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Assessment of microbial diversity of soil exposed to nicosulfuron

ABSTRACT

Nicosulfuron belongs to the sulfonylurea pesticides, which are widely used for weeds control. Except of benefits in plant production, long-term application of nicosulfuron may have toxic effect for living organisms, including microorganisms. The aim of this paper was to determined impact of nicosulfuron on microbial diversity of soil. Sampling of soil (0-20 and 20-40 cm) treated with nicosulfuron at village Trenica (Novi Travnik municipality, Bosnia and Herzegovina) was performed in autumn 2017. Determination of microbial diversity (total number of bacteria, ammonification bacteria, fungi and actinomycetes) was performed using standard methodology, whilst nicosulfuron-tolerant bacteria were isolated using enrichment method. Soil without nicosulfuron application was used as a control.

The results showed that bacteria were most abundant microbial population. In all experiments, reduction of microbial diversity in nicosulfuron-treated soil compared to untreated was observed. This reduction was most expressed in fungal number, which is reduced from 38 to 60% compared to control.

Several nicosulfuron-tolerant isolates were isolated by enrichment method. By microscopic observation and using API test kits and APIWEB database, isolates 17cs, and 22wl and 5 wl were identified as Pseudomonas fluorescens, and Bacillus subtilis, respectively. These bacterial isolates could be applied in remediation of environments polluted by nicosulfuron.

Keywords: *microbial diversity, nicosulfuron, Bacillus, Pseudomonas.*

1. INTRODUCTION

Nicosulfuron is one of the sulfonylurea herbicides that is widely used to control weeds, especially in corn cultivation [1,2]. This sulfonylurea group is characterized by good selectivity and control of wide spectrum of weeds in agricultural production [3]. Because of huge amount of its widespread use in agriculture and hazard potential [4], as well as high mobility [5], nicosulfuron may easily cause the environmental pollution [6]. Several recent studies showed the toxicity of nicosulfuron on living organisms [7,8], due to changes in peptide structure and protein denaturation [9].

Except of impact on environmental function and soil health [10], pesticides affect the soil biodiversity [11], where microorganisms plays a key role. Environmental pollution may change the microbial abundance in soil [12]. In general, organic contaminants are responsible for reduction of microbial prevalence in soil [13]; however, some microbial populations are capable of using the organic pollutants as unique carbon and energy sources [14]. According to Šantrić et al. [15], the influence of pesticides on microbial diversity of soil depends on various factors, such as pesticide characteristics, soil properties, environmental conditions etc. Nevertheless, studies about the long-term impact of pesticides on microbial diversity of soil are comparatively rare. Thus, the aim of this paper was to assess the microbial diversity of soil after long-term application of nicosulfuron.

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2. MATERIAL AND METHODS

This research was conducted in village Trenica (Novi Travnik municipality, Bosnia and Herzegovina) in autumn 2017. Sampling of surface (0-20 cm) and subsurface (20-40 cm) layers was performed from soil. In five successive vegetation seasons, cultivation of corn was performed on this experimental field. Every year, treatments of nicosulfuron were applied in order to inhibit the growth of weeds.

Several soil samples were taken from experimental field, from which composite sample was obtained. Similar procedure was used for sampling of control sample (without nicosulfuron application). The samples were stored at 4ºC until determination of microbial diversity.

Microbial diversity of soil was determined using standard methodology. Total number of bacteria was detected using 0.1xTSA (Torlak, Serbia), ammonification bacteria on nutrient agar (Torlak, Serbia) fungal number on Rose bengal streptomycin agar [16], and actinomycetes on starch-ammonia agar. Number of microorganisms was expressed as colony forming units (CFU) per g of absolutely dry soil sample.

Nicosulfuron-tolerant bacteria were isolated using modification of enrichment method [17], where nicosulfuron, in concentrations of 0.4 and 1%, was used as sole carbon and energy source. Isolated bacterial strains, after purification, were morphologically characterized using microscopic method (Leica, Germany). Identification of these strains was performed by API and APIWEB technique (BioMerieux, France).

3. RESULTS AND DISCUSSION

Microbial diversity in soil was influenced by long-term nicosulfuron treatment. Abundance of microorganisms depends on soil depth and pesticide application (table 1).

Table 1. Microbial prevalence in surface soil layer (0-20 cm)

Tabela 1. Zastupljenost mikroorganizama u površinskom sloju zemljišta (0-20 cm)

Sample	Total number of bacteria	Ammonification bacteria	Fungi	Actinomycetes
	(x 10^5 CFU/g)		(x 10^3 CFU/g)	
Treated soil	17.4	9.2	34.3	18.2
control	22.7	11.8	55.1	28.3

As can be seen form table 1, bacteria were most dominant microbial population. Long-term effects of nicosulfuron treatment, taking into account the microbial diversity, were obvious in our research; in control, higher prevalence of microorganisms were detected compared to soil treated with nicosulfuron. Abundance of all experimental groups of microorganisms decreased due to application of nicosulfuron.

Quantitative changes in microbial prevalence in subsurface layer (20-40 cm) were also recorded. All microorganisms showed an inhibition in soil treated with nicosulfuron compared to control (Table 2). Compared to surface layer (0-20 cm), the microbial abundance was lower in subsurface layer. In all experiments, highest number of microorganisms was detected for total number of bacteria, whilst actinomycetes presence was lower compared to other groups of microorganisms. This observation was confirmed by other authors [18], who suggest that bacterial growth is stimulated by high organic matter content, aeration and fertility presented in surface layer of soil. Bacteria are involved in various enzymatic transformation, which support their growth in agricultural soils.

Table 2. Microbial prevalence in subsurface soil layer (20-40 cm)

Tabela 2. Zastupljenost mikroorganizama u podpovršinskom sloju zemljišta (20-40 cm)

Sample	Total number of bacteria	Ammonification bacteria	Fungi	Actinomycetes
	(x 10^5 CFU/g)		(x 10^3 CFU/g)	
Treated soil	10.3	7.1	20.0	12.2
control	12.7	9.2	49.4	20.5

Microorganisms play an important role in soil ecosystems. With metabolic activity, they contribute to the nutrient cycling and transformation of organic matter in soil, i.e. into ecosystem functioning [19]. Soil microorganisms are most abundant in surface soil layer, which is confirmed in our study. Eilers et al. [20] found that variability of microbial diversity was highly expressed in soil surface compared to depth layers.

Several recent studies have addresses various impact of pesticides on microbial activity of soil [21,22]. Treatment of pesticides may kill various groups of microorganisms [23]. Many researches have shown decrease in soil microbial activity [3] after application of nicosulfuron, which is in accordance with our observations. In addition, microbial prevalence in soil is linked with pesticide characteristics, such as toxicity, concentration, and availability for living organisms [24].

Fungi were more sensitive to application of nicosulfuron compared to bacteria. Decrease of fungal abundance was 38% in surface layer and about 60% in subsurface compared to control. This detrimental effect could be explained by inhibitory effect of pesticide on acetohydroxyacid synthase gene found in most of fungal species [25].

By enrichment method, several nicosulfuron-tolerant bacterial isolates on nutrient agar were observed. In surface layer of soil, two colony morphologies were recorded: creamish small (isolate 17cs) and white larger (isolate 22wl) colonies. In subsurface layer one white large colony (isolate 5wl) were noticed. By microscopic observation, creamish small colony belongs to Gram-negative, nonspore-forming rods, which indicates the possible similarity with *Pseudomonas* sp. White large colonies found in surface and subsurface layer are morphologically similar to *Bacillus* sp., due to Gram-positive staining and spore-forming rods. Using API test kits and APIWEB database, isolate 17cs showed maximal similarity with *Pseudomonas fluorescens*, whilst both other isolates showed similarity with *Bacillus subtilis*.

Pseudomonas' capability in tolerance and removal of organic pollutants is well documented [26,27]. Several researches have addressed the potential of *Pseudomonas* sp. strains in removal of nicosulfuron. Zhao et al. [28] found that *Pseudomonas nitroreducens* NSA02 was able to tolerate the nicosulfuron concentration of 600 mg/L and degrade this pesticide. In first 24 h of incubation, *P. fluorescens* SG-1 was able to reduce 77.5% of nicosulfuron [29]. On the other hand, tolerance of *Bacillus* sp. on nicosulfuron and degradation capability of *Bacillus* sp. strains were was observed in several researches. Petric et al. [30] showed that using of this pesticide may affect the selection of nicosulfuron-tolerant bacteria. *Bacillus subtilis* YB1 showed high degradation rate (87.9 to 98.8%) of nicosulfuron [31]. *Bacillus sp.* strains were also responsible for degradation of other sulfonyl-urea pesticides, such as tribenuron methyl [32].

4. CONCLUSION

Our results showed that long-term application of nicosulfuron has detrimental impact on microbial diversity of soil. However, several bacterial strains showed capability of tolerance on nicosulfuron and could serve promising strains in removal of nicosulfuron and improvement of environmental quality. Further research will be focused on practical application of isolated strains in nicosulfuron-polluted environments.

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IZVOD

ISPITIVANJE MIKROBNOG DIVERZITETA ZEMLJIŠTA TRETIRANOG NIKOSULFURONOM

Nikosulfuron pripada grupi pesticide sulfonil urea, koji imaju široku primenu u uništavanju korova. Iako je njegova primena korisna sa aspekta uspešne biljne proizvodnje, njegova višegodišnja upotreba može imati toksične efekte za živi svet, uključujući i mikroorganizme. Cilj ovog rada bio je ispitivanje uticaja nikosulfurona na mikrobni diverzitet zemljišta. Uzorkovanje zemljišta (0-20 i 20-40 cm) tretiranog nikosulfuronom na području sela Trenica (Opština Novi Travnik, Bosna i Hercegovina) obavljeno je u jesen 2017. godine. Određivanje mikrobnog diverziteta (ukupnog broja bakterija, amonifikatora, gljiva I aktinomiceta) izvršeno je korišćenjem standardnih metoda, dok su bakterije tolerantne na prisustvo nikosulfurona određene metodom obogaćenja. Kontrolu je predstavljalo zemljište koje nije tretirano nikosulfuronom.

Rezultati ukazuju da su bakterije bile najbrojnija populacija mikroorganizama. U svim varijantama ogleda konstatovana je redukcija mikrobnog diverziteta u zemljištu koje je tretirano nikosulfuronom u odnosu na kontrolni uzorak. Stepen redukcije bio je najveći kod gljiva, čija je brojnost redukovana za 38-60% u odnosu na kontrolu.

Nekoliko izolata bakterija tolerantnih na prisustvo nikosulfurona je izolovano iz uzoraka zemljišta metodom obogaćenja. Mikroskopskim ispitivanjem I pomoću API i APIWEB metode, izolati 17cs, odnosno 22wl i 5wl, su identifikovani kao Pseudomonas fluorescens, odnosno Bacillus subtilis. Ovi bakterijski izolati bi mogli da imaju potencijalnu primenu u remedijaciji ekosistema kontaminiranih nikosulfuronom.

Ključne reči: *mikrobni diverzitet, nikosulfuron, Bacillus, Pseudomonas.*

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