Bull. Environ. Contam. Toxicol. (2004) 72:504–513

DOI: 10.1007/s00128-004-0273-8

Environmental Contamination and Toxicology

Studies on Biodegradation of 2,4-D and Metribuzin in Soil Under Controlled Conditions

Z. M. Getenga,[1] V. Madadi,[2] S. O. Wandiga[2]

[1] Western University College of Science and Technology, Department of Chemistry, Post Office Box 190, Kakamega, Kenya
[2] University of Nairobi, Department of Chemistry, Post Office Box 30197, Nairobi, Kenya

Received: 3 October 2003/Accepted: 30 November 2003

Composting is a developing alternative for municipal solid waste management which should develop in near future. It provides an organic amendment useful to improve soil structure and nutrient status, with effects on physical, chemical and biochemical soil properties (Giusquiani et al. 1994). The addition of organic amendments increases the soil organic matter content and generally stimulates the soil microbial activity(Alvey and Crowley 1995). The first effect of amendment addition to soil is increase of pesticide sorption, thus decreasing leaching (Davis Carter and Burgra 1993). This may limit pesticide pollution but can reduce pesticide efficiency, mainly for pesticides applied directly to the soil, such as root absorbed herbicides. On the other hand, some organic amendments produce soluble organic matter, which promotes pesticide desorption and enhances their apparent water solubility through stable interactions solution between pesticide and soluble organic matter. Pesticide degradation in soils amended with organic materials can also be modified, depending on the organic amendment and pesticide properties (Barriuso et al. 1992). A reduction in degradation is usually explained by the decrease of pesticide availability after sorption increase (Doyle et al. 1978). In contrast, an increase in degradation may be explained by soil microbial activation, which favors pesticide by co-metabolism (Hance 1973).

In the present study the effect of compost on the biodegradation of two herbicides metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5(4H)-one)] and 2,4-D (2,4-dichlorophenoxyacetic acid), which are heavily used to control weeds in the sugarcane plantations in Kenya was carried out. The influence of natural light on the biodegradation of the two herbicides in soil was also investigated. The sorption of the pesticides by soil was evaluated by batch equilibrium method in order to help explain the results of biodegradation of the herbicides in soil. Both ^{14}C-labeled and non-labeled solution of each pesticide was applied to soil.

MATERIALS AND METHODS

Both uniformly ring-labeled ^{14}C-2,4-D and ^{14}C-metribuzin (International Isotopes, Munich) with specific activities of 51.6 and 30.0mCi/mmol, and radiochemical purity 95 and 98%, respectively, were determined by TLC. Formulated 2,4-D of

Correspondence to: Z. M. Getenga

the dimethylamine salt of active ingredient (a.i.=41%) and non-labeled metribuzin were obtained from local suppliers. Both labeled and non-labeled compounds were used together in biodegradation studies. Quicksafe A, 2,5-diphenyloxazole (PPO) and 1,4-bis[5-phenyl-2-oxazolyl]-benzene; 2,2'p-Phenylene-bis[5-phenyloxazole] (POPOP) in toluene and Harvey Carbon-14 Cocktail (Zinsser Analytic (UK) Ltd) were used in Liquid Scintillation Counting. All solvents used were re-distilled in all-glass apparatus. The clay soil had the following characteristics, organic content (OC) 2.07%, pH 6.08, clay content 60%, sand content 28%, silt 12%, N 0.19%, P 80ppm, Na 0.95%, K 1.85%, Ca 10.5%, Mg 4.95%, Mn 0.51%, Fe 226.96ppm, electrical conductivity (EC) of 0.62μS/cm) and compost had (N 1.14%, P 0.72ppm, Cu 140ppm, Mn 2217ppm, Fe 1272ppm and Zn 755ppm. A liquid Scintillation Counter (Tricarb-1000) and Biological Materials Oxidizer (OX-600 model) were used for radio-assaying.

Two parallel experiments were conducted at the same time for 2,4-D and metribuzin. In the incubation experiment, 50g of sieved soil samples in replicas of three were placed in biometer flasks (Bell Co. Glass Inc.) after the air-dried and homogenized soil was sieved though a 2-mm sieve. The soil was equilibrated in the laboratory at 25°C for one week after being moistened to 75% of the field water capacity. The soil samples in biometer flasks were subjected to different treatments before they were incubated at 30°C in the darkness under aerobic conditions. The first set of the soil samples was autoclaved at 121°C for 45 minutes at the pressure of 1.2 bars for three consecutive days and then mixed with $HgCl_2$ at a rate of 1000ppm. The second set of the soil samples was not amended with compost. The third set of the soil samples was amended with compost at concentrations of 1000μg/g, 2500μg/g and 5000μg/g (compost/soil). 10ml of solution of each of the herbicide in distilled water was applied to the 50g-soil sample in each biometer flask giving an initial herbicide concentration of 100μg/g and initial radioactivity of 0.3μCi in soil. The soil was homogenized with a spatula to ensure uniform distribution of the herbicide in soil. The side arm of each biometer flask was filled with 10ml of 0.1N NaOH to trap the $^{14}CO_2$ gas released during mineralization by soil microorganisms. The inlet of each biometer flask was filled with Ascarite to exclude carbon dioxide from entering the system. The biometer flasks were placed in an incubator to monitor the progress of mineralization. At different time intervals, the 10ml of 0.1N NaOH solution from the side arm was sampled from which one ml of the solution aliquot was taken and mixed with 5ml of Quicksafe A cocktail in a 20-ml scintillation vial before it was radio-assayed. After sampling, the side arm was refilled with fresh 10ml of 0.1N NaOH solution. The experiment was run for 120 days. The amount of accumulated $^{14}CO_2$ over the 120-day period was computed from the amount of $^{14}CO_2$ obtained in each sampling.

Sorption (adsorption and desorption) experiments of both 2,4-D and metribuzin were conducted using a batch-equilibrium method. 4g of homogenized soil samples were weighed and placed in 40ml-centrifuge tubes. 10ml of the pesticide solution prepared in 0.01M $CaCl_2$ at different concentrations of 1.0, 5.0, 10.0, 15.0 and 25.0ppm was added to 4g of soil in the centrifuge tubes. The resulting

pesticide concentration in soil in each centrifuge tube was determined after adsorption at different periods of shaking. The centrifuge tubes were shaken on an orbital shaker at 200rpm for 4 hours, 8 hours and 24 hours respectively. Thereafter, the centrifuge tubes were transferred to a centrifuge machine and centrifuged at 3600rpm for 30 minutes. 1ml of the supernatant was taken and mixed with 5ml of the quick safe A cocktail solution and radio assayed. The difference between the amounts of pesticide in the 0.01M $CaCl_2$ solution before and after shaking was the amount of pesticide adsorbed by soil. At equilibrium, all the 0.01M $CaCl_2$ solution was removed from the centrifuge tube and replaced with a fresh pesticide-free solution of 0.01M $CaCl_2$. Desorption was carried out for 24 hours when the pesticide in the two phases (soil and 0.01M $CaCl_2$ solution) was assumed to have attained equilibrium. The amount of pesticide desorbed from the soil was computed at different concentrations.

Effects of natural light on the degradation of both 2,4-D and metribuzin in soil were conducted in biometer flasks in the open at the rooftop of a two-storey building. One experiment consisted of biometer flasks, which were exposed to natural light at 27°C and another set of flasks was covered with aluminium foils to shield them from natural light. 50g of sieved and homogenized soil, moistened to 75% of the water holding capacity was placed in a flask. The soil in each flask was spiked with a solution of the pesticide (labeled and non-labeled) to give an initial total radioactivity of 0.62μCi and initial pesticide concentration of 100μg/g of soil. Each experiment was replicated three times. At different time intervals, 1ml of the 0.1NaOH solution was taken from the side arm of each flask, mixed with 5ml of Quicksafe cocktail and radioassayed. The experiment was discontinued after 111 days.

^{14}C-mass balance of both 2,4-D and metribuzin were determined in the soil samples from both photolysis and biodegradation experiments. 10g of soil in three replicates from the biodegradation experiment was mixed with 20ml of methanol:buffer solution (4:1) mixture in 40-ml centrifuge tubes. The buffer solution was made by dissolving 2.5M chloroacetic acid and sodium acetate in the ratio of 39:25. The tubes were shaken on an orbital shaker for 12 hours. The mixture in the tubes was centrifuged at 4000rpm for 30 minutes and decanted. 1ml of the supernatant was mixed with 5 ml of the Quicksafe A cocktail for scintillation counting. The extracted soil was re-extracted five times. The same procedure was adapted to soil from the photolysis experiment. The separate LSC readings were added up to get the total extractable amount of residue in the soil for each pesticide. The extracted soil was air-dried from which sub-samples of 1.5g in five replicates were combusted in a Biological Materials Oxidizer (OX-600). The $^{14}CO_2$ released was trapped in Harvey carbon-14 cocktail, which was scintillation counted. All the readings were corrected according to the efficiency results from the oxidizer.

RESULTS AND DISCUSSION

Figures 1 and 2 show mineralization curves for 2,4-D and metribuzin,

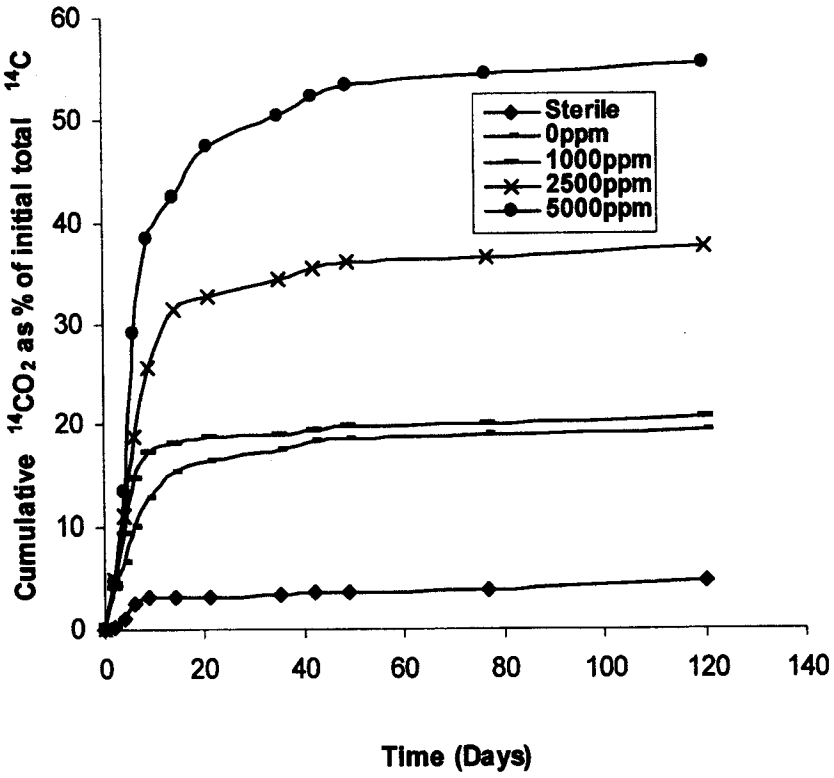

Figure 1. Mineralization of 2,4-D in soil with different amounts of compost amendment.

respectively. The mineralization curves for the two herbicides did not exhibit a lag phase. There was rapid phase, which lasted for a maximum of 14 days for both compounds. Thereafter, the curves levelled off, attaining plateaus. For both herbicides, the addition of compost to soil stimulated microbial degradation of the compounds. Increase in compost concentration from 1000ppm to 2500ppm and then to 5000ppm resulted in substantial increase in the rate of $^{14}CO_2$ production for the two compounds. The microbial activity in the sterile set of soils was not totally eliminated; 4.8% and 0.85% of the initially applied radioactivity was recovered in $^{14}CO_2$ in the soils spiked with 2,4-D and metribuzin, respectively. Compared with the compost-amended soils, the soil without compost amendment had the least mineralization of both herbicides. Metribuzin resisted mineralization when compared with mineralization of 2,4-D at all the compost concentrations used in the study. At the maximum compost concentration of 5000ppm, only 5.8% of metribuzin was mineralized to $^{14}CO_2$, when 55.5% of 2,4-D was mineralized after 120 days of pesticide application. The lower mineralization of

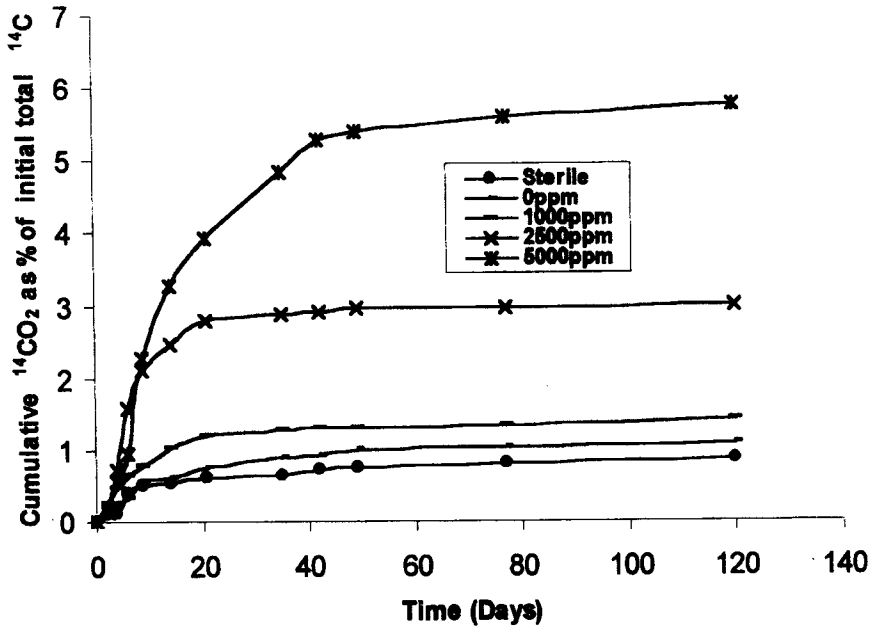

Figure 2. Mineralization of metribuzin in soil with different amounts of compost amendment.

metribuzin than that of 2,4-D could be related to the high adsorption of metribuzin by soil and that, the sorbed state of metribuzin could not be mineralized. Compared with metribuzin, 2,4-D exhibited low adsorption by soil with subsequent high desorption rate back to solution during sorption experiments. Jeong-Hun et al. (2001) showed that 2,4-D could be degraded both in liquid phase and in the sorbed state. The mineralization curve with no lag phase for 2,4-D has also been observed in other studies (Uan-Boh et al.1998), in soils where 2,4-D and other pesticides were previously applied.

Figures 3 and 4 show the adsorption curves of 2,4-D and metribuzin at equilibrium concentrations of the pesticide solutions. The data was fitted into the logarithmic form of Freundlich equation: $\log x/m = \log K_f + N\log C_e$, where x/m (μg/g) is the sorbed concentration and Ce (μg/ml) is the equilibrium solution concentration. The values K_f and N, which are Freundlich coefficients were computed for 2,4-D and metribuzin from the equation, $\log x/m = \log K_f + N\log C_e$. The K_f and N values for 2,4-D were 1.035 and 0.7963, respectively. The corresponding values for metribuzin were 1.376 and 0.8837 for K_f and N, respectively. K_f is a measure of sorption intensity by soil for each pesticide. The value of N is used as an index of isotherm linearity (Guangwei et al. 2002). Metribuzin had a higher value of K_f due to higher adsorption by soil. The amount of metribuzin adsorbed by soil from solution decreased from 41.2% of metribuzin

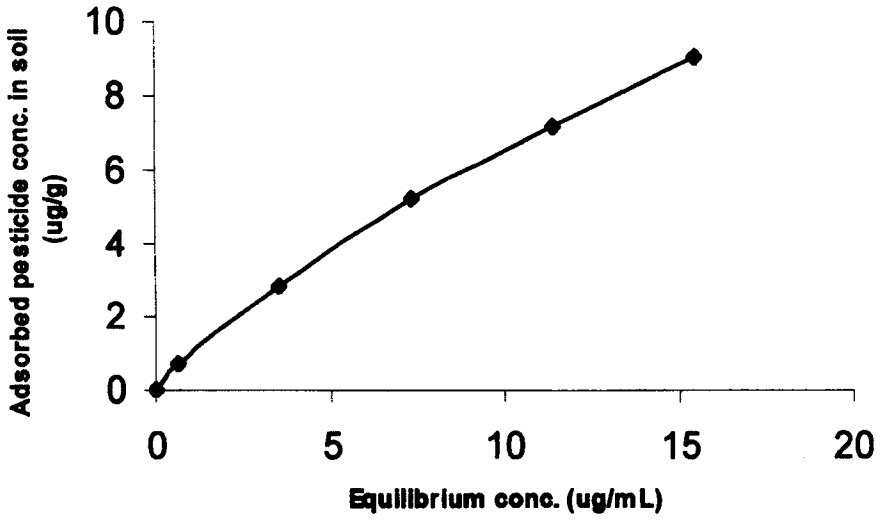

Figure 3. Adsorption curve for 2,4-D.

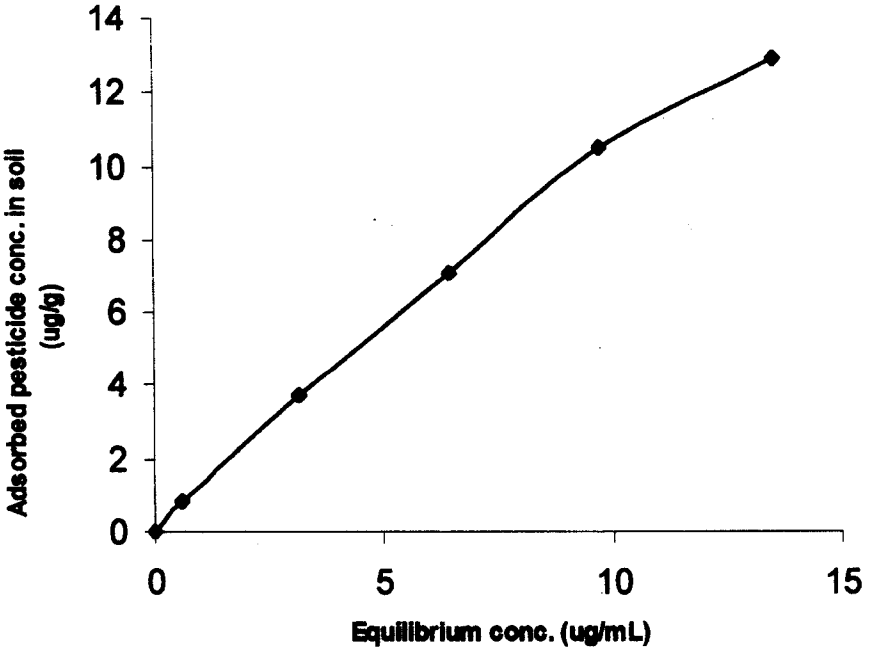

Figure 4. Adsorption curve for metribuzin.

in solution at a concentration of 1ppm to 32.3% at the solution concentration of metribuzin of 20ppm. For 2,4-D adsorption decreased from 36.1% at 1ppm to 22.6% at 20ppm. The decrease in % of the amount of adsorbed pesticide with increase in pesticide solution concentration was reflected in this study by the Freundlich coefficients of N, which were all less than one. The decrease has been explained by an increased difficulty to access the adsorption sites when pesticide concentrations are elevated (Konda et al. 2002). During desorption of the herbicides, the K_{des} and N values were also computed where K_{des} stands for the K_f for desorption. The values for 2,4-D were 0.648 and 0.3825 for K_{des} and N, respectively. The values for metribuzin were 1.452 and 0.8261 for K_{des} and N, respectively. The results showed that it was easier for 2,4-D than metribuzin to be desorbed back to solution as indicated by a lower value of K_{des} (0.648) for 2,4-D than that of metribuzin, which was 1.452. The amount of metribuzin, which was desorbed into solution as % of initially, adsorbed metribuzin ranged from 52.2% to 61.4%, while for 2,4-D the amount desorbed back to solution from the adsorbed state ranged from 82.7% to 87.2%.

Figure 5 shows the effect of natural light on the mineralization of 2,4-D and metribuzin. The amount of $^{14}CO_2$ evolved from mineralization of 2,4-D and metribuzin from covered soil was higher than from exposed soil. The amount of $^{14}CO_2$ evolved from mineralization of metribuzin was lower than from mineralization of 2,4-D in both exposed and covered soils. Moorman and Harper (1989) found that more $^{14}CO_2$ was evolved from surface soil with metribuzin at 0.1μg/g than soil treated with 1.0 μg/g of metribuzin. The low mineralization of metribuzin in the present study could be attributed to the high concentration (100 μg/g) of metribuzin in soil, which affected the microbial activity in soil. Some toxic effects of metribuzin towards soil microflora have been described (Evans etal. 1965; Hill and Stratton 1991; Junnila et al. 1993). The amount of accumulated $^{14}CO_2$ evolved from 2,4-D in covered soil was 45.7% but only 20.2% was evolved from 2,4-D in exposed soil. The mineralization of metribuzin was very low from both covered and exposed soils, being 9.85% and 6.32%, respectively. Mineralization of both pesticides in exposed soil was lower probably due to adverse effects of UV-radiation on microbes in soil. The negative effect of natural light on microbes was also reflected in the higher amount of extractable residue of the pesticides in exposed soil than in covered soil. The lower amount of the non-extractable residue of 2,4-D in covered soil than in the exposed soil could be as a result of mineralization to $^{14}CO_2$, which may have not occurred in the exposed soil where microbial activity seemed to have been reduced by the natural light. Li-tse ou et al. (1978) compared the biodegradation of formulated and technical grade 2,4-D at different concentrations. Results showed that biodegradation was higher in formulated 2,4-D than from the technical grade. Furthermore, higher concentrations of 2,4-D in soil enhanced biodegradation. This has been attributed to higher solubility of the formulated 2,4-D than the technical grade in water. Biodegradation occurs normally in the aqueous phase. However, other studies (Olson and Lindwall 1991) showed that under laboratory conditions, elevated concentrations of 2,4-D reduced microbial activity as measured by nitrification.

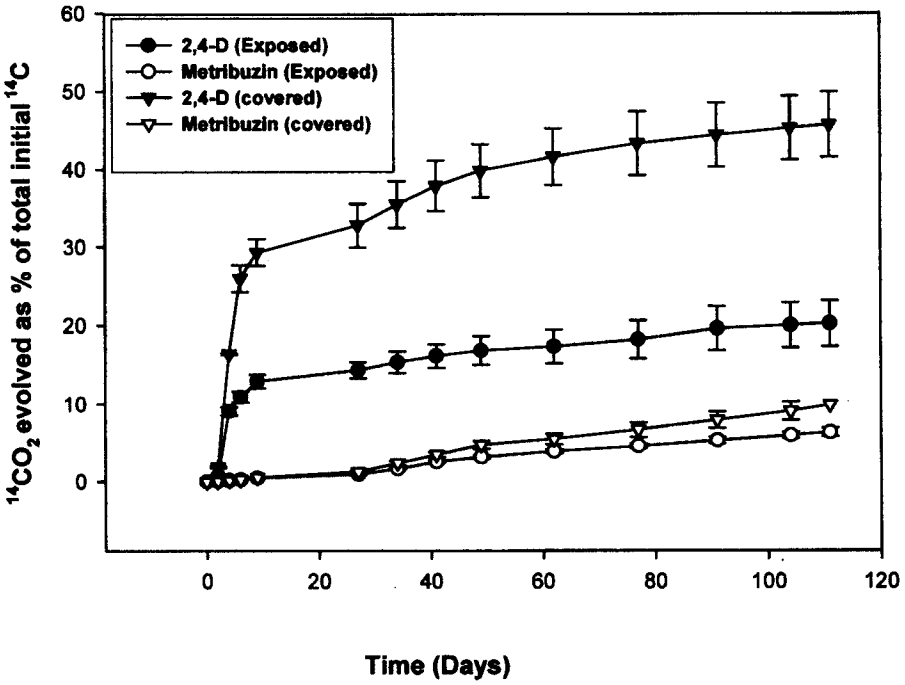

Figure 5. Mineralization of 2,4-D and metribuzin in covered and exposed soils.conditions

Tables 1 and 2 show the ^{14}C-mass balance of the pesticides in soil during the photolysis and biodegradation studies, respectively. The mass balance for both pesticides shows that at end of the incubation period of 120 days, there were losses of the ^{14}C, which could not be accounted for in this study. The loss is partially attributed to volatile materials, which were not trapped and quantified. There were higher losses from metribuzin than from 2,4-D pesticide. This could be due to higher amount of the extractable residue of metribuzin than that of the 2,4-D at any given time in the corresponding different soil treatments. Extractable residue was more vulnerable than the non-extractable residue to the many degradative and dissipative processes in soil. Non-extractable residue formation increased as mineralization of the pesticides also increased. Soil microorganisms were responsible for both non-residue formation and mineralization. It is only in the soil with 5000ppm of compost where the amount (19.9%) of non-extractable residue formation was drastically reduced. This could be due to the mineralization of the non-extractable residue. It was possible because this is the soil where there was enhanced mineralization of 2,4-D, with 55.5% of the initial ^{14}C having been converted to $^{14}CO_2$.

The results showed higher mineralization of 2,4-D than that of metribuzin for all the compost concentrations used. Mineralization curves of both pesticides did not show a lag phase showing that the microbes in the soil had adapted due to the

Table 1. ^{14}C-mass balance for metribuzin and 2,4-D in soil during photolysis

Component (%)	2,4-D		Metribuzin	
	Exposed	Covered	Exposed	Covered
$^{14}CO_2$ evolved	20.2	45.7	6.3	9.9
Extractable residue	44.7	36.5	83.5	73.2
Non-extrac. residue	25.3	15.6	6.7	8.4
Total residues	90.2	97.8	96.5	91.5

Table 2. ^{14}C-mass balance for metribuzin and 2,4-D in soil during biodegradation.

Pesticide	Treatment	Component of the pesticide (%)			
		$^{14}CO_2$	Ext. residue	Non-ext. res.	Total ^{14}C
2,4-D	Sterile	4.8	55.0	15.3	75.1
	0ppm	19.4	13.3	33.5	66.2
	1000ppm	20.8	8.6	34.4	63.8
	2500ppm	37.5	6.6	36.6	80.7
	5000ppm	55.5	6.4	19.9	81.8
Metribuzin	Sterile	0.9	59.0	5.7	65.6
	0ppm	1.1	41.5	18.2	60.8
	1000ppm	1.4	36.9	20.5	58.8
	2500ppm	3.0	34.8	24.2	62.0
	5000ppm	5.8	33.3	27.6	66.7

repeated applications of the pesticides to the soil. Although sorption studies of the two pesticides showed that 2,4-D was less adsorbed to soil than metribuzin, and that the latter could not easily be desorbed to solution as 2,4-D, the difference in the extent of mineralization between the two pesticides is too big. It is therefore concluded that metribuzin is difficult to biodegrade even under enhanced conditions of biodegradation.

Acknowledgments. I thank the International Foundation for Science (IFS) for financial support of the research work, through Agreement contract No.C/3192-1.

REFERENCES

Alvey S, Crowley DE (1995) Influence of organic amendments on biodegradation of atrazine as a nitrogen source. J Environ Qual 24: 1156-1162

Barriuso E, Baer U, Calvet R (1992) Dissolved organic matter and adsorption-desorption of dimefuron, atrazine and carbetamide by soils. J Environ Qual 21: 359-367

Carter JG, Burgra B (1993) Atrazine run off and leaching losses from soil in tilted beds as influenced by three rates of lagoon effluent. J Environ Sci Health 28: 1-18

Doyle RC, Kaufman DD, Burt GW (1978) Effect of dairy manure and sewage on ^{14}C-pesticide degradation in soil. J Agric Food Chem 26: 987-989

Evans WC, Ferney HN, Griffiths E (1965) Oxidative metabolism by soil Pseudomads: The ring fission mechanism. Biochem J 95: 819-821

Giusquieni PL, Pagiali M, Gigliotti G, Businelli D, Bebetti A (1994) Urban waste compost: effects on physical, chemical and biochemical soil properties. J Environ Qual 17: 257-262

Guangwei D, Jeffrey MN, Stephen H, Baoshan X (2002) Long-term tillage effects on soil metolachlor sorption and desorption behaviour. Chemosphere 48:897-904

Hance RJ (1973) the effect of nutrients on the decomposition of the herbicides atrazine and linuron incubated with soil. Pestic Sci 4: 817-822

Hill TL, Stratton GW (1991) Interactive effects of the fungicide chlorothlonil and the herbicide metribuzin towards the fungal pathogen. *Alternaria solani.* Bull Environ Contam Toxicol 47: 97-103

Jeong-Hun P, Denise K, Xianda Z, Stephen AB, Thomsa CV (2001) Kinetic modeling of bioavailability for sorbed-phase 2,4-D. J Environ Qual 30: 1523-1527

Junnila, Heinonen-Tanski SH, Ervio LR, Laitinen P (1993) Phytotoxic persistence and microbiological effects of metribuzin in different soils. Weed Res 33:213-223

Konda CN, Furleky G, Morovjan G, Csoken P (2002) Sorption behaviour of acetochlor, atrazine, cabendazin, diazinon, imidacroprid and isoproturon in Hungarian agricultural soil. Chemosphere 48: 545-552

Li-tseou, Rothwell DF, Wheeler WB, Davidson JM (1978) The effect of high 2,4-D concentrations on degradation and carbon dioxide evolution in soils. J Environ Qual 7:241-246

Moorman TB, Harper SS (1989) Transformation and mineralization of metribuzin in surface and subsurface horizons of a Mississippi delta soil. J Environ Qual 18: 302-306

Olson BM, Lindwall CW (1991) Soil microbial activity under chemical fallow conditions: Effects of 2,4-D and glyphosate. Soil Biol Biochem 23:1071-1075

Uan-Boh C, Ralph CK, Keng-Yeang L (1998) Degradation of four commonly used pesticides in Malaysian agricultural soils. J Agric Food Chem 46: 1217-1223