Bioactivity, adsorption and persistence of two herbicides in tropical soils

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Summary

The effects of environmental factors on bioactivity, adsorption and persistence of two herbicides, viz. alachlor and terbuthylazine, were studied in the laboratory and greenhouse using cucumber (*Cucumis sativus* L.) as the bioassay species. The bioactivity of both herbicides was inversely correlated with the organic matter content of the soil and increased with increasing herbicide concentrations. Alachlor adsorption by sand-peat mixture increased proportionately with an increase of soil organic matter content and with increasing herbicide concentration. Half-life of alachlor decreased from 15.2 to 6.2 days as temperature increased from 25 to 35°C in the Selangor Series soil and from 14.5 to 6.2 days in the Serdang Series soil. An increase of temperature from 25 to 35°C also reduced the half-life of terbuthylazine in both soils. The half-life of alachlor decreased at moisture level of 70% field capacity in both soils. However, the dissipation rate of terbuthylazine was significantly faster at 50% field capacity (9.4 days) in Serdang Series than at 70% (11.4 days).

Introduction

The fate of herbicides in soils is greatly influenced by their interaction with the soil environment. It is now well established that phytotoxicity and persistence of most herbicides are influenced by the organic matter content of soil (e.g. Blumhorst *et al.* 1990). Generally, adsorption and decomposition are the two major factors that influence the bioactivity of most of soil-applied herbicides (Aldrich 1984). Phytotoxicity is inversely related to the soil organic matter content (Okafor *et al.* 1983, Peter and Weber 1985, Oppong and Sagar 1992). It has been suggested that variations in the phytotoxicity of the herbicides in soil are caused by differences in the adsorption capacity of the soil (Okafor *et al.* 1983). An inverse relationship between phytotoxicity and soil adsorption of various herbicides has been reported by Doherty and Warren (1969).

Herbicides are degraded in the soil by a combination of microbiological and chemical processes. Microbial degradation of herbicides increases with increasing soil temperature (Bouchard *et al.* 1982) and soil moisture levels (Hurle and Walker 1980). Temperature can, therefore, be an important factor affecting the persistence of herbicides in the soil, especially in the tropics. High temperatures would favour herbicide loss through volatilization and photodecomposition and by increasing microbial activity (Klingman and Ashton 1982). Soil moisture is also known to influence the dissipation of herbicides in soil (Aldrich 1984). Herbicide degradation in soil generally increases with increasing moisture levels up to field capacity, probably reflecting increased microbial activity (Hurle and Walker 1980).

Alachlor (2-chloro-2,6-diethyl-N-[methoxy methyl]-acetanilide) is a selective pre-emergence or early post-emergence herbicide used in many countries for the control of annual grasses and several broad-leaved weeds in soybean, maize, cotton, peanut, brassica, oilseed rape, sugar cane and potato crops (Sharp 1988). Terbuthylazine is a broad-spectrum herbicide with high selectivity for many coniferous and deciduous forest trees. Terbuthylazine (2-tert.buthylamine-4-chloro-6-ethylamino-5-triazine) enters the plants through the roots and inhibits photosynthesis. It is used as a pre-emergence or early post-emergence herbicide in sorghum, citrus and maize crops, pod forests and vineyards (Worthing and Hance 1991). Alachlor and terbuthylazine are primarily degraded microbiologically in soil, and the rate of degradation is influenced by temperature and soil moisture content (Zimdahl and Clark 1982, Walker and Brown 1985, Sun *et al.* 1990).

As both alachlor and terbuthylazine are soil-applied herbicides, it is important to study their behaviour and interaction with various soil components. High organic matter content in soil increases adsorption, while conditions of high temperature and moisture that favour herbicide degradation, may result in reduced herbicide efficacy. Although many reports have been published on the persistence of herbicides in soil, only limited information is available on the persistence of these herbicides in tropical soils. Therefore, experiments were conducted to determine the persistence of alachlor and terbuthylazine in two soils, viz. Selangor Series and Serdang Series, at various temperatures and moisture levels. The effects of soil organic matter content on the bioactivity and adsorption of both these herbicides were also examined.

Materials and methods

Soils

Sand and peat soil were used in the phytotoxicity and adsorption studies in a greenhouse. In the persistence study, two soils, Selangor Series (silty clay) and Serdang Series (sandy clay loam), were used. Samples of Selangor Series soil was collected from Jenderata Estate, Teluk Intan, while the samples of Serdang Series came from the experimental plot at Universiti Pertanian Malaysia, Serdang. Soil characteristics are shown in Table 1. The soils were collected from the 0-10 cm depth, air-dried and screened through a 1.0 cm sieve prior to use.

Herbicides

Two herbicides were used in the study, viz. alachlor containing 480 g a.i. L^{-1} aqueous solution; and terbuthylazine containing 500 g a.i. L^{-1} aqueous solution.

Phytotoxicity study

Sand, peat or their mixtures containing 5, 10, 20 or 50% (v/v) peat were treated to obtain a final concentration of 0, 2, 4 or 6 ppm for alachlor and 0, 5, 10 or 15 ppm for terbuthylazine. The treated soils were placed in 6.5 cm diameter plastic pots into each of which ten cucumber (*Cucumis sativus* L.) seeds were sown at a depth of 0.5 cm. The soil in each pot was watered to 80–90% field capacity and maintained at this level throughout the experiment by watering twice a day. After emergence, the seedlings were thinned to four per pot. Seven and nine days after emergence for alachlor and terbuthylazine respectively, the plants were harvested by cutting at soil level and fresh weights were recorded. The data were expressed as a percentage of the untreated control value.

Adsorption study

Two gram samples of peat (100% organic matter), 5 g samples of sand or peat-sand mixtures containing 5, 10 or 20% (v/v) peat and 4 g sample of 50% (v/v) peat-sand mixture were weighed into 50 mL conical flasks, to which 20 mL of 0, 2, 4 or 6

Table 1. Some characteristics of the two soils used in these studies.

Soils	pH	% sand	% silt	% clay	% organic matter	CEC (Meq $100g^{-1}$)
Selangor Series	4.01	3.1	53.5	43.4	4.33	23.7
Serdang Series	4.57	50.6	14.6	34.8	0.78	4.7

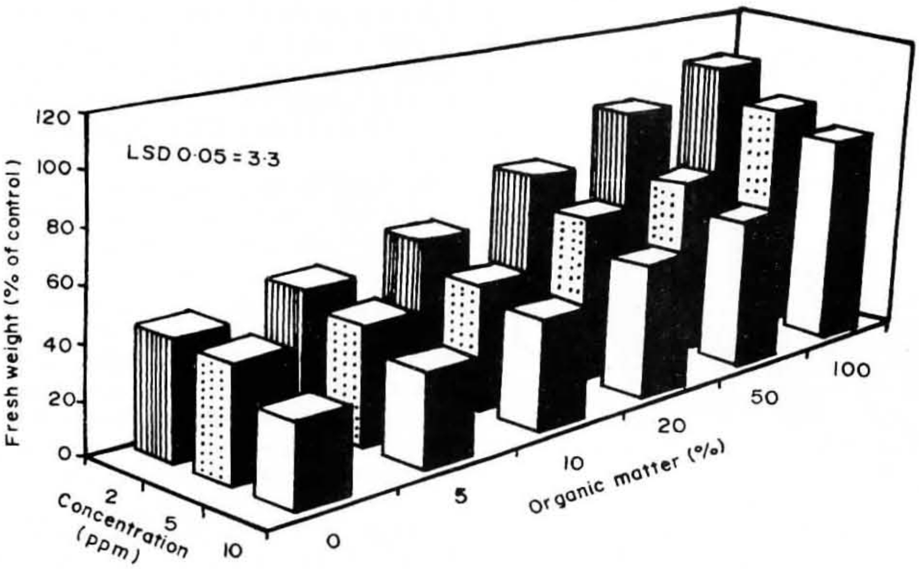

Figure 1. Fresh weights of cucumber seedlings (per cent control 7 days after sowing) grown in soils with varying organic matter content and treated with varying concentrations of alachlor.

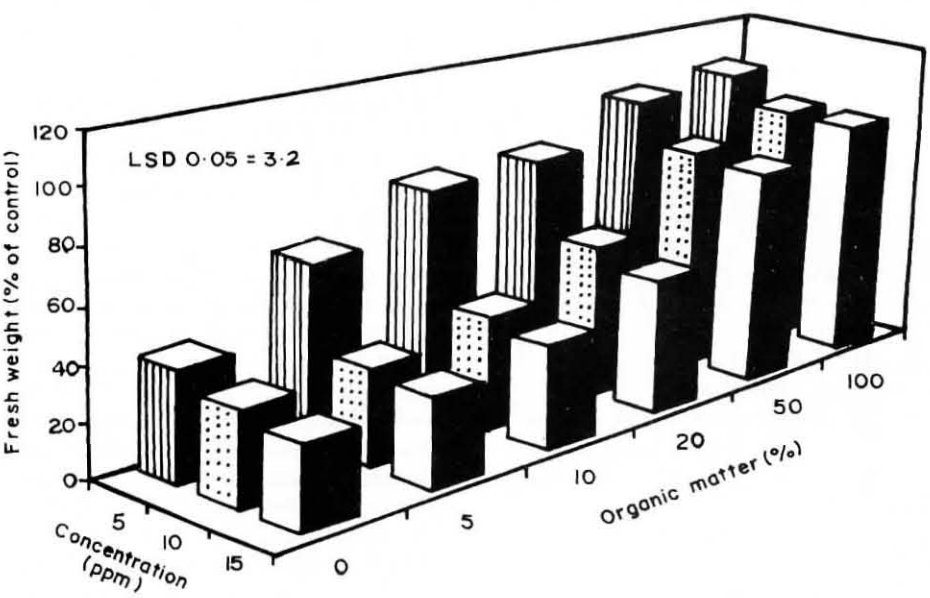

Figure 2. Fresh weights of cucumber seedlings (per cent control 9 days after sowing) grown in soils with varying organic matter content and treated with varying concentrations of terbuthylazine.

ppm (w/w) alachlor and 0, 5, 10 or 15 ppm (w/w) terbuthylazine were then added. Control samples were prepared without adding any soil. The flasks were covered immediately with aluminium foil. The samples were allowed to equilibrate for 12 hours on an orbital shaker at 150 rpm and then were centrifuged for 20 minutes at 4000 rpm to obtain a clear solution, then 7 mL of the supernatant were transferred to 9 cm diameter petri dishes, each lined with one sheet of Whatman No. 3 filter paper. Six cucumber seeds were placed in each petri dish and incubated at 28°C for seven and nine days for alachlor and terbuthylazine respectively. After incubation, the length of the longest root was measured and expressed as a percentage of the untreated control value.

Standard curve for bioassay

Cucumber was used to define a linear relationship of the concentration range of the two herbicides in the soils used for the persistence study in the greenhouse. Concentrations used for alachlor in the two soils were 0, 0.1, 0.3, 1.0, 3.0, 5.0, 8.0 and 10.0 ppm (w/w). For terbuthylazine, the concentrations used were 0, 0.1, 0.3, 1.0, 3.0, 5.0, 8.0, 10, 15.0 and 20.0 ppm (w/w). The required volume of either herbicide was thoroughly mixed with air-dried soil to obtain a concentration of 10 and 20 ppm for alachlor and terbuthylazine, respectively. Other required concentrations were then prepared by diluting the treated soil with untreated soil. The soil samples were bioassayed in the greenhouse under natural light. Five pots were each filled with 200 g of treated soil, in which five cucumber seeds were planted at a depth of 0.5 cm in each pot. Soil moisture was maintained at about 90% field capacity throughout the experimental period. After emergence the seedlings were thinned to two per pot. Eight days after emergence, the plants were harvested by cutting at soil level and fresh weights were recorded. The fresh weight of five replicates (expressed as a percentage of the control value) was plotted against log herbicide concentration.

Persistence study

For each herbicide, 45 kg of air-dried soils were treated to obtain final concentrations of 10 ppm (w/w) for alachlor and 20 ppm (w/w) for terbuthylazine. For the control treatment, soils were not treated with any herbicide. After mixing, 1.5 kg samples of treated soil was kept in each of 30 polyethylene bags. The bags were then divided into three groups and incubated at either 25, 28 or 35°C. The final soil moisture level was maintained at about 50% of field capacity by watering after weekly weighing. One 1.5 kg bag of soil for each herbicide-soil combination at each temperature level was kept at 8°C on Day 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63. After Day 63 of incubation, samples were thawed and air-dried overnight. All soil samples were bioassayed in the greenhouse under natural light. Each approximately 1.5 kg soil sample was then split into four portions, each of which was then placed in a pot into which ten cucumber seeds were then planted at a depth of 0.5 cm. The soils were watered twice a day to maintain the moisture level at about 90% of field capacity. After emergence, plants were thinned to four per pot. Seven days after emergence for alachlor-treated soil and nine days for terbuthylazine-treated soil, the plants were cut at soil level, fresh weights were recorded and expressed as a percentage of the control value. The concentration of herbicide in the soil was estimated by referring to a dose-response curve developed concurrently. The herbicide concentrations calculated for each data point using the dose-response curve were converted to log and plotted against time, from which the half-life was calculated by assuming first-order kinetic behaviour.

In another set of experiments, the soil samples were divided into three groups with soil moisture levels of either 30, 50 or 70% field capacity and kept in a room at 28°C. The bags were weighed weekly and, when necessary, water was added to restore the initial moisture level. One 1.5 kg bag of soil for each herbicide-soil

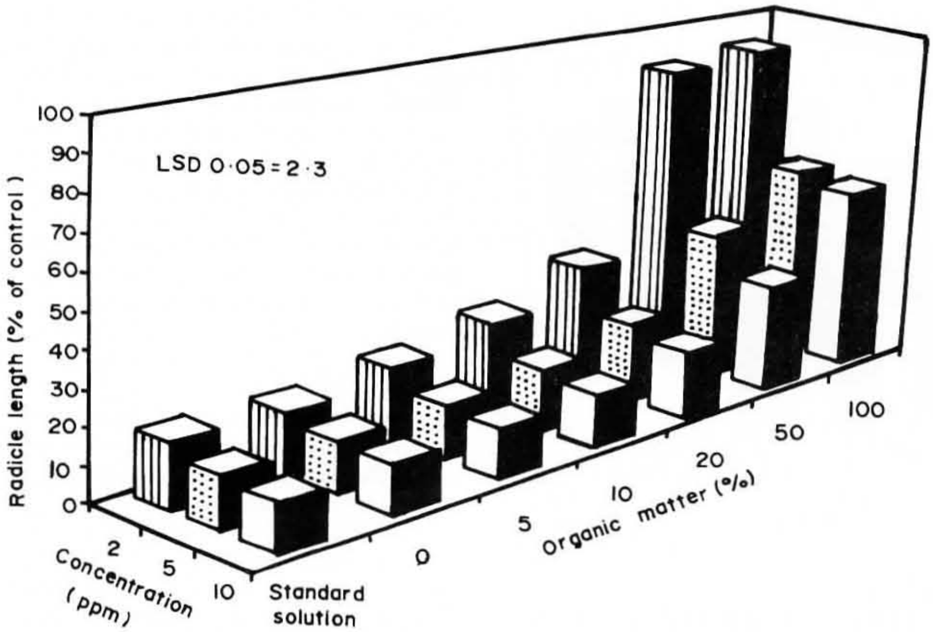

Figure 3. Radicle length of cucumber seedlings (per cent control 7 days after sowing) grown in soils with varying organic matter content and treated with varying concentrations of alachlor.

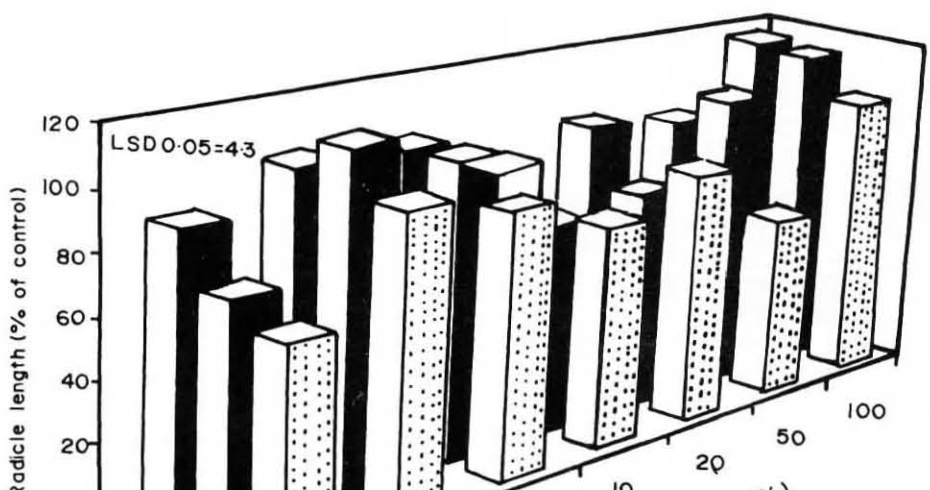

Figure 4. Radicle length of cucumber seedlings (per cent control 9 days after sowing) grown in soils with varying organic matter content and treated with varying concentrations of terbuthylazine.

combination at each temperature level was kept at 8°C on Day 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63. After Day 63 of incubation, the soil samples were thawed and air-dried overnight. All soil samples were bioassayed in a greenhouse as before to determine the half-lives of alachlor and terbuthylazine.

The experimental design was a randomized block with four replications. Data were subjected to analysis of variance and means were compared with an LSD test followed by Duncan's multiple range test at the 5% level of significance.

Results

Phytotoxicity study

The phytotoxicity of alachlor decreased with increasing levels of soil organic matter (Figure 1). An increase of the herbicide concentration to 10 ppm reduced fresh weights in each soil mixture. The results indicated that the fresh weight of cucumber seedlings increased significantly as soil organic matter content increased between 10, 20, 50 and 100%. However, an increase in the organic matter content from 0 to 5% did not show any significant effect on the fresh weight for the three concentrations of alachlor used. Generally, an increased herbicide concentration decreased fresh weights of cucumber seedlings as percentage of control in each of the sand-peat mixtures.

Similarly, an increase in the soil organic matter content reduced the phytotoxic effect of terbuthylazine at each concentration used (Figure 2). The fresh weight of cucumber seedlings was significantly higher in soil containing 5% organic matter than in the sandy soil (0% organic matter). A significant increase in percentage fresh weight of cucumber was also recorded between each level greater than 5% of organic matter. Phytotoxicity of terbuthylazine also increased with increasing herbicide concentration. An increase from 5 to 10 ppm significantly reduced the fresh weight of seedlings but not when the concentration was increased from 10 to 15 ppm. At 100% organic matter, an increase in the concentration only slightly affected the fresh weight.

Adsorption study

Generally an increase in the concentration of herbicide in each soil mixture reduced the radicle length of seedlings. The length of roots of plants treated with alachlor but not equilibrated with soil did not differ markedly from those treated with equivalent solutions which had been equilibrated with sand. An increase in the level of soil organic matter reduced the phytotoxicity of alachlor in the supernatant and increased the radicle length of cucumber seedlings (Figure 3). Radicle length of seedlings reduced with increasing terbuthylazine in standard solution but not in sand-peat mixture. Figure 4 shows that organic matter content did not significantly affect the adsorption of terbuthylazine onto soil particles.

Persistence study

The degradation of alachlor and terbuthylazine increased at higher temperatures, and a significant effect of temperature was observed in both soil types (Table 2). Half-lives of alachlor and terbuthylazine were decreased by 8.3 and 6.9 days respectively in Serdang Series soil when temperature increased from 25 to 35°C. Similarly, in Selangor Series soil, the half-lives of alachlor and terbuthylazine decreased by 9.0 and 9.7 days respectively as the temperature increased from 25 to 35°C. Statistical analyses of data indicated that the half-life of terbuthylazine, but not of alachlor, was significantly different between the soil types.

The degradation rate of alachlor increased significantly with increasing soil moisture levels (Table 3). At 70% field capacity, the half-lives of alachlor in Selangor and Serdang Series soil were 8.5 and 7.0 days respectively. The degradation rate of

Table 2. Half-lives (days) of alachlor and terbuthylazine in Selangor Series and Serdang Series soils at different temperatures.

Temperature (°C)	Alachlor			Terbuthylazine		
	Selangor Series	Serdang Series	LSD P=0.05	Selangor Series	Serdang Series	LSD P=0.05
25	15.2	14.5	0.9	16.9	15.3	0.4
28	10.2	10.1	0.6	8.3	9.4	0.1
35	6.2	6.2	0.2	7.2	8.4	0.1
LSD P=0.05	0.8	0.3	–	0.3	0.2	–

Table 3. Half-lives (days) of alachlor and terbuthylazine in Selangor Series and Serdang Series soils at different moisture levels.

Moisture levels (%)	Alachlor			Terbuthylazine		
	Selangor Series	Serdang Series	LSD P=0.05	Selangor Series	Serdang Series	LSD P=0.05
30	19.8	14.1	0.5	17.7	19.1	0.4
50	10.2	10.1	0.6	8.3	9.4	0.1
70	8.5	7.0	0.3	8.5	11.4	0.3
LSD P=0.05	0.5	0.3	–	0.3	0.2	–

terbuthylazine also increased with increasing moisture level from 30 to 50% field capacity in both soils studied. In Selangor Series soil, an increase in soil moisture from 50 to 70% field capacity did not show any significant effect on the half-life of terbuthylazine.

Discussion

Growth of cucumber seedlings was greatly reduced by treatment with alachlor when sandy soil was used as growing medium, but an increase in the level of organic matter reduced its phytotoxicity. These results are in line with Peter and Weber (1985), who found an inverse relationship between bioactivity of alachlor and organic matter levels. Results of a field study by Rahman *et al.* (1978) also showed that more alachlor was required for weed control when organic matter content increased from 8.0 to 19.3% in 13 trial sites. In the soil adsorption studies, the bioactivity of alachlor in the supernatant also decreased as the amount of organic matter in the soils was increased. This suggests that there was a correlation between phytotoxicity and adsorption of alachlor onto soil particles. The simplest hypothesis to account for our results is that adsorption onto organic matter reduced the amount of herbicide available for bioactivity, and the more organic matter present, the greater the adsorption. This agrees with previous reports which showed that organic matter strongly reduces the phytotoxicity of alachlor due to adsorption by organic matter (Weber and Peter 1982). The acetanilide herbicides are probably adsorbed to Ca-organic matter in a planar fashion through multifunction H-bonds between the carbonyl O of the molecule and the H atoms of carboxyl and hydroxyl groups of the organic surfaces and through charge transfer bonds between the aromatic nucleus of the acetanilide molecules and aromatic rings in the organic matter surfaces (Weber and Peter 1982).

Although the level of organic matter influenced the phytotoxicity of terbuthylazine in soil, there was no significant correlation between organic matter and adsorption (Figure 4). In the phytotoxicity study, an increase in the organic matter content was found to reduce the phytotoxicity of terbuthylazine, but the bioactivity of the supernatant on radicle length was not affected in a similar fashion. It is possible that terbuthylazine caused less effect on the radicle length than on plant growth in general. The properties of terbuthylazine may also influence its adsorption onto soil particles. Terbuthylazine (a triazine herbicide) is a weak base and can become cationic, in contrast to most other herbicides which remain uncharged (Hance 1988). Therefore, adsorption of triazines is dominated by clays (ion exchange) rather than organic matter (Hance 1988).

Degradation of both alachlor and terbuthylazine was the highest at 35°C in both soil types. This observation is in line with Briggs (1983), who suggested that an increase of 10°C in temperature decreased the half-life of herbicides by 2–3 times. Increasing temperature probably increases both biological and non-biological activity of herbicides. The increased dissipation of herbicides at higher temperatures could also be attributed to volatility and photodecomposition (Aldrich 1984, Ashton and Monaco 1991). Similar results for alachlor were reported by Zimdahl and Clark (1982), who reported increased herbicide dissipation with increasing temperature. Degradation of alachlor at 50% field capacity moisture level was higher at 35°C than at 25 and 28°C in our study. Positive correlations between temperature and degradation have also been confirmed in previous studies (Zimdahl *et al.* 1970, Zimdahl and Gwynn 1977). The primary factors affecting soil degradation of acetanilide herbicides are adsorption and microbial decomposition (Zimdahl and Clark 1982). Alachlor was found approximately 50 times more persistent in sterile than in non-sterile soil (Beestman and Deming 1974). In our study the degradation rate of terbuthylazine also increased at higher temperature levels. Higher temperatures favour the hydrolysis of chloroatriazines and are also favourable for microbial growth. McCormick and Hiltbold (1966) also showed that inactivation of *s*-triazines was correlated to conditions optimum for microbial growth.

Dissipation of both herbicides was also influenced by the soil moisture level. The half-lives of alachlor and terbuthylazine decreased with increasing moisture levels from 30 to 50% field capacity in both soils. A decrease in degradation at higher soil moisture levels is expected as a result of the consequent weaker adsorption of herbicide molecules by the soil particles. At higher soil moisture levels, water molecules compete with the herbicide for adsorption sites on soil colloids and increased herbicide concentration in the soil solution would make them more readily available to soil microbes. Under aerobic conditions, degradation rate increased with increasing soil moisture content and tended to plateau at high moisture content and saturation levels (Hamaker 1972). The aeration of the soil is closely linked with its moisture levels (Sethunathan *et al.* 1982).

Our results show that the half-lives of alachlor were longer in the Selangor Series soil at 30 and 70% moisture levels than in the Serdang Series soil, which has a lower organic matter content (Table 1). Similar observations for alachlor have been reported by Zimdahl and Clark (1982) and Fishel and Coats (1993). The half-lives of terbuthylazine , on the other hand, were shorter in Selangor Series than in Serdang Series soil, as shown in Tables 2 and 3. The difference may be attributed to the lower pH value and higher organic matter content in Selangor Series (Table 1). Obein and Green (1969) reported lower pH value and higher organic matter were suitable for hydrolysis of chloroatrazine herbicides.

The results of this study clearly demonstrate that in warm and moist soils breakdown of both alachlor and terbuthylazine is more rapid than in cooler and drier soils. The herbicide residues would therefore tend to decrease faster in tropical regions where both soil temperature and soil moisture are usually high. Thus, application of these herbicides at normal use rates would not be expected to have any significant detrimental effect on soil ecology and fertility or cause any damage to subsequent crops.

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