Journal of Environmental Sciences (China), Vol. 1, No. 2, pp 98–103, 1989 ISSN 1001-0742

Fate of the herbicide metolachlor in aerobic and anaerobic soils*

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(Received December 14, 1988)

Abstract—The fate of the herbicide metolachlor in aerobic and anaerobic soils with repeated applications of the metolachlor over a period of 5 years was studied. After 12 weeks incubation, cumulative $^{14}CO_2$ evolution from the soil accounts for 8.01% in aerobic condition versus 1.5% of $^{14}CO_2$ in the soil had not been treated with metolachlor. The total ^{14}C recovery in the methanol–water extract and in the non-extractable portion of this aerobic soil accounted for 73.1% and the total metolachlor recovery in the methanol–water extract was 46.7% but 86.9% of ^{14}C was accounted for in the γ-irradiated control soil. There are no differences in the recovery of ^{14}C between non-sterile and γ-irradiated control soil under anaerobic conditions. The results show that there was some active metolachlor-degrading population in the Virginia soil which had been previously received repeated applications of the metolachlor but only under aerobic condition.

Keywords: metolachlor; aerobic soil; biodegradation.

Metolachlor (2–chloro–N(2–ethyl 6 - methylphenyl) N (2 methoxy - 1 - methylethyl) acetamide) is the active ingredient of the important selective herbicide 'Dual' which is widely used for the control of several annual grass weeds, yellow nutsedge and certain broadleaf weeds in maige, soubeans, peanuts, sunflowers, sugarbeets and other crops. Because of the large quantities of this herbicide applied to soil and the potential toxicity to nontarget organisms, particularly its persistency in the soil environment (Zimdahl, 1982), the fate of metolachlor in terrestrial ecosystems has attracted considerable attention. Studies published over the last decade have shown that metolachlor activity can be diminished by volatilization, photodecomposition absorption and leaching (Hill, 1978; Kozak, 1983; Parocheffi, 1973). Microbial degradation may be a major influence upon the enviornmental fate of metolachlor, yet this process is poorly understood. Ellgehausen (1976) found that 12 weeks after application of the herbicide, 30% of metolachlor was dechlorinated in aerobic sterile soil, while in aerobic or anaerobic nonsterile soil about 18% of the radioactivity was oxidized at the acetyl group to form an oxalic acid derivative. Analysis of the soil, Ellsohausen indicated that only 10% of the residual activity in nonsterile soil was the parent compound versus 65% of metolachlor from soil were identified by Sumner *et al.*(1976), but altogether these comprised less than 4% of the total radioactivity. Zimdhl and Clark (1982) reported that metolachlor persisted longer than alachlor under both laboratory and field condition. Both alachlor and metolachlor have been reported to be degraded by a soil fungus (Liu, 1988). Our experiments demonstrate that microbial activity is responsible for the mineralization of metolachlor in a soil perfusion system.

The half life of metolachlor in soil has been reported to be from 15 to 50 days under field conditions (WSSA, 1979). The rate of $^{14}CO_2$ evolution from soil, however was slow, 4.8% over

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*Most experiments and research work have been done in Soil Microbiology Lab, the Pennsylvania State University, USA, under the guidance of Professor Jean Marc Bollag.

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12 weeks under laboratory conditions (Ellgehausen, 1976). McGahen and Tiedge (1978) investigated metabolism of metolachlor by resting cells of *Chaetomium globosum* and found 45% disappearance of the herbicide after 144 hours of incubation. Eight extractable products were found, and four were identified. In 1982, the same authors studied the anaerobic metabolism of metolachlor with lake sediment and found two products, the dechlorinated metolachlor with and without a thiomethyl moiety. Krause *et al.*(1985) reported eight metolachlor metabolites were isolated in the pure culture and identified as it showed the monochlorine isotopic pattern. However, dechlorinated metabolites of alachlor (Bollag, 1982), butachlor (Chen, 1978), metolachlor (McGahen, 1978) and propachlor (Kaufman, 1971; Lee, 1982) have been reported. We have found strains of *Bacillus, Bacillus megaterium, Fusarium sp., Mucor racemosus*, and an actinomycete to transform metolachlor (Adesh, 1987). To summarize, then, possible microbial transformation reactions of metolachlor include dechlorination, dehydrogenation, dealkylation, hydroxylation and indoline ring formation, and mineralization.

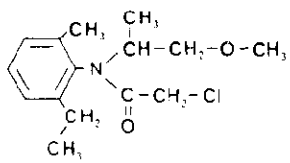
Although a few authors reported the fate of metolachlor in the sterile or unsterile soil under aerobic or anaerobic condition, little is known about the mineralization of this herbicide, especially the comparison of the fate of metolachlor in anaerobic soil to in aerobic soil and the comparison of the transformation of metolachlor in the soil which has been previously exposed to metolachlor with in unexposed metolachlor soil.

The present study was designed to examine whether or not soils previously exposed to metolachlor have active metolachlor–degrading population and to compare metolachlor biodegradation under aerobic and anaerobic conditions.

MATERIAL AND METHODS

Chemicals

Metolachlor (technical grade) of 95.4% of purity and uniformly ring–labelled (^{14}C) metolachlor (specific activity 2.63 μCi/mg, 97%pure) were supplied by Ciba–Geigy Corp., Agricultural Division, Greensboro, N.C. The structure of the non–labelled reference substances used as following:



Soil

The soil used was a sandy loam collected from Virginia State in USA. The soil had been received repeated applications of the metolachlor over a period of 5 years.

Soil incubation under aerobic and anaerobic conditions

Twenty grams of the soil with 20% moisture content was put into 125ml glass jars. To each jar, 0.4 ml of 50% ethanol containing 3 mg of metolachlor, 2.7×10^5 dpm (120 nCi) ring–UL–^{14}C–metolachlor, and 1 ml of sterilized H_2O were added and mixed thoroughly. The final concentration of metolachlor was 150 ppm. For aerobic samples, all jars were closed with foam plugs. For anaerobic study, the jars were prepared in anaerobic chamber (Loy laboratories product Inc.) in the following way: after air was withdraw by a vacuum pump, the chamber was filled with nitrogen, which was subsequently replaced by a gas mixture (95% nitrogen, 5% hydrogen). A glass vial containing 6 ml of 2 mol/L NaOH was placed inside each jar, and the jars were closed with rubber stoppers. All samples were incubated at 28°C in the dark. For aerobic samples, sterilized water (1.5 ml) was added every 4–5 days to maintain approximately

the same mixture level.

Trap $^{14}CO_2$ from aerobic incubation soil

100 g of the soil with 20% moisture content was added to 250 ml flask and the mixture content was adjusted to 50% of the water holding capacity using soil extraction (pH 6.8). The flask was connected at both ends to a CO_2 trap which contained 15 ml of NaOH solution. The incoming air was humidified and free of CO_2, while the outgoing air contained CO_2 liberated into the outgoing trap.

The outgoing CO_2 trap contained standardized 0.5 mol/L NaOH solution. The tubes were periodically removed for analysis and replenished with fresh 0.5 mol/L NaOH solution (Fig. 1).

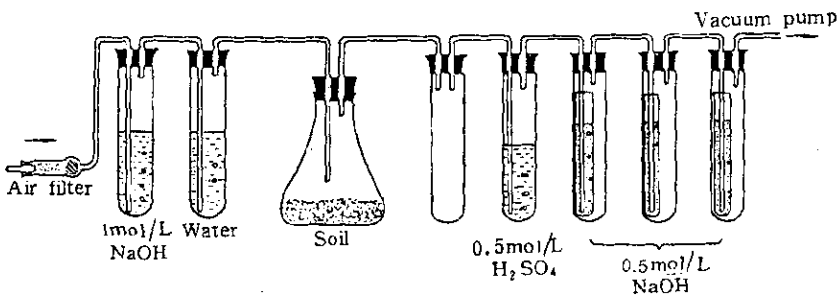


Fig. 1 Apparatus for trapping $^{14}CO_2$ from aerobic incubation soil

Radioactivity was measured with a Beta Trac 6895 liquid Scintillation Counter (Tracor Analytic, EIK Grove Village, IL). Samples were counted in Scinti-Verse universal liquid scintillation cocktail (Fisher Scientific Company, Fair Lawn, NJ).

Extracted soils were mixed thoroughly and air dried. Nonextractable radioactivity in the soil was determined by dry combustion of the soil using a Packard Model Tri-Carb 306 sample oxidizer. 500 mg of soil were mixed with 300 mg of cellulose powder and the entire content was placed in a combusto-cone sample holder (Pachard), and a pellet was made using a Parr press. $^{14}CO_2$ liberated from soil was absorbed in 7 ml of Carbo-sorb T.M. and 12 ml of a cocktail (toluene-based, containing 91% PPO and 9% bis-MESB Pachard) was added for radioactivity counting.

Sampling and analytical techniques

To determine the extent of degradation of metolachlor at each sampling interval, the soil of each jar extracted twice using 50 ml of 80% methanol and then with 50 ml of 100% methanol for extraction of metolachlor. After each extraction, the methanolwater soil slurry was filtered through a Whatman No.4 filter paper in a Buchner funnel. All filtrates were combined and condensed on a rotary vaccum evaporator at 65°C. The remaining water phase was collected and adjusted to volume of 50 ml with water. Aliquots (4 ml) of the water samples were extracted with an equal volume of methylene chloride which subsequently was evaporated to dryness and resuspended in 2 ml of hexane. 2 μl of sample in hexane were injected onto a GC for metolachlor analysis, and 0.5 ml of the sample were counted for radioactivity.

Gas-liquid chromatographic determinations of metolachlor were done on a Pachard Model 7424 gas chromatograph equipped with a flame-ionization detector. A 1.2 m, 2 mm i.d. glass column packed with 10% SP-2100 on 80-100 mesh Supel Coport was used. Operating temperatures were: injection port, 240°C; column, 210°C and detector, 230°C. Nitrogen (carrier gas) flow rate was 40 ml/min, hydrogen and air 40 and 400 ml/min, respectively.

RESULTS AND DISCUSSION

After 12 weeks incubation, cumulative $^{14}CO_2$ evolution from the soil which had been exposed to metolachlor over a period of five years account for 8.01% under aerobic conditions

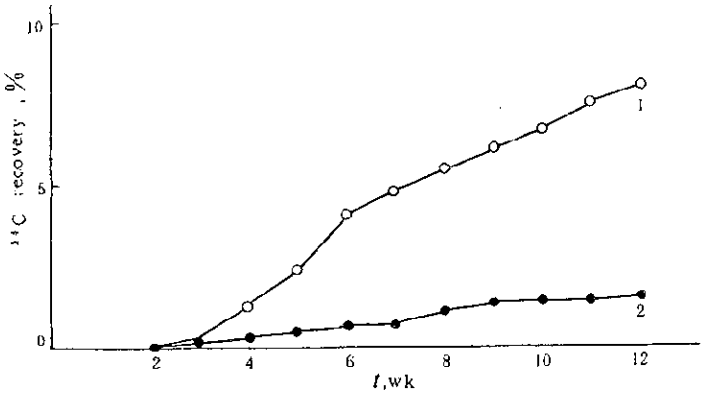


Fig. 2 Cumulative $^{14}CO_2$ evolution from metolachlor-amended Virginia soil 1. Virginia soil previously treated with metolachlor; 2. Virginia soil never treated with metolachlor

versus 1.5% of $^{14}CO_2$ in the soil had not been treated with metolachlor (Fig.2).

The amount of $^{14}CO_2$ evolved from no-metolachlor treated soil indicating the possible microbial involvement in the mineralization of metolachlor in the metolachlor exposed soil under aerobic condition.

The total ^{14}C recovery in the methanol-water extract and in the non-extractable portion of aerobic soil accounted for 73.1% and 86.9% in non-sterile and γ-irradiated metolachlor-exposed soil, respectively. The recovery of extractable ^{14}C in the sterile soil was always higher than that of non-sterile soil. The total metolachlor recovered in the hexane extract from methanol-water extract of aerobic soil was 46.7% and 60.7% respectively (Fig.3); thus, some binding, even to γ-irradiated soil, occurred. The recovery of metolachlor from non-sterile soil was always lower than from γ-irradiated soil and the non-extractable ^{14}C from the non-sterile soil was always higher than that from γ-irradiated soil, indicating possible microbial enhancement of metolachlor-soil binding and transformation. The microbial transformation included the transformation and mineralization. The total ^{14}C recovered is always higher than the recovery of metolachlor measured by gas-chromatography, therefore this phenomenon indicated some organic products may have been produced.

The results showed that there was some active metolachlor-degrading population in the Virginia soil which had been previously received repeated applications of the metolachlor over a period of five years. This microorganism community increases the rate of transformation of herbicide metolachlor as a result of a period exposure to it. Previous work has shown that microorganisms could develop the ability to degrade pesticide or herbicide either by gene transfer, chance mutation of enzyme induction and adaptation (Hill, 1978; Spain, 1980). It can play a major role in determining biodegradation rates.

The decrease of metolachlor concentration in sterile control (Fig. 3) may be due to several factors, including absorption (Bartha, 1971). The soil organic matter is the principal component governing the availability of pesticides to microbial metabolism, but the major role that soil organic matter plays in adsorption of organic chemicals by soil is well recognized (Carringe, 1975; Kozak, 1983). Metolachlor is nonionic, according to Shin *et al.*(1970), the adsorption of nonionic pesticides by organic meterials increases with degree of humification. If sterilization did not drastically alter the humic structure of soil, significant adsorption could occur (Bartha, 1971).

As shown in Fig. 4, there are some differences in the recovery of ^{14}C between non-sterile

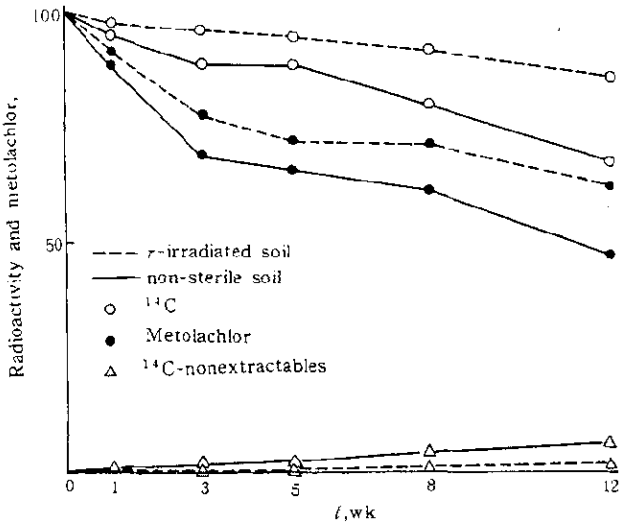


Fig.3 Degradation of metolachlor in aerobic soil which had been exposed to metolachlor over a period of 5 years

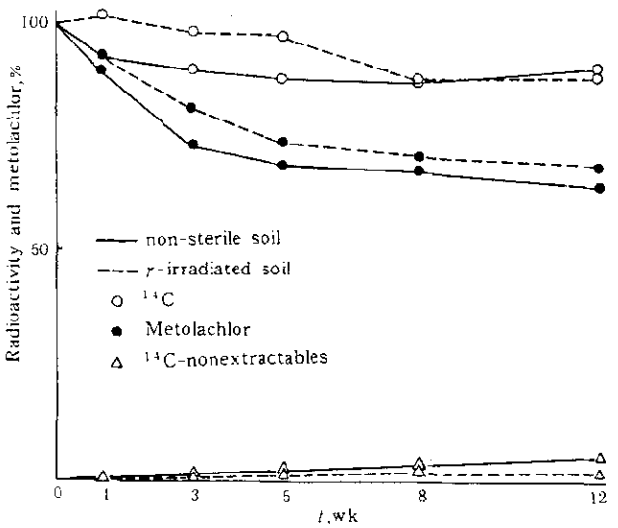


Fig.4 Degradation of metolachlor in anaerobic soil exposed to metolachlor over a period of 5 years

and γ-irradiated soil during the first five weeks of incubation in anaerobic soil.

During the entire incubation period, approximately 11% of ^{14}C was lost in both soils, 2% of which was trapped in 2 mol/L NaOH. There was no significant lost of ^{14}C in case of anaerobic condition besides some absorption. The results have not shown microbial involvement in the mineralization of metolachlor in the anaerobic soil. Although the microbial activity in anaerobic soil was lower, the recovery of metolachlor in the non-sterile soil is always lower than that of γ-irradiated soil indicating possible microbial involvement in the transformation of metolachlor in the non-sterile soil, even though it does not serve as an energy source for the microorganism in the non-sterile soil.

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