		Media at pH 4.5	
	Soil	Water	Soil	Water
Propachlor	*-	-	-	-
CDAA	-	-	-	-
Alachlor	-	-	-	-
Propanil	+	+	+	+
3'-Chloroacetanilide	+	+	+	+
Propionanilide	+	+	+	+
N-Methyl acetanilide	+	+	+	+
Acetanilide	+	+	+	+
Oxanilic acid	+	+	+	+

* -, no growth; +, growth.

released in the aqueous medium at the same time (Table 3), suggesting that the chlorine atom of the parent molecule was removed during metabolism by *A. niger*. The relatively small amount of alachlor degraded in this experiment (12.7%) could be due to the fact that the fungus was not growing during metabolism. No metabolites were detected by GLC in this experiment.

Growing mats of *A. niger* showed a small increase in alachlor degradation with approximately 18% unaccounted for (Table 4). Again no metabolites were detected by GLC. Acetanilide was added to *A. niger* mat media in an attempt to

Table 2 - Metabolism of alachlor by a resting mat of *Aspergillus niger*

Treatment	Incubation time (hr)	Alachlor in medium (ppm)	Alachlor in medium corrected* for 100% extraction (ppm)	Alachlor from mat (ppm)	Total alachlor (ppm)
Alachlor & mat	0	91.0	93.8	0.0	93.8
Alachlor sterile control	0	97.8			
Mat control	0	0.0		0.0	
Alachlor & mat	5	92.0	94.8	4.6	99.4
Alachlor sterile control	5	94.4			
Mat control	5	0.0		0.0	
Alachlor & mat	10	93.0	94.9	6.8	101.7
Alachlor sterile control	10	99.8			
Mat control	10	0.0		0.0	
Alachlor & mat	24	88.6	91.3	7.4	98.7
Alachlor sterile control	24	96.0			
Mat control	24	0.0		0.0	
Alachlor & mat	168	76.8	79.1	8.2	87.3
Alachlor sterile control	168	97.0			
Mat control	168	0.0		0.0	

* Extraction efficiency correction factor derived from the amount of alachlor added, 100 ppm, divided by the average amount of alachlor recovered from the sterile controls.

Table 3 - Chloride released from alachlor by resting mats of *Aspergillus niger*

Incubation time (hr)	Chloride from mat* (µg)	Chloride from sterile alachlor control medium (µg)	Chloride released from alachlor by fungus (µg)	Percent chloride released
0	36.3	36.3	0.0	0.0
5	62.5	0.0	49.8	3.7
10	36.3	0.0	64.8	4.9
24	17.5	17.5	86.0	6.5
168	36.3	0.0	237.3	18.0

* Inorganic chloride in water used for second wash of mats.

Table 4 - Metabolism of alachlor after a 17 day incubation period by growing mats of *Aspergillus niger* and *Chaetomium sp.*

Treatment	Alachlor in medium (ppm)	Alachlor in medium corrected for 100% extraction* (ppm)	Alachlor from mat (ppm)	Total alachlor (ppm)
Aspergillus (1)†	69.0	75.9	5.0	80.9
Aspergillus (2)	70.0	77.0	5.0	82.0
Aspergillus + Acetanilide (1)	61.0	67.1	8.0	75.1
Aspergillus + Acetanilide (2)	75.4	82.9	7.0	89.9
Chaetomium (1)	6.0	6.6	0.0	6.6
Chaetomium (2)	2.5	2.7	0.0	2.7
Aspergillus (mat control)	0.0	0.0	0.0	0.0
Chaetomium (mat control)	0.0	0.0	0.0	0.0
0 time control (average)	90.7			

* Extraction efficiency correction factor was derived from the amount of alachlor added 100 ppm, divided by the average amount of alachlor recovered from the 0 time control.

† (1) and (2) represents replicates of the same treatment.

induce enzymes capable of breaking the amide linkage of alachlor. The results showed little difference in the amount of alachlor metabolized (17.5%) due to the presence of acetanilide.

Growing mats of *Chaetomium sp.*, however, metabolized an average of 95.4% of the alachlor added but again no metabolites were detected (Table 4). In a second growing mat experiment (Table 5), replicate flasks containing *Chaetomium sp.* degraded 69.1% and 43.9% of the alachlor. In this experiment two possible metabolites were detected (Figure 1), both of which were shoulder peaks on the solvent tail. The presence of these peaks in this experiment and not in the previous appeared to be due to slightly earlier elution of the solvent in this case, which was not the case in the previous experiment and thus masked small peaks of this retention time. Since no mat control was included in this experiment it is not certain that the peaks are from the herbicide. No attempt was made to identify the possible metabolites. The difference in the amount of alachlor metabolized by the replicates of *Chaetomium sp.* could be attributed to the difference in fungal mat masses, *Chaetomium* (1) weighing 0.053 g and *Chaetomium* (2) weighing 0.041 g. Extraction of the aqueous media after adjusting the pH to 2 did not yield any extractable metabolites detectable by gas chromatography.

Table 6 provides information on the fate of the ^{14}C-labelled alachlor used in this same growing mat experiment. Essentially none (0.08% and 0.06%) of the radioactivity was

Table 5 - Metabolism of alachlor after a 21 day incubation period by growing mats of *Chaetomium sp.*

Treatment	Alachlor in medium (ppm)	Alachlor in medium corrected for 100% extraction* (ppm)	Alachlor from mat (ppm)	Total alachlor (ppm)
Chaetomium (1)†	28.0	29.4	1.5	30.9
Chaetomium (2)	50.0	52.6	3.5	56.1
Sterile control	94.0	98.9	0.0	98.9
0 time control	97.0	101.8	0.0	101.8

* Extraction efficiency correction factor was derived from the amount of alachlor added, 100 ppm, divided by the average amount of alachlor recovered from the 0 time and the sterile controls.

† (1) and (2) represents replicates of the same treatment. Weight of Chaetomium (1) mat = 0.053 g; weight of Chaetomium (2) mat = 0.041 g.

Figure 1. Gas chromatography traces showing alachlor and possible metabolites.

A and B = possible metabolites

Tpm = triphenylmethane (internal standard)

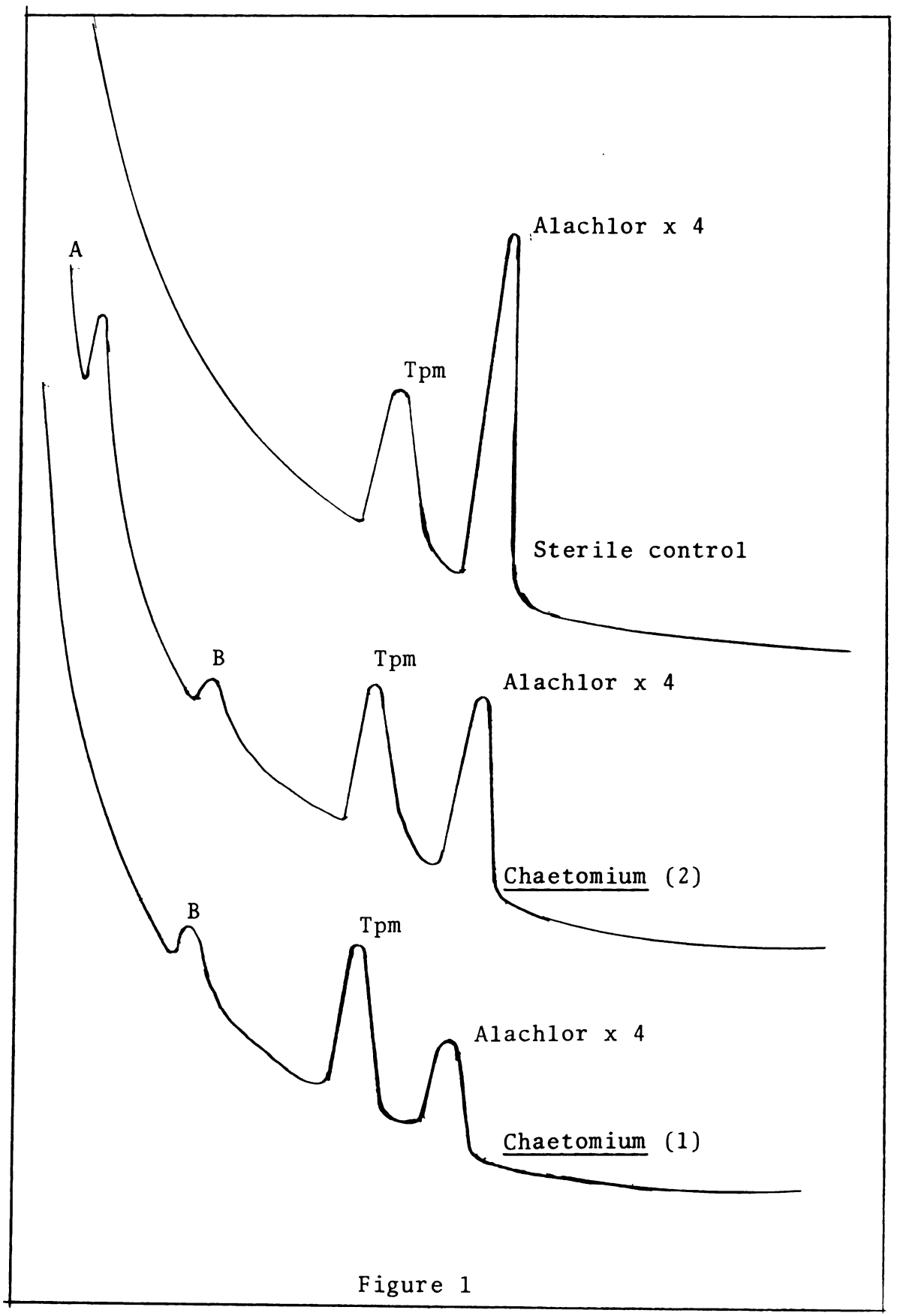
A
Alachlor x 4
Tpm
Sterile control
B
Tpm
Alachlor x 4
Chaetomium (2)
B
Tpm
Alachlor x 4
Chaetomium (1)

Figure 1

Table 6 - Fate of the ^{14}C-label of alachlor following metabolism by growing mats of *Chaetomium sp.* after a 21 day incubation period

Treatment	Percentage of total counts added				
		Neutral extraction		Acidic extraction	
	NaOH trap	Aqueous	Ethyl acetate	Aqueous	Ethyl acetate
Chaetomium (1)*	0.08	11.4	81.4	6.9	2.6
Chaetomium (2)	0.06	6.4	90.1	4.4	2.4
Sterile control	0.07	0.5	99.3	1.6	0.6
0 time control	-	1.0	89.4	1.4	0.6
Mat (1)	-	-	4.1	-	-
Mat (2)	-	-	4.3	-	-

* (1) and (2) represents replicates of the same treatment.

present in the NaOH trap showing that the ring was not cleaved and degraded to $^{14}CO_2$. Almost all (81.4% and 90.1%) of the radioactivity was extracted by the ethyl acetate. Since the GLC analysis showed that *Chaetomium* (1) and (2) did not metabolize 30.9% and 56.1%, respectively, of the alachlor in the medium, 50.1% and 46.0% of the radioactivity in the respective ethyl acetate extracts was not alachlor and this supports the suggestion that the two products detected are alachlor metabolites. Also, the finding of most of the radioactivity in the ethyl acetate extract suggests that the majority (3/4) of the metabolites from cometabolism of alachlor by *Chaetomium sp.* are non-polar. The remaining radioactivity (11.4% and 6.4%) found in the aqueous media suggests that there may be some water soluble metabolites also. This finding appears to be contrary to work on alachlor metabolism in plants in which the majority of metabolites are water soluble (Armstrong, 1972). The amount of radioactivity extracted by ethyl acetate from the acidified solution was minor.

Acetone-water (80:20) was used to extract alachlor from the soil because of its high efficiency of extraction for non-polar molecules, such as alachlor, which possess functional groups capable of forming hydrogen bonds with metal ions on the clay surfaces via water bridges.

The results of the experiment on alachlor degradation in soil are shown in Tables 7 and 8. At zero time, the GLC analysis (Table 7) indicated that 72.1% of the alachlor was recovered in the ethyl acetate extract. After 21 days only

Table 7 - Recovery of alachlor* from soil

Treatment	Incubation Time (days)	Alachlor extracted (ppm)
Soil	0	72
Sterile soil	21	16.0
Soil 1	21	16.1
Soil 2	21	16.5

* 100 ppm of alachlor was added to each soil.

Table 8 - Fate of ^{14}C-label from alachlor* incubated with soil

Treat-ment	Incubation time (days)	Percent of total counts added			
		NaOH trap	Acetone water extract	Ethyl acetate extract	Aqueous phase
Soil	0	-	91.0	93.3	4.0
Soil	2	0.2	93.7	-	-
Soil	7	0.2	90.8	-	-
Soil	21	0.7	43.9	32.8	12.5
Soil	21	0.9	44.1	30.8	14.3
Sterile Soil	21	0.7	49.9	42.9	3.6

* 100 ppm containing 0.078 µCi ^{14}C-alachlor was added to each soil.

16% of the original alachlor could be recovered from the sterile control and both soil replicates. The low recovery in the case of the sterile control could be due to destruction of the alachlor parent molecule by gamma radiation, chemical degradation or degradation by soil enzymes which are not usually destroyed by the dosage of radiation used in this experiment (Peterson, 1962). Thus because of the uncertainty of explanation for the poor alachlor recovery from the sterilized soil it is not possible to make any conclusion on the extent of its biodegradation in soil. Gas-liquid chromatography analysis of all four soil extracts showed another peak as a shoulder on the tail of the alachlor peak. No attempt was made to identify this peak. The possibility exists that this shoulder peak is a degradation product of alachlor or it may be a compound extracted from the soil and unrelated to the alachlor addition.

The 72% of the original alachlor present in the ethyl acetate extract as determined by GLC analysis (Table 7) does not appear to agree with the 93.3% of the ^{14}C-label in the same ethyl acetate extract (Table 8). This discrepancy could be due to experimental error or to rapid catalysis of alachlor in soil. Liquid scintillation counting showed that 91% of the ^{14}C-label was extracted at zero time in the acetone-water extract and approximately the same amount was extracted in the ethyl acetate extract. After 21 days, however, the soil replicates and sterile control contained only 43.9%, 44.1% and 49.9% of the ^{14}C-label, respectively, in the acetone-water extract. The apparent loss of alachlor

here could be due to microbial or chemical degradation or irreversible adsorption to the surface of the clay particles. Degradation by the acidic soil water film surfaces (Hargrove and Merkle, 1971) was apparently not important here since the soil was very moist throughout the experiment.

The ethyl acetate extracts of the soil and sterile control contained 32.8%, 30.8% and 42.9% of the ^{14}C-label, respectively. This also did not agree with the GLC analysis of the same solutions. This discrepancy could be explained by the possibility of a non-polar metabolite(s) in the extract. Radioactive analysis of the water extract (Table 8) indicates that there may also be a water-soluble metabolite since 12.5% and 14.3% of the ^{14}C-label remained in the aqueous portion of the soil replicates. In this case, however, both the zero time and sterile controls showed less radioactivity in the water extracts.

The aromatic ring of alachlor was not metabolized to $^{14}CO_2$ (Table 8) in the soil, suggesting that the apparent degradation would have resulted in the transformation or removal of at least one functional group.

In summary, the herbicide alachlor was cometabolized by pure cultures of *A. niger* and *Chaetomium sp.* The latter fungus produced two possible non-polar metabolites. No conclusion could be made as to the extent of the biodegradation of alachlor in soil, though apparent disappearance of the herbicide occurred with the production of both non-polar and polar metabolites indicated.

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