# Influence of propanil and glyphosate on soil microbial activity

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#### **Abstract**

Potential environmental pollutants such as herbicides, may affect soil fauna and flora and microbial activity, depending upon their quantity and frequency of application. Therefore, the current study was undertaken to assess the effect of two herbicides, propanil and glyphosate, on soil microbial activity, as measured by carbon mineralization.

Propanil was added at the rate of 0.0224 (recommended rate), 0.224 and 2.24  $\mu$ g/g soil and the corresponding rates of glyphosate were 0.3546 (recommended rate), 3.546, and 35.46  $\mu$ g/g soil. A Completely Randomized Design (CRD) was used for the experiment with four replicates. Carbon mineralization was determined at 1, 3, 5, 7, 10, 14, 21, and 35 days after propanil and glyphosate application and a further reading was taken at 56 days after glyphosate application, considering their half life.

Carbon mineralization decreased significantly ( $P \le 0.05$ ) by both herbicides during the early stages of incubation period. The initial reductions were higher (81% and 47% for propanil and glyphosate at the rate of 2.24 and 35.46 µg/g soil respectively) with the increasing concentrations. Carbon mineralization was increased after 5 days (propanil) and 10 days (glyphosate). The reduction of carbon mineralization was higher in propanil compared to glyphosate treatments, indicating that propanil was more toxic than glyphosate. However, no significant changes occurred, for both herbicides at recommended rates (0.0224 and 0.3546 µg/g soil for propanil and glyphosate respectively). Therefore, recommended application rate of both propanil and glyphosate to red yellow podzolic soils could be recommended, without affecting the microbial activity.

Key words: Carbon mineralization, red yellow podzolic soils

### Introduction

The intensive use of synthetic herbicides has raised increasing concerns mainly due to their contribution to pollution of the soil environment. The widespread use of herbicides over several dacades has led to a decrease in agricultural productivity of soils at an alarming rate. Therefore, with regard to sustainable use of soils for agricultural crops, it is important to maintain their fertility and productivity. Many herbicides have been found to have no detectable effect on soil microorganisms at the recommended application rates (Anderson, 1993). However, in Sri Lanka, little information is available on the effect of herbicides on the productivity of agricultural soils. Generally, farmers decisions on the use of herbicides are made mainly on their own experience.

It is vitally important to study the effects of herbicides on soil microbial activity under Sri Lankan conditions. Therefore, the objective of this study was to assess the effect of two herbicides, namely propanil and glyphosate on soil microbial activity as measured by carbon mineralization. These two herbicides were selected for the study, considering their widespread usage in Sri Lanka for controlling a variety of annuals, biannual and perennial weeds such as sedges, broad-leaved weeds and woody shrubs.

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#### Materials and Methods

# Soil and soil sampling

The soil used in this study belonged to red yellow podzolic great soil group and classified as Hapludults, according to the USDA soil taxonomy (Mapa et al., 1999). Soil samples were drawn randomly from several selected locations at the research farm of the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya. Random samples were taken from 0 – 15 cm depth using a soil auger and then mixed well to make a composite sample, to be used for the incubation study. Soil placed in glass bottles were pre - incubated for a week at 60 % water holding capacity prior to the application of herbicides.

## Propanil treatment

Propanil (3.4 Dichloropropionamide/3.4.DPA – 360g active ingredient/L) was added to 100g of soil in the form of solution at concentrations of 2.24  $\mu$ g/ml, 22.4  $\mu$ g/ml and 224  $\mu$ g/ml equivalent to 0.0224  $\mu$ g/g soil, 0.224  $\mu$ g/g soil and 2.24  $\mu$ g/g soil, corresponding to field rate, 10 times, and 100 times of the recommended field rate. The conversion of the field rate application to  $\mu$ g of propanil per g soil was calculated assuming an even distribution of propanil in the 0 – 15 cm layer and a bulk density of 1.5 g / cm<sup>-3</sup>.

### Glyphosate treatment

Glyphosate (phosphomethyl glycine/roundup – 360g active ingredient/L) was added to 100g of soil in the form of solution at concentrations of 35.46, 354.6 and 3546 $\mu$ g/ml equivalent to 0.3546, 3.546 and 35.46  $\mu$ g/g soil, corresponding to field rate, 10 times, and 100 times of the recommended field rate. The conversion of the field rate application to  $\mu$ g of glyphosate per g soil was calculated assuming an even distribution of glyphosate in the 2 mm soil layer and a bulk density of 1.5 g/cm<sup>3</sup>.

#### Carbon mineralization

Control and herbicide treated soil samples were placed in gas-tight glass containers along with a vial containing 10ml of 1M NaOH to trap CO<sub>2</sub> and a vial of water to maintain humidity. Soil was incubated at room temperature in the dark (25 °C) with NaOH traps replaced after 1, 3, 5, 7, 10, 14, 21, and 35 days in propanil treatment and after 1, 3, 5, 7, 10, 14, 21, 35 and 56 days in glyphosate treatment. Unreacted alkali in the NaOH traps was titrated with 0.5 M HCl to determine CO<sub>2</sub>-C released from the soil (Anderson, 1982).

### Statistical analysis

Data were subjected to analysis of variance procedure (ANOVA) using SAS software (SAS Institute, 1988). Least significant difference at P d" 0.05 was used to separate the means.

### **Results and Discussion**

# Effect of different rates of propanil on carbon mineralization

Results showed that carbon mineralization was not affected significantly (P e" 0.05) by propanil at the recommended field application rate (0.0224  $\mu$ g/g soil). However, when the application rate was increased to 10X and 100X, carbon mineralization was significantly (P  $\leq$  0.05) lower, compared to the control (Table 1). This trend of carbon mineralization continued during the entire incubation period for all treatments (Fig. 1).

Table 1. Effect of different rates of propanil on carbon (mg/kg soil) mineralization

Incuba. period (days)	Co	C1	C2	C3	LSD (P d" 0.05)*
1	99 ° (4.5) y	95°(1.8)	80 b (1.7)	31°(1.0)	4.0
3	86° (2.7)	83 a (3.1)	57 <sup>b</sup> (1.1)	21°(0.6)	3.3
5	74 a (4.2)	70°(2.5)	44 b (0.7)	14°(0.7)	3.9
7	97°(1.8)	95°(1.8)	69 <sup>b</sup> (1.1)	21°(1.1)	2.3
10	101°(0.8)	100°(1.0)	87 <sup>b</sup> (0.6)	47°(2.1)	1.9
14	114*(2.3)	112°(0.6)	97 <sup>b</sup> (1.5)	82°(0.6)	2.1
21	114°(2.3)	112°(1.0)	97 <sup>b</sup> (1.5)	84°(0.6)	2.3
35	114° (1.5)	113 a (0.6)	97 <sup>b</sup> (0.6)	83°(0.7)	1.4

<sup>\*</sup>Means followed by the same letter (in each row) are not significantly different at  $P \le 0.05$ .

Co – Control (Without herbicide), C1 – 0.0224  $\mu$ g/g soil, C2 - 0.224  $\mu$ g/g soil, C3 – 22.4  $\mu$ g/g soil

It was observed that, carbon mineralization decreased at the initial stage (up to 5 days) of the incubation period. After this reduction, carbon mineralization increased up to 14 days and then remained constant. It should be noted that C mineralization for 10X and 100X treatments always remained significantly ( $P \le 0.05$ ) lower than the control. Results further revealed that C mineralization decreased with increasing rates of propanil application (Table 1).

According to the results when propanil was applied at the recommended field rate (0.0224  $\mu$ g/g soil), carbon mineralization was reduced in the range of 1.0 to nearly 5.5 % compared to the control during the 35 days incubation period (Fig. 2). Carbon mineralization reduction observed for the other two rates were higher. The ranges of reduction observed for 10X and 100X were 14 to 40 % and 27 to 81 % respectively (Fig. 2).

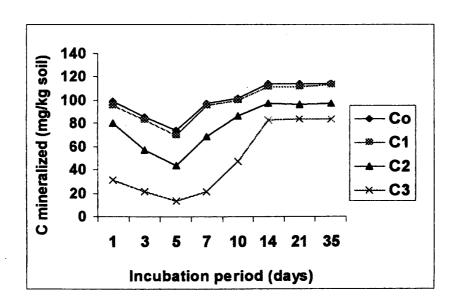


Fig. 1: Effect of different rates of propanil on carbon mineralization

YValue in parenthesis is the standard deviation of means.

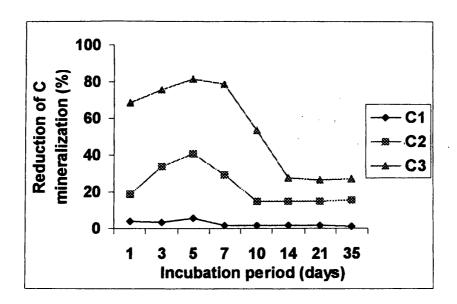


Fig. 2: Effect of different rates of propanil on reduction of carbon mineralization

Co – Control (Without herbicide), C1 – 0.0224  $\mu$ g/g soil, C2 - 0.224  $\mu$ g/g soil, C3 –2.24  $\mu$ g/g soil

At the initial stage of incubation period, it was observed that reductions of mineralized carbon were significant  $(P \le 0.05)$ , compared to the control. The highest reduction (81%) was observed at the application rate of 100X followed by 40% and 5.5% for the application rates of 10X and recommended rate, respectively.

# Effect of different rates of glyphosate on carbon mineralization

Compared to the control, carbon mineralization was not affected significantly ( $P \le 0.05$ ) when the recommended field rate of glyphosate (0.3546 µg/g soil) was applied to the soil (Table 2). However, when the application rate was increased from the recommended field rate to 10X and 100X, significant ( $P \le 0.05$ ) reductions of carbon mineralization were observed, compared to the control and the field rate. It was also observed from the analysis of the data that, during the first 7 days of the incubation, carbon mineralization reduction by the treatment 100X was significantly (P d'' 0.05) higher than that of treatment 10X compared to rest of the incubation period (Fig. 3).

Table 2. Effect of different rates of glyphosate on carbon (mg/kg soil) mineralization

Incuba. period	Со	Cl	Œ	C3	LSD	
(days)				•	$(P \le 0.05)^{x}$	
1	110°(20.6) y	108*(18.6)	88 <sup>b</sup> (0.0)	66°(5.7)	20.2	
3	100 ° (5.7)	98 • (6.6)	81 <sup>b</sup> (0.0)	67° (5.7)	7.8	
5	87*(10.4)	87° (5.4)	75 <sup>b</sup> (0.0)	63° (6.3)	10.3	
7	91*(5.8)	88*(9.5)	65 <sup>b</sup> (9.5)	51° (5.8)	12.1	
10	85*(5.8)	82*(11.2)	58 <sup>b</sup> (11.1)	44 <sup>b</sup> (9.5)	15.1	
14	88°(14.7)	86*(25.2)	71 ab (14.7)	54 <sup>b</sup> (5.8)	25.2	
21	96*(9.8)	90 ab (15.5)	78 ab (20.8)	63 <sup>b</sup> (6.0)	21.8	
35	102*(12.0)	96 • (0.0)	84 <sup>bc</sup> (9.8)	75° (6.0)	12.8	
<b>5</b> 6	105*(6.0)	102*(6.9)	87 ab (22.7)	78 <sup>b</sup> (6.9)	19.6	

<sup>\*</sup>Means followed by the same letter (in each row) are not significantly different at  $(P \le 0.05)$ .

YValue in parenthesis is the standard deviation of means.

Co - Control (Without herbicide),  $C1 - 0.3546 \mu g/g$  soil,  $C2 - 3.546 \mu g/g$  soil,  $C3 - 35.46 \mu g/g$  soil

The results revealed that when glyphosate was added at the field rate (0.3546  $\mu$ g/g soil), it reduced the carbon mineralization nearly in the range of 0.01 to 6% compared to the control, during the incubation period (Fig. 4). A marked decline in carbon mineralization was also observed at 10X treatment, ranging from 14 to 31%, compared to the control. However, the reductions were in the range of 26 to 47% compared to the control for the highest rate (100X) applied to the soil.

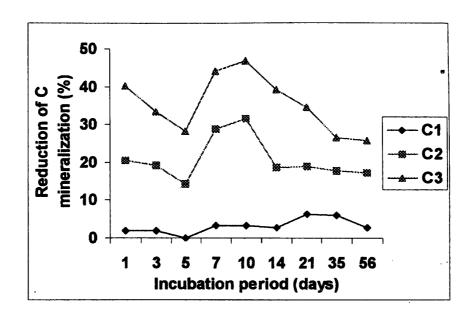


Fig. 3: Effect of different rates of glyphosate on carbon mineralization

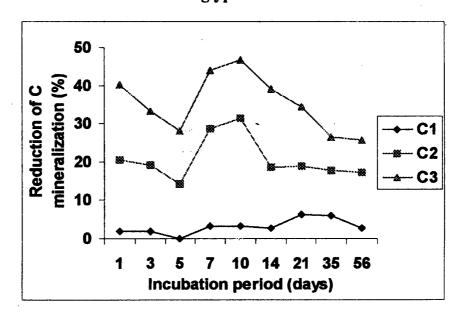


Fig. 4: Effect of different rates of glyphosate on reduction of carbon mineralization

Co - Control (Without herbicide)

 $C1 - 0.3546 \mu g/g soil$ 

 $C2 - 3.546 \mu g/g soil$ 

 $C3 - 35.46 \mu g/g soil$ 

Significant decreases in carbon mineralization, compared to the control, were observed during the initial period (10 days) of the incubation. The maximum decrease (47 %) in the carbon mineralization was observed in the soil, applied with the highest glyphosate (100X) concentration. These decreases did not exceed 31 % and 5.9 % for the concentrations of 10X and recommended rate respectively.

Results showed that all propanil and glyphosate treatments caused a decrease in soil microbial activity initially. This may be explained by the slight inhibitory effects due to the surfactant (fatty acids) that soil microbes may or may not completely overcome. The influence of the surfactant (chemical compounds that facilitate the movement of the active herbicide ingredient in to the plant) may be partially responsible for the initial carbon mineralization reduction, possibly from surfactant lysis of microbial cells or from surfactant herbicide interaction. Haney et al., (2000) also observed that C mineralization lag at the higher rate of application due to surfactant.

This inhibition was further confirmed by the greater reduction of the carbon mineralization in the soil samples treated with the higher rate of propanil and glyphosate such as 100X compared with the lower rates such as 10X and recommended rate.

Similar results were reported in a separate experiment with product grade glyphosate and analytical grade glyphosate, where it was apparent that surfactant suppressed C mineralization at the higher rate, initially in the product grade, compared with the higher rate of analytical grade (234  $\mu$ g/g soil) material, that contained no surfactant (Haney *et al.*, 2000).

The carbon flush after 5 days (propanil) and 10 days (glyphosate) may be due to these herbicides being readily or directly utilized by soil microbes after the initial adaptation period. Forlani *et al.*, (1999) also found that carbon flush after 7 days of incubation and suggested that this was due to herbicide utilization by soil microbes or made other resources available. Studies of Tu (1981a, 1981b) however suggested that at normal application rates and doubled rates, any changes in various measures of microbial activity (when they occur) are temporary and the soil recovers quickly.

Considering the fact that the highest reduction of C mineralization (81 %) was observed in the soil treated with propanil at the rate of 100X, it is clear that propanil was more toxic than glyphosate where the reduction was just 47 % at the application rate of 100X. As far as toxicity is concerned, more evidence would be given by other application rates also saying propanil is more toxic than glyphosate in terms of C mineralization of soil.

After pre-incubated for 7 days C mineralization reached a peak at the end of the 7<sup>th</sup> day, both in control and field application rate and then gradually decreased. Dry soil samples used for the experiment were moistened just before the pre-incubation, and thus C mineralization was enhanced during this period. Therefore, no significant difference could be found between the control and field application rate in terms of C mineralization.

# **Conclusions**

Carbon mineralization was reduced by both herbicides ( $P \le 0.05$ ) during the early stages of the incubation. These initial reductions increased with increasing concentrations. Carbon mineralization was increased after 5 days (propanil) and after 10 days (glyphosate).

Carbon mineralization reductions were higher in propanil compared to glyphosate treatments, suggesting that propanil would be more toxic than glyphosate. However no significant changes were observed, when used at field rates. Further research is required on the retention of these herbicides in soil and effect of repeated application on soil microbial processes.

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