Effects of High Rate of Alachlor and Metolachlor on Microbial Activities in Soil

ISMAIL SAHID and ZAYDA KASIM

Department of Botany, Faculty of Life Sciences
Universiti Kebangsaan Malaysia UKM, Bangi, Selangor Darul Ehsan
43600 Malaysia

ABSTRAK

Satu kajian telah dijalankan untuk melihat kesan dua herbisid asetanilid, iaitu alachlor dan metolachlor ke atas akitiviti mikrob dalam tanah loam berpasir. Pengaruh herbisid ke atas pembebasan CO_2 telah dijalankan selama 50 hari dalam keadaan ambien. Pada amnya, hasil kajian mendapati yang alachlor dan metolachlor menyebabkan pembebasan CO_2 berkurangan di peringkat awal tetapi selepas 25 hari ia meningkat sama seperti kawalan. Pada kepekatan yang rendah, kedua-dua herbisid tersebut kurang berkesan terhadap pembebasan CO_2. Populasi kulat dan bakteria dalam tanah didapati menurun pada kepekatan herbisid yang tinggi. Pada amnya kedua-dua herbisid, kecuali metolachlor pada 20 ppm didapati mempengaruhi aktiviti phosphatase di dalam tanah.

ABSTRACT

A study was carried out to investigate the effects of the two acetanilide herbicides alachlor and metolachlor on microbial activities in sandy loam soil. Effects of the herbicides on CO_2 evolution were monitored for 50 d in ambient conditions. The results showed that alachlor and metolachlor generally caused an initial decrease in CO_2 release, which subsequently increased to control level after 25 d of incubation. Both herbicides exert less effect on CO_2 evolution at lower concentrations. Fungal and bacterial populations in the soil also declined in the presence of either herbicide at higher concentrations. Phosphatase activity was generally affected in the presence of either herbicide except in soil treated with metolachlor at 20 ppm.

INTRODUCTION

Soil fertility often depends on the very delicate balance of the various types of micro-organisms whose activities determine the efficiencies of the various metabolic cycles, e.g. nitrogen, carbon and mineral. It is clear that the addition to the soil of any potentially toxic herbicide, or for that matter, any compound such as insecticide, fungicide or nematicide, constitutes a threat to this equilibrium and hence to the future fertility of the soil (Edwards 1989; Moorman 1989). The equilibrium can be altered by direct toxic action on microorganisms in the soil, by selective toxicity for certain groups of microorganisms thus indirectly altering the population equilibrium, or by promoting the growth of one or more types of soil organism. These depressive or stimulating effects depend upon the kind of chemical and its concentration, possibly moderated by environmental conditions (Moorman 1989).

The acetanilide herbicides alachlor and metolachlor are registered for control of most annual grasses and certain broadleaf weeds in many crops such as corn (*Zea mays* L.), soybean (*Glycine max* [L.] Merr.), rice (*Oryza sativa* L.), and peanut (*Arachis hypogaea* L.) (Weed Science Society of America 1989). In Malaysia, these two herbicides are used for weed control in corn and peanut. While much is known about their activity and mode of action, little quantitative information is available concerning their effects on soil microbial activities under local conditions.

There are several ways of assessing the effects of herbicides on microbial activities, including detection of CO_2 evolution, measurement of O_2 uptake, estimation of microbial population lev-

els, and assay of soil enzyme activities. Each method has its advantages and disadvantages. In recent years, the measurement of soil biological activity has increasingly relied on assays for soil-borne microbial enzymes such as phosphatase (Marsh 1980; Davies and Greaves 1981; Schinner and Mersi 1990). The present study measured carbon dioxide evolution, phosphatase activity and microbial populations in order to assess potential detrimental effects of herbicides on the soil microflora.

MATERIALS AND METHODS

Soil Samples

Soil samples were obtained from the top 5 cm of an uncultivated plot at the Universiti Kebangsaan Malaysia, Bangi, Selangor. The soil was a sandy loam (45% sand, 35% silt and 20% clay) with 0.38% organic C and pH 4.5. Before use, the soil was passed through a 3-mm sieve, placed in black polythene bags and stored at 4ºC.

Herbicides

The two acetanilide herbicides tested were metolachlor (Dual[R], Ciba Geigy) containing 720 g L^{-1} of 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide, and alachlor (Lasso[R], Monsanto) containing 480 g L^{-1} of chloro-2',6'-diethyl-N-methoxymethyl.

Soil Treatment

Soil samples were treated with either alachlor or metolachlor according to the commercial formulation of either Lasso[R] or Dual[R]. Moist soil equivalent to 4 kg oven-dried soil was placed in a cylindrical metal drum (30 cm x 27.5 cm) lined with a polythene sheet. Each herbicide was applied separately by spraying onto the soil to give a mean final concentration of 0, 20 or 150 ppm of the active ingredient (calculated on an oven-dried basis). The mixture was mixed thoroughly in a rotating drum. The moisture content was then adjusted to 80% of field capacity as described by Grossbard and Wingfield (1975). Field capacity was determined at a suction pressure of 75 cm of water on tension tables (Clements 1966). Three 4-kg replicates per treatment were prepared and incubated in air-filled double polyethylene bags at 27ºC. The bags were opened once a week to prevent the soil becoming oxygen-deficient. The

soil moisture levels were checked regularly by weighing and adjusted to 80% of field capacity by adding deionized water as necessary.

Carbon Dioxide Evolution

Carbon dioxide evolution was measured using a continuous gas flow system as described by Grossbard and Marsh (1974). Two samples of 100 g of soil each were taken from each replicate one day after spraying and incubated in 500 ml respiration flasks attached to a manifold supplying a slow flow of moist CO_2-free air. This was passed through the layer of soil in the flask from an inlet close to the bottom of the vessel and the CO_2 was absorbed in 40 ml M-NaOH in a Drechsel bottle and measured periodically by titration with 0.05 M H_2SO_4. The soil samples were incubated at 27ºC. By replacing fresh NaOH in the Drechsel bottles, the CO_2 evolved from the soil was recorded during 50 d incubation.

Microbial Count and Phosphatase Activity

Soil samples were removed from the polyethylene bags on day 0, 2, 5, 15, and 45 for microbial population counts and on day 2, 10, 17, 28 and 42 for phosphatase assay.

Soil suspensions were prepared by homogenizing 5 g soil in 50 ml of quarter-strength Ringer solution (Harrigan and McCance 1966) for 15 min at 300 rpm. A series of ten-fold dilutions between 10^{-3} and 10^{-5} of the suspension was made with sterile Ringer solution. Each dilution was gently agitated throughout the plating procedure for 15 min. A preliminary experiment had indicated that 10^{-4} dilution was suitable for the study; so this dilution was used throughout for enumerating bacterial population. About 0.1 ml of this suspension was transferred to each of five petri dishes containing nutrient agar to examine bacterial behaviour, with the addition of cyclohexamide at 50 ug/ml for suppression of fungi. For enumeration of fungi, the above dilutions were transferred onto potato dextrose agar with addition of streptomycin (30 ug/ml) to avoid bacterial growth. Plates were incubated at 30±3ºC. Dilutions of soil samples were made in triplicate.

Phosphatase activity was measured on 1 g samples of soil by the method of Tabatabai and Bremner (1969). The production of p-nitrophenol was determined spectrophotometrically at 420 nm.

RESULTS AND DISCUSSION

Evolution of CO_2 is the most commonly used measurement of microbial activity, especially the decomposition of organic matter. Any great reduction should be regarded as a preliminary warning of interference with the metabolism of the soil.

In laboratory experiments, adverse effects are more frequent when high concentrations are used. These are sometimes preceded by a temporary increase in carbon dioxide (Bartha *et al.* 1967). In this study, the rate of CO_2 released was greatly reduced in soil treated with 150 ppm of either alachlor or metolachlor during the first 7 d of incubation (*Fig. 1*). However, CO_2 evolution increased to and exceeded control levels in soil treated with either herbicide (except alachlor at 150 ppm) on day 25. This excess of carbon was perhaps derived from the decomposition of the herbicide. It has been suggested that certain species of fungi may use herbicide as a source of carbon (Smith *et al.* 1976).
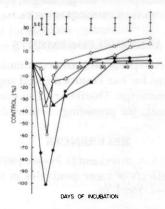
DAYS OF INCUBATION

Fig. 1: The effect of alachlor and metolachlor on carbon dioxide evolution of soil incubated under laboratory condition for 50 days. Each point on this graph corresponds to the amount of carbon dioxide, expressed as % of the control. ○ 20 ppm alachlor; ● 150 ppm alachlor; ▲ 20 ppm metolachlor; ▲ 150 ppm metolachlor

The lower CO_2 evolution early in incubation may be due to the lower microbial population, increasing later as the population recovered. As shown in Table 1, fungal and bacterial numbers decreased in soil treated with either herbicide as compared to control from day 2 of incubation. The increase of microbial populations after further incubation may be due to decreased toxic effect of herbicide due to adsorption or degradation of the herbicide residue.

Alachlor is degraded in soil mainly by microbial activity (Weed Science Society of America 1989). The half-life of alachlor and metolachlor in tropical soil has been reported to be 8 to 22 d depending on soil moisture (Ismail and Wei 1993). These herbicides have quite similar behaviour in soil since they both belong to the acetanilide group; however, metolachlor was found to persist longer than alachlor (Zimdahl and Clark 1982). The difference in persistence may reflect both the change in microbial populations as well as microbial activities.

TABLE 1

Fungal and bacterial populations (x10⁵) in sandy loam soil after treatment with alachlor and metolachlor.

Herbicides (ppm)	Days after treatment				
	0	2	5	15	45
			Fungi		
Control	3.5	2.3	1.5	1.9	1.7
Alachlor					
20	2.5	1.9	1.4	1.9	1.3
150	2.1	1.6	1.1	1.9	0.9
Metolachlor					
20	1.5	0.9	0.8	1.3	0.9
150	0.9	1.0	0.5	0.6	0.4
S.E.	0.28	0.16	0.12	0.31	0.20
			Bacteria		
Control	4.2	3.6	2.3	3.2	2.1
Alachlor					
20	3.2	3.3	2.0	2.7	1.5
150	2.5	3.2	1.3	1.9	1.0
Metolachlor					
20	2.2	2.7	1.4	2.1	1.7
150	1.4	3.6	2.2	2.9	2.7
S.E.	0.22	0.21	0.22	0.27	0.35

The populations of soil fungi and bacteria were affected by treatment with either alachlor or metolachlor (Table 1). Generally metolachlor gave greater reduction in fungal numbers in soil than alachlor at comparable concentrations. An increase of alachlor from 20 to 150 ppm resulted in a very slight additional reduction of fungal population. Fungal population seemed to fluctuate during the incubation period but generally decreased following a pattern similar to that in the control. Alachlor at 150 ppm treatment ap-

peared to cause a greater reduction in bacterial than in fungal numbers, while metolachlor at the same concentration did not seem to affect bacterial populations during the incubation period after day 0. The result clearly shows that metolachlor was more toxic to fungi than to bacteria while alachlor was more toxic to bacteria than to fungi. Some fungi such as *Rhizoctonia solani* can degrade alachlor in soil (Smith and Phillips 1975; Tiedje and Hagedorn 1975), perhaps eliminating alachlor residue faster than metolachlor from the soil. The difference in degradation rates between the two herbicides may be explained in part by their different susceptibilities to microbial degradation.
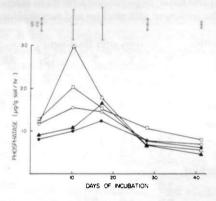

Fig.2: The effect of alchlor and metolachlor on phosphatase activity in sandy loam soil, □ control soil: ○ 20 ppm alachlor: ● 150 ppm alachlor: □ 20 ppm metolachlor: ▲ 150 ppm metolachlor

Phosphatase activity was generally lower in soil treated with either herbicide than in the control (*Fig. 2*). The level of phosphatase in soil treated with 20 ppm metolachlor was 150% of the production of phosphatase in the control soil at day 10, reducing to slightly more than control on day 15. By day 28, phosphatase activity was lower in soil treated with either herbicide than in untreated soil. In general, phosphatase activity in soil treated with either herbicide inversely correlated with microbial population. Earlier reports have shown that herbicides may enhance or inhibit soil enzyme activities (Quilt *et al.* 1979; Davies and Greaves 1981). For instance, Quilt *et al.* (1979) showed that after an initial inhibition, there was a consistent increase in phosphatase activity in sandy loam soil treated with barban at 200 ppm. In contrast, atrazine was found to significantly reduce enzymatic activity

in loamy sand soil (Voets *et al.* 1974). Earlier reports testing the herbicides glyphosate, paraquat, trifluralin and atrazine have shown that none of the herbicides markedly reduce enzyme activities in sandy loam soil (Davies and Greaves 1981). Direct comparison of our results with those described in the literature is difficult. Undoubtedly, the wide range of soil type used with greatly differing enzyme activities is responsible for the frequently contradictory results.

Under field conditions, it has been suggested that the reduction of soil enzyme activity results partially from the indirect effects of the application of herbicide, namely the elimination of the direct vegetative cover and the concomitant decrease in the soil organic matter (Voets *et al.* 1974). Organic content is one of the factors that has an effect on soil microbe populations (Marsh *et al.* 1978). It should be noted that the soil was not supplied with nutrients or organic sources during the incubation period. The reduction in phosphatase activity observed in control soil when the incubation period was prolonged may be due to the depletion of nutrient sources.

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