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Article

The Effects of Nicosulfuron and Glyphosate on Microbial Activity of Different Soils

Efeitos do Nicosulfuron e do Glifosato na Atividade Microbiana em Solos Diferentes

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ABSTRACT - The effects of the nicosulfuron and glyphosate herbicides on microbial activity in two soils with different physical and chemical properties (loam and sand) were investigated. Nicosulfuron was applied at the rates of 0.3, 0.6, 3.0 and 30.0 mg kg^{-1} soil and glyphosate at 32.6, 65.2, 326.0 and 3260.0 mg kg^{-1} soil in the laboratory. Changes in dehydrogenase and urease activity, as well as in microbial biomass carbon, were examined. Samples for the analysis were collected at 3, 7, 14, 30 and 45 days after herbicide application. The results showed that the effects of nicosulfuron and glyphosate depended on treatment rate, duration of activity, test parameters and soil types. In general, application of the herbicides significantly increased the activity of dehydrogenase and urease. Nicosulfuron had a stimulating activity on microbial biomass carbon in loam, while both herbicides demonstrated negative effects on the parameter in the sandy soil.

Keywords: herbicides, loam; sand, dehydrogenase, urease, biomass carbon.

RESUMO - Foram investigados os efeitos dos herbicidas nicosulfuron e glifosato na atividade microbiana em dois solos com diferentes propriedades físicas e químicas (argiloso e arenoso). O nicosulfuron foi aplicado em laboratório nas doses de 0,3, 0,6, 3,0 e 30,0 mg kg^{-1} solo, e o glifosato, a 32,6, 65,2, 326,0 e 3.260,0 mg kg^{-1} solo. Foram examinadas alterações na atividade da desidrogenase e da urease, bem como no carbono de biomassa microbiana. As amostras para análise foram recolhidas aos 3, 7, 14, 30 e 45 dias após a aplicação do herbicida. Os resultados mostraram que os efeitos do nicosulfuron e do glifosato dependeram da dose de tratamento, da duração da atividade, dos parâmetros do teste e dos tipos de solo. Em geral, a aplicação dos herbicidas resultou em aumento significativo da atividade da desidrogenase e da urease. O nicosulfuron teve uma atividade estimulante sobre o carbono da biomassa microbiana no solo argiloso, e ambos os herbicidas demonstraram efeitos negativos quanto a esse parâmetro no solo arenoso.

Palavras-chave: herbicidas, argila, areia, desidrogenase, urease, carbono da biomassa.

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INTRODUCTION

Soil is essentially a non-renewable resource, which performs many functions and vitally supports human activities and survival of ecosystems. A large number of physical, chemical, microbiological and biochemical properties influence soil processes, and their spatial and temporal variations contribute

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to the concept of soil quality (Gimsing et al., 2004). Herbicide application is an integral and economically essential component of modern agricultural practices. Despite the beneficial effects of herbicides on agricultural productivity, the exposure of soil to potential contaminants, such as herbicides, represents a considerable side-effect of agricultural practices. As a consequence, the interaction between soil microorganisms and biologically active herbicides may influence the quality and fertility of soil (Lone et al., 2014). The impact of herbicides on soil microorganisms and their activity are governed not only by chemical and physical properties of the herbicides themselves but by type of soil, soil properties and prevailing environmental conditions as well**.** Soils contain organic matter and clay particles that control herbicide adsorption and water relations as well as provide different environments for microbial activity (Joergensen and Emmerling, 2006). Soil is a living dynamic system that contains many free enzymes, which are soil quality indicators. They participate in adsorption, oxidation, reduction, hydrolysis and complexation reactions, converting organic substances into other products to maintain a balance in each soil environment (García-Ruiz et al., 2008). Also, microbial biomass is an active component of each soil organic pool, which is responsible for organic matter decomposition that affects soil nutrient content and, consequently, the primary productivity in most biogeochemical processes in terrestrial ecosystems (Kara and Bolat, 2008).

This investigation was conducted to study the effects of nicosulfuron and glyphosate on soil enzymes (dehydrogenase and urease) and microbial biomass carbon in two different types of soil (loamy soil and sandy soil). Nicosulfuron1-(4, 6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridyl sulfonyl) urea) is a sulfonylurea herbicide used for post-emergence treatments in maize crops. This herbicide has a broad and super-high activity against annual gramineous plants, such as broad-leaved and sedge weeds. It has been shown that microbial degradation is one of the most important forms of decomposition of sulfonylurea herbicide residues in the environment. Glyphosate (N-phosphonomethylglycine) is a systemic herbicide commonly used to control a broad spectrum of weeds in crops and on pastures worldwide. This is the most common herbicide today and has long been considered as environmentally safe, as a result of its rapid inactivation in soil, both by degradation and adsorption (Benslama and Boulahrouf, 2013). The effects of herbicides on soil microorganisms can be clarified in studies of functional parameters, such as carbon and nitrogen mineralization, which are governed by enzyme activities. Many studies have shown that enzyme activity and microbial biomass are sensitive enough to detect herbicide impact (Riah, 2014; Das and Day, 2014).

Soil properties that affect the availability and activity of herbicides include soil texture, organic matter level, and pH. These properties also have a significant role in determining microbiological activity in soil. Therefore, the objective of this paper is to assess the effects of different rates of nicosulfuron and glyphosate on certain microbiological variables (dehydrogenase, urease, biomass carbon). In addition, the object is a comparison of the effects of these herbicides on microbial activity in soils with different physical and chemical properties.

MATERIALS AND METHODS

The herbicide nicosulfuron tested in the experiment was the product Motivell, manufactured by BASF (Germany) and its rates of application were: 0.3, 0.6, 3.0 and 30.0 mg kg^{-1} soil. The herbicide glyphosate was the product Roundup, manufactured by Monsanto (USA) and the rates of application were: 32.6, 65.2, 326.0 and 3260.0 mg kg^{-1} soil. The lowest tested concentrations equalled the recommended rates, while the other three were double, 10-fold and 100-fold higher than the recommended rates. The two highest concentrations (10-fold and 100-fold) have been used to assess the potential hazards for microorganisms in the soil during undesirable events, such as spilling of pesticides (in our case, herbicides) in high quantities into the soil, which can occur during the damage of devices used for application, as well as through transport or industrial accidents (Cycon and Piotrowska-Seget, 2015). The laboratory experiment was carried out in two agricultural soils. The loamy soil (Zemun Polje, Belgrade) and the sandy soil (Tavankut, Subotica) chosen for the study had never been treated with pesticides before. Physico-chemical characteristic of the loamy soil were: sand 49.80%, silt 33.40, clay 16.80, total carbon 2.30%, total nitrogen 0.25%, organic matter 3.96% and pH 7.64. The properties of the sandy soil were: sand 91.44%, silt 1.32%, clay 7.24%, total carbon 0.53%, total nitrogen 0.06%, organic matter

0.91% and pH 8.04. According to WRB (IUSS Working Group WRB, 2015) the first type of soil belongs to the Chernozems group while the sandy soil is classified into the group of Arenosols.

Soil samples were collected from the upper layer (0-10 cm), and were carefully dried, sieved to pass a 5 mm mesh, and stored at 4 ^{o}C. Before they were used, the soils were air-dried at room temperature for 24 hours. Each herbicide concentration was pipetted to the surface of 1 kg of soil before homogenization on a rotary stirrer for 30 minutes. After homogenization by mixing, the soil was portioned out in pots. Untreated soil served as control. The experiments were conducted in four replications. The pots were kept in a controlled-environment chamber at 20 ± 2 ^{o}C, 50% air humidity and 12/12 h day/night photoperiod throughtout the experiment. The samples were collected for analysis 3, 7, 14, 30 and 45 days after herbicides application.

Dehydrogenase activity was measured as described by Tabatabai (1982). The soil samples were prepared by incubation with triphenyltetrazolium chloride (TTC) at 37 ^{o}C for 24 h. Triphenylformazan (TPF), which is derived from triphenyltetrazolium chloride (TTC) as a product of enzyme activity, was determined spectrophotometrically. Measurements were performed at the wavelength of 485 nm (Gilford stasar III model 2400), and enzyme activity is presented as µg TPF g^{-1} soil.

Urease activity was measured as described by Tabatabai and Bremner (1972). The method involves determination of ammonium released by urease activity when soil is incubated with tris(hydroxymethyl)aminomethanes (THAM buffer, pH 9.0), 0.2 M urea solutions and toluene at 37 ^{o}C for 2 h. Ammonium release was determined by a rapid procedure involving treatment of the incubated soil samples with 2.5 M KCl containing a urease inhibitor ($AgSO_4$) and steam distillation of an aliquot of the resulting suspension with MgO for 3 minutes. Enzyme activity is presented as µg $NH_4^+ g^{-1}$ soil h^{-1}.

Microbial biomass carbon in herbicide-treated and control soil samples was determined by the fumigation-extraction method (Jenkinson et al., 1979). The samples were fumigated with non-alcoholic chloroform ($CHCl_3$) under moist conditions for 24 h. After incubation, carbon was extracted with a 0.5 M solution of potassium sulphate (K_2SO_4) and its content was determined by titration with 0.0333 M solution of Mohr salt ($(NH_4)_2Fe(SO_4)_2$) in the presence of phenylantranil acid as the indicator. Non-fumigated samples were extracted under the same conditions. Microbial biomass carbon was calculated based on a difference between carbon in the fumigated and non-fumigated samples using the factor 0.45 (Vance et al., 1987). The results are presented in µg Cg^{-1} soil.

Data were statistically processed in Statistica 8.0 software. A three-way analysis of variance was used to compare means of the examined microbial parameters: enzyme activity and microbial biomass carbon. The LSD test was used to compare treatments and assessments of each parameter when differences in F-values were statistically significant.

RESULTS AND DISCUSSION

Dehydrogenase plays a significant role in biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors and it is one of the most sensitive bioindicators of soil fertility (Järvan et al., 2014). In the loamy soil, a statistically significant decrease in dehydrogenase activity was detected for the concentration of 0.6 mg kg^{-1} soil on the 3rd and the 30th days after treatment (Figure 1). However, there was an increase in dehydrogenase activity, which ranged from 5.8 to 24.1%, and it peaked on the 3rd day after treatment with 30.0 mg kg^{-1} soil of nicosulfuron.

Application of this herbicide to the sandy soil also led to a significant increase in dehydrogenase activity (74.6-105.5%) compared to the untreated control of soil sample. A full factorial analysis of variance (Table 1) showed that all of its three factors and their interactions had a highly significant ($p<0.01$) impact on dehydrogenase activity, whereas type of soil had a higher influence.

The effect of time on dehydrogenase activity showed that its activity was at a peak from the 3rd until 30th day, while it decreased after 45 days of incubation (Figure 1). This data may indicate that microorganisms in both types of soil were tolerant to nicosulfuron and have participated in

Table 1 - Three-way ANOVA for determining the effects of herbicides, soil type and days after treatment (DAT) on dehydrogenase, urease and microbial biomass carbon

Source of variance	F-value(1)					
	Dehydrogenase (µg TPF g^{-1} soil)		Urease (µg $NH_4^+ g^{-1}$ soil h^{-1})		Microbial biomass carbon (µg Cg^{-1} soil)	
	Nicosulfuron	Glyphosate	Nicosulfuron	Glyphosate	Nicosulfuron	Glyphosate
Soil type	6993.01**	5446.93**	13722.13**	12055.17**	2645.67**	1017.32**
Concentration	212.23**	72.42**	360.89**	44.06**	7.45**	66.94**
DAT	133.22**	193.02**	277.81**	103.10**	36.69**	88.81**
Soil *x* Concentration	5.00**	4.24**	120.70**	12.86**	28.43**	10.98**
Soil *x* DAT	48.46**	47.34**	123.19**	82.25**	55.93**	77.27**
Concentration *x* DAT	10.72**	6.70**	33.68**	15.09**	6.02**	8.76**
Soil *x* Concent. *x* DAT	7.82**	8.49**	19.10**	14.11**	8.89**	5.10**

(1) F-value- calculated for 4 petri dishes; *, ** Significant at p<0.05 and 0.01 respectively.

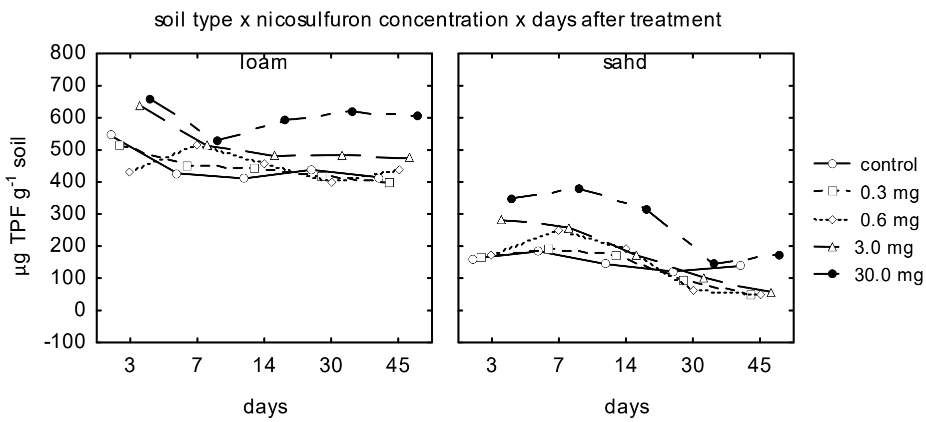

Figure 1 - Effects of nicosulfuron on dehydrogenase (µg TPF g^{-1} soil).

its degradation. Accinelli et al. (2002) also reported a stimulation of soil dehydrogenase activity by rimsulfuron at low concentrations. Their research indicated that sulfonylurea herbicides applied at lower doses stimulated dehydrogenase activity, while high agricultural rates had decreasing effects. However, Radivojevic et al. (2012) showed that different concentrations of nicosulfuron (0.3-3.0 mg kg^{-1} soil) decreased dehydrogenase activity from 5.1 to 42.7%.

The application of glyphosate to the loamy soil led to a significant increase in dehydrogenase activity (Figure 2). In this study, dehydrogenase activity increased with the glyphosate application rates of 326.0 mg kg^{-1} of soil and 3260.0 mg $kg^{-1}$of soil (7.1-15.7%). Both concentrations increased the activity of this enzyme throughout the 45 days of the experiment. The results also revealed a significantly higher (94-114%) dehydrogenase activity after glyphosate application to the sandy soil. This stimulating effect was achieved by the two highest concentrations (326.0 and 3260.0 mg kg^{-1} of soil) from the 3rd until 7th day. However, dehydrogenase activity decreased at 30 days after glyphosate application (Figure 2).

Consequently, statistical analysis of our data showed that soil type, different concentrations of glyphosate, number of days after treatment and interactions between them had an impact on dehydrogenase activity (Table 1). Partoazar et al. (2011) reported similar findings, showing that glyphosate increased dehydrogenase activity at 3 days after application. Also, Gomez et al. (2009) found that dehydrogenase activity was significantly higher after glyphosate treatments than in the control at the beginning of incubation. Treatment with 0.48 L a.i ha^{-1} presented the highest value after 45 days, while the dose of 3.84 L a.i ha^{-1} had the lowest dehydrogenase activity. This may be due to an increase in microbial population which has a potential for utilizing glyphosate as a source of nutrients. Conversely, some studies (Bennicelli et al., 2009; Sebiomo et al., 2011)

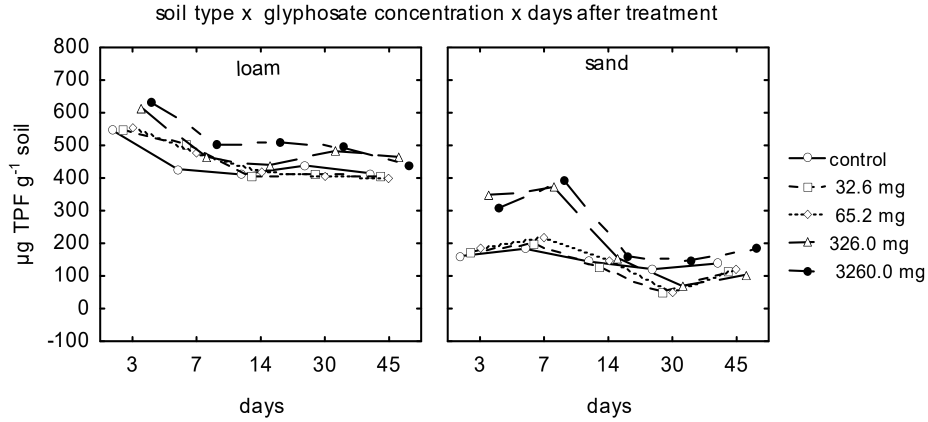

***Figure 2* -** Effects of glyphosate on dehydrogenase (μg TPF g^{-1} soil).

reported reductions in dehydrogenase activity compared with the control samples after application of glyphosate.

The urease enzyme activity in soil is important for releasing simple carbon and nitrogen sources that are used for the growth and multiplication of soil microorganisms (Riah et al., 2014). The effect of nicosulfuron on urease activity shows that the activity increased in both soil types (Figure 3; Table 1). The activity of this enzyme in loamy soil increased significantly over 30 days at all concentrations and the increase ranged from 45.5 to 136.0%. Compared to the untreated control, its maximum value was reached on the 7th day. Higher urease activity in the nicosulfuron-treated sandy soil was detected from 3 to 14 days after application. Maximum enzyme activity was found 7 days after treatment with 30.0 mg kg^{-1} of soil of nicosulfuron. The other nicosulfuron treatments had no significant effects on urease activity in either soil (Figure 3).

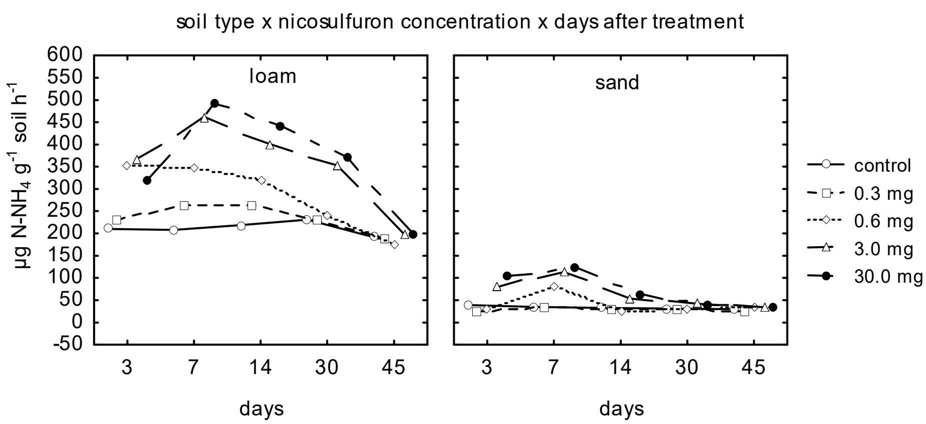

***Figure 3* -** Effects of nicosulfuron on urease (μg $NH_4^+ g^{-1}$ soil h^{-1}).

The application of glyphosate to loam led to a significant increase in urease activity in the initial fourteen days of the experiment (15-45%). However, urease activity in that soil was decreased from the 30th to 45th day (13.4-79.5%). The effect of glyphosate on urease activity in the sandy soil was minimal. A significant increase was detected on the 7th day only when the two highest concentrations (326.0 and 3260.0 mg kg^{-1} of soil) were applied. All other values were at the control level (Figure 4). Similar results were reported by Vandana et al. (2012), who found butachlor and cyhalofop-butyl to stimulate urease activity from 0 to 60 days. Also, urease activity in pyrozosulfuron-treated soil showed an increasing trend from the 7th to 28th day of incubation (Baboo et al., 2013). Most referenced studies report no effect or reduced effects of herbicides on urease activity (Romero, 2010; Bacmaga et al., 2012), but in the work of Tejeda (2009), urease activity in a clayey soil and sandy loam soil was inhibited to 58% and 49% after glyphosate application.

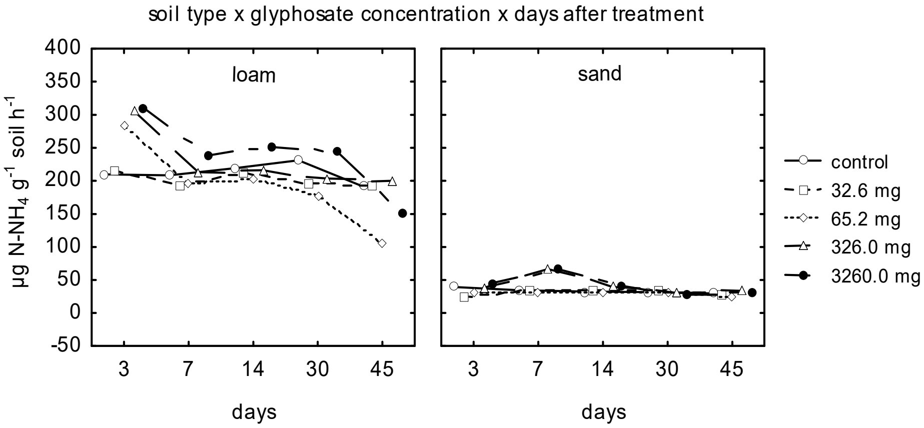

Figure 4 **-** Effects of glyphosate on urease (µg $NH_4^+g^{-1}$ soil h^{-1}).

Soil microbial biomass is an active component of soil organic pools, which is responsible for organic matter decomposition affecting soil nutrient content and primary productivity in most biogeochemical processes in terrestrial ecosystems (Kara and Bolat, 2008). The effect of nicosulfuron on microbial biomass carbon was positive in the loamy soil. Between the 7th and 14th days, an increase in biomass carbon (23.4-42.2%) was detected in this soil. Considering the effect of time on microbial biomass carbon, its maximum activity was reached at 14 days after treatment with 3.0 mg of nicosulfuron. There were no significant differences between all other treatments. However, nicosulfuron applied to the sandy soil reduced microbial biomass carbon. The reduction ranged from 9.2% to 18.2%, and the lowest value was found at 7 days after treatment with 3.0 mg nicosulfuron. The values in all other trial variants stayed at the control level (Figure 5).

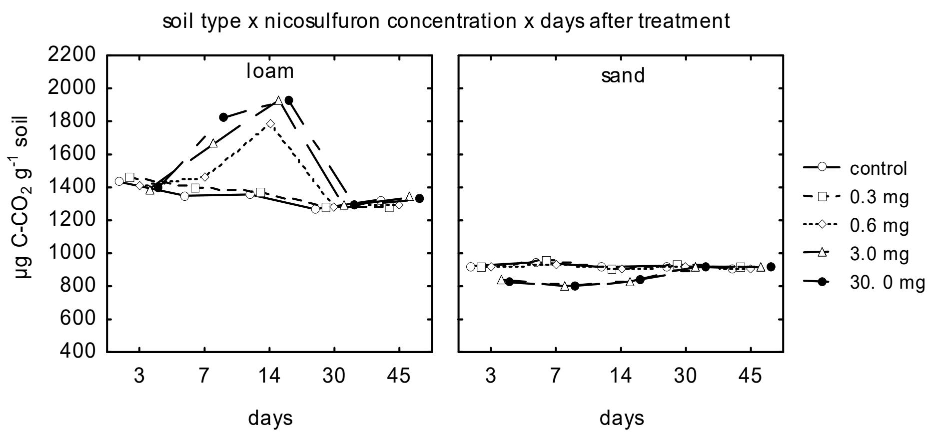

Figure 5 **-** Effects of nicosulfuron on microbial biomass carbon (µg Cg^{-1} soil).

Glyphosate treatment decreased microbial biomass carbon in both types of soil. A negative effect in the loamy soil was found between 7 and 30 days after application, and it ranged from 21.3% to 39.8%. The value was the lowest at 7 days after treatment with 3260.0 mg kg^{-1} of soil glyphosate. In the sandy soil, the two concentrations (326.0 and 3260.0 mg kg^{-1} soil) of glyphosate reduced microbial biomass carbon from the 3rd until the 45th day and the reduction ranged from 6.7% to 28.9% (Figure 6). Therefore, all three factors and their interactions had highly significant ($p<0.01$) effects on microbial biomass carbon (Table 1). Sofo et al. (2012) investigated the effects of four sulphonylurease herbicides on soil microbial biomass. They found that the decrease of microbial biomass carbon ranged from 25% for cinosulfuron at the normal field dose to a 54% reduction in the case of thifensulfuron-methyl at ten-fold the field dose. Lupwayi et al. (2004) reported similar results from treatments with metsulfuron-methyl and triasulfuron. However, Panettieri et al. (2013) reported high values of microbial biomass carbon after application of glyphosate between the 18th and 37th day.

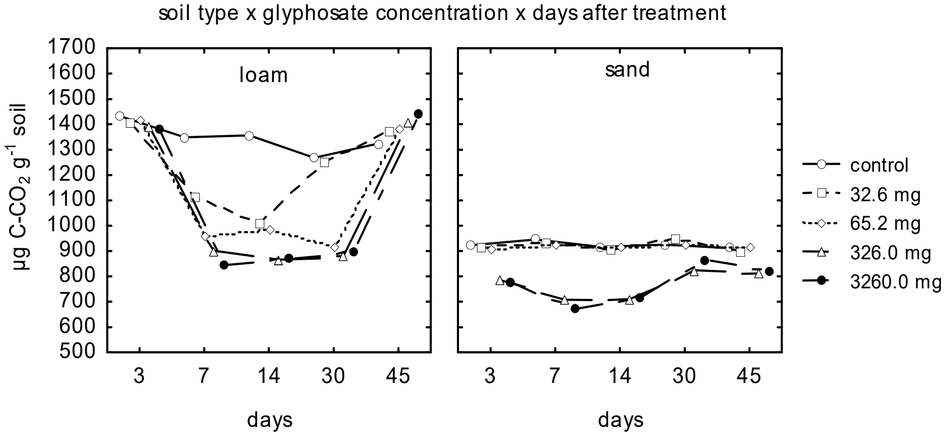

Figure 6 **-** Effects of glyphosate on microbial biomass carbon (μg Cg^{-1} soil).

The results showed that the effects of nicosulfuron and glyphosate depended on treatment rate, duration of activity, test parameters and soil type. Dehydrogenase activity was higher after glyphosate treatment in both types of soil, while urease activity was significantly affected by nicosulfuron. Both herbicides demonstrated negative effects on microbial biomass carbon in the sandy soil, while nicosulfuron increased its values in loam. Studies have shown that microbiological activity in loamy soil is generally higher compared to sand, as a result of superior physicochemical properties. The present study indicated that the application of these herbicides, either at the recommended or multiplied doses, influences temporary changes in character and intensity, which suggests that there is no real risk of causing a disruption of the existing balance of the soil biochemical processes.

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