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Metsulfuron Methyl - Effect On Soil Microflora

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----------ABSTRACT----------
Effect of soil microflora on metsulfuron methyl was investigated in loamy sand soil under laboratory condition. Metsulfuron methyl was incubated in loamy sand soil over a period of 28 days for carbon transformation and 42 days for nitrogen transformation at concentrations of 0.02 µL and 0.2 µL/kg soil dry weight. Soil nitrate content deviation from the control determined on day 42 after the application of test item treated groups compared to control was 4.0 % and 18.8% for 0.02 and 0.2 µL of metsulfuron methyl/kg soil dry weight, respectively. The difference between control and treatments was statistically significant for both concentrations. The rate of nitrate formation between 28 and 42 days after application of the test item to soil differed from controls by -4.6 % and -19.5 % for 0.02 µL metsulfuron methyl/kg soil dry weight and 0.2 µL metsulfuron methyl/kg soil dry weight, respectively. In soil respiration study at the end of the 28 day study, deviations in respiration rates compared to controls after applying the test item to soil were 2.6% and 6.6% for the test concentrations of 0.02 and 0.2 µL of metsulfuron methyl/kg soil dry weight, respectively.

KEY WORDS: *Soil microflora, Metsulfuron methyl, Loamy sand, Carbon, Nitrogen, Respiration.*

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I. INTRODUCTION

Herbicides are valuable tools to manage early emerging weeds. However, if these herbicides are applied without care, they could damage the crop or contaminate the groundwater. The goals of controlling weeds are to prevent or minimize yield loss, minimize future weed problems, and prevent interference at harvest. To ensure effective and safe applications, it is important to understand the different factors affecting the fate of soil-applied herbicides. The movement of herbicides through soil is an important process that determines their fate in both soil and aquatic environment. Protecting groundwater from pesticide contamination is a high priority. Industrial effluents entering the water bodies not only affect the quality of drinking water but also have deleterious impact on the soil microflora and aquatic ecosystem[15]. Coarse textured soils with low organic matter and clay content also tend to have low microorganism populations thereby reducing herbicide microbial degradation [14]. Soil persistence is dependent on factors such as herbicide chemical structure, herbicide physical properties (i.e. water solubility and volatility), soil adsorptive properties, soil temperature and moisture conditions, soil composition and soil microbial content [16]**.** Of the four soil properties organic matter, total clay, Cation Exchange Capacity and pH, organic matter content is often considered to be the best single predictor of herbicide adsorption [9]**.** The most important pathways for degradation of sulfonylurea herbicides in soil are chemical hydrolysis and microbial degradation, while other dissipation processes such as volatilization and photolysis are relatively insignificant [3,5,7]**.** The degradation of sulfonylurea herbicides in soil is primarily dependent on chemical and biological degradation, which is influenced by temperature, pH, and moisture content [4, 5]. In general, soil pH and organic matter are the main factors affecting the mobility of the sulfonylurea herbicides, with mobility being reduced as organic matter content and pH increase [5, 16]. Metsulfuron-methyl is sulfonylurea herbicide, with high herbicidal activity and very low mammalian toxicity and is widely used in agriculture [11]. The half-life of metsulfuron-methyl at different soil water content and temperatures is 8 to 36 days [12]. The microbial biomass in clay loam soil increased with herbicide (metsulfuron-methyl) treatment during the first 9 days of incubation, but declined from day 19 onward was reported. However, in sandy loam soil, the biomass decreased with an increase of herbicide concentration on day 1, but increased thereafter [10]. In particular, several studies have been carried out on concerns relating to microbial degradation of sulfonylureas [13].

The maintenance of soil fertility depends on the size and activity of soil microbial biomass [2], which is of fundamental importance in the biological cycles of almost all major plant nutrients [15]. Weak acid herbicides such as the sulfonylureas will be mostly anionic and less likely to be adsorbed to soil. Microbial breakdown is the breakdown of chemicals by microorganisms such as fungi and bacteria**.** The degradation of soil microorganisms on the benzene ring of the sulfonylurea hydrolysis product was reported [18]. Factors such as soil temperature, humidity, pH, and organic concert affecting the degradation of sulfonylureas in soil has also been reported [1, 8, 17, 19, 20]. Microbial degradation of metsulfuron methyl in soil microorganisms is an important factor for the complete degradation of metsulfuron methyl in the field [6]. Microbial breakdown tends to increase when:

- Temperatures are warm
- Soil pH is favourable
- Soil moisture and oxygen are adequate
- Soil fertility is good

The purpose of this study was to describe the effects of sulfonylureas application on the population of microorganisms in soil. The sulfonylurea chosen was metsulfuron-methyl [2-(4-methoxy-6-methyl-1,3,5-triazin-2-yl carbamoyl sulfamoyl) benzoicacid], a herbicide commonly used for the control of weeds in cereal crops field. The effect of metsulfuron methyl on soil microflora was conducted under laboratory condition that measured nitrogen and respiration following an application of the sample to loamy sand soil. The compound was incubated in loamy sand soil over a period of 28 days for carbon transformation[22] and 42 day for nitrogen transformation[21] at concentrations of 0.02 and 0.2 µL of formulation /kg soil dry weight. The concentrations tested were based on the maximum recommended single field application rate of 80 g of metsulfuron methyl/ha.

II. EXPERIMENTAL

Materials

BOD meter supplied by Lovibond, Germany
Expandable ion analyzer, Model EA 940, Orion Research Inc., USA
Nitrate electrode, Model 93-07, Orion Research Inc.
Reference electrode, Model 90-02, Orion Research Inc.
Water bath supplied by Lakshmi Enterprises, Chennai
Incubation chamber supplied by Thermo scientific and Bioscientific, Chennai
pH meter - Supplied by Eutech Instruments Private Limited, Singapore India.
Vacuum desiccator supplied by Shakthi Agencies, Chennai
Hot Air Oven supplied by Lab mate and Lab serve, Chennai
Laboratory balance, Sartorious Mechatronics India Private Limited, Bangalore, India.

Experimental Procedure: Loamy sand soil was taken from an agricultural field with the sampling depth of 0-20 cm. For at least four years prior to test initiation, no pesticides had been used on the soil. No organic or mineral fertilizer had been applied to the soils for two years prior to study initiation, respectively. The sieved soil (mesh 2 mm) was pre-incubated at 50% of its maximum water holding capacity in ventilated boxes at about 20°C before use in the experiment. The soil parameters were analysed before the initiation of the study.

Preparation of Soil : Prior to the initiation of the study, the moisture content of the soil was determined and the amount of water needed to bring the soil moisture content to approximately 50% of the WHC_{max} (maximum water holding capacity) was calculated. For nitrogen transformation, the soil was thoroughly mixed with the ground lucerne meal before applying metsulfuron methyl. The final concentration of the dried lucerne meal was 0.5% of the soil dry weight. For respiration, the amount of glucose needed to obtain maximum rates of respiration in the test soil was determined prior to the beginning of the test. A concentration of 3.2 g glucose/kg soil dry weight was found to be optimum and was used in the test.

Preparation of Stock Solution in Deionised Water :50 mg homogenized test item was weighed and transferred into a 100 mL standard volumetric flask. Then it was dissolved and diluted up to the mark with deionized. The concentration of this stock solution was 1 mg metsulfuron methyl in 1.96 mL of the test item solution.

Analysis of Samples : At each sampling date, the required amount of soil sample was taken from the corresponding vessel, and the following parameters were determined:

Dry weight (one representative sample per treatment and sampling date)
pH (one representative sample per treatment and sampling date)
Nitrate content (three replicates per treatment in every sampling date)
Short-term respiration (three replicates per treatment in every sampling date)
At each sampling date, the following analysis was performed
The nitrate content was determined using Expandable Ion Analyzer with nitrate selective ion electrode.
The short-term respiration was determined using the BOD meter system in a 300 g soil sample after adding 3.2 g glucose/kg soil dry weight to the soil sample.
Dry weight was determined with 2 to 3 g of soil sample by accurately weighing the samples before and after drying at 105°C for 2 h.

Nitrogen Turnover : For the determination of nitrate content, soil samples were taken within 6 hours after application and afterwards at each sampling date (7, 14, 28 and 42 days). The nitrate content was determined in each sample of treated and control soils. Soil nitrification was determined by measuring the NO_3^- contents of aqueous soil extracts using calibrated ion sensitive electrodes and the Orion expandable ion analyzer. The concentrations of NO_3^- in the soil were then calculated from the measured values.The volume of the bottles containing fifty grams soil from each treatment was brought up to a final volume of 200 mL with 0.1% alum solution (Aluminum-potassium-sulfate, $AlK(SO_4)_2$ 12 H_2O). The samples were vigorously shaken for approximately 30 minutes. The aqueous supernatant was filtered through whatman 41 filter paper and fifty milliliter aliquot was used for the nitrate measurements using the NO_3^- selective electrode. The ion electrode was calibrated with freshly prepared standard solution. The concentrations of $NO3^-$ in the soil were expressed as mg NO_3^- per kg soil dry weight. For all the test samples, the contents of NO_3^- were determined and calculated in the respective treatments. Deviations from treatment and control were reported based on the comparison of nitrate content between treatment and controls.

Nitrate
Nitrate content = milligram of nitrate / Soil DW x 1000

Rate of formation of nitrate formation (mg nitrate/kg soil dry weight/day):
The rate was calculated for each time interval and treatment as follows:
Rate of nitrate formation =
[(mg nitrate / kg soil dry weight on sampling day 'a') - (mg nitrate / kg soil dry weight on previous sampling day)]/ 'a' days
Where 'a' = 7, 14, 28, 42 days.

Short- term Respiration
The glucose induced respiration rate was determined in all the treated and control soil samples after 0 (within 6 hours), 7, 14 and 28 days. For each interval, approximately 300 g of soil samples were collected in three replicates from the representative boxes on each occasion. Then a 4 mL volume of glucose solution (4 mL of a solution of 12.5 g glucose/50 mL deionized water i.e. end concentration 3.2 g of glucose/kg soil dry weight.) was added in to each box and mixed thoroughly. The amount of glucose to give the highest respiration rates was determined in a pre-test.

The glucose amended soil samples were incubated at 20 ± 2 °C. The oxygen consumption was measured up to 24 consecutive hours using the BOD meter, and the carbon dioxide release was calculated. The linear part of the respiration curve after 2 hours and up to 14 hours was used for the calculation of the respiration rate,
The amount of CO_2 produced during short-term respiration was calculated from the consumed O_2; based on the stoichiometry of O_2 consumption and CO_2 production during respiration (1 mg of consumed O_2 corresponds to 1.375 mg of respired CO_2).

III. RESULTS AND DISCUSSION

The soil parameters such as water holding capacity, pH, soil texture, Cation Exchange Capacity, microbial biomass were analysed before the initiation of the study and reported, (Table 1).

Nitrogen turn Over : The test item metsulfuron methyl was incubated in a loamy sand soil over a period of 42 days (nitrogen transformation) at concentrations of 0.02µL metsulfuron methyl /kg soil dry weight and 0.2µL metsulfuron methyl /kg soil dry weight (maximum single application rate equivalent to 8 L of metsulfuron methyl /ha and 80 L of metsulfuron methyl /ha). After 42 days, the mean percent deviation in the rate of nitrate formation between treated and untreated soils was less than 25%. The soil nitrate content, deviation from the

control, determined on day 42nd after application of the test item treated groups compared to control was 4.0 % and 18.8 % for 0.02 and 0.2 µL metsulfuron methyl /kg soil dry weight, respectively. The difference between controls and treatments was statistically significant for both concentrations, (Table 2).The rate of nitrate formation between 28 and 42 days after application of the test item to soil differed from controls by -4.6 % and -19.5 % for 0.02 µL metsulfuron methyl /kg soil dry weight and 0.2 µL metsulfuron methyl /kg soil dry weight, respectively. The difference between treatment and controls was statistically significant for both concentrations. After 42 days, deviations between treatments and control did not exceed the 25%.A summary of the analytical results of the nitrate formation rate in soil treated with 0.02 µL metsulfuron methyl /kg soil dry weight and 0.2 µL metsulfuron methyl /kg soil dry weight is shown, (Table 3). The limit of quantification (LOQ) for nitrate was calculated on the basis of the dry substance of sample of all sampling dates by spiking the known concentration into the soil, (Table 4)**.**

Short-term Respiration : The test item was incubated in a loamy sand soil over a period of 28 days at rates of 0.02 µL metsulfuron methyl/kg soil dry weight and 0.2 µL metsulfuron methyl /kg soil dry weight. The test item at soil concentrations of 0.02 µL metsulfuron methyl /kg soil dry weight and 0.2µL metsulfuron methyl /kg soil dry weight had no long term influence on the short-term, substrate-induced respiration. The difference in soil respiration between the treated and control soils was below the 25% trigger value at the end of 28th day given by the OECD 217 guideline[23]. At the end of the 28 day study, deviations in respiration rates compared to controls after applying the test item to soil were 2.6% and 6.6% for the test concentrations of 0.02 µL metsulfuron methyl /kg soil dry weight and 0.2 µL metsulfuron methyl /kg soil dry weight, respectively, (Table 5). The respiration curves from each sampling date are presented in Figures 1 to 4, respectively. The respiration rates in treated soil were statistically significantly different from control.

IV. CONCLUSIONS

Soil microflora study was conducted to investigate the effects of metsulfuron-methyl herbicide on the soil microbial biomass in loamy sand soil. The herbicide was applied to the soil at two concentrations: control, 0.02, 0.20 µL/kg soil. Determinations of microbial biomass-C and microbial biomass-N contents were carried out at 0, 7, 14, 28 for carbon and 0, 7, 14, 28 and 42 days for nitrogen after herbicide application. Compared to the untreated control, a marked increase in the microbial biomass for C: N ratio was observed in the herbicide treated soil. The soil microbial effect was significant upto 42 days of incubation for nitrogen and 28 days for carbon. There is no long term influence on soil microbial properties.

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Table 1: Soil Parameters

PARAMETER	RESULT	METHOD
Soil texture	Loamy sand	Method : International pipette method Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi
pH	6.40	Method : CIPAC MT 75.3 Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi
Total Org. C (%) based on soil dry weight	0.81	Method : Walkley and Black, Ref: Brian A. Schumacher United States Environmental Protection Agency, Environmental Sciences Division National Exposure Research Laboratory NCEA-C- 1282 EMASC-001 April 2002 Year: Apr. 2002
Microbial biomass (% of total organic carbon)	14.33	Method : Walkley and Black, Ref: Brian A. Schumacher United States Environmental Protection Agency, Environmental Sciences Division National Exposure Research Laboratory Year: Apr. 2002
NH_4^+-N (mg/kg dry weight)	1.024	Method: Expandable Ion Analyzer Ref: Model EA940, manufactured by Orion Research Incorporated, USA
NO^-_3–N (mg/kg dry weight)	12.814	Method: Expandable Ion Analyzer Ref: Model EA940, manufactured by Orion Research Incorporated, USA
N_{min}-N (mg/kg dry weight)	13.864	Method: Expandable Ion Analyzer Ref: Model EA940, manufactured by Orion Research Incorporated, USA
CEC* (mmol Ba/kg dry weight)	78	Method : Titration method Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi

Soil Parameters (Contd.,)

PARAMETER	RESULT	METHOD
WHC_{max}** (%)	30.1	Method : Double end Open glass tube method Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi
Clay (%)	4	Method : International pipette method Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi
Silt (%)	28	Method : International pipette method Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi
Sand (%)	68	Method : International pipette method Ref: Jackson M.L.1973. Soil Chemical analysis Prentice Hall of India Private Limited, New Delhi
*CEC = cation exchange capacity **WHC_{max} = maximum water holding capacity		

Table 2: Metsulfuron methyl on soil nitrate content (mean values)

DAY	CONTROL		0.02 µL METSULFURON METHYL /KG SOIL DRY WEIGHT			0.2 µL METSULFURON METHYL /KG SOIL DRY WEIGHT		
	MEAN NO_3^- MG/KG DRY WEIGHT[1]	CV[2] (%)	MEAN NO_3^- MG/KG DRY WEIGHT[1]	DEV[3] (%)	CV[2] (%)	MEAN NO_3^- MG/KG DRY WEIGHT[1]	DEV[3] (%)	CV[2] (%)
0	45.33	0.361	49.92	10.1	0.993	50.80	12.1	0.259
7	47.29	0.156	52.52	11.1	0.829	53.23	12.6	0.189
14	48.47	0.233	52.79	8.9	0.820	53.48	10.3	0.194
28	53.73	0.819	57.50	7.0	0.625	71.22	32.6	0.371
42	73.01	0.690	75.90	4.0	0.119	86.75	18.8	1.583

[1]: Nitrate content [mg of nitrate content/kg dry weight soil] Mean of 3 replicates
[2]: Co-efficient variation = SD / mean value * 100
[3]: Deviation from control = [(Treatment-Control) / Control] x 100
(Negative value =% inhibition, positive value =% stimulation)

Table 3: Metsulfuron Methyl - Rate of Nitrate Formation

INTERVAL[2] SAMPLING DAYS	MEAN MG OF NITRATE/KG SOIL DRY WEIGHT PER DAY[1]							
	CONTROL		0.02 µL METSULFURON METHYL/KG SOIL DRY WEIGHT			0.2 µL METSULFURON METHYL/KG SOIL DRY WEIGHT		
	MG/DAY	CV[3] (%)	MG/DAY	DEV[4] (%)	CV[3] (%)	MG/DAY	DEV[4] (%)	CV[3] (%)
0 – 7	0.280	6.107	0.371	32.6	11.103	0.348	24.4	3.640
7 – 14	0.169	3.316	0.039	-76.7	12.454	0.035	-79.3	5.080
14 – 28	0.380	6.213	0.337	-11.3	8.534	1.267	233.9	1.309
28- 42	1.378	4.622	1.314	-4.6	2.178	1.109	-19.5	8.142

[1]: Calculated from the mean values of NO_3^- content between the sampling date and previous sampling day
[2]: Time interval from test start (day 0) until measurement
[3]: Co-efficient variation = SD / mean value * 100
[4]: Deviation from control = [(Treatment-Control) / Control] x 100
(Negative value =% inhibition, positive value =% stimulation)

NITRATE VALUES

Table 4: Limit of Quantification For Nitrate Content (mg/kg soil dry weight)

LOD OF INSTRUMENT = 6.2 MG/KG					
SOIL TYPE	**LOWEST VALUE OF NITRATE CONTENT (MG/KG SOIL DRY WEIGHT)**				
	DAY 0	**DAY 7**	**DAY 14**	**DAY 28**	**DAY 42**
Loamy sand soil	33.44	34.02	35.47	35.84	36.20

Table 5: Metsulfuron methyl - Soil Respiration Rates (Mean Values)

DAY	**CONTROL**			**0.02 µL METSULFURON METHYL /KG SOIL DRY WEIGHT**				**0.2 µL METSULFURON METHYL/KG SOIL DRY WEIGHT**			
	RESPIR[1]	**SD[2]**	**CV[3] (%)**	**RESPIR[1]**	**SD[2]**	**DEV[4] (%)**	**CV[3] (%)**	**RESPIR[1]**	**SD[2]**	**DEV[4] (%)**	**CV[3] (%)**
0	44.80	4.34	9.692	47.24	0.39	5.5	0.830	50.87	0.34	13.5	0.674
7	49.02	0.20	0.416	52.43	0.21	6.9	0.405	54.97	0.45	12.1	0.810
14	56.70	0.09	0.165	61.71	0.29	8.8	0.471	67.31	0.42	18.7	0.629
28	58.70	0.32	0.541	60.20	0.06	2.6	0.095	62.60	0.19	6.6	0.308

[1]: Soil respiration [mg CO_2/hr/kg dry weight], mean of 3 replicates
[2]: Standard deviation
[3]: Co-efficient variation = SD / mean value * 100
[4]: Deviation from control = [(Treatment-Control) / Control] x 100
(Negative value =% inhibition, positive value =% stimulation)

Figure 1: Soil Respiration Curve – 0 Day

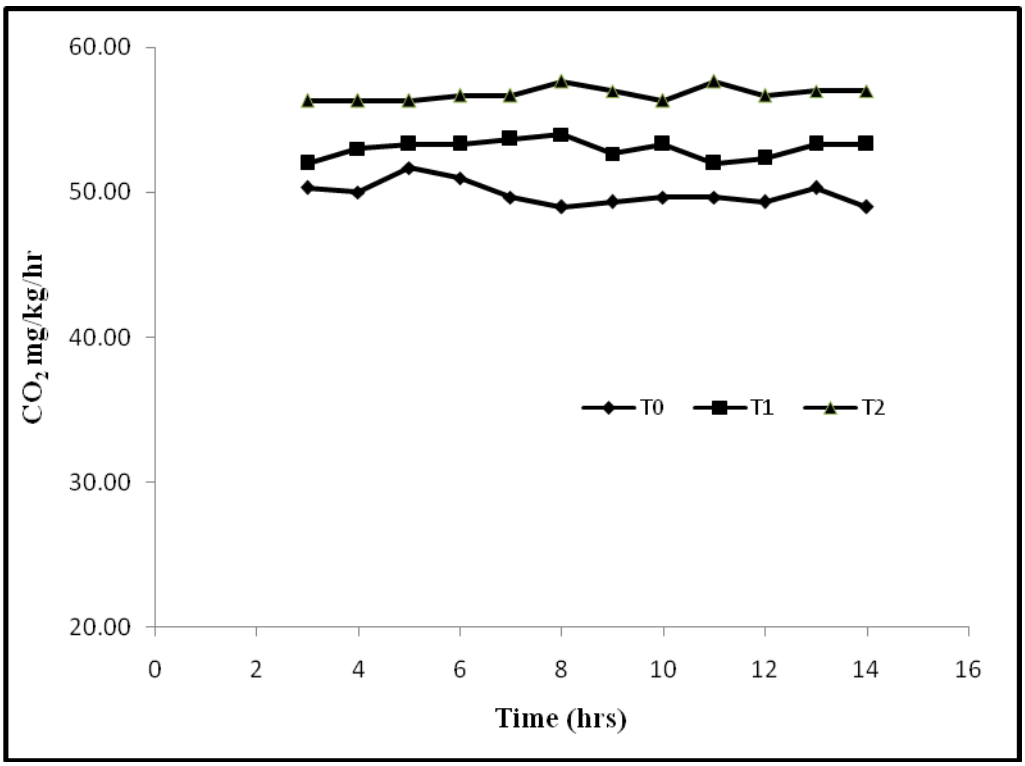

Figure 2 : Soil Respiration Curve –Day 7

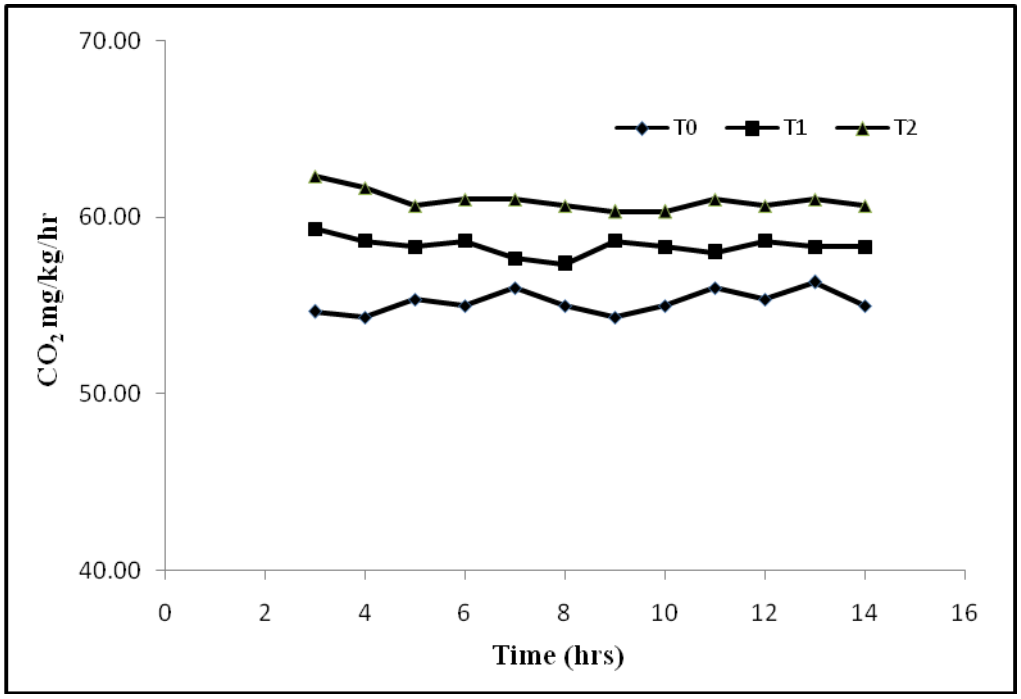

Figure 3 : Soil Respiration Curve – Day 14

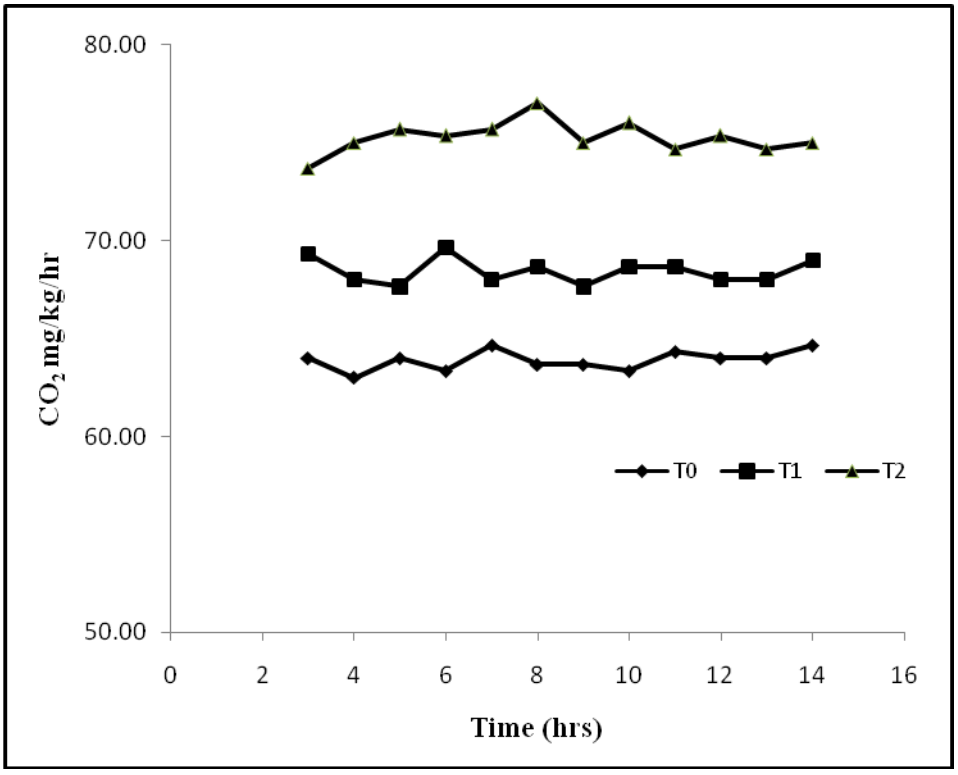

Figure 4: Soil Respiration Curve – Day 28