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Original scientific paper

The effect of metribuzin on the density of proteolytic microorganisms and proteolytic activity in different types of soil

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SUMMARY

Soil texture and other physical and chemical characteristics of soil are important factors influencing the retention of herbicides in soil ecosystems. A laboratory experiment was conducted to estimate the response of proteolytic microorganisms to applications of metribuzin in different types of soil (loamy and sandy) in terms of density and protease activity. The following concentrations were tested: 12.0, 24.0, 120.0 and 1200.0 mg a.i.kg^{-1} soil. Samples were collected 7,14 and 30 days after treatment with metribuzin.

Metribuzin did not affect the number of proteolytic microorganisms in loamy soil. In sandy soil, their number was reduced 26.7% by the highest concentration 7 days after application. Protease activity was reduced in both types of soil on the 7th and 14th day and the percentage of reduction was 21% for loamy soil and 29.9% for sandy soil. Statistical analysis of data showed that the correlation between test parameters was positive in both types of soil (r^2=1 for loamy soil, and r^2=0.81 for sandy soil). The study shows that metribuzin causes a passable impact on microbial population and enzymatic activity which depends on the type of soil.

Keywords: Metribuzin; Soil; Proteolytic microorganisms; Protease

INTRODUCTION

Soil is a complex multiphase and dynamical system, functionally making a system in which various physical, chemical, biochemical and biological processes are continually evolving. These processes are of crucial importance for the functioning of ecosystem (Marhan et al., 2007). Soil type may be the key factor determining the bacterial community and presence of other microorganisms (Girvan et al., 2003). Considering the factors involved in transformation of organic matter, circulation of nutrients, and in creation of soil structure, microorganisms play the key role. Moreover, soil enzymes are important catalysts in biochemical transformations of matter. They are constantly synthetized, accumulated or decomposed, which is of great importance for agricultural production (Deborah, et al., 2013).

Proteolytic microorganisms belong to the group of aminoheterotrophs, which require proteins as a source of N. Proteases have an important role in mineralization of nitrogen, making it available to plants and microorganisms. (Caldwell, 2005). Proteolytic activity in soil can be influenced by various factors, such as organic C concentration, total N concentration, clay content and other soil factors. Some studies suggest that proteolytic activity may be inhibited when the number of proteolytic microorganisms is reduced (Sardans et al., 2008; Baboo et al., 2013).

In agricultural practice, herbicide applications have an important place in plant protection. The behavior and destiny of these compounds in soil depend on their chemical and physical properties and on how they interact with biotic and abiotic soil components (Pal et al., 2006; Maqueda et al., 2009). The use of these compounds can influence the number of microorganisms, their composition, as well as their enzymatic activity. The extent to which herbicides will influence microbiological activity depends on the compound itself, the type and composition of soil, as well as microbiological parameters (Zaid et al., 2014). Metribuzin is a herbicide used in the production of potato, soybean, tomato and other crops. Metribuzin is a member of the substituted as-triazinone group of herbicides. Its activity is due to an interference with photosystem II electron transport in plant chloroplasts. This herbicide can affect the balance of microbiological parameters which crucially ensure the fertility and ecosystems of soil (Šantrić et al., 2016).

The objective of this paper was to assess the effects of different rates of metribuzin application on certain microbiological variables (number of proteolytic microorganisms and protease activity). Another goal was to compare herbicide effects on the microbial activity in soils with different physical and chemical properties.

MATERIALS AND METHODS

The herbicide metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one) tested in the experiment was the product Sencor, manufactured by Bayer, and its rates of application were: 12.0, 24.0, 120.0 and 1200.0 mg kg^{-1}soil. The lowest tested concentrations equaled the recommended rates, while the other three were double, 10-fold and 100-fold higher than the recommended rates. The two largest concentrations (10-fold and 100-fold) have been used to assess the potential hazard for microorganisms in soil during undesirable events, such as spilling of pesticides (in this case a herbicide) in high quantities into soil, which may occur through damage of devices used for their application, as well as in transport or industrial accidents (Cycoń & Piotrowska-Seget, 2015).

The laboratory experiment was carried out in two agricultural soils. The loamy soil (from Zemun Polje location in Belgrade) and sandy soil (Tavankut) chosen for the study had never been treated with pesticides before. 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. According to the WRB (International Union of Soil Sciences, 2015), the former type of soil belongs to the group of chernozems, 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 5 mm mesh, and stored at 4°C. Before using them, the soil samples were air-dried at room temperature for 24 hours. Each herbicide concentration was pipetted on 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 a control. The experiments were conducted in four replications. The pots were kept in a controlled-environment chamber at 20±2°C, 50% air humidity and 12/12 h day/night photoperiod throughtout the experiment. The samples were subsampled for analysis 7, 14 and 30 days after treatment (DAT).

Proteolytic microorganisms were counted by a soil dilution plate technique using agar medium supplemented with gelatin. Inoculated agar plates (three replicates) were incubated at 28°C for 5 days. The counts are presented as the number of microorganisms per 1 gram of dry soil (Jarak & Đurić, 2006). Protease activity was determined by Romeiko's (1969) method. It is based on titrimetric determination of protease activity in gelatin medium. Proteolytic activity of soil was calculated from an applied 4% $FeCl_3$. Protease activity is expressed as the number of gelatin units per 1 g of air dry soil. Ten gelatin units required 0.2 ml of $FeCl_3$.

Data were statistically processed in Statistica 8.0 software. A two-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 AND DISCUSSION

The data presented in Figure 1 show that metribuzin caused no statistically significant changes ($p>0.05$) in the density of proteolytic microorganisms in loamy soil. Their density was minimal (log 4.79) 7 DAT with the highest concentration (1200.0 mg a.i.kg^{-1}soil) and 2.3% lower than the control data. Similarly, proteolytic microorganisms in sandy soil reacted to metribuzin application without a significant change. Their density decreased around 26.7% only 7 DAT with the highest concentration and differences were statistically significant ($p<0.05$) (Figure 1b).

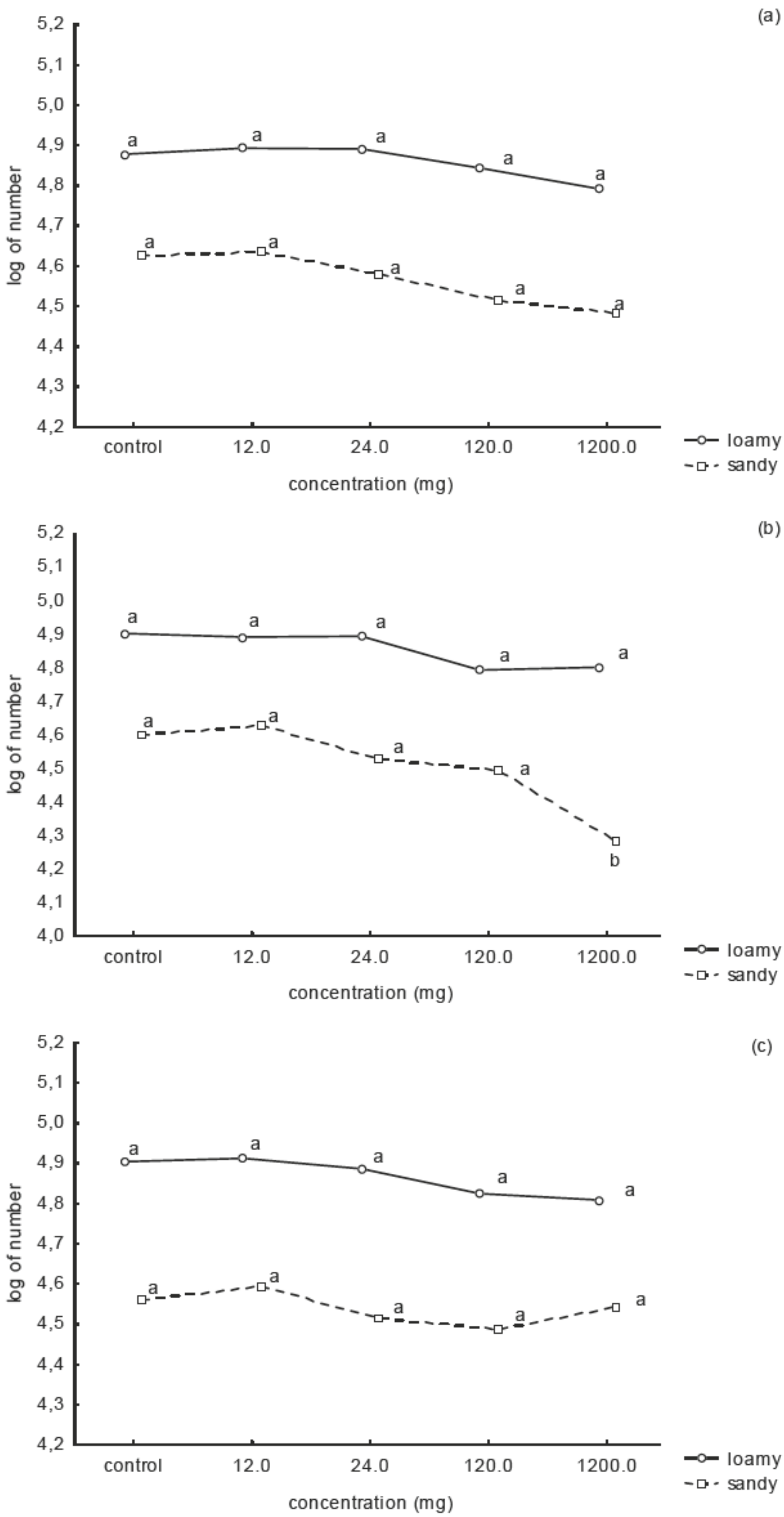

Figure 1. Effects of metribuzin on proteolytic microorganism counts in different types of soil: a) 7 days after treatment; b) 14 days after treatment; c) 30 days after treatment

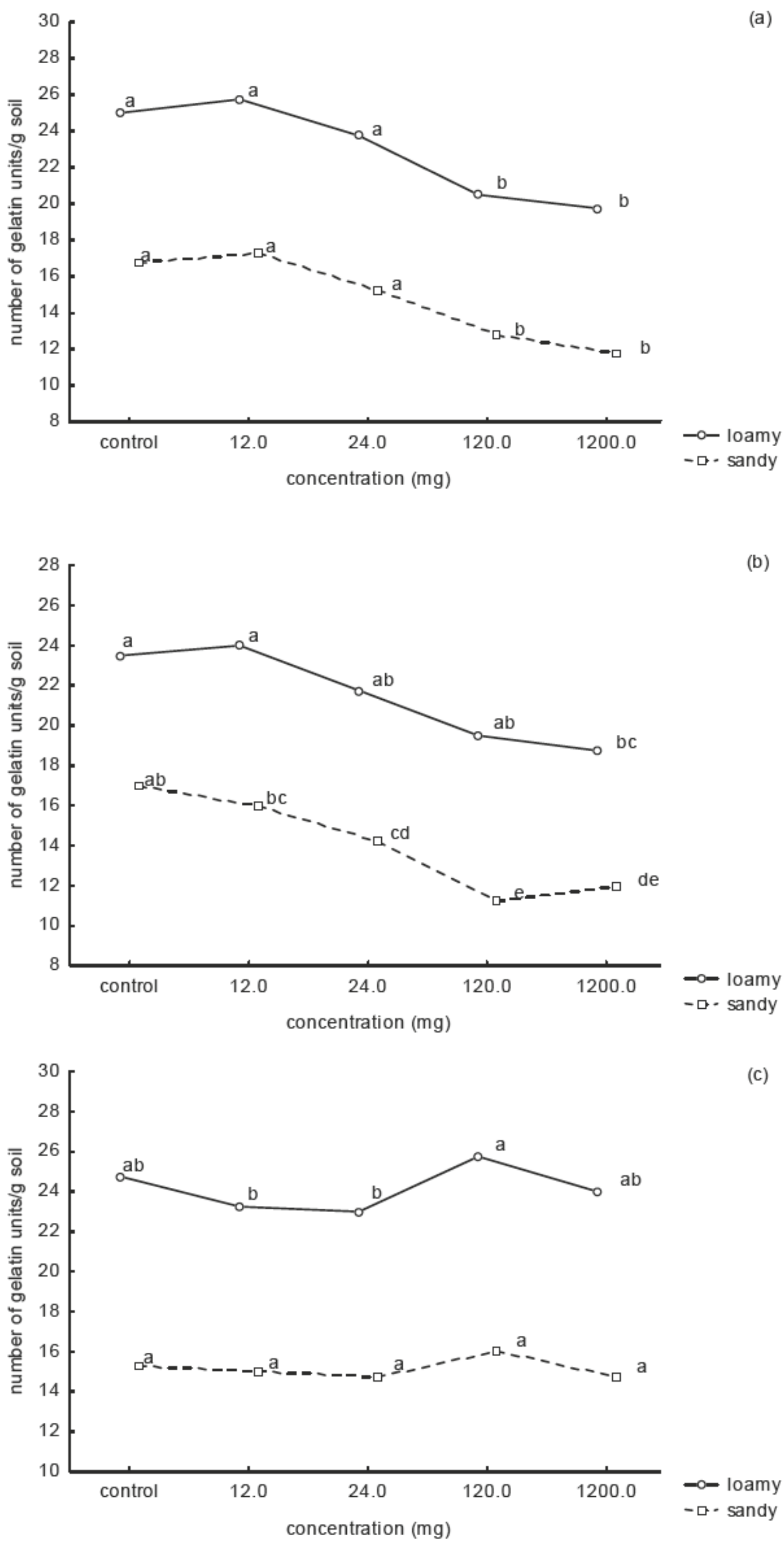

Figure 2. Effects of metribuzin on proteolytic activity in different types of soil:
a) 7 days after treatment; b) 14 days after treatment; c) 30 days after treatment

However, metribuzin affected the activity of protease both in loamy and sandy soils (Figure 2). A significant decrease in the activity of that enzyme was detected 7 and 14 DAT ($p<0.05$) in both types of soil. In loamy soil 7 days after metribuzin application (120.0 and 1200.0 mg a.i. kg^{-1}soil), proteolytic activity was minimal (19.75 gelatin units/g soil), i.e. 21.0% lower than the control data. Also, these concentrations decreased the activity of protease in sandy soil by 29.9% and the minimal value was 11.75 gelatin units/g soil (Figure 2a).

The data in Figure 2b show that metribuzin (1200 mg a.i.kg^{-1}soil) significantly decreased ($p<0.05$) proteolytic activity in loamy soil 14 DAT. The minimum recorded value (18.75 gelatin units/g soil) was 20.2% lower than the control. In sandy soil, three concentrations (24, 120 and 1200 mg a.i.kg^{-1}soil) significantly decreased the activity of protease (33.8%). The lowest recorded value of proteolytic activity in that soil was 11.25 gelatin units/g soil. The results presented in Figures 1 and 2 showed that proteolytic activity is a more sensitive parameter than counts of proteolytic microorganisms after metribuzin application.

Several studies have shown so far that enzymes are more sensitive parameters than microbial density for detecting changes in microbial populations (Weaver et al., 2004; Abbas et al., 2015). Those research studies noted an inactivation of most soil enzymes due to herbicide attachment to the active site of enzymes, thus preventing substrate attachment to the enzymes. Beilińska and Pranagal (2007) also reported a negative effect of triazine herbicides on the activity of enzymes catalyzing the most essential processes of soil organic matter transformation. This process continues to deepen over the years and is expected to gradually cause humus deficit in soil. However, in a study testing several herbicides (butachlor, pyrozosulfuran, paraquat and glyphosate), Baboo at al. (2013) noticed a trend of increasing protease activity from the 7 th to 28th day of incubation in all treatments. Šantrić et al., (2014) showed that nicosulfuron caused no statistically significant changes in the number of proteolytic microorganisms in loamy and sandy soil. However, Przybulewska and Taborska (2008) reported that the use of triazine herbicides over many successive years had increased the abundance of proteolytic microorganisms resistant to that group of herbicides.

Further statistical analysis (Table 1) revealed a very significant difference in counts of proteolytic microorganisms between the test soils ($p<0.01$). However, the results showed that the effects of different concentrations of metribuzin on proteolytic microorganism counts were significant only 14 DAT. Statistical analysis also revealed a very significant difference in protease activity between the tested soils ($p<0.01$). The effects of different metribuzin concentrations on proteolytic activity were very significant ($p<0.01$) 7 and 14 DAT. There were no statistical differences 30 DAT. The influence that soil texture has both on microbial function and microbial community composition has been examined by many researchers. Soil texture has been shown to be a major contributor to differences in microbial activity and microbial community composition (Banks et al., 2014; Girvan et al., 2003).

Correlations between microbial groups and soil enzyme activity may provide information about how microorganism community composition influences the production of enzymes and cycling of specific nutrients. In the present study (Figure 3 and 4), protease activity was positively correlated with proteolytic microorganisms in loamy soil ($r^2=1$; $p<0.05$) and in sandy soil ($r^2=0.81$; $p<0.05$). The results revealed that proteolytic activity could be used as an indicator of the status of soil bacterial community, and show that proteases in the test soils were mainly produced by soil bacteria. Also, some

Table 1. Two-way ANOVA test revealing the level of significance of interactions between soils and treatments

	Proteolityc microorgansms				Protease activity			
days	F_{soils}	p	$F_{treatment}$	p	F_{soils}	p	$F_{treatment}$	p
7	35.66	0.0000	0.97	0.4373	341.90	0.0000	26.55	0.0000
14	66.30	0.0000	3.59	0.0166	225.04	0.0000	18.61	0.0000
30	56.83	0.0000	0.67	0.6181	376.74	0.0000	2.39	0.0724

Non-significant ($p>0.05$);* ($0.01<p<0.05$); ** ($p<0.01$)

researchers have proposed that protease activity depends on the distribution of proteolytic microorganisms and NH_4-N accumulation in soil organic matter (Sardans et al., 2008; Baboo et al., 2013). Therefore, examination of soil microorganisms and enzymatic activity is important for understanding the effects of management practices on soil quality, and enables the development of sustainable agricultural practices (Sun et al., 2018)

In this study, the effects of metribuzin on the examined microbial parameters were not great and mostly depended on soil properties and herbicide concentration. Also, the parameter of proteolytic activity was more sensitive than the density of proteolytic microorganisms.

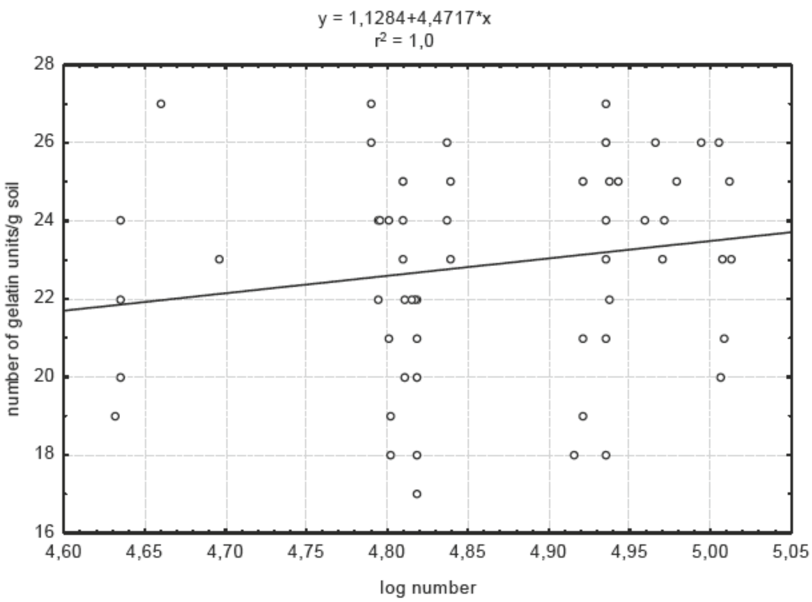

Figure 3. Relationship between protease activity (number of gelatin units/g soil) and density of proteolytic microorganisms (log number) in loamy soil

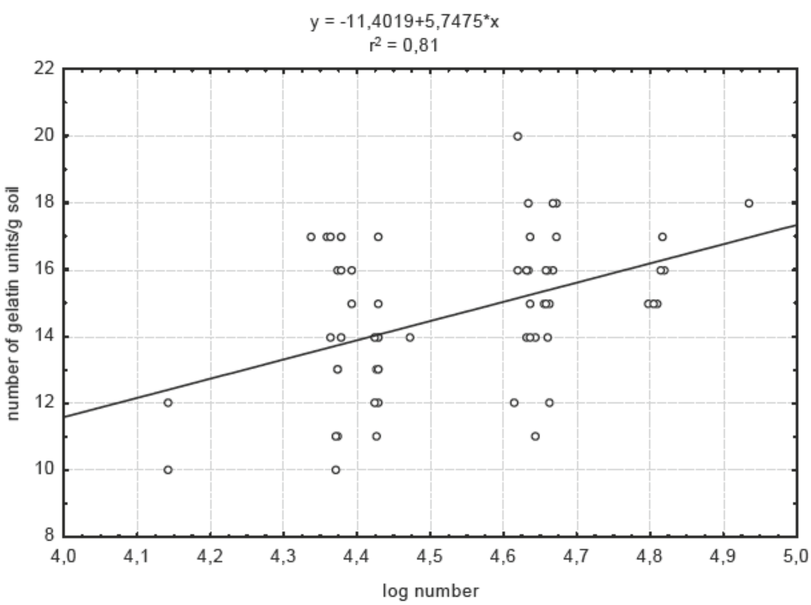

Figure 4. Relationship between protease activity (number of gelatin units/g soil) and density of proteolytic microorganisms (log number) in sandy soil

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Efekat metribuzina na broj proteolitičkih mikroorganizama i proteolitičku aktivnost različitih tipova zemljišta

REZIME

Tekstura zemljišta i druge fizičke i hemijske karakteristike zemljišta predstavljaju važne faktore koji utiču na zadržavanje herbicida u zemljišnim ekosistemima. Postavljen je laboratorijski ogled da bi se procenio odgovor proteolitičkih mikroorganizama i aktivnosti proteaze na primenu metribuzina u različitim tipovima zemljišta (ilovača i peskuša). Ispitivane su sledeće koncentracije. 12,0, 24,0; 120,0 i 1200,0 mg a.s. kg^{-1}zemljišta. Uzorci su uzeti 7, 14 i 30 dana nakon primene metribuzina.

Metribuzin nije uticao na broj proteolitičkih mikroorganizama u ilovači. U peskuši broj je smanjen za 26,7% sedam dana nakon primene najveće koncentracije. Aktivnost proteaze bila je smanjena u oba tipa zemljišta sedmog i četrnaestog dana, u ilovači procenat smanjenja je iznosio 21% a u peskuši 29,9%. Statistička analiza podataka je pokazala da je korelacioni odnos između ispitivanih parametara bio pozitivan za oba tipa zemljišta (za ilovaču r^2=1; za peskušu r^2=0,81). Ispitivanja su pokazala da je metribuzin imao prolazni uticaj na mikrobiološku populaciju i enzimatsku aktivnost i zavisio je od tipa zemljišta.

Ključne reči: Metribuzin; Zemljište; Proteolitički mikroorganizmi; Proteaza