RESPONCES OF THE SOIL MICROBIAL BIOMASS CARBON TO THE HERBICIDES PROPANIL AND GLYPHOSATE

B.C. Walpola[1], S.D. Wanniarachchi[1] and J.A. Liyanage[2]

ABSTRACT

The effect of propanil and glyphosate on Soil Microbial Biomass Carbon (SMBC) was assessed in the study. The experiment comprised three propanil (i.e. 0.0224, 0.224 and 2.24 μg/g soil) and glyphosate (i.e. 0.3546, 3.546, and 35.46 μg/g soil) treatments. A Completely Randomized Design (CRD) was used for the experiment with four replicates. Determination of SMBC was carried out 1, 3, 5, 7, 10, 14, 21, and 35 days after propanil and glyphosate application and an additional reading of 56 days after glyphosate application was also taken.

Results showed that SMBC decreased at all the propanil treatments during the first 5 days of incubation, followed by a gradual recovery. For glyphosate this reduction was extended for further 5 days. During the period, both herbicides reduced the microbial biomass in a dose-dependent manner. Toxicity of the herbicides could be the key contributor for the initial biomass reduction as the microorganisms often take a considerable period of time for the adaptation. As it proved that SMBC was increased continuously after the initial shock, recommended field rate application of both propanil and glyphosate on Red Yellow Podzolic soils sounds no adverse effects on soil micro-fauna.

Key words: *Soil microbial biomass carbon, Red Yellow Podzolic, Propanil, Glyphosate*

INTRODUCTION

The intensive use of synthetic herbicides has raised increasing concern as it could lead to the massive pollution of the environment including the soil systems. The widespread use of herbicides over the past several decades has led to decrease the agricultural productivity of soils at an alarming rate. It is therefore of prime important to maintain the fertility of agricultural soils in order to make sure a sustainable production.

Soil microbial biomass is of fundamental importance in the biological cycles of almost all major plant nutrients (Robert and Chenu, 1992). Maintenance of soil fertility depends on the size and activity of microbial biomass of the soil (Alexander, 1977). Although it represents only a small fraction of the total C, N, P and S of the soil, it has a relatively rapid turnover (Marumoto *et al.*, 1982). The biomass has a multiple role in soil, affecting the decomposition and turnover of organic matter, nutrient immobilization and cycling, root physiology and soil structure (Vaughan and Malcolm, 1985). Microbial biomass in soil also plays a key role in biodegradation of pesticides and other toxic compounds added to soil via agricultural and industrial practices.

Many herbicides have been found to have no detectable effects on soil microorganisms at field-relevant application rates (Anderson, 1993). Some herbicides, despite at excessive application rates could be degraded by

[1]*Department of Soil Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka*
[2]*Department of Chemistry, Faculty of Science, University of Kelaniya, Sri Lanka*

soil microorganisms without adversely affecting them (Haney *et al.*, 2000). However, only a little information is available on the effect of herbicides on agricultural soils in Sri Lanka, where farmers depend widely on their own experience when decisions on using pesticides are made. Generally pesticides are being used at excessive rates by most of the farmers. Therefore, it is of primly important in assessing the effect of herbicides on soil microbial biomass and activity under Sri Lankan conditions. Therefore, the objective of the present study was to determine the effect of propanil and glyphosate on soil microbial biomass carbon (SMBC).

MATERIALS AND METHODS

Climate and soil

The present investigation was carried out at the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka, from October 2002 to December 2003. According to the agro-ecological classification (Panabokke, 1980), the region of investigation comes under agro-ecological region WL_2 (low country wet zone). The soil used in this study belongs to Red Yellow Podzolic great soil group and is classified as Hapludults according to the USDA soil taxonomy (Mapa *et al.*, 1999). The climate of the area is tropical monsoonal (Panabokke, 1980), with a warm wet period (April to June) and a relatively dry period (January to March). The area receives an annual rainfall of around 2500 mm. The distribution of rain is bi-model. Annual mean air temperature of the area is 22 - 30 ^{0}C with the relative humidity of 80 %.

Soil sampling

Soil samples were drawn randomly from several selected locations at the research farm of the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka. After removing the surface litter, soil sampling was performed from 0 – 15 cm depth using an auger. They were then mixed thoroughly in order to make a composite sample. Samples placed in glass bottles were moistened until they reach to the field capacity and pre-incubated for a week before being treated with herbicides.

Treatments

Here we selected . two herbicides; propanil and glyphosate considering their widely usage in Sri Lanka for controlling a variety of annuals, biannual and perennial weeds including sedges, broad-leaved weeds and woody shrubs.

Propanil treatment

Commercially available propanil (3.4 Dichloropropionamide/3.4.DPA – 360 g active ingredient/L) was diluted in methanol to prepare the initial concentrations (i.e. 2.24, 22.4 and 224 µg/ml). The experimental concentrations [i.e. corresponding to field rate (0.0224 µg/g), 10 times of field rate (0.224 µg/g) and 100 times of field rate (2.24 µg/g)] were prepared by properly diluting the initial solutions. The conversion of the field rate application to µg of propanil per g soil was calculated assuming an even distribution of the propanil herbicide in the 0 – 15 cm layer and a bulk density of 1.5 g / cm^3 (Perucci and Scarponi 1996).

Glyphosate treatment

Three initial concentrations (i.e. 35.46, 354.6 and 3546 µg/ml) were prepared using commercially available glyphosate (phosphomethyl glycine/roundup – 360g active ingredient/L) and methanol. The concentrations required for the experiment [i.e. corresponding to field rate (0.3546 µg/g), 10 times of field rate (3.546 µg/g) and 100 times of field rate (35.46 µg/g)] were prepared using initial glyphosate solutions. The conversion of the field rate application to µg of glyphosate per g soil was calculated as described in propanil tteatments. A shallow 2 mm soil interaction depth is due to glyphosate's high adsorptivity and low leachability (Haney et al., 2000).

Herbicide treated soil samples along with the controls (free from herbicides) were then incubated in the dark at room temperature (25 ±1°C). Constant moisture content of the soil was maintained by daily monitoring and adding water when necessary. Soil microbial biomass was determined 1, 3, 5, 7, 10, 14, 21 and 35 days after propanil treatment and 1, 3, 5, 7, 10, 14, 21,3 5 and 56 days after glyphosate treatment considering their half lives. Measurements of SMBC were made using a modified fumigation-extraction procedure of Voroney *et al.* (1993).

Statistical analysis

Data generated were subjected to analysis of variance (ANOVA) for a Completely Randomized Design (CRD) with four replicates using SAS software (SAS Institute, 1988). Least significant difference at $P \leq 0.05$ was used for mean comparison.

RESULTS AND DISCUSSION

Effect of propanil on soil microbial biomass carbon

At the end of day-one, no significant differences ($P \leq 0.05$) in SMBC content could be found between soils treated with the recommended field rate (0.0224 µg/g soil) and the control. However, at 0.224 and 2.24 µg/g soil (respectively 10 and 100 times of the field rate), the SMBC contents were significantly lower ($P \leq 0.05$) than that of the control and the field rate. A similar trend of reduction at 0.224 and 2.24 µg/g soil was observed until day-five of the incubation. At the end of the seventh-day, the only propanil treatment, which significantly affected on SMBC was 2.24 µg/g soil. However, SMBC resulted in the treatments of 2.24 and 0.224 µg/g soil were not significantly different ($P \leq 0.05$) from each other.

Among the treatments, the reductions of SMBC were dominated by the treatment of 2.24 µg/g soil throughout the 35-day incubation period. Furthermore, the SMBC of the soil treated at 2.24 and 0.224 µg/g soil, remained significantly low compared to the control during the entire period of the incubation, whereas, the figures for the treatments of 0.0224 and 0.224 µg/g soil were proved to be not significantly different from the control. (Figure 01).

Results further revealed that propanil at the recommended field rate (0.0224 µg/g soil), proved to reduce the SMBC contents by 0.5-10 % during the course of experiment. The respective SMBC reductions at 0.224 and 2.24 µg/g soil were 10.5 - 46 % and 12 - 76 % indicated the dose-dependent manner of microbial response to the herbicide.

Being pre-incubated for 7 days, SMBC reached to the peak at the end of the 7th 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 SMBC was enhanced during this period. Therefore, no significant difference can be found between control and field application rate.

The toxicity of the herbicide would be the apparent contributor to the reduction of microbial biomass. The gradual increased in SMBC as incubation progressed imply that a considerable period of time is needed for the adaptation of microorganisms to the applied propanil. During the adaptation period, some sort of reductions in microbial biomass carbon could simply be expected as showed during the first five days of incubation (Figure 01).

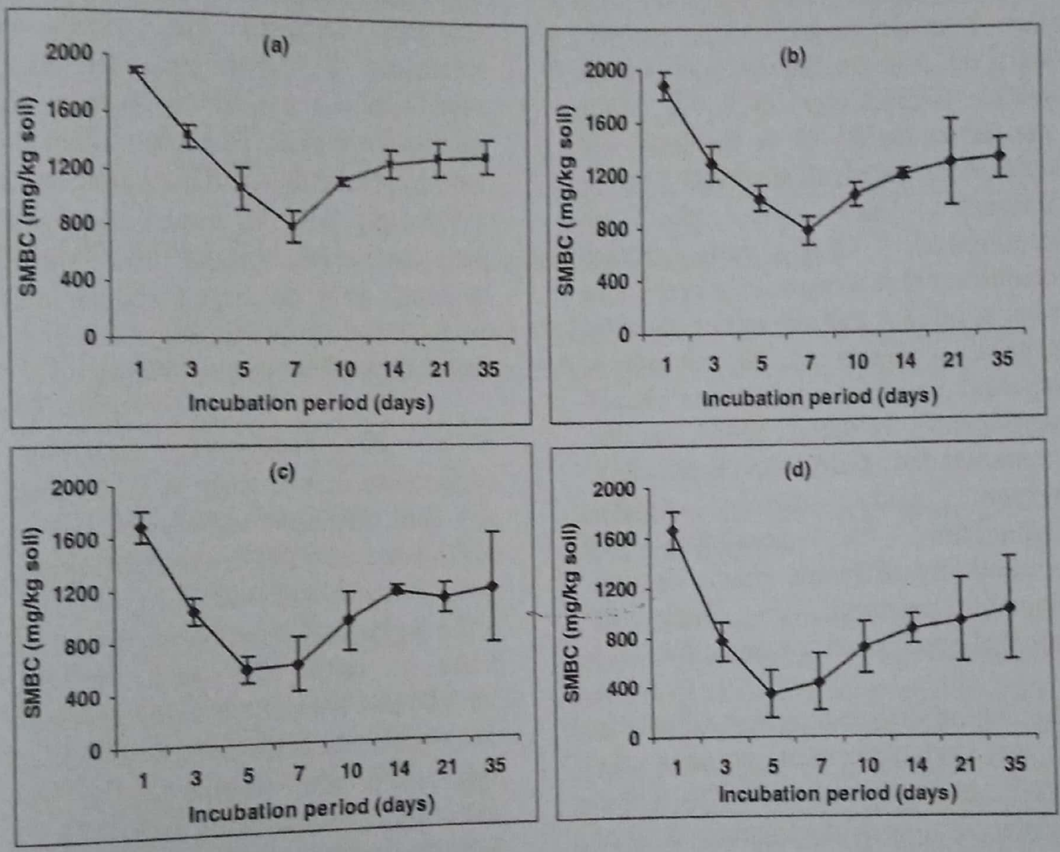

Figure 01: Effect of propanil on soil microbial biomass carbon

(a) Control (without herbicide), (b) (c) and (d) represent 0.0224, 0.224 and 2.24 µg/g soil of propanil treatments respectively. Values given here are the means (n = 4) ± standard deviation.

The involvement of the toxicity was further confirmed by the greater reductions in SMBC contents reported at higher propanil concentrations such as 2.24 μg/g soil, compared to those of lower rates such as 0.224 and 0.0224 μg/g soil (field rate).

Present findings are in agreement with Perucci and Scarponi (1996) who observed reduced microbial biomass in response to rimsulfuron herbicide application in a clay loam soil at the rate of 20, 200 and 2000 μg/kg soil corresponding to field rate, 10 times of the field rate and 100 times of the field rate. Vischetti *et. al.* (2002) was also reported that benfluralin reduced the initial SMBC by 64.7 % while imazamox by 25 % at the field rate application (equivalent to 100 g/ha for imazamox and 500 g/ha for benfluralin). They have further mentioned that though some herbicides such as MCPA and simazine proved no detectable effects on the microflora, repeated paraquat applications caused significantly lowered SMBC at the recommended field rate application. Yentum and Johnson, (1986) highlighted the possibility of substantially different effects on soil biomass produced by single or repeated applications of pesticides.

The degradation rate and the half-life of the herbicide could explain the significant decreased in SMBC during the early stages of the incubation (first 5 days) reported in the present study. The half-life is simply the time taken for a half of the herbicide applied to the soil to dissipate, and for propanil it generally ranged from 1-3 days under warm, moist conditions (WSSA, 1994; USDA, 1990). A considerable amount of applied propanil could therefore be remained in soil within the first 5 days leading to drastic changes in soil microbial biomass carbon in particular, at higher concentrations. However, the half-life gives only a rough estimation of the persistence of an herbicide as it can vary significantly depending on herbicide, soil characteristics, weather (especially temperature and soil moisture) and the vegetation of the site.

Effect of glyphosate on soil microbial biomass carbon

No significant differences in SMBC contents between the glyphosate treatment of 0.3546 μg/g soil (field rate) and the control throughout the incubation period. However, when the application rates were increased up to 3.546 μg/g soil (10 times) and 35.46 μg/g soil (100 times), the SMBC contents were decreased significantly ($P \leq 0.05$), in particular, during the first 7 days of incubation (Figure 02).

After 10 days of glyphosate application, it was only at 35.46 μg/g soil that displayed significantly ($P \leq 0.05$) lower SMBC content compared to the control, while the rest (35.46 and 3.546 μg/g soil) were not significantly different until the end of the incubation. The results also revealed that glyphosate at the field rate (0.3546 μg/g soil), has contributed to the reduction of SMBC contents by 0.4 - 7 %. The percentage reductions in SMBC were ranged 12 - 27 % and 23 - 43 % respectively for the treatments of 3.546 and 35.46 μg/g soil compared to the control.

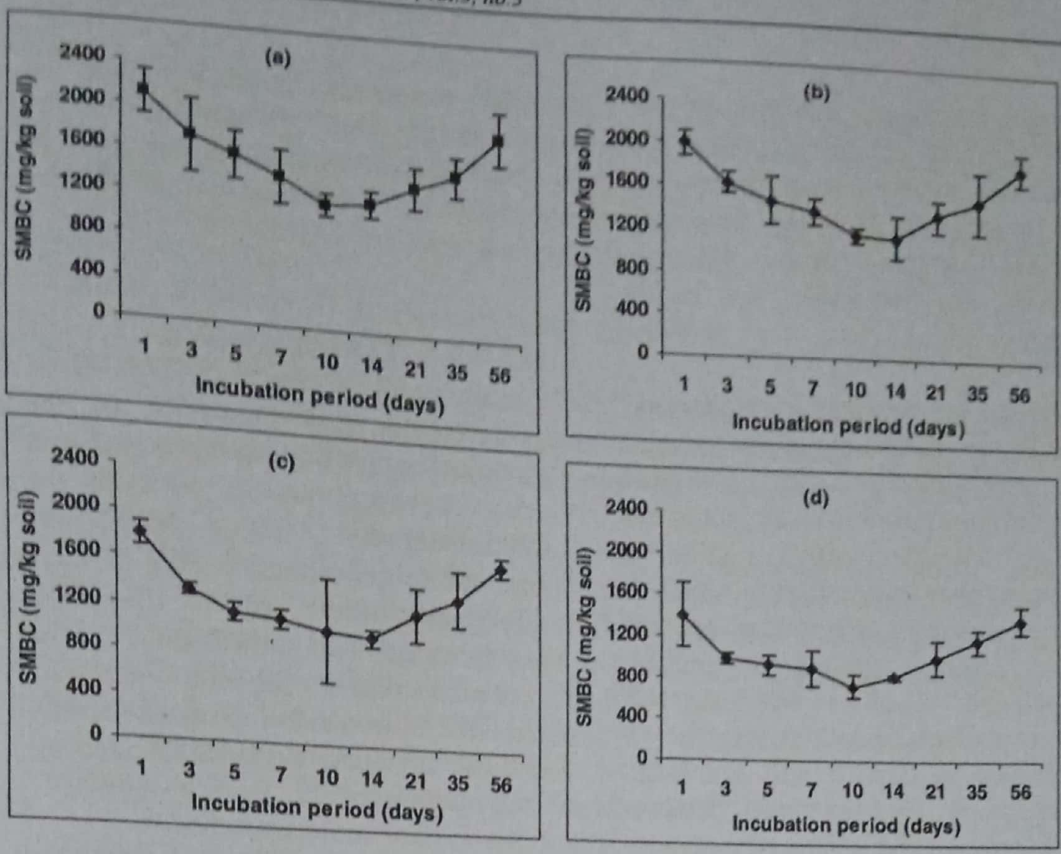

Figure 02: Effect of glyphosate on soil microbial biomass carbon

(a) Control (without herbicide), (b) (c) and (d) represent 0.3546, 3.546 and 35.46 µg/g soil of glyphosate treatments respectively. Values given here are the means (n = 4) ± standard deviation.

It is apparent from the results that all the glyphosate treatments caused reductions in soil microbial biomass contents during the early stage of the incubation, regardless the concentration. This may be explained by the slight inhibitory effects of glyphosate due to the surfactant (fatty acids) that soil microbes may or may not completely over come. Average concentrations of surface applied herbicides to soil may be as low as 1- 4 mg/Kg (Moorman, 1989). These concentrations however are usually calculated based on a 15 cm furrow slice soil depth. Concentrations based on 15 cm soil depth could sometimes be misleading, because soil penetration of glyphosate may be only a few millimeters of its high adsorptivity (Sprankle *et al.*, 1975).

Glyphosate is commonly used in minimum and zero tillage systems. According to studies conducted in field and laboratory conditions little or no ecological effect of glyphosate have been observed, when used at field rates (Carlisle and Trevors, 1988). Carlisle and Trevors (1986) showed that glyphosate can either stimulate or inhibit soil organisms depending on the soil and herbicide concentration used. Wardle and Parkinson (1990) and Hart and Brookes (1996) observed no effects of glyphosate on soil microbial biomass or activity. Glyphosate rates used in their studies were probably too low for detecting effects by the methods employed. However, Wardle and Parkinson (1990) reported that glyphosate application at the rate of 200 µg g^{-1} soil, increased microbial biomass carbon from day-three onwards, but that effect was highly transitory.

Discrepancies between laboratory and field studies can be explained partially by unrealistically high herbicide concentrations used in many laboratory studies (Wardle, 1995) and by herbicide chemistry. Most information on the non-target effects of glyphosate comes from agricultural studies. Knowledge of forest soil microorganisms and their response to glyphosate is limited and somewhat contradictory. For example, Stratton and Steward (1992) found no change in microbial biomass in mineral soil and a stimulation of microbial biomass in litter, following the addition of glyphosate to an acidic forest soil. Mycorrhizal infection of red fine (*Pinus resinosa*) was also unaffected by glyphosate application (Chakravarty & Chatarpaul, 1990). Busse *et al.*, (2001) assessed the effect of repeated glyphosate application on soil microbial communities from poderosa pine (*Pinus ponderosa*) plantations. They found that long term repeated field rate application of glyphosate had minimal affect on seasonal microbial communities in ponderosa pine plantations. As it is generally believed that pesticides are being used at excessive rates by most of the Sri Lankan farmers, the present findings initiate an insight into what we felt lack.

CONCLUSIONS

During the first five days of the incubation, SMBC was decreased in a dose-dependent manner by both the herbicides ($P \leq 0.05$). SMBC was proved to be increased after 5 days (propanil) and after 10 days (glyphosate) of incubation. The possible reason for the increased SMBC as incubation progressed may be due to utilization of herbicides by soil microbes.

The reductions of SMBC were considerably higher in propanil compared to glyphosate for all concentrations tested indicating the greater toxicity of propanil over glyphosate. However, at field rates (0.0224 µg/g soil for propanil and 0.3546 µg/g soil for glyphosate), no significant differences could be observed between two herbicides.

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