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Short-time effects of the herbicide nicosulfuron on the biochemical activity of Chernozem soil

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Abstract: Short-time effects of the herbicide nicosulfuron on the biochemical activity of soil were investigated. Nicosulfuron rates of 0.3, 1.5 and 3.0 mg kg^{-1} of soil were laboratory-tested on Chernozem soil. The change in the dehydrogenase activity, in microbial biomass carbon, soil respiration and the metabolic coefficient (q_{CO_2}) were examined. Samples were collected for the analysis 1, 7, 14, 21, 30 and 60 days after nicosulfuron application. The obtained results indicated that the effect of nicosulfuron on the soil biochemical activity depended on its application rate and duration of activity, and the effect was either stimulating or inhibiting. However, the changes detected were found to be transient and, therefore, there is no real risk of the compound disrupting the balance of biochemical processes in Chernozem soil.

Keywords: nicosulfuron; Chernozem soil; dehydrogenase; biomass carbon; respiration.

INTRODUCTION

The perfect pesticide should be toxic only to the target organisms, be totally biodegradable to CO_2 and H_2O, and should not leave intermediate compounds in environment or be leached into the groundwater. Unfortunately, this is rarely the case and the widespread use of pesticides in contemporary agriculture is of increasing concern. The main problems in a real system arising from the use of pesticides in agriculture are their toxicity to non-target organisms and the environment, and their persistence in soil.[1]

The toxicity of pesticides has been examined individually in a variety of soils under different conditions and there is mounting evidence that the biological

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parameters of soil may be used as early and sensitive indicators of soil ecological stress.[2–4]

Some researchers[5] proposed a set of soil quality indicators that are sensitive to changes in soil management, and integrate biological, physical and chemical properties. In discussions about soil quality indicators, other researchers[6] included microbial biomass carbon, enzyme activity and soil respiration as biological indicators.

Sulfonylureas are class of herbicides characterized by high biochemical activity at low application rates. Modern pesticides tend to be applied at much lower doses than older compounds, but this does not mean they are less harmful to non-target organisms, as environmental risk arises from dose and activity, not just from the dose alone.[7] Sulfonylurea herbicides were introduced in the 1980s and have become valuable tools for weed management in agricultural production. Depending on crop type and local legislation, the application rates of these herbicides range from 2 g to 150 g a.i ha^{-1}. Although the mode of action of this herbicide class has been reported,[8–10] little additional information is available on the overall effects of this herbicide class on the biochemical properties of soil.

Nicosulfuron, 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridyl sulfonyl) urea), a member of this class, is a common agricultural herbicide used to control most annual and perennial grasses and several broad-leaved weeds in maize.

Microbial degradation critically affects the fate and behavior of pesticides in soil. The microbial population in soil constitutes a complex biochemical system capable of producing unique enzymes that degrade a large number of pesticides. Establishment of the degradation pathway of a pesticide in soil is difficult, but the use of various biochemical indicators can help the impact of a pesticide on soil to be better understood Biochemical indicators, such as soil enzymes, biomass, respiration, *etc*., are often used to characterize the effects of pesticides on the environment.[11]

The objective of the present study was to investigate, under laboratory conditions, the effects of nicosulfuron on the biochemical properties of soil by measuring different parameters, *i.e.*, microbial biomass carbon, dehydrogenase activity, soil respiration and the microbiological metabolic coefficient (q_{CO_2}).

EXPERIMENTAL

The pesticide (herbicide) nicosulfuron, 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea, tested in the experiment was a technical grade product of BASF, Germany. The rates of application were 0.3, 1.5 and 3.0 mg kg^{-1} soil. The lowest concentration tested was the label rate (0.3 mg kg^{-1}), while the other two were five and ten times higher than the recommended dose. The experiment was performed in Chernozem soil with a clay loam texture (pH 7.10, organic matter, 3.32 %, sand, 21 %, silt, 49 %, and clay, 30 %) at Zemun Polje, Belgrade. The soil chosen for the study had never been previously treated

with pesticides. Various management practices would have otherwise affected the soil microbial populations. In this way, it was possible to control the effects of the chosen pesticide (nicosulfuron).

The dehydrogenase activity, microbial biomass carbon and soil respiration were examined as relevant biochemical indicators.[12-15]

The soil samples were collected from the upper layer (0–10 cm), carefully dried, sieved to <5 mm mesh and stored at 4 °C. Before use, the soil was air-dried at room temperature for 24 h. Each herbicide concentration was pipetted onto the surface of 1 kg of soil before homogenization on a rotary stirrer for 30 min. After homogenization, the soil was portioned into pots. Untreated soil served as the control. The experiments were conducted in four replicates. The pots were kept in a controlled-environment chamber at 20±2 °C, 50 % air humidity and a 12/12 h day/night photoperiod throughout the experiments. The soil humidity was maintained at 50 % field capacity. Samples were collected for analysis 1, 7, 14, 21, 30 and 60 days after nicosulfuron application.

The activity of the enzyme dehydrogenase was determined according to Tabatabai.[16] The soil samples were prepared by incubation with triphenyltetrazolium chloride (TTC) under moist conditions at 37 °C for 24 h. Determination of triphenylformazan (TPF), which is derived from triphenyltetrazolium chloride (TTC) as a product of enzyme activity, was realized spectrophotometrically. The measurements were performed at a wavelength of 485 nm (Gilford stasar III, model 2400) and the enzyme activity is given as μg TPF g^{-1} soil.

Fumigation-extraction[17] was employed to determine the microbial biomass carbon. The samples were fumigated with alcohol-free chloroform ($CHCl_3$) under moist conditions for 24 h. After incubation, the carbon was extracted with a 0.5 M solution of potassium sulfate (K_2SO_4) and its content determined by titration with a 0.0333 M solution of Mohr salt ($(NH_4)_2Fe(SO_4)_2$) in the presence of phenylanthranilic acid as the indicator. Non-fumigated samples were extracted under the same conditions. The microbial biomass carbon was calculated based on a difference between carbon in the fumigated and non-fumigated samples using the factor 0.38.[18] The results are presented in μg C g^{-1} soil.

The Walter method[19] was employed to determine the soil respiration. The soil samples were incubated with sodium hydroxide under moist conditions at room temperature for 24 h. The carbon dioxide (CO_2) released during soil respiration was absorbed by 0.1 M solution of sodium hydroxide (NaOH), and CO_2 content was determined by titration with 0.1 M hydrochloric acid (HCl) in the presence of an appropriate indicator (phenolphthalein, Methyl Orange). The results are presented in μg CO_2 g^{-1} soil.

The microbiological metabolic coefficient (q_{CO_2}) was computed from the ratio of the intensity soil respiration and the microbial biomass.[20]

Statistical data processing was realized using PC Anova software. *F*-test was applied to all variables and their interactions and, in the case of a significant result in the individual comparisons, the LSD test was applied. Probability levels of 0.05 and 0.01 were used as significance criteria.

RESULTS AND DISCUSSION

Dehydrogenase activity is a measure of microbial metabolism and thus of the microbial oxidative activity in a soil. The soil enzyme activity is believed to be sensitive to pollution and has been proposed as an index of soil degradation. Dehydrogenase is thought to be an indicator of the overall microbial activity, because it occurs intracellularly in all living microbial cells and is linked with

microbial oxidation–reduction processes. It is a specific kind of enzyme that plays a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. The dehydrogenase activity of a is considered a valuable parameter for assessing the side effects of herbicide treatments on the soil microbial biomass.[21]

The effect of nicosulfuron on the enzyme activity is shown in Fig. 1, from which it can be seen that the dehydrogenase activity decreased for all applied nicosulfuron concentrations from the 1st to the 30th day. The decrease ranged from 5.1–25.8 % for the 0.3 mg concentration to 3.4–30.1 % for 1.5 mg kg^{-1} of soil and 4.4–42.7 % for 3.0 mg kg^{-1} of soil, and the found differences were statistically significant ($P < 0.01$). The decreased dehydrogenase activity was the result of the impact of the herbicide on the soil microorganisms. Actually, any toxicant from the external environment added to a soil may inhibit the microorganisms and thus the dehydrogenase enzymes. The altered enzyme activity depended on the concentration and duration of nicosulfuron activity. There was an increase in enzyme activity from 30th to 60th day, and the values of treated and untreated soils were similar at the end of the examination period.

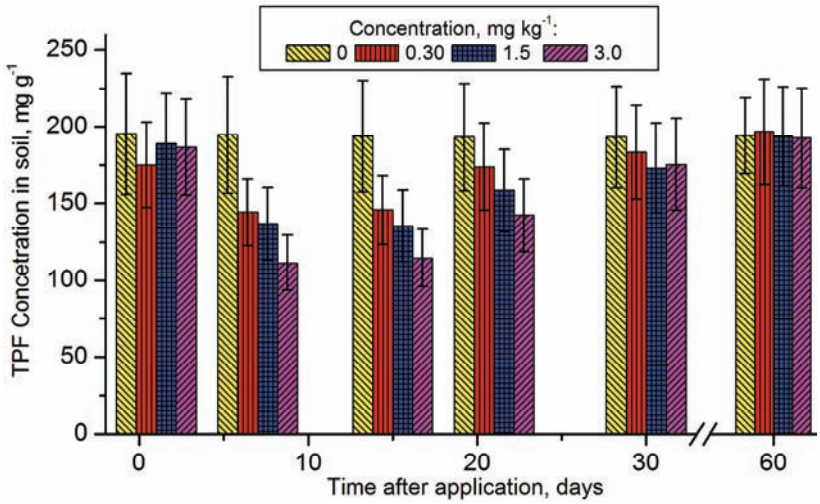

Fig. 1. Effect of nicosulfuron on the dehydrogenase activity.

These results are in accordance with the results of other authors who investigated the effects of different pesticides on dehydrogenase activity.[22–27] However, Ming *et al.*[28] reported that butachlor stimulated the soil microorganisms as well as the dehydrogenase activity. Radivojević *et al.*[29] investigated the effects of the herbicide metribuzin on the activity of some enzymes in soil. They found that the effect depended on the treatment rate, exposure time, enzyme group and that it initially inhibited but finally became stimulating at the end of the experiment.

Soil microbial biomass is defined as the living part of the organic matter of a soil. The composition of the soil microbial biomass varies depending on the soil characteristics. The soil microbial biomass increases or decreases in response to changes in soil management. Therefore, biomass measurement can indicate the effects of a pesticide on a soil and it is an important parameter in ecological tests. Data showing the effect of nicosulfuron on the biomass carbon are presented in Fig. 2. The highest biomass carbon (2033.2 μg C g^{-1} soil) was found for a nicosulfuron concentration of 3.0 mg kg^{-1} of soil (7 days after application) and the lowest (408.7 μg C g^{-1} soil) for a concentration of 3.0 mg kg^{-1} of soil (1 day after application).

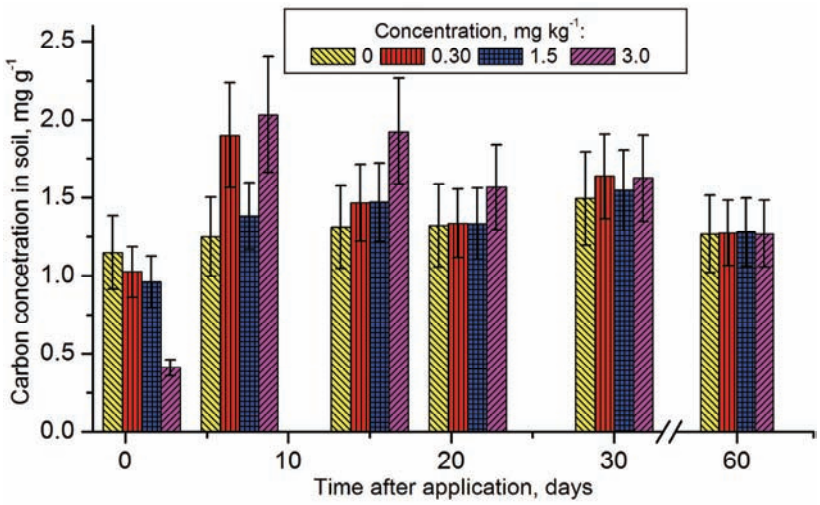

Fig. 2. Effect of nicosulfuron on the microbial biomass carbon.

These results indicate that nicosulfuron influenced the microbial biomass carbon, particularly at the beginning of the experiment, as a significant decrease in the microbial biomass carbon was observed on the first day. However, after one week and until the end of experiment (60th day), an increase in the biomass in relation to the applied concentration was recorded. This is not strange as it was assumed that the most dramatic decrease in the biomass carbon would occur immediately after pesticide application, when the concentration of the compound in the soil solution was the highest.[30] Many authors believe[31–34] that the biomass carbon later increases primarily due to restored populations of living organisms that are able to adapt to the particular pesticide present in the soil. Therefore, a new biomass that is metabolically very active and participates in various biochemical processes in the soil is formed. There have been other reports on the activity of different pesticides in relation to biomass carbon. The effects of long-term cumulative field application of the pesticides benomyl, chlorfenvinphos, aldicarb,

triadimefon and glyphosate on soil microbial biomass and mineralization of the soil organic matter were investigated. The addition of aldiocarb consistently increased the microbial biomass, due to its beneficial effect on crop growth, but this effect was not influenced by the rate of organic matter mineralization. However, in general, the continued application of these pesticides for up to 19 years, at slightly higher than the recommended rates, had very little effect on the soil microbial population.[35] On the contrary, Duah-Yentumi and Johnson[36] reported a dramatic reduction in the soil biomass following vinclosolin application, but for other pesticides, such as carbofuran, cabosulfan, simasine, paraquat, *etc*., they concluded that there were substantially different effects on soil biomass production by single or repeated application. It can be concluded that almost every pesticide has a different impact on the microbial biomass, as there is no general rule for their behavior. Still, it is very important to know the influence as microbial biomass reflects the effects of pesticide contaminants on the overall microbial population.

The biochemical activity of a soil, therefore, can be quantified by measuring CO_2 evolution. Carbon dioxide evolution is often used to characterize the effects of pesticides on the soil microflora. Soil respiration is one of the oldest and still the most frequently used parameter for quantifying activity in soil.[37] Soil respiration, as indicated by oxygen consumption and CO_2 evolution is considered as an indicator of microbiological activity, although it should be interpreted with caution. The rate of soil respiration depends on the physiological condition of the organisms and the edaphic conditions, such as temperature and soil moisture. Soil respiration measurements are often useful when made in conjunction with other response parameters, as was the case in this investigation.

The effect of nicosulfuron on soil respiration primarily depended on the pesticide concentration (Fig. 3). The respiration intensity at a nicosulfuron concentration of 3.0 mg kg^{-1} of soil ranged between 2.8 (1 day after application) and 6.9 μg CO_2 g^{-1} soil (15 days after application). At all concentrations, the respiration was reduced 2.5–40.2 % one day after application. Between the 7^{th} and 15^{th} day, an increase in respiration (21.7–56.4 %) was observed for all applied concentrations. A statistically significant increase in respiration ($P<0.01$) was detected for a concentration of 3.0 mg kg^{-1} of soil until 21 and 30 days after application. The degree of respiration inhibition increased with increasing nicosulfuron concentration at the beginning of the experiment but there was an increase in respiration at the end of the investigation. Thus, the more drastic the effect, the greater is the potential for recolonization of the treated soil.

Respiration gives a measure of the overall microbial activity and is considered as a bioindicator of soil quality.[38] Soil respiration has been frequently used for assessing the side effects of pesticides, such as atrazine, pentachlorophenol, 4-chloroanile and chloroacetamide, and it was found that atrazine caused minor

effects but the other pesticides stimulated respiration.[39] Araujo *et al.*[40] studied changes in microbial activity caused by glyfosate application and the result was an increase of 10–15 % in the respiration. Other researchers[41,42] reported that primsulfuronmethyl had no effect on respiration but that rimsulfuron greatly reduced the intensity of this process.

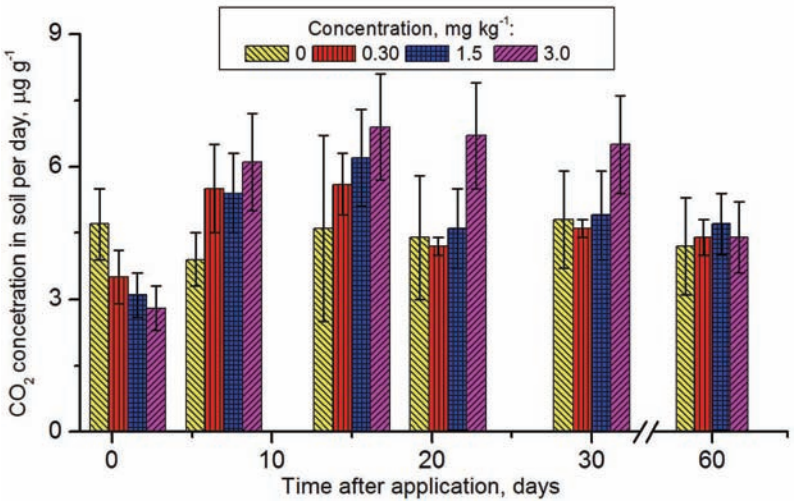

Fig. 3. Effect of nicosulfuron on soil respiration.

Based on literature data, it is not clear enough if the degree of soil respiration is influenced by the catabolic activity of the microbiological community or by changing microbial biomass. Based on the results obtained in the present study, it could be concluded that changes in biomass carbon influences changes in respiration.

The ratio between soil respiration and the soil microbial biomass of the microorganisms presents a safe way to evaluate microbial activity. This ratio, named metabolic coefficient (q_{CO_2}), was proposed by Anderson and Domsch[43] and is directly related to the fact that the biomass of the soil microorganisms becomes more efficient in utilizing the ecosystem resources. This coefficient is indicative of the activity of the microorganisms in soil. The degree of disturbance of the microbial community by anthropogenic impacts can be comparable to that caused by natural stress (drying–wetting and freeze–thawing), yet the duration of anthropogenic exposure is much longer, which makes it more harmful. An increase in the soil q_{CO_2} was observed after various anthropogenic disturbances: heavy metal contamination[44,45] or long term exposure to pesticides.[46,47] Therefore, the metabolic coefficient is used as a measure of microorganism stress because of different harmful influences. Thus, soil under stress would present higher q_{CO_2}

values than non-stressed soils.[48] The comparison of the metabolic coefficients in the affected and intact soil could be used to quantify different impacts.

In this investigation, increased values of the metabolic coefficient were recorded on the 1st (3.0 mg kg^{-1} of soil), 15th (1.5 mg kg^{-1} of soil), 21st (3.0 mg kg^{-1} of soil) and 30th (0.3 and 3.0 mg kg^{-1} of soil) day of the experiment (Fig. 4). Reduced coefficient values were recorded after the 1st (0.3 and 1.5 mg kg^{-1} of soil), 7th (0.3 and 3.0 mg kg^{-1} of soil) and 21st (0.3 mg kg^{-1} of soil) days. All differences detected were statistically significant ($P < 0.01$ or $P < 0.05$).

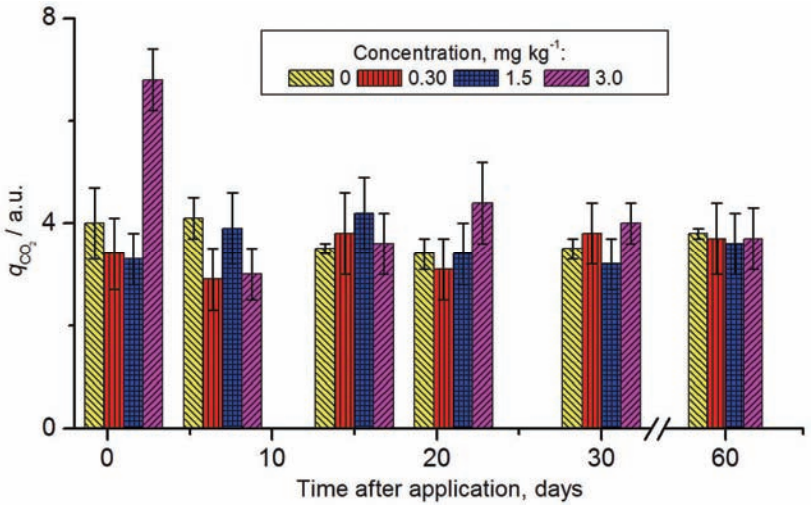

Fig. 4. Variation of the metabolic coefficient (q_{CO_2}) in the presence of nicosulfuron.

The increased values of the metabolic coefficient were induced by the adverse effects of nicosulfuron on the biochemical activity of soil. The period in which an increase in the metabolic coefficient was recorded was in accordance with the time when significant changes in dehydrogenase activity, microbial biomass carbon and intensity of soil respiration were registered. Bearing in mind the fact that the level of the metabolic coefficient reached the control level after 30 days, it was concluded that the harmful effect of nicosulfuron decreased with time. We are of the opinion that this occurred because of the microbial degradation of the nicosulfuron and restoration of the microbial community with microorganism groups that could use nicosulfuron as a source of nutrients and energy for physiological processes.

The results obtained in the present study are in accordance with the results of other authors. Increased values of the metabolic coefficient were found 21 days after application of glyphosphate and dinoseb.[47] Larger values were found in the case of dinoseb compared to glyphosphate, which is not strange bearing in mind the greater toxicity of dinoseb. Radivojevic *et al.*[15] showed that atrazine had in-

creased the values of the coefficient q_{CO_2} 30 days after application. In addition, it was found that there was an increase by 20–55 % in the metabolic coefficient after application of metalaxyl.[45]

Finally, it should be mentioned that the data presented herein suggest difficulties in the employment of biochemical parameters as indicators of nicosulfuron impact on soil, as different results were acquired depending on the biochemical parameter examined, the rate of application and the post-treatment time. In the literature, contrasting and opposing results of the impact of different pesticides on the biochemical parameters are reported. According to the present investigation, it seems that, of the examined parameters, the dehydrogenase activity was the most useful indicator of nicosulfuron impact on soil.

CONCLUSIONS

This short-term study, which lasted for 60 days, showed that soil microbial activities, such as soil respiration, dehydrogenase, soil biomass carbon and the metabolic coefficient changed on application of the herbicide nicosulfuron. Under the employed experimental conditions, the short-term use of nicosulfuron caused different effects on the biochemical activity in soil. The influence of nicosulfuron depended on the rate of application and the duration of activity, and was either stimulating or inhibitory. The impact of nicosulfuron on the dehydrogenase activity was consistently negative for each herbicide concentration and depended on the rate of application. Based on the microbial biomass carbon, soil respiration and the metabolic coefficient (q_{CO_2}), non consistent positive or negative effects of nicosulfuron were observed and the effects persisted until the 60th day.

The present study indicated that the application of nicosulfuron, 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. The microbial activities seemed to recover after the application. However, regarding pesticide application, laboratory results may not necessarily reflect the situation under field conditions, because in the field many factors could mask or reduce the potential toxicity of pesticides. Therefore, field studies would be a more realistic approach before general conclusions on the effect of nicosulfuron on the biochemical activity in soil are made.

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ИЗВОД

КРАТКОРОЧНО ДЕЛОВАЊЕ НИКОСУЛФУРОНА НА БИОХЕМИЈСКУ АКТИВНОСТ ЧЕРНОЗЕМА

ЉИЉАНА РАДИВОЈЕВИЋ[1], СЛАВИЦА. ГАШИЋ[1], ЉИЉАНА. ШАНТРИЋ[1], ЈЕЛЕНА. ГАЈИЋ УМИЉЕНДИЋ[1] и ДРАГАНА МАРИСАВЉЕВИЋ[2]

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У раду је испитивано краткорочно деловање хербицида никосулфурон на биохемијску активност земљишта. Оглед је постављен у лабораторијским условима на земљишту типа глиновита иловача. Никосулфурон је примењен у количинама од 0,3, 1,5 и 3,0 mg kg^{-1} земљишта. Праћени су следећи биохемијски параметри: активност ензима дехидрогеназе, промене микробиолошке биомасе угљеника, респирација (дисање) земљишта као и метаболички коефицијент (q_{CO_2}). Узорци за анализе узимани су 1, 7, 14, 21, 30 и 60 дана после примене никосулфурона. Добијени резултати су показали да је утицај никосулфурона на биохемијску активност земљишта зависио од примењене количине и дужине деловања, те је у зависности од тога, било стимулативно или инхибиторно. Међутим, утврђене промене су биле пролазног карактера, тако да може да се сматра да нема реалног ризика од нарушавања равнотеже биохемијских процеса у земљишту под утицајем овог једињења.

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