

Microbial activity in different soils in response to metribuzin treatment

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SUMMARY

The effect of metribuzin on soil microorganisms and their enzymatic activity, as well as the amount of metribuzin residues, greatly depend on the type of soil and its physico-chemical properties. A laboratory experiment was set up to determine the effects of metribuzin on different groups of microorganisms and dehydrogenase activity in loamy and sandy soils. The amounts of metribuzin residues in those soils were also determined. The following concentrations were tested: 12.0, 24.0, 120.0 and 1200.0 mg a.i. kg⁻¹soil. Samples were collected 3, 7, 14, 30 and 45 days after metribuzin application. Metribuzin acted inhibitory on total bacteria counts in both types of soil: 6.9% in loamy and 7.9% in sandy soils. Actinomycetes counts decreased over the first 14 days after metribuzin treatment by 15.6% in loamy and 8.1% in sandy soil. However, starting with the 30th post-treatment day, their number either increased or remained at the control level. Fungi counts increased by 6.0% seven days after the application of the two highest concentrations. In sandy soil, fungi counts were significantly reduced by 29%. Metribuzin treatment significantly reduced the activity of dehydrogenase enzyme in both types of soil. Only the highest test concentration applied to the sandy soil showed a stimulating effect (33.2 %). Degradation data showed that the measured amounts of metribuzin decreased over time in both types of soil at all four test concentrations. The results showed that the extracted amounts of metribuzin active ingredient at all its test concentrations were greater in the sandy type of soil. Loamy soil was shown to have a better sorption capacity due to its higher contents of organic matter and clay.

Keywords: herbicides; metribuzin; residues; soil microorganisms; dehydrogenase

INTRODUCTION

Metribuzin [4-amino-6-tertbutyl-3-methylthio-1,2,4-triazin5-(4H)-one] is a selective triazinone herbicide for soil treatment which is intended for controlling annual broad-leaved weeds in potato, tomato, soybean and new alfalfa crops (Majumdar & Singh, 2006). Degradation

(DT₅₀) period of this active ingredient is between one and two months in soil, and around seven days in water. Adsorption and desorption, which depend on physico-chemical properties of soil, are important factors which affect degradation, transition and leaching of herbicides in soil (Đurović, 2011). Also, soil is a complex system with many components, including many organic and

inorganic compounds of different contents and types of activity, and it impacts the behavior of herbicides that are incorporated in it (Rigi, et al., 2015; Gamiz et al., 2017). Microorganisms and their activity are one of such components.

The abundance, content and activity of microorganisms greatly depend on the type of soil (Torsvik & Øvreås, 2002). Some studies have shown that the activity of certain enzymes in soil, such as urease, invertase or alkaline phosphatase, is higher in sludge and clay, while the activity of xylanase is elevated in sand fractions (Kandeler et al., 1999; Stemmer et al., 1998). Similarly, microbial communities are very important for the development and maintaining of soil texture (Martin et al., 2012). Interactions between soil components and herbicides further affect the biochemical processes initiated by microorganisms (Banks et al., 2014; Jacobsen & Hjelmsø, 2014).

Microorganisms provide the main pathway of metribuzin degradation in soil (Mehdizadeh et al., 2019). In that process, the herbicide may affect their abundance, content and activity. The extent and direction of such influence depend on a variety of factors. Those are primarily the rate of metribuzin application, duration of exposure, climatic and edaphic factors, and type of microbiological parameters under scrutiny (Niewiadomska et al., 2018).

Having in mind the impact of various physico-chemical properties of loamy and sandy soils, the following targets were set to our experiments: to learn more about the influence of different concentrations of metribuzin on the abundance of microbial communities, the activity of dehydrogenase enzyme, and metribuzin fate in those soils.

MATERIAL AND METHODS

A laboratory experiment was carried out in two agricultural soils. The loamy (from location Zemun Polje) and sandy (location Tavankut) soils chosen for the study had never been treated with pesticides before. The physico-chemical characteristics 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. Soil samples were collected from the upper layer (0-10 cm), then carefully dried, sieved to pass 5 mm mesh, and stored at 4°C. According to the World Reference Base (FAO, 2015), the former type of soil belongs to the group of

Chernozems, while the sandy soil is classified into the group of Arenosols. Before using them, both soils were 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 minutes. After homogenization, the soil was portioned out in pots. Untreated soil served as a control. The experiments were conducted in four replications. The pots were kept in a controlled-environment chamber at 20±2°C, under 50% relative humidity and 12/12 h day/night photoperiod throughout the experiment. Samples were collected for analyses 3, 7, 14, 30 and 45 days after herbicide application.

The herbicide metribuzin, which was tested in the experiment, was the product Sencor WG-70, manufactured by Bayer CropSciences, and the rates of application were: 12.0, 24.0, 120.0 and 1200.0 mg a.i. kg⁻¹ soil. The lowest test concentration equalled the recommended rate, while the upper three were double, ten-fold and hundred-fold. The ten-fold and hundred-fold concentrations were chosen in accordance with a standard ecotoxicological practice for determining possible negative impact of a substance on the environment (Cycoń & Piotrowska-Seget, 2015).

Total bacterial population in a microbial colony, along with actinomycetes and fungi, was determined by soil sample serial dilutions using fresh soil. Total bacteria were counted on soil agar, while fungi were counted on Chapek agar, and actinomycetes on synthetic agar supplemented with sucrose. After incubation, the number of bacteria and fungi were converted into colony forming units/g of soil dry weight (CFU/g of soil DW), and presented as log of number (Jarak & Đurić, 2006).

Dehydrogenase activity was measured as describe by Tabatabai (1982). Soil samples were prepared by incubation with triphenyltetrazolium chloride (TTC) at 37°C for 24 h. Triphenylformazan (TPF), which is derived from TTC as a product of enzymatic 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.

Metribuzin residues in soil were determined in test samples. Soil samples (10 g) were homogenized twice using 15 ml of methanol:acetone mixture at 1:1 ratio (30 min on rotary mixer), adding 2 g Na₂SO₄ (dried for 24 h at 130 °C). After centrifugation (15 min, 4000 rpm) and extract filtering, the filtrate was evaporated to dryness (rotary evaporator, 35 °C), and then dissolved in 5 ml of ethyl:acetone = 4:1 mixture. One ml of the obtained solution was inserted in a chromatographic column filled

with 1 g Na₂SO₄ (anh., dried for 24 h at 130 °C) and 5 g of sorbent (inserted in the column with 30 ml of ethyl acetate:acetone=4:1 mixture). Metribuzin was eluted from the column by 30 ml of ethyl acetate:acetone = 4:1. The eluent was evaporated to dryness, then dissolved in 5 ml acetone, and finally 1 µl of the solution was injected in the gas-chromatography mass spectrometry device (GC-MS) (Đurović- Pejčev et al., 2012).

The data were statistically processed in Statistica 8.0 software. A one-way analysis of variance was used to compare means of the examined microbial parameters. The LSD test was used to compare treatments and assessments of each parameter when differences in F values were statistically significant ($p < 0.05$).

RESULTS

Assessment of microorganism abundance

Metribuzin application caused statistically significant changes regarding total bacterial counts in both types of soil (Table 1). Three, 7 and 45 days after treatment (DAT), the lowest metribuzin concentration (12.0 mg a.i.kg⁻¹soil), representing the recommended application rate, caused a significant increase in total bacterial count in loamy soil (log 7.41 - log 7.56). In all other test variants, the herbicide caused statistically significant reductions in bacterial counts in that type of soil. The greatest decrease

of 6.9% was noted 7 days after metribuzin treatment with 120.0 mg a.i.kg⁻¹ soil. Over the initial 14 days after metribuzin treatment of sandy soil, it stimulated the total bacterial count. The highest count (log 7.33) was noted 14 days after treatment with the top product concentration (1200.0 mg a.i.kg⁻¹soil) and it was 6.1% higher than control data. Thirty and 45 DAT, bacterial counts were significantly reduced. The lowest count (log 6.13) was noted 30 days after treatment with the 100-fold concentration (1200.0 mg a.i.kg⁻¹soil) of metribuzin, and it was 7.9% lower than the control count.

Over the period 3-14 DAT, metribuzin treatment reduced the number of actinomycetes in loamy soil (log 4.54 to log 5.09). The minimum actinomycetes count was found 14 days after treatment with the herbicide concentration of 120.0 mg a.i.kg⁻¹soil, which was 15.6% lower than control data. A statistically significant increase in actinocetes count was noted 30 and 45 DAT. The maximum count of actinomycetes (log 5.63) was found after treatment with 12.0 mg a.i.kg⁻¹soil. Over the initial 14 days of the experiment in sandy soil, the two highest concentrations of metribuzin caused statistically significant reductions in actinomycetes counts. The lowest count (log 5.24) was noted seven days after metribuzin treatment with the concentration of 1200.0 mg a.i.kg⁻¹soil, which was 8.1% lower than control data. There were no statistically significant changes between actinomycetes counts in the other test variants (Table 1).

Table 1. Effects of metribuzin on total bacteria, actinomycetes and fungi counts in different types of soil

Treatments	Microbial group	Loam					Sand				
		Days after incubations					Days after incubations				
		3	7	14	30	45	3	7	14	30	45
Control	Total bacteria	7.39 b	7.38 b	7.35 a	7.43 b	7.29 b	6.93 c	6.91 b	6.91 c	6.85 c	6.82 a
	Actinomycetes	5.29 b	5.33 a	5.38 a	5.38 d	5.39 c	5.68 bc	5.70 b	5.68 bc	5.72 a	5.73 a
	Fungi	5.26 ab	5.17 c	5.30 a	5.22 a	5.19 bc	4.38 b	4.80 b	4.79 b	4.86 b	4.80 a
12 mg kg ⁻¹	Total bacteria	7.47 a	7.56 a	7.34 a	7.38 b	7.41 a	6.90 c	7.01 b	6.85 c	6.78 cd	6.56 b
	Actinomycetes	5.25 bc	5.09 b	4.85 b	5.55 a	5.63 b	5.70 bc	5.64 b	5.70 bc	5.70 a	5.74 a
	Fungi	5.15 bc	5.18 c	5.19 a	5.15 a	5.04 c	4.56 b	4.75 b	4.75 b	4.89 b	4.82 a
24 mg kg ⁻¹	Total bacteria	7.29 d	7.05 d	7.33 a	7.17 c	7.24 c	6.89 c	7.02 b	6.92c	6.73 d	6.46 bc
	Actinomycetes	5.26 bc	5.15 b	4.75 bc	5.47 c	5.62 b	5.74 b	5.65 b	5.74 b	5.75 a	5.78 a
	Fungi	5.19 bc	5.18 c	5.22 a	5.27 a	5.12 bc	4.60 bc	4.78 b	4.80 b	4.87 b	4.70 a
120 mg kg ⁻¹	Total bacteria	7.09 c	6.87 c	7.05 c	7.01 a	6.92 e	7.05 b	7.15 a	7.01 b	6.34 a	6.41 bc
	Actinomycetes	5.17 a	4.74 c	4.54 c	5.42 a	5.45 a	5.54 c	5.30 a	5.54 c	5.70 a	5.73 a
	Fungi	5.18 bc	5.42 b	5.34 a	5.26 a	5.28 ab	4.15 a	4.15 a	4.08 a	4.14 a	4.72 a
1200 mg kg ⁻¹	Total bacteria	7.31 d	7.02 d	7.18 b	7.11 c	7.03 d	7.23 a	7.22 a	7.33 a	6.13 b	6.29 c
	Actinomycetes	5.10 a	4.58 c	4.70 c	5.37 d	5.34 c	5.31 a	5.24 a	5.31 a	5.68 a	5.71 a
	Fungi	5.35 a	5.48 a	5.32 a	5.26 a	5.22 ab	3.90 a	3.90 a	3.40 a	3.89 a	4.57 a

Values in columns marked by different letters indicate significant difference between treatments at $p < 0.05$.

The results in Table 1 show that metribuzin had no significant influence on the abundance of fungi in loamy soil. A statistically significant increase of 6.0% was caused by the two highest concentrations of metribuzin 7 DAT (log 5.42 and log 5.48). However, the application of metribuzin to sandy soil had an inhibitory activity. The two highest concentrations significantly reduced fungi counts 3-30 DAT. The lowest value (log 3.40) was noted 14 days after treatment with the metribuzin concentration of 1200.0 mg a.i.kg⁻¹soil, which was 29% lower than control data.

Dehydrogenase activity

Metribuzin treatment of loamy soil at the recommended application rate (12.0 mg a.i.kg⁻¹soil) had no statistically significant effect on the activity of dehydrogenase. A significant reduction was noted only 30 DAT (357.6 µg TPF/g soil), which was 18.4 % compared to control data. In sandy soil, the same

metribuzin concentration mostly had a negative effect on dehydrogenase activity. The lowest dehydrogenase value was found 14 DAT (81.1 µg TPF/g soil) and it was 43.6 % lower than in untreated control soil (Figure 1a).

In loamy soil, the application of metribuzin at 24.0 mg a.i.kg⁻¹ soil had a negative impact on dehydrogenase activity over the entire 45-day experimental period. Its lowest value of 365.6 µg TPF/g soil (16.5% compared to control) was noted 30 DAT. In sandy soil, the same metribuzin concentration increased dehydrogenase activity over the first seven days, while it caused a statistically significant decrease from 14 DAT, when the lowest value (98.0 µg TPF/g soil) was noted, which was 31.8% lower than the control (Figure 1b).

The application of 100-fold concentration of metribuzin (1200.0 mg a.i.kg⁻¹soil) caused a reduction in dehydrogenase activity in loamy soil over the entire experimental period, and the lowest value (333.4 µg TPF/g soil) was noted 30 DAT, when it was 23.8 % lower than control data. In sandy soil, the same concentration

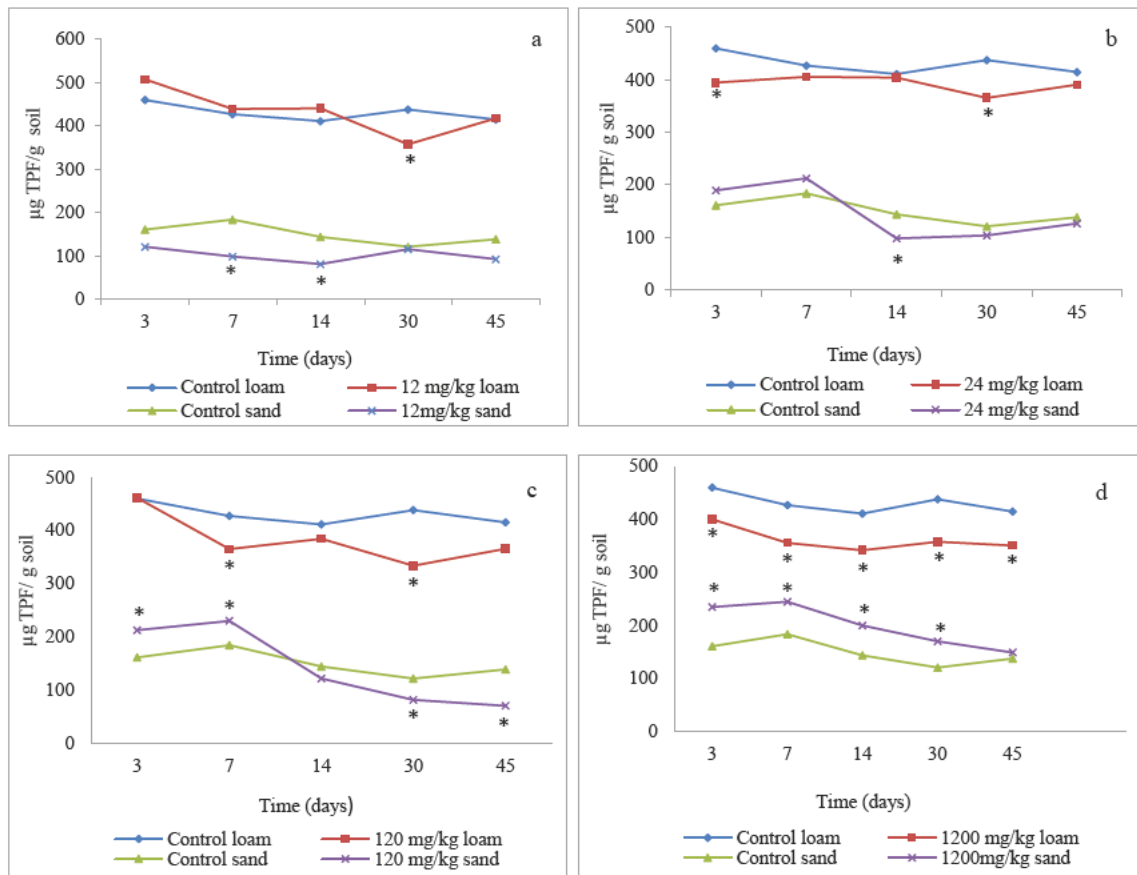


Figure 1. Effects of different concentrations of metribuzin on soil enzyme dehydrogenase in different types of soil: a) 12.0 mg/kg soil; b) 24.0 mg/kg soil; c) 120.0 mg/kg soil; d) 1200.0 mg/kg soil

reduced dehydrogenase activity from 14 DAT onwards, achieving the lowest value ($69.6 \mu\text{g TPF/g soil}$) 45 DAT, which was 49.6% lower than in control soil (Figure 1c).

The highest concentration of metribuzin ($1200.0 \text{ mg a.i.kg}^{-1}\text{soil}$) caused a statistically significant decrease in dehydrogenase activity over the entire period of 30 days of the experiment. The lowest value was found 14 days after application ($342.0 \mu\text{g TPF/g soil}$), when it was 16.8% lower than in the control. Unlike the findings in loam, this concentration had a stimulating impact on the parameter in sandy soil. Seven days after application, it significantly increased dehydrogenase value to $245.0 \mu\text{g TPF/g soil}$, which was 33.2% higher than in control soil (Figure 1d).

Metribuzin degradation in loamy and sandy soils

Data presented in Figure 2 a-d show that the amounts of metribuzin, applied at all four concentrations, decreased over time in both types of soil. At the lowest concentration (12 mg kg^{-1}), the difference between metribuzin amounts extracted by loamy and sandy soils increased from the initial 5.9%, measured immediately after herbicide application (3 h) to 41.2% after 45 days (Figure 2a). At the double dose, the difference in extracted amounts of metribuzin immediately after

application (3 h) was 27.1%, while it decreased to 25.7% after 45 days of the experiment. Considering the concentration that was ten-fold the recommended dose, the difference was initially 27.2%, and it decreased to 16.7% at the end of the experiment (Figures 2b and 2c). At the 100-fold herbicide concentration, the difference in metribuzin amounts extracted by loamy and sandy soils was almost the same over the experimental period, i.e. 7.4% immediately after herbicide application and 7.1% on the 45th day of the experiment. This result is attributable to sorption saturation of metribuzin molecules in both types of soil (Figure 2d).

DISCUSSION

The findings in the present study show that all tested parameters had higher values in loamy than in sandy soil, which may be attributed to better physico-chemical characteristics of the former type of soil. Girvan et al. (2003) found soil type to be the principle factor determining the composition of bacterial communities, rather than crop management practices in those soils. Bacteria make the most abundant group of microorganisms in soil. Metribuzin treatment had an inhibitory impact on their abundance in many treatment variants in both tested soils. Over the initial 14 days,

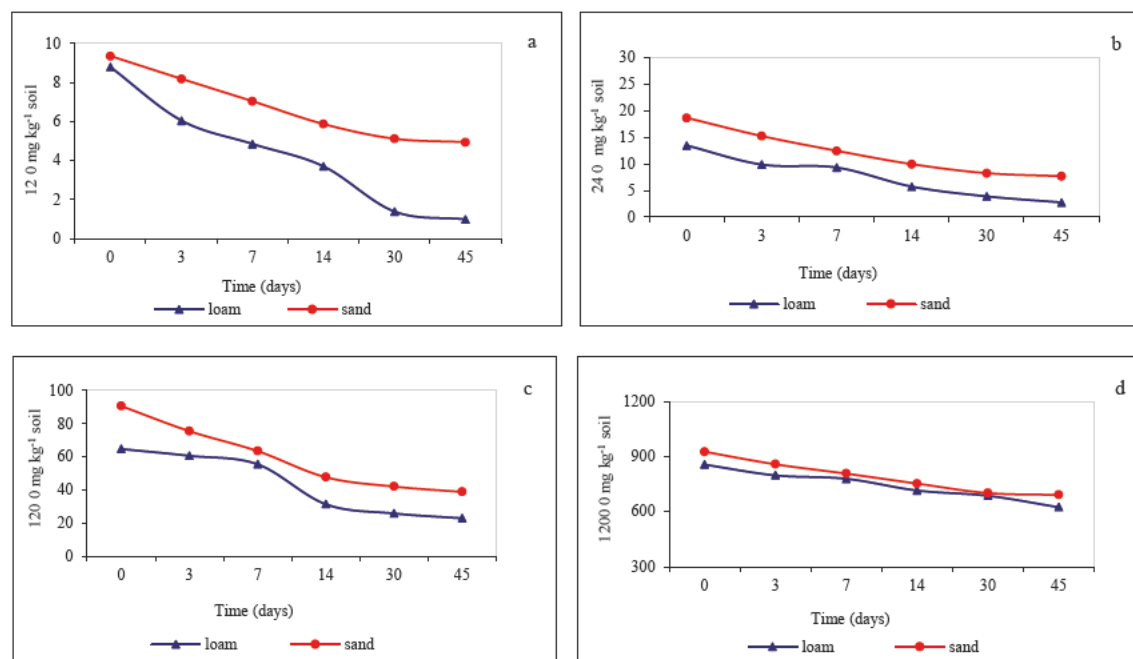


Figure 2. Extracted quantities of metribuzin in loamy and sandy soils: a) for $12.0 \text{ mg kg}^{-1}\text{soil}$; b) for $24.0 \text{ mg kg}^{-1}\text{soil}$; c) for $120.0 \text{ mg kg}^{-1}\text{soil}$; d) for $1200.0 \text{ mg kg}^{-1}\text{soil}$

metribuzin had a stimulating effect in both types of soil. However, their total counts dropped significantly after 30 days, and the negative effect was more prominent in sand. Mohiuddin & Mohammed (2013) also reported a significant reduction in bacteria counts in their experiments with herbicides (metribuzin and 2,4-D) and a fungicide (carbendazim). The strongest inhibitory effect was found on the 14th day of the experiment and it was noted in all treated pots. Similar results were also reported by Lone et al., (2014), and their conclusion was that toxic effects of metribuzin increased at higher concentrations, but also that they were transitory.

Metribuzin significantly affected the number of actinomycetes in both types of soil. The results showed that their counts significantly decreased in both soils over the initial 14 days, compared to control pots. After an initial negative response to the toxic activity of metribuzin, actinomycetes recovered, causing herbicide degradation by their enzymatic activity. Thirty and 45 days after soil treatment, the number of actinomycetes either significantly increased or remained at the control level. In contrast, a negative response was reported by Niewiadomska et al. (2018) in their study using the yellow sweet clover. They applied metribuzin in combination with diflufenican, which reduced the number of actinomycetes by 46% and the negative trend persisted in all experimental variants. Radivojević et al. (2003) found that temperature was also an important factor for the activity of herbicides against microorganisms. Their results showed that the toxic effect of metribuzin to actinomycetes significantly increased at higher temperature (30°C).

Fungi participate in many biochemical processes, and their role is especially important in the process of degradation of complex organic molecules such as herbicides (Imberger & Chiu, 2002). The results of the present study show that metribuzin did not have a significant impact on fungal counts in loamy soil. However, the number of fungi in sandy soil was significantly reduced when the two highest concentrations were applied. A positive response of fungi to herbicide application (metribuzin and propyzamid) was also reported by Zaid et al. (2014) based on an experiment with peas.

Dehydrogenase is an enzyme that belongs to the group of oxidoreductases and it is an important indicator of the state and changes occurring in soil. Metribuzin treatment significantly reduced its activity in both types of soil. Only the highest concentration of metribuzin applied to the sandy soil had a stimulating effect. Similar responses were observed by Sebiomo et al. (2011) in a study with atrazine, and by Pampulha et al. (2007) in experiments with glufosinate-ammonium. However, Šantrić et al.

(2018) observed an elevated activity of dehydrogenase in a study with nicosulfuron and glyphosate in loamy and sandy soils.

After herbicides are incorporated in soil, their behavior and fate depend on many factors (soil properties, herbicide properties, weather conditions, etc.) (Parker et al., 2018). Our results show that the amounts of metribuzine decreased over time in both types of soil at all four concentrations tested. The findings result from an increased sorption of metribuzine molecules by soil particles and/or heightened herbicide degradation over time. Also, the extracted amounts of active ingredient (metribuzin) at all test concentrations were higher in the sandy type of soil. It indicates that loamy soil has a better sorption capacity due to its greater content of organic matter and clay. Li et al. (2003) also pointed at organic matter and clay contents as the two most important components in soil-herbicide interaction. Furthermore, the extracted amounts of metribuzin in loamy and sandy soils depended on herbicide concentrations. At the lowest concentration, the difference was most prominent over time, while it significantly decreased at the double and ten-fold concentrations, and the amounts of metribuzin extracted by loamy and sandy soils at the 100-fold concentration were almost the same. The findings may be attributed to gradual sorption saturation with metribuzin molecules in both types of soil. Bouchard et al. (1982) found that adsorption and degradation in a test loamy soil were greater at the depth of 10-20 cm than at 40-50 cm, where the content of organic matter was significantly smaller. Other authors have come to a similar conclusion. After testing different types of soil and organic fertilization, they found a significant relation between adsorption and mobility of metribuzin, and content of organic matter (Henriksen et al, 2004; Majumdar & Singh, 2007).

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Mikrobiološka aktivnost u različitim tipovima zemljišta kao reakcija na primenu metribuzina

REZIME

Uticaj metribuzina na zemljišne mikroorganizme, njihovu enzimatsku aktivnost kao i nivo ostataka ovog herbicida u velikoj meri zavisi od tipa zemljišta i njihovih fizičko-hemijskih karakteristika. Postavljen je laboratorijski ogled da bi se utvrdio uticaj metribuzina na različite grupe mikroorganizama i dehidrogenaznu aktivnost u ilovači i peskuši. Takođe je određen nivo ostatka metribuzina u ovim zemljištima. Ispitivane su sledeće koncentracije. 12,0, 24,0, 120,0 i 1200,0 mg a.s. kg⁻¹zemljišta. Uzorci su uzeti 3,7, 14,30 i 45 dana nakon primene metribuzina. Uticaj metribuzina na ukupan broj bakterija je bio inibitornog karaktera kod oba tipa zemljišta, i kretao se od 6,9% za ilovaču do 7,9% za peskušu. Aktinomicete su u prvih četrnaest dana nakon primene metribuzina imale smanjenu brojnost za 15,6% u ilovači i 8,1 u peskuši. Međutim, od tridesetog dana broj se povećao ili bio na nivou kontrole. Broj gljiva u ilovači je bio povećan za 6,0% sedam dana nakon primene dve najveće koncentracije. U peskuši je brojnost ove grupe bila značajno smanjena za 29%. Primena metribuzina je značajno smanjila aktivnost enzima dehidrogenaza kod oba zemljišta. Jedino je najveća koncentracija u peskuši ispoljila stimulativan efekat (33,2 %). Rezultati degradacije su pokazali da su izmerene količine metribuzina opale sa vremenom kod oba tipa zemljišta i pri primeni sve četiri koncentracije. Može se zaključiti da je ekstrahovana količina aktivne materije metribuzina za sve primenjene koncentracije veća za zemljište tipa peskuša. Ovo ukazuje da je zbog većeg sadržaj organske mase i gline, zemljište tipa ilovače ispoljilo bolje sorpcione sposobnosti.

Ključne reči: herbicidi; metribuzin; ostaci; zemljišni mikroorganizmi; dehidrogenaza