LABORATORY ASSESSMENT OF ^{14}C-PHENYL METSULFURON-METHYL DEGRADATION IN AN OIL PALM PLANTATION SOIL

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ABSTRACT

The ^{14}C-phenyl metsulfuron-methyl is a selective systemic sulfonylurea herbicide. Degradation studies in soils are essential for the evaluation of the persistence of pesticides and their breakdown products. The objective of this study was to investigate the degradation of metsulfuron-methyl in an oil palm plantation soil under laboratory conditions. The soil used was both sterilised and non-sterilised soil in order to observe the involvement of soil microbes. The estimated DT_{50} and DT_{90} values of metsulfuron-methyl in a non-sterile system were approximately 13 and 44 days, whereas in sterilised soil, the DT_{50} and DT_{90} were 31 and 70 days, respectively. The principal degradation product after 60 days is CO_2. The higher cumulative $^{14}CO_2$ in ^{14}C-phenyl in the non-sterilised soil compared to that in the sterile system suggests that biological degradation by soil micro-organisms significantly contribute to the dissipation of the compound. In some the major routes of degradation are O-demethylation, sulfonylurea bridge cleavage and triazine ring-opened. In vitro *study in order to identify the microbes involve in the degradation of the compound should be carried out in the future study.*

Keywords: metsulfuron-methyl, degradation, oil palm soil, DT_{50}.

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INTRODUCTION

The environmental fate of pesticides in the soil is viewed with great concern today, due to the potential effects on surface and ground water quality. The rate of mobility and degradation in the soil are the most important processes that determine the fate of pesticides in soils (Ye *et al.*, 2003). Therefore, degradation studies in soils are essential for the evaluation of the persistence of pesticides and their breakdown products. Data on the rate of degradation are extremely important as they permit prediction of the levels likely to remain in soil and on which assessment could be made of the potential risk associated with exposure to them.

In an ideal situation, microbial degradation of pesticides should be studied under field conditions but this is often dogged by problems due to the interplay of several factors of spatial and temporal variability. Laboratory degradation studies have the merit of being carried out under controlled conditions which allow for the measurement of one factor at a time. Therefore, under controlled conditions it is possible to detect which factor is more responsible for the dissipation of a particular pesticide within a given environment and soil. When properly planned, laboratory studies have been found to yield good data that can be employed in modelling pesticide degradation in the field. Sulfonylurea herbicides degrade in soils primarily

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by chemical hydrolysis and microbial metabolism and there have been several publications, which elucidate the significance of microbial degradation (Tan *et al.*, 2013; Marie *et al.*, 2014; Rafqah *et al.*, 2015). Chemical hydrolysis of metsulfuron-methyl has been shown to be very rapid at low pH which means it is less persistent in low pH soils. Its persistency at 45°C increased from 2.1 days at pH 5 to 33 days at pH 7 (Marie *et al.*, 2014; Tayeb *et al.*, 2015). The degradation rate of metsulfuron-methyl is affected by soil temperature, moisture, pH, and soil microbial viability. The half-life of metsulfuron-methyl ranges from 2.5 days [soil conditions: pH 3.1, 35°C, 80% field water holding capacity (FC)] to 36 days (soil conditions: pH 5.7, 10°C, 60% FC) depending on the above mentioned factors (Tan *et al.*, 2013; Xiangwen *et al.*, 2014). The degradation rate of metsulfuron-methyl has been positively correlated with microbial biomass (Song *et al.*, 2013). Although several researchers have reported the effects of environmental conditions on the degradation rates of metsulfuron-methyl, there have been only a few attempts to identify the degradation products (Yu *et al.*, 2005). Some aqueous hydrolysis products, plant metabolites and degradation products of metsulfuron-methyl in soil minerals and humic acids have been identified (Da Silva *et al.*, 2013; Chuah *et al.*, 2015). In Malaysia, metsulfuron-methyl is being used extensively to control weeds in oil palm plantations. It is used in low dosage compared to the value of other herbicides used. However, not many studies have being carried out to study its fate under the oil palm agro-ecosystem. It is an acknowledged fact that oil palm is the major crop in Malaysia and there is no doubt that metsulfuron-methyl has been used in large quantities to combat weeds. Therefore, it is important to study the dissipation of this compound in the oil palm soil.

The objective of the study is to determine the degradation rate and pattern of ^{14}C-labelled metsulfuron-methyl in sterile and non-sterile plantation soil of the oil palm (*viz*. Bernam soil series) under laboratory conditions. The Bernam soil series was selected for this study, as the observations from this study will be further verified in the field with the same soil series. The ^{14}C-radiollabelled metsulfuron-methyl labelled at the 2-C in the phenyl ring [phenyl-^{14}C] was used in this study to identify the metabolic pathway of metsulfuron-methyl in the soil.

MATERIALS AND METHODS

Chemicals

The herbicide selected for this study was metsulfuron-methyl. The purity of the technical sample used as the analytical standard was about 99%. The ^{14}C-radiolabelled metsulfuron-methyl (methyl 2-[[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino] carbonyl] amino] sulfonic] benzoate) was synthesised at DuPont New England Nuclear (NEN) Research Products (Boston MA, USA). It was labelled uniformly at the phenyl ring [phenyl-^{14}C] with a specific activity of 1.42 MBq mg^{-1} and 38.28 μCi mg^{-1}. The ^{14}C-labelled compound had radiochemical purity higher than 99% as determined by high performance liquid chromatography (HPLC). Unlabelled reference standards of the test substance and expected degradation products were synthesised at the DuPont Agricultural Products, E.I. du Pont de Nemours and Company (Wilmington, DE, USA).

All organic solvents and water used in the study were of HPLC grade while all other chemicals were of deionised grade. The radioactivity in samples was determined by LSC in scintillation fluid. The potassium hydroxide and ethylene glycol trap solution was used to trap $^{14}CO_2$ and organic volatiles released during combustion.

Soil Sample

Soil samples (Bernam soil series) were collected from the top 15 cm layer at the Sungai Buloh Oil Palm Plantation, Selangor, Malaysia. Soil physico-chemical characteristics were determined at Harris Laboratories, Inc. (Lincoln, NE, USA). The soil was classified as clay soil consisting of 27.6% sand, 27.2% silt, and 45.2% clay with pH of 4 and organic matter content of 4.3%, organic carbon 2.4%, bulk density 0.92 g cm^{-3} and cation exchange capacity 25.6 Meq 100 g^{-1}. The fresh soil was sieved through a 2 mm sieve and used immediately for the study. The soil was then used for microbial and degradation studies.

Microbial Counts

The soil samples taken from the biometer flasks which were identical to those used in the degradation studies were evaluated for their total bacterial counts so as to ascertain the effect of the closed system of the flask on the microbial population of the soils. The soil was sampled at 0 (immediately after treatment), 30 and 60 days after treatment. The plating process commenced with the weighing of 23.0 g of nutrient agar (from Difco Laboratories, Detroit MI 48232-7058, USA) consisting of Bacto beef extract (3 g), Bacto peptone (5 g) and Bacto agar (15 g) which was then added to 800 ml of sterilised water. It was heated on a hotplate magnetic stirrer until uniform solubility was achieved and the final solution was made up to 1 litre with sterilised water. An autoclave (All American Electric Pressure Steam Steriliser Model No. 25X) was used to sterilise the agar solution at 15 KPa for 15 min and at a temperature of 121°C. The agar solution was left to cool at room

temperature soon after autoclaving and while still warm, plating was done. Agar plating was carried out using 25 ml of agar solution per plate and left to solidify overnight. Soil samples (10 g) collected at specified intervals of time were each added to a conical flask each containing 90 ml of sterilized water and shaken on a rotary shaker (Stuart Flask Shaker; Stuart Scientific Co. Ltd, England) for 1 hr. This was followed by serial dilution of 10-fold steps and the appropriate dilutions plated out onto nutrient agar plates and incubated for a period of 24 - 48 hr at 30°C, before counting of colonies was carried out. Results of microbial colonies counting were expressed in colony forming units (CFU).

Dissipation Studies

The soil sample (100 g) was placed in a sample bottle, which was connected to a trap bottle. A 190 ml aliquot of stock solution was mixed with 100 g of soil in each designated vessel to produce a concentration in the soil of approximately 1.4 ppm. The trap bottle was then connected to the ethylene glycol (25 ml) bottle to trap any organic volatiles. Lastly, the ethylene glycol bottle was connected to a 0.1 M KOH (25 ml) bottle to trap $^{14}CO_2$ evolved as a result of microbial activity. The flask was corked with a rubber stopper. The soil was maintained at the water holding capacity of 50% throughout the experiment. This was achieved by periodically weighing the flask and adding water as and when required. The experiment was conducted in a controlled environment chamber with the temperature maintained at 30°C (±1°C) and the humidity at 80% (±2%) in total darkness. The experiment was replicated three times.

For the sterile system, the same number of flasks was prepared as described above except that each flask containing 100 g dry weight equivalent of moist soil was autoclaved at 15 KPa and at a temperature 120°C for 1 hr for four separate days to kill off the microorganisms. The Market Forge Sterilmatic Autoclave Model STM-EL was used to sterilise the soil. The sterile test system was connected to a volatile trapping system as described above with the addition of a sterile Gelman ACRO 50 PTFE 0.2 mm filter inserted before the test system flask to prevent microbial contamination.

Degradation Study of the Phenyl Ring [phenyl-^{14}C] Metsulfuron-methyl

A 1035 μg ml^{-1}primary stock solution (specific activity 38.28 μCi mg^{-1}) was prepared by mixing 10.35 mg of neat material (99.99% purity) into 10 ml sterilised water that was adjusted to a pH of 7. This primary stock solution was stored frozen when not used.

A 10.35 μg ml^{-1}secondary stock solution (specific activity 38.28 μCi mg^{-1}) was prepared by diluting a 1 ml aliquot of the primary stock solution (1035 ppm) to a final volume of 100 ml in sterile deionised water. A 190 μl aliquot of this solution was mixed with 100 g of soil in each designated vessel to produce a concentration in the soil of approximately 1.4 ppm. This concentration allowed for adequate detection levels of parent and metabolites.

Reference Standard Solutions

The metsulfuron-methyl solution and reference chemicals were prepared by dissolving 5 mg of the mixtures in 5 ml of water: acetonitrile (3:2, v:v). Dilutions and mixtures of the standards were prepared in reagent water prior to HPLC analysis.

Mixtures of the following standards were prepared to evaluate the HPLC resolution of degradation from the phenyl radiolabelled test substance and for performing chromatographic analysis: IN-581, IN-B5685, IN-D5803, IN-B5067, IN-F5438, IN-MU717, and DPX-T6373.

Sample Collection and Handling

Sampling was conducted at various intervals by taking the three replicate biometer flasks at 0 (immediately after treatment), 1, 3, 7, 10, 14, 21, 30, 45, and 60 days after treatment (DAT). One ml aliquots in triplicates of 0.1 M KOH and ethylene glycol solutions used to trap $^{14}CO_2$ and organic volatiles were sampled at each of the specified intervals. The solutions were then combined with the scintillation fluid and analysed for total radioactivity by the Liquid Scintillation Counter (LSC).

Extraction of Soil Radioactivity

At each sampling time, two flasks (one from each of the two ^{14}C metsulfuron-methyl treatments) were taken. A two-step extraction method was used as described below:

Step 1. The soil in each test flask was extracted with 100 ml of acetonitrile: 2 M ammonium carbonate (9:1, v:v) and shaken for 1 hr on a platform shaker at room temperature (23°C). The solution was centrifuged at approximately 2500 rpm for 15 min. The supernatant was decanted to a graduated cylinder. The extraction was conducted three times and the extracts pooled; 1 ml aliquots were analysed in triplicate by LSC. The recovery study was conducted by spiking a known amount of two ^{14}C metsulfuron-methyl on the soil sample following the same procedure. The sample was left for 1 hr before it was analysed by LSC.

After step 1 extractions were done, the bound residue (%) in the extracted soil was estimated from the equation:

$$\text{Estimated bound residue (\%)} = 100 - \text{\% Applied radioactivity in traps} - \text{\% Applied radioactivity in extracts}$$

If the estimated bound residue was >10% of the applied radioactivity, step 2 extraction was carried out.

Step 2. After the first extraction, the remaining residues were extracted from the soil samples with 100 ml of CH_2Cl_2: methanol: 2 M ammonium carbonate (3:4:1, v:v:v) and shaken for 1 hr at room temperature (23°C). The extraction was conducted three times, the extracts pooled and 1 ml aliquots were analysed in triplicate by LSC. The LSC aliquots were withdrawn in the homogenous phase.

After completing step 2, the extracts of steps 1 and 2 were pooled. The extracts were concentrated by vacuum rotary evaporation and re-dissolved in water. Aliquots were analysed in triplicate by LSC and an aliquot was removed and analysed by HPLC. The extracts were stored in a freezer at - 4°C.

Analysis of Soil Extracts

Soil extracts were analysed using a HPLC equipped with both a UV-detector and an on-line radio-chemical detector (Ramona, Raytest Inc.). The HPLC method 1 used the Supelco Discovery column (250 x 4.6 mm, 5 mm), a gradient with mobile phase A, a phosphate buffer H_2O (pH = ± 7), and B, methanol (from 0 to 3.5 min, 100% B; at 19 min, 90% B; at 19.5 to 29.5 min, 80% B; at 30 to 37 min, 65% B; at 40 to 42 min; 0% B and 45 min, 100% B). The HPLC method 2 used a PRP-1 column (305 x 7.0 mm, 10 μm) with the same mobile phase as the HPLC method 1 but with a different gradient (from 0 to 3 min, 10% B; at 10 min, 20% B; at 20 min, 40% B; at 30 min, 90% B at 35 min, 100% B). The mobile phase flow rate was 1.5 ml min^{-1} in both methods. The oven and injector temperature was set to 35°C. The HPLC method 1 was used for all the sample analyses and HPLC method 2 was used for confirmatory analyses.

The standard mix (a mixture of available standards) was used to verify that the above HPLC conditions adequately separated metsulfuron-methyl from its expected metabolites and also to confirm the retention times of the standards during the course of the study.

Aliquots of concentrated extracts of 250 μl were injected into the column and the elution of radioactivity was monitored and quantitated by fraction collection and LSC.

Determination of Soil Bound Residues

Post-extracted soil samples were air-dried in the laboratory hood. When dried, the samples were homogenised and weighed. Aliquots in triplicate were combusted using a Harvey biological oxidiser (Harvey Instrument Inc., model OX 500). The $^{14}CO_2$ released from the combustion process was trapped in 15 ml of ^{14}C-cocktail scintillation fluid and radioactivity was measured by the LSC analysis.

Determination of DT_{50} and DT_{90}

The first-order model was used to estimate the DT_{50} (the time required for 50% of the applied chemical to degrade) and DT_{90} (the time required for 90% of applied chemical to degrade) values. The first-order model equation used is as follows (Yi *et al.*, 2013):

$$C = C_0.e^{-K*t}$$

where,

C_0: initial concentration (mg kg^{-1});
C: concentration at time *t* (mg kg^{-1}); and
K: degradation rate constant.

RESULTS AND DISCUSSION

Assay for Total Microbial Population

Microbial plate counts of the Bernam soil series samples conducted at 0, 30 and 60 days after treatment (DAT) are presented in *Table 1*. The soil microorganisms remained viable throughout the study in the non-sterile flasks. There was a slight increase of microbial mass (8.23 CFU/g soil x 10^{-5}) at 60 DAT. These observations indicated that the non-sterile system for the degradation studies that were conducted had no adverse effect on the microbial population. An earlier study by Abdullah *et al.* (2001) demonstrated that application of metsulfuron-methyl would not adversely affect soil microbial biomass and population once soil microbes adapted to the presence of metsulfuron-methyl.

Dissipation of Radiolabelled Phenyl Treated Soil Samples

Data on the recovery and distribution of radioactivity from the non-sterile soils treated with ^{14}C-phenyl metsulfuron-methyl have been presented as percentage of applied radioactivity as shown in *Table 2*. The total recovery of applied radioactivity (mass balance) for the ^{14}C-phenyl non-sterile system soil samples ranged from 93.3% to 101.1% with an average of 95.6 ± 4.6%.

TABLE 1. TOTAL BACTERIAL COUNTS IN THE NON-STERILE SOIL SAMPLES

Incubation period (day)	Bacterial counts (CFU g^{-1} soil x 10^{-5})
0	1.33 (± 0.25)
30	5.36 (± 0.07)
60	8.23 (± 0.13)

Note: CFU - colony forming units.

TABLE 2. DISTRIBUTION AND RECOVERY OF RADIOACTIVITY IN BERNAM SOIL SERIES SAMPLES TREATED WITH ^{14}C-PHENYL METSULFURON-METHYL IN THE NON-STERILE SYSTEM

Time (day)	Percentage of applied radioactivity (concentration in ppm*)				
	Extracted	Non- extracted	CO_2 volatiles	Organic volatiles	Total recovered
0	99.3 (0.139)	1.5 (0.002)	ND (<0.001)	ND (<0.001)	100.8 (0.141)
1	96.6 (0.135)	3.3 (0.005)	ND (<0.001)	ND (<0.001)	99.9 (0.140)
3	91.1 (0.128)	4.1 (0.006)	0.9 (0.001)	ND (<0.001)	96.1 (0.135)
7	84.0 (0.118)	12.3 (0.017)	1.2 (0.002)	ND (<0.001)	97.5 (0.137)
10	72.9 (0.102)	16.5 (0.023)	5.6 (0.008)	ND (<0.001)	95.2 (0.133)
14	75.5 (0.106)	18.2 (0.025)	7.4 (0.010)	ND (<0.001)	101.1 (0.142)
21	59.3 (0.083)	23.7 (0.033)	10.3 (0.014)	ND (<0.001)	93.3 (0.131)
30	53.2 (0.074)	27.5 (0.039)	15.3 (0.021)	ND (<0.001)	96.0 (0.134)
45	45.3 (0.063)	31.6 (0.044)	22.8 (0.032)	ND (<0.001)	99.7 (0.140)
60	34.6 (0.048)	35.6 (0.050)	28.3 (0.040)	ND (<0.001)	98.5 (0.138)
Average recovery					97.8 ± 4.6

Note: * Average of three replicates.
Values in parentheses are the concentration of the compound reported as parts per million (ppm), rounded to the nearest 0.001 ppm.
ND - not detected.

In the non-sterile system treated with ^{14}C-phenyl metsulfuron-methyl, the extracted radioactivity steadily decreased with time from 100% to 34.6% at 60 DAT. The formation of $^{14}CO_2$ corresponded to the decline of the parent; $^{14}CO_2$2 increased to 28.3% of the applied radioactivity at 60 DAT in non-sterilised soil (*Figure 1*). The total recovery of applied radioactivity (mass balance) for the ^{14}C-phenyl sterile system soil samples ranged from 86.8% to 99.7% with an average of 95.6 ± 6.8% applied radioactivity indicating that mass balance was maintained (*Table 3*).

The results show that the extractable residues increased with time to about 36% of the applied radioactivity at 60 DAT. Less than 0.1% of the applied radioactivity was found in the ethylene glycol and foam plug traps (collectively assumed as organic volatiles). The extracted radioactivity in the sterile soil treated with ^{14}C-phenyl metsulfuron-methyl also steadily decreased with time from 100% to 58.7% at 60 DAT. The $^{14}CO_2$ was only produced at 60 DAT at about 14% of the applied radioactivity. Non-extractable residues accounted for 4.5% at 21 DAT and steadily increased to 28.1% at 60 DAT (*Figure 2*).

In general, in the non-sterile system, the rate of extraction from applied radioactivity degraded faster (from 99.3% at 0 DAT to 34.6% at 60 DAT) than in the sterile system (from 99.7% at 0 DAT to 58.7%

TABLE 3. DISTRIBUTION AND RECOVERY OF RADIOACTIVITY IN BERNAM SOIL SERIES SAMPLES TREATED WITH ^{14}C-PHENYL METSULFURON-METHYL IN THE STERILE SYSTEM

Time (day)	Percentage of applied radioactivity (concentration in ppm*)				
	Extracted	Non- extracted	CO_2 volatiles	Organic volatiles	Total recovered
0	99.7 (0.140)	ND (<0.001)	ND (<0.001)	ND (<0.001)	99.7 (0.140)
1	98.7 (0.138)	ND (<0.001)	ND (<0.001)	ND (<0.001)	98.7 (0.138)
3	97.8 (0.137)	ND (<0.001)	ND (<0.001)	ND (<0.001)	97.8 (0.137)
7	97.3 (0.136)	ND (<0.001)	ND (<0.001)	ND (<0.001)	97.3 (0.136)
10	96.9 (0.136)	ND (<0.001)	ND (<0.001)	ND (<0.001)	96.9 (0.136)
14	94.7 (0.133)	ND (<0.001)	ND (<0.001)	ND (<0.001)	94.7 (0.133)
21	91.7 (0.128)	4.5 (0.006)	ND (<0.001)	ND (<0.001)	96.2 (0.135)
30	88.7 (0.124)	9.3 (0.013)	ND (<0.001)	ND (<0.001)	98.0 (0.138)
45	70.4 (0.099)	19.3 (0.027)	ND (<0.001)	ND (<0.001)	89.7 (0.126)
60	58.7 (0.082)	28.1 (0.039)	13.8 (0.019)	ND (<0.001)	86.8 (0.122)
		Average recovery			95.6 ± 6.8

Note: * Average of three replicates.
Values in parentheses are the concentration of the compound reported as parts per million (ppm), rounded to the nearest 0.001 ppm.
ND - not detected.

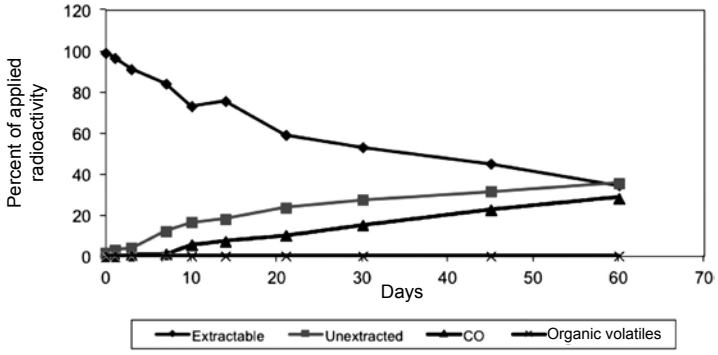

Figure 1. Distribution and recovery of radioactivity in the non-sterile system treated with ^{14}C-phenyl metsulfuron-methyl.

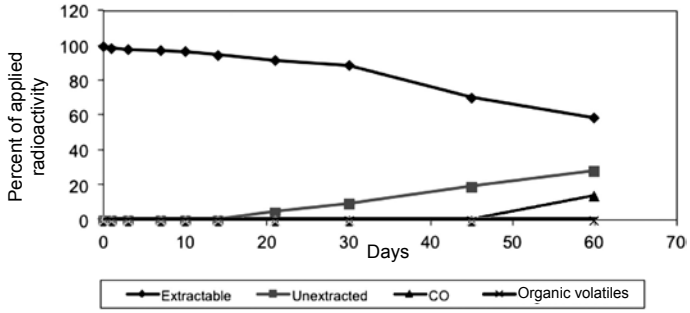

Figure 2. Distribution and recovery of radioactivity in the sterile system treated with ^{14}C-phenyl metsulfuron-methyl.

at 60 DAT). The results showed that $^{14}CO_2$ was also released faster in the non-sterile system compared to the sterile system. This was due to soil microbial activity in the non-sterile system. Microbial degradation is the main pathway for degradation of metsulfuron-methyl in the soil.

Rate of Dissipation of ^{14}C-Phenyl Metsulfuron-methyl in Bernam Soil Series

The first-order model was used to determine the DT_{50} and DT_{90} values of ^{14}C-phenyl metsulfuron-methyl in both the non-sterile and sterile systems (*Figure 3*). The DT_{50} was estimated by using the equation: $DT_{50} = \ln (2)/k$. The degradation rate constant *(k)* was determined using linear regression of $\ln (C/C_0)$ (C = concentration) over time and was the slope of the linear regression line.

Based on the first-order model, the ^{14}C-phenyl non-sterile system had DT_{50} and DT_{90} of 12 days and 41 days respectively, while the ^{14}C-phenyl sterile system had DT_{50} and DT_{90} of 19 and 64 days respectively (*Table 4*). These results are similar to those of (Da Silva *et al*., 2013; Xiangwen *et al*., 2014) who found the half-lives to be between 8 to 36 days (based on the first-order model) in an acidic soil (pH 5.7 and 7.3% organic carbon) at various soil moistures and temperature levels.

The DT_{50} value of metsulfuron-methyl in the non-sterile system was lower than that in the sterile system, suggesting that biological degradation (by soil microorganisms) significantly contributed to the dissipation of this compound. The dissipation of metsulfuron-methyl in the soil involves both chemical and microbial processes and this has been observed in other sulfonylurea herbicides as well (Barraclough *et al*., 2005; Marie *et al*., 2014). The degradation of metsulfuron-methyl in an acidic soil environment is relatively rapid because of sulfonylurea bridge hydrolysis which diminishes in alkaline soils.

Degradation of Metsulfuron-methyl

The composition of the radioactivity in the soil samples treated with ^{14}C-phenyl metsulfuron-methyl has been presented as a percentage of the applied radioactivity in the non-sterile system (*Table 5*) and sterile system (*Table 6*).

In the ^{14}C-phenyl metsulfuron-methyl non-sterile system, based on the relative retention time (RRT), six metabolites were detected. The identities (code number) of the metabolites were 581, NC 148, B5685, D5803, B5067 and F5438. Out of the six metabolites, chemical structures of five metabolites were able to be identified, with the exception of NC 148. Six metabolites were also detected in the sterile system. Four out of six metabolites, 581, NC148, D5803 and B5067 were detected as well as in non-sterile system. However, one new metabolite, namely MU717 was only detected in the sterile system but the chemical structure of this metabolite was not available. For another metabolite, SP3 both name and structure could not be identified.

Radiolabelled Phenyl Treated Soil Samples

The analysis at 0 DAT soil extracts from the fraction collector indicated that 92.5% and 96.5% of the applied radioactivity was recovered as

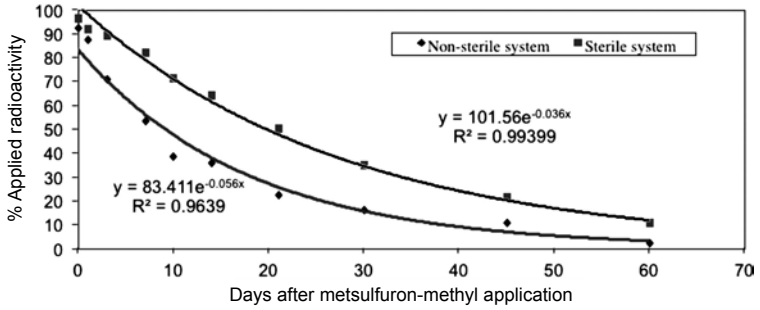

Figure 3. Dissipation of ^{14}C-phenyl metsulfuron-methyl over time in soil.

TABLE 4. DEGRADATION OF ^{14}C-PHENYL METSULFURON-METHYL IN BERNAM SOIL SERIES – FIRST-ORDER MODEL

System	First-order model		DT_{50} (day)	DT_{90} (day)
	K (slope)	r^2		
Non-sterile	0.0563	0.96	12	41
Sterile	0.0360	0.99	19	64

TABLE 5. DEGRADATION OF ^{14}C-PHENYL METSULFURON-METHYL IN THE BERNAM SOIL SERIES SAMPLES IN THE NON-STERILE SYSTEM

Time (day)	Peak I.D. (RRT)*	T6373 (1.00)	Applied radioactivity (%) P1 IN-581 (0.62)	P2 IN-NC148 (0.68)	P3 IN-B5685 (0.73)	P4 IN-D5803 (0.80)	P5 IN-B5067 (0.83)	P6 IN-F5438 (0.86)
0		92.5 (± 8.33)	ND	ND	2.3 (± 0.09)	1.8 (± 0.12)	2.7 (± 0.31)	ND
1		87.3 (± 5.21)	ND	ND	ND	3.2 (± 0.28)	3.5 (± 0.44)	2.6 (± 0.05)
3		70.6 (± 3.38)	3.2 (± 0.14)	2.3 (± 0.06)	ND	3.3 (± 4.21)	6.5 (± 0.53)	5.2 (± 0.50)
7		53.4 (± 2.83)	4.6 (± 0.22)	ND	ND	10.5 (± 0.42)	11.3 (± 0.62)	4.2 (± 0.47)
10		38.4 (± 2.01)	6.3 (± 0.53)	2.8 (± 0.10)	ND	13.2 (± 0.53)	10.1 (± 0.36)	2.1 (± 0.09)
14		35.7 (± 1.82)	8.0 (± 0.64)	10.4 (± 0.57)	ND	12.5 (± 0.45)	5.3 (± 0.20)	3.6 (± 0.11)
21		22.2 (± 1.03)	9.3 (± 0.78)	7.2 (± 0.72)	3.1 (± 0.13)	ND	8.2 (± 0.83)	9.3 (± 0.41)
30		16.3 (± 0.83)	7.6 (± 0.68)	9.1 (± 0.83)	2.8 (± 0.11)	2.9 (± 0.30)	9.8 (± 0.86)	4.7 (± 0.53)
45		10.9 (± 0.35)	6.3 (± 0.54)	16.2 (± 1.08)	ND	2.6 (± 0.23)	5.1 (± 0.42)	4.2 (± 0.32)
60		2.1 (± 0.05)	5.2 (± 0.42)	19.5 (± 1.11)	ND	ND	4.2 (± 0.39)	3.6 (± 0.25)

Note: *Relative retention time (RRT) - retention time of degrade/retention time of parent (min).
ND - not detected.
T6373 = 2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl-ureidosulfanoyl)-benzoate acid methyl ester (metsulfuron-methyl).
581= 1,2-benzisothiazol-3(2H)-one, 1,1-dioxide.
NC148 = Not available.
B5685 = Methyl 2-[[(aminocarbonyl) amino]sulfonyl]benzoate.
D5803 = Methyl 2-(aminosulfonyl) benzoate.
B5067 = Methyl 2 [[[[(4-hydroxy-6-methyl-1, 3, 5-triazine-2y 1) amino] carbonyl] amino]sulfonyl]benzoate.
F5438 = Methyl 2 [[[[(4-methoxy-6-methyl-1,3,5-triazine-2y 1) amino]carbonyl]amino]sulfonyl]benzoate acid.

^{14}C-phenyl metsulfuron-methyl in the non-sterile and sterile test systems respectively. *Figures 4* and *5* show the radio chromatogram of ^{14}C-phenyl metsulfuron-methyl for 0 DAT samples in the non-sterile and sterile soils. These results demonstrated that the test substance was stable in the extraction systems.

In the non-sterile system, six metabolites were observed over the duration of the study of which one was < 5% (P3). The 30 DAT chromatogram of the fraction collected from the HPLC analysis is presented in *Figure 6*.

In the sterile system, six metabolites were observed over the duration of the study of which two were < 5% (SP2 and SP3). The 30 DAT chromatogram of the fraction collected from the HPLC analysis is presented in *Figure 7*.

The metabolites PS1, PS2, PS4, PS5 and PS6 peaked corresponding to IN-581, IN-NC148, IN-D5803, IN-B5067 and IN-MU717 by RRT comparison. IN-581 reached a maximum of 20.8% at 60 DAT. The IN-D5803 reached a maximum of 10.2% at 10 DAT and declined to 5.5% at 60 DAT. IN-B5067 reached a maximum of 18.5% at 30 DAT and declined to 9.3% at 60 DAT. The IN-MU717 reached a maximum of 14.5% at 45 DAT. IN-581, IN-D5803 and IN-B5067 were observed in both the non-sterile and sterile systems.

Metabolic Pathway of Radiolabelled Metsulfuron-methyl in Non-sterilised Soil

The proposed degradation pathway of metsulfuron-methyl in soil under aerobic conditions is summarised in *Figure 8*. The major metabolite in the non-sterile system for phenyl moieties is IN-B5067, IN-F5438 and IN-NC148. Metabolite IN-B5528 was found only in the triazine moiety and metabolites IN-581 and IN-D5803 were found only in the phenyl moiety. Both the ^{14}C-phenyl and

TABLE 6. DEGRADATION OF ^{14}C-PHENYL METSULFURON-METHYL IN THE BERNAM SOIL SERIES SAMPLES IN THE STERILE SYSTEM

Time (day)	Peak I.D. (RRT)*	T6373 (1.00)	Applied radioactivity (%) SP1 IN-581 (0.62)	SP2 IN-NC148 (0.68)	SP3 UN** (0.76)	SP4 IN-D5803 (0.80)	SP5 IN-B5067 (0.83)	SP6 IN-MU717 (0.96)
0		96.5 (± 8.73)	ND	ND	ND	ND	3.2 (± 0.16)	ND
1		92.1 (± 8.06)	ND	ND	ND	2.1 (± 0.03)	4.5 (± 0.21)	ND
3		89.3 (± 7.56)	ND	ND	ND	3.2 (± 0.09)	5.3 (± 0.31)	ND
7		82.0 (± 5.28)	ND	ND	ND	6.3 (± 0.36)	7.3 (± 0.58)	1.8 (± 0.02)
10		71.1 (± 4.52)	4.2	ND	ND	10.2 (± 0.65)	8.9 (± 0.63)	2.5 (± 0.05)
14		64.1 (± 3.59)	9.3 (± 0.63)	ND	ND	8.3 (± 0.47)	4.2 (± 0.28)	8.8 (± 0.63)
21		50.2 (± 2.73)	13.2 (± 0.93)	ND	2.1 (± 0.04)	5.5 (± 0.40)	8.2 (± 0.60)	12.5 (± 0.93)
30		34.9 (±1.68)	10.6 (± 0.71)	ND	3.3 (± 0.10)	9.6 (± 0.65)	18.5 (± 1.06)	11.8 (± 0.85)
45		21.7 (± 1.25)	18.9 (± 1.03)	ND	2.3 (± 0.06)	5.5 (± 0.28)	7.5 (± 0.55)	14.5 (± 1.02)
60		10.6 (± 0.83)	20.8 (± 1.15)	2.3 (± 0.07)	3.5 (± 0.12)	ND	9.3 (± 0.68)	12.2 (± 0.93)

Note: *Relative retention time (RRT) - retention time of degraded / retention time of parent (min).
** UN – unknown.
ND = not detected.
T6373 = 2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl-ureidosulfanoyl)-benzoate acid methyl ester (metsulfuron-methyl).
581 = 1,2-benzisothiazol-3(2H)-one, 1,1-dioxide.
NC148 = Not available.
D5803 = Methyl 2-(aminosulfonyl) benzoate.
B5067 = Methyl 2 [[[[(4-hydroxy-6-methyl-1,3,5-triazine-2y 1) amino]carbonyl]amino]sulfonyl]benzoate.
MU717 = Not available.

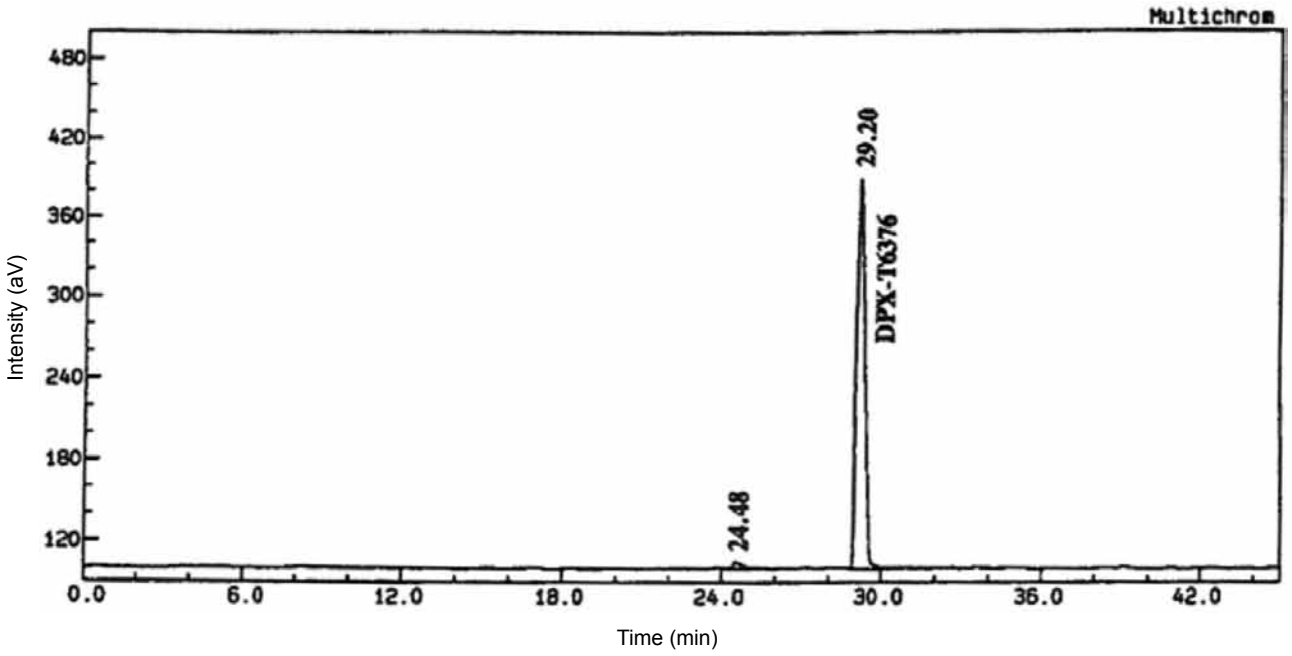

Figure 4. The ^{14}C-radiochromatogram of ^{14}C-phenyl metsulfuron-methyl at 0 days after treatment (DAT) samples in the non-sterile system.

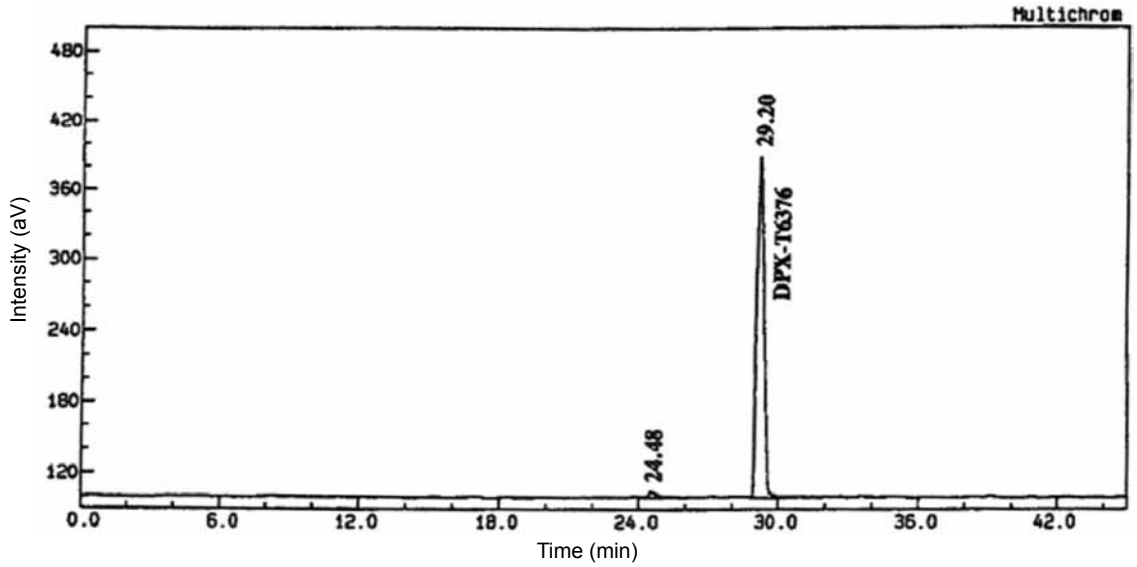

Figure 5. The ^{14}C-radiochromatogram of ^{14}C-phenyl metsulfuron-methyl at 0 days after treatment (DAT) samples in the sterile system.

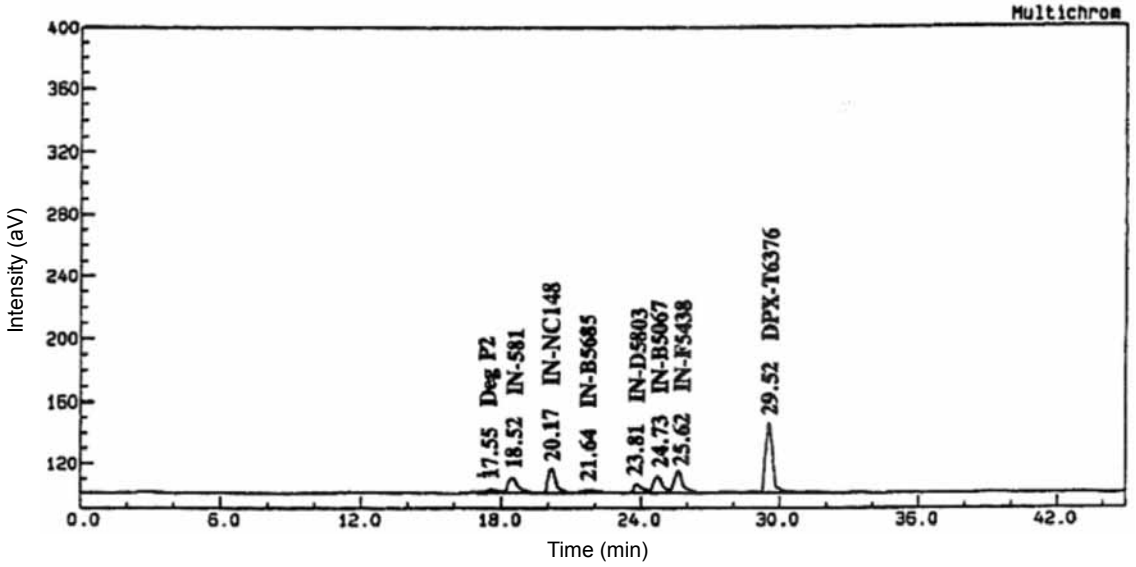

Figure 6. The ^{14}C-radiochromatogram of ^{14}C-phenyl metsulfuron-methyl at 30 days after treatment (DAT) samples in the non-sterile system.

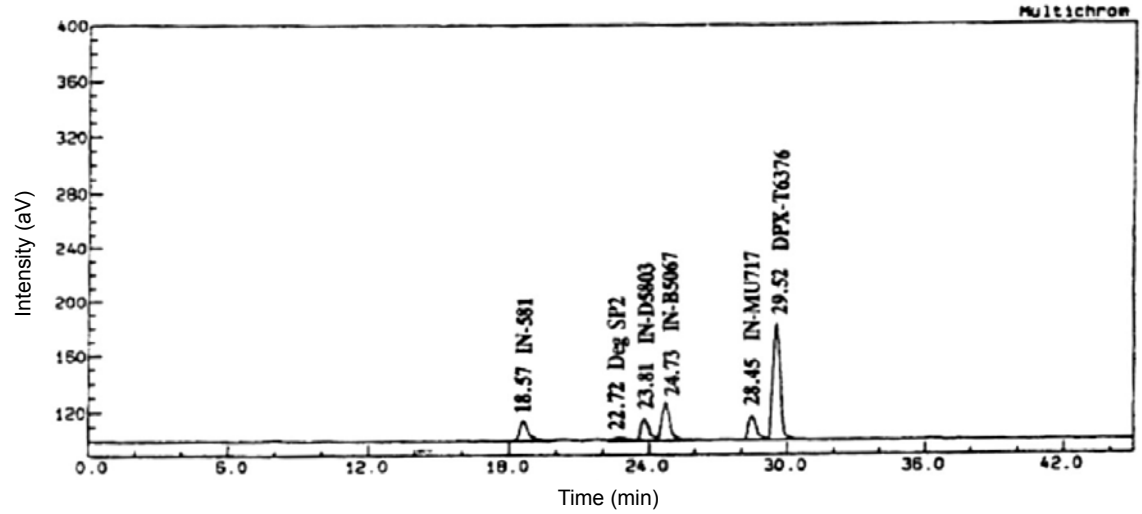

Figure 7. The ^{14}C-radiochromatogram of ^{14}C-phenyl metsulfuron-methyl at 30 days after treatment (DAT) samples in the sterile test system.

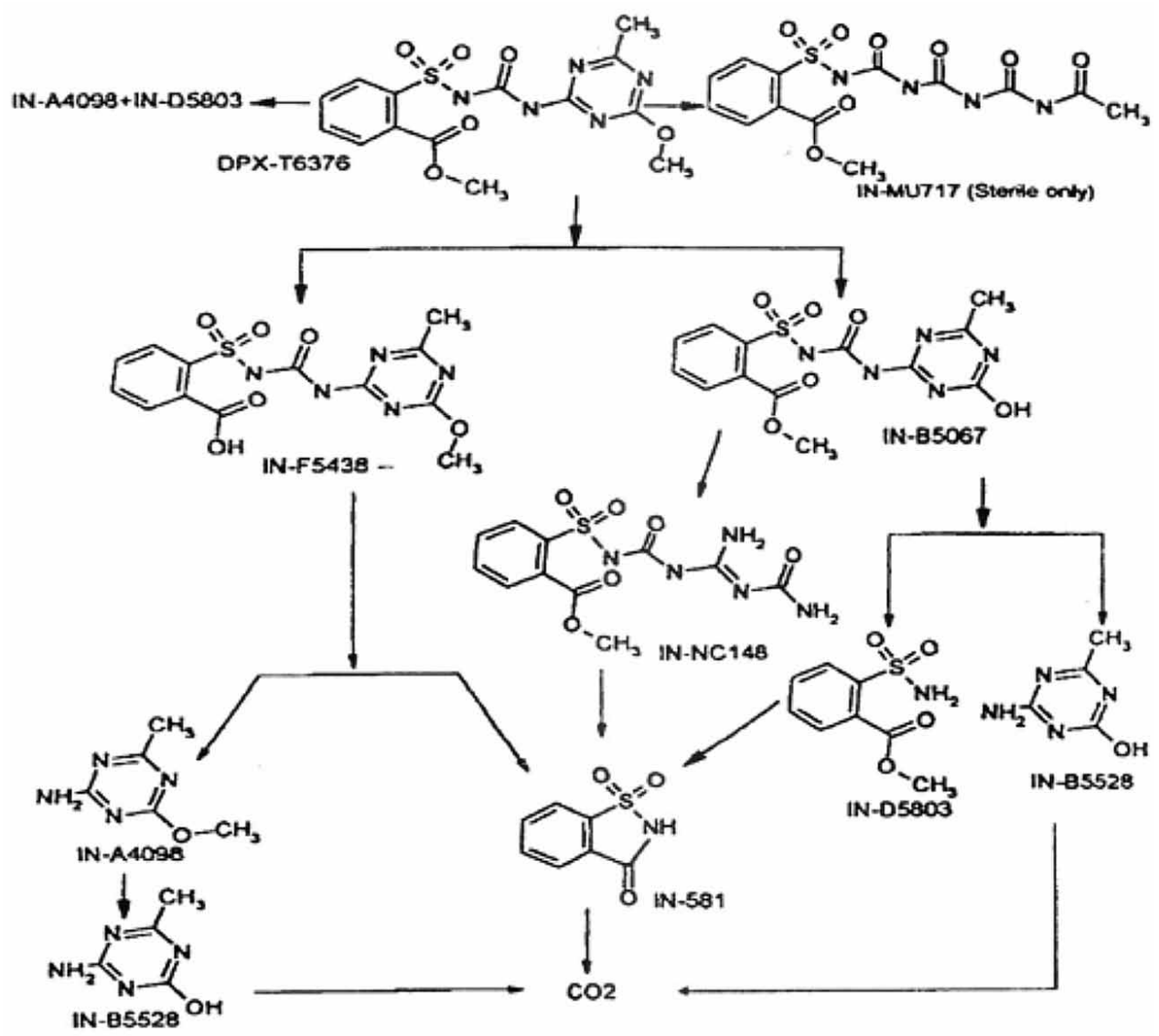

Figure 8. Proposed metabolic pathways of metsulfuron-methyl in Bernam soil series.

^{14}C-triazine metsulfuron-methyl were mineralised to $^{14}CO_2$ extensively in the non-sterile soils. In the sterile system, the major metabolites that included both the phenyl and triazine moieties together were IN-B5067 and IN-MU717. Metabolites IN-A4098 and IN-B5528 were found only in the triazine moiety and metabolites IN-581 and IN-D5803 in the phenyl moiety.

Cleavage of the sulfonylurea linkage, which has been commonly found in other sulfonylurea herbicides (EI-Ghamry *et al.*, 2000; Ananpattarachai and Kajitvichyanukul, 2015; Ismail *et al.*, 2015), results in the formation of metabolites IN-B5528 and IN-D5803. The urea bridge cleavage occurs rapidly in acidic aqueous solutions in the absence of micro-organisms, but slow down at neutral pH. In the non-sterile soil, this cleavage proceeds through both the abiotic bridge hydrolysis as well as the microbially mediated hydrolysis (Gevao *et al.*, 2000; Yi *et al.*, 2013).

The *O*-demethylation of the triazine moiety of metsulfuron-methyl may be a chemical or microbial process (or both) since metabolites IN-B5067 was found in both the sterile and non-sterile soils. The *O*-demethylation of the ester on the phenyl moiety of metsulfuron-methyl is probably a microbial degradation process since the metabolite IN-F5438 was found only in the non-sterile soil.

Primisulfuron-methyl and metsulfuron-methyl are degraded both by chemical hydrolysis and by soil micro-organisms at low pH (Braschi *et al.*, 2000). Degradation also depends on temperature and soil water content. The chemical degradation products are 1-[2X-benzene-1-sulfonyl]-7-acetyltriuret, where X is $COOCH_3$ for metsulfuron-methyl and Cl for chlorsulfuron. They derive from the triazine ring cleavage (Perreau *et al.*, 2007), the same product obtained from chlorsulfuron, sulfonylurea, prosulfuron and thifensulfuron-methyl. The biodegradation of metsulfuron-methyl and chlorsulfuron is

operated by *Pseudomonas fluorescens* B2 strain. Yu *et al.* (2005) isolated a strain of *Curvularia* sp. which is able to be used as a sole source of carbon and energy and studied several features of herbicide degradation in pure culture and soils. He *et al.* (2006) isolated four bacteria, nine filamentous fungi and 20 actinomycetes capable of degrading metsulfuron methyl and selected an unknown strain of *Penicillium* sp. Chemical and/or photochemical processes under typical environmental conditions cannot degrade most of the pesticides (Lagana' *et al.*, 2000).

Metabolite IN-MU717 was unique to the sterile soil suggesting that it is an abiotic hydrolysis product. The triazine ring-opened degradation products analogous to IN-MU717 have been reported for other sulfonylurea herbicides such as prosulfuron (Ghani and Wardle, 2001; Ismail *et al.*, 2015), thifensulfuron-methyl (Azcaratea *et al.*, 2015) and chlorsulfuron (Rosenkrantz *et al.*, 2013a) in hydrolysis studies. A triazine ring-opened degradation product of metsulfuron-methyl was reported in an aqueous hydrolysis study at pH 5 and in sterile soil in a soil degradation study (Haizhen *et al.*, 2010; Rosenkrantz *et al.*, 2013b; Rafqah *et al.*, 2015). Both hydrolysis and soil studies reported the same triazine ring-opened degradation products with an elemental composition of $C_{13}H_{14}N_4O_8S$ which is equivalent to metabolite IN-MU717 in the present study. Metabolite IN-MU717 was not observed in the non-sterile soil. Study by Li *et al.* (1999) suggested two possible explanations. The microbial enzymatic reaction from metabolite IN-B5067 to IN-NC148 may be faster than the hydrolysis reaction to IN-MU717. Secondly, metabolite IN-MU717 may have been formed but got degraded microbially to undetectable levels by the time the samples were taken.

Metabolite IN-NC148 appears to be a biotransformation product since it was not detected in the sterile soil. The same finding was reported by Li *et al.* (1999). An analogous metabolite had been observed in chlorsulfuron soil dissipation (Strek, 1998; Olivera *et al.*, 2001). A mechanism for the formation of IN-NC148 can be proposed as follow: the first step appears to be *O*-demethylation of the triazine ring to form the metabolite IN-B5067, since this metabolite was observed initially, then it declined while the presence of IN-NC148 gradually increased (*Figure 8*). Enzyme-mediated hydrolytic bond cleavage of two of the triazine ring C-N bonds is necessary to arrive at the carbamoyl guanidine structure of IN-NC148.

CONCLUSION

Metsulfuron-methyl degraded faster in acidic soil in the dark at 30°C under the non-sterile system as compared to that in the sterile system. The estimated DT_{50} and DT_{90} values of metsulfuron-methyl in the non-sterile system using the first-order model were approximately 13 and 44 days, respectively. In the sterile system, the estimated DT_{50} and DT_{90} values of metsulfuron-methyl were approximately 31 and 70 days, respectively The fact that the DT_{50} values of metsulfuron-methyl in the non-sterile system were lower than the sterile system suggested that biological degradation (by soil micro-organisms) significantly contributed to the dissipation of the compound. The principal degradation product after 60 days is carbon dioxide. The major routes of degradation are *O*-demethylation, sulfonylurea bridge cleavage and triazine ring-opened. Microbial metabolism best explains the formation of IN-NC148 while chemical hydrolysis leads to the formation of IN-MU717. The results indicate that the triazine ring opening did occur and that hydrolytic and microbial mechanisms were operable.

Therefore, *in vitro* study should be carried out in order to identify the microbes involve in the degradation of the compound.

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