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Jelena B. MARINKOVIĆ[1*], *Ivan P. ŠUŠNICA*[2], *Dragana Đ. BJELIĆ*[1], *Branislava B. TINTOR*[1], *Mirjana A. VASIĆ*[1]

[1] Institute of Field and Vegetable Crops,
Maksima Gorkog 30, 21000 Novi Sad, Serbia
[2] University of Novi Sad, Faculty of Sciences – Department of Biology and Ecology,
Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

SOIL MICROBIAL ACTIVITY UNDER CONVENTIONAL AND ORGANIC PRODUCTION OF BEAN AND MAIZE

ABSTRACT: The objective of this study was to compare the effects of conventional and organic production system on microbial activity in the soil cultivated with bean and maize crops. The trial in Đurđevo was set up according to the conventional farming system, while organic farming system was used in Futog. Two maize hybrids and two bean cultivars were used in the trial. Soil samples were collected in two periods during 2014 (before sowing, at flowering stage of bean crops, and at 9–11 leaf stage of maize) at two depths, at both locations. The following microbiological parameters were tested: the total number of microorganisms, number of ammonifiers, *Azotobacter* sp., free nitrogen fixing bacteria, fungi, actinomycetes, and activity of dehydrogenase enzyme. The results showed that the total number of microorganisms, number of free N-fixers and dehydrogenase activity were higher within organic production, while *Azotobacter* sp. was more abundant in conventional production. Variations in the number of ammonifiers, fungi and actinomycetes in relation to the type of production were not obtained. Significant differences in microbial activity were also obtained between period and depths of sampling.

KEYWORDS: bean, conventional and organic production, dehydrogenase activity, maize, microbial abundance

INTRODUCTION

Microorganisms account for 0.1 to 3.0% of total soil organic matter, and their biomass in soil ranges from 1 to 5 t ha^{-1} on average. Microorganisms play the key role in the mineralization of organic compounds to inorganic and mobilization of less soluble inorganic compounds in the soil, thus providing plants with nutrients. In addition to the mineralization and nutrient cycling, soil microorganisms

* Corresponding author E-mail: jelena.marinkovic@nsseme.com

are also involved in other important ecosystem functions, such as the formation and preservation of soil structure [Bloem and Breure 2003].

Routine monitoring of soil from the biological aspect was launched in several countries in the early nineties. One or more microbiological parameters were included in these monitoring programs in most countries [Stenberg 1999]. Today, due to the increasing pollution, the European Union and many countries around the world are working on introducing laws that would impose microbiological monitoring of soil as an obligation for the most effective protection of the environment [Bloem *et al*., 2006].

For each soil type there are characteristic communities of microorganisms with a specific number and proportion of different physiological groups [Marinković *et al*., 2007]. Cultivation practices lead to the disturbance of these relationships, which is manifested by a reduced number and enzymatic activity of microorganisms, especially because modern agricultural production involves the use of large amounts of pesticides and fertilizers [Đurić *et al*., 2006].

Information about the general microbiological activity, potential soil fertility and general causes of a certain condition of soil can be obtained by determining the presence of certain systematic and physiological groups of microorganisms, the abundance of some genera and species as well as the activity of microbial enzymes [Milošević 2008].

The objective of this study was to compare the effects of conventional and organic production system on microbial number and dehydrogenase activity in the soil cultivated with bean and maize crops.

MATERIALS AND METHODS

Field trials were set up during 2014 at production plots in Futog and Đurđevo. The trial in Đurđevo was set up according to the conventional farming system, while organic farming system was used in Futog. Trials at both localities were set on chernozem soil using a randomized block design with three replications. Two maize hybrids and two bean cultivars (Institute of Field and Vegetable Crops, Novi Sad) were used for the trial. Maize hybrid NS 444 and bean cultivar "Dvadesetica" were used in Futog, while maize hybrid NS 609B and bean cultivar "Maksa" were used in Đurđevo. Sowing was conducted during the optimal sowing period, using all the necessary cultivation practices. Bean seeds were inoculated with *NS-Nitragin* for beans and string beans (Institute of Field and Vegetable Crops, Novi Sad).

Soil microbial properties were determined according to the number of different systematic and physiological groups of microorganisms and the activity of the enzyme dehydrogenase (EC 1.1.1.). At both localities, soil samples for microbial analyses were taken before sowing (March) and once during the vegetation period – at the stage of flowering in beans and at the 9–11 leaf stage in maize. Soil samples were taken from two depths, 0–30 cm and 30–60 cm. Before sowing, the main soil chemical properties were determined at both locations.

The number of microorganisms was determined using the method of agar plates on a suitable nutrient medium, while soil suspension was prepared using a dilution series. The total number of microorganisms was determined on an agarised soil extract, and the number of ammonifiers on the meat-peptone agar (MPA) [Pochon and Tardieux 1962]. The presence of free N-fixers was determined on a N-free agar, and "fertile drops" method was used for the number of *Azotobacter* sp. [Anderson 1965]. The number of actinomycetes was determined on synthetic agar [Krasilnikov 1965], and the number of fungi on Czapek-Dox agar. Incubation temperature was 28 °C, while incubation time depended on the tested group of microorganisms [Jarak and Đurić 2006]. All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1.0 g of absolute dry soil.

Dehydrogenase activity was determined using the spectrophotometric method according to the standard [Casida *et al*., 1964], which is based on measuring the extinction of triphenyl formazan (TPF) created by the reduction of TTC (2,3,5-triphenyltetrazolium chloride).

The data were analyzed in accordance with three–way model of analysis of variance (ANOVA) using Statistica software (StatSoft Inc. 2012), followed by mean separation according to Fisher's LSD test.

RESULTS AND DISCUSSION

The chemical soil properties of experimental fields are presented in Table 1. According to pH reaction of soil solution, soils from both localities can be placed into the group of slightly alkaline soils. Soil at Đurđevo locality is humic and contains an optimal supply of easily accessible phosphorus, while the content of easily accessible potassium is high. Soil at Futog locality is slightly humic, poorly supplied with easily accessible phosphorus, and supplied with an optimal level of easily accessible potassium.

Table 1. Soil chemical properties

Experimental Field	pH		$CaCO_3$ %	Humus %	Total N %	AL-P_2O_5 mg/100g	AL-K_2O mg/100g
	in KCl	in H_2O					
Đurđevo	7.26	8.07	1.51	3.14	0.215	24.8	48.0
Futog	7.48	8.31	6.78	2.08	0.155	6.7	21.8

Microorganisms are one of the indicators of the overall soil biogeny since they are actively involved in the processes of transformation of organic matter, assimilation of mineral elements, and formation of humus [Đukić *et al*., 2003]. Some microbial groups can be used as indicators of soil fertility because of their great sensitivity to changes of nutrient concentration in soil solution, water content, etc. [Jarak *et al*., 2010].

The largest parts of soil enzymes have a microbial origin. Their activity is primarily related to catalysis of the reaction of synthesis and mineralization of organic matter, which can be used as a valid indication of soil fertility. Since dehydrogenases are constitutive enzymes of most microorganisms, a general assessment of microbial activity in soil can be given on the basis of dehydrogenase activity [Jarak and Đurić 2006].

In these trials, the number of microorganisms and dehydrogenase activity depended on the farming system, period and depth of soil from which the samples were taken. Soil samples from deeper soil layers had a lower number of microorganisms of the tested microbial groups and a weaker dehydrogenase activity in both sampling periods (Tables 2 and 3). Numerous previous studies [Govedarica *et al.*, 2000; Tintor *et al.*, 2007; Marinković *et al.*, 2008; Milošević *et al.*, 2010] have confirmed that the number of microorganisms decreases with the depth of sampling, which is in accordance with the obtained results. The same conclusion was reached by Samuel *et al.* [2008]. Microbial activity is higher at the soil surface (0–30cm) which contains more organic matter, as well as enough moisture and oxygen. Aerobic microorganisms are most commonly found, and their activity is the most significant to agriculture [Jarak and Čolo 2007]. Deeper soil layers have less favourable ecological conditions, which results in the lower number and weaker enzymatic activity of microorganisms.

The number of microorganisms and microbial activity are seasonal in our climatic region. They are the highest in spring and early autumn, when soil moisture is at a suitable level, and when temperatures range between 20–30 ºC. High temperatures and low soil moisture level during summer, as well as low temperatures during winter, cause decrease in the number of microorganisms and microbial activity.

Sampling period significantly affected the number of microorganisms within the studied groups, and enzymatic activity in soil. Favourable climatic factors during second sampling period caused the increase in the number of microorganisms within the studied groups, while greatest differences were observed in the system of organic farming at the soil surface (0–30 cm). A higher number of ammonifiers, azotobacters, free nitrogen fixers, actynomicetes, and the total number of microorganisms, was determined in soils under bean and maize crops, whereas the number of fungi was not significantly changed. Dehydrogenase activity was significantly higher in second sampling period, in both conventional and organic production, when compared with the period before sowing (Tables 2 and 3).

In the period before sowing, the total number of microorganisms and the number *Azotobacter* sp., free N-fixing bacteria and actinomycetes was higher at the surface of soil under conventional production compared with the organic production system. However, dehydrogenase enzymatic activity at the soil surface layer was significantly higher under organic production system (Tables 2 and 3).

Table 2. Number of microorganisms and dehydrogenase enzymatic activity in the soil cultivated with bean crops at the flowering stage

Production system		Microbial group (CFU ml^{-1} g^{-1} absolutely dry soil)						Dehydrogenase activity (µg TPF g^{-1} soil 24 h^{-1})
		Total microbial number x 10^7	Ammonifiers x 10^6	*Azotobacter* x 10^2	Free N-fixers x 10^6	Fungi x 10^6	Actinomycetes x 10^4	
Before sowing								
Conventional	0–30 cm	92^{b}	42^{abc}	99^{cd}	80^{b}	17^{bc}	10^{a}	306^{cd}
	30–60 cm	47^{c}	31^{cde}	62^{e}	33^{cd}	7^{e}	1^{cd}	151^{d}
Organic	0–30 cm	43^{cd}	36^{bcd}	68^{e}	48^{c}	20^{ab}	6^{b}	717^{b}
	30–60 cm	22^{d}	23^{de}	38^{e}	19^{d}	11^{cde}	0^{d}	253^{cd}
During the vegetation period								
Conventional	0–30 cm	51^{c}	53^{ab}	173^{a}	46^{c}	16^{bcd}	11^{a}	675^{b}
	30–60 cm	44^{c}	36^{cd}	149^{ab}	28^{cd}	9^{cde}	2^{cd}	235^{cd}
Organic	0–30 cm	217^{a}	56^{a}	127^{bc}	224^{a}	26^{a}	11^{a}	914^{a}
	30–60 cm	104^{b}	19^{e}	62^{e}	78^{b}	8^{de}	3^{bc}	313^{c}

The different letter above the number indicates a significant difference at $P < 0.05$

Table 3. Number of microorganisms and dehydrogenase enzymatic activity in the soil cultivated with maize crops at the 9–11 leaf stage

Production system		Microbial group (CFU ml^{-1} g^{-1} absolutely dry soil)						Dehydrogenase activity (µg TPF g^{-1} soil 24 h^{-1})
		Total microbial number x 10^7	Ammonifiers x 10^6	*Azotobacter* x 10^2	Free N-fixers x 10^6	Fungi x 10^6	Actinomycetes x 10^4	
Before sowing								
Conventional	0–30 cm	92^{b}	42^{b}	99^{c}	80^{bc}	17^{bc}	10^{a}	306^{c}
	30–60 cm	47^{c}	31^{bcd}	62^{d}	33^{de}	7^{d}	1^{c}	151^{d}
Organic	0–30 cm	43^{cd}	36^{bc}	68^{d}	48^{de}	20^{ab}	6^{b}	717^{a}
	30–60 cm	22^{d}	23^{d}	38^{e}	19^{e}	11^{cd}	0^{c}	253^{c}
During the vegetation period								
Conventional	0–30 cm	85^{b}	54^{a}	185^{a}	57^{cd}	26^{a}	12^{a}	483^{b}
	30–60 cm	41^{cd}	23^{d}	163^{ab}	30^{de}	14^{bcd}	3^{bc}	162^{d}
Organic	0–30 cm	206^{a}	65^{a}	156^{b}	158^{a}	13^{bcd}	11^{a}	690^{a}
	30–60 cm	107^{b}	28^{cd}	79^{cd}	98^{b}	8^{d}	2^{c}	308^{c}

The different letter above the number indicates a significant difference at $P < 0.05$

The total number of microorganisms and free nitrogen fixers was significantly higher at both surface and deeper layers of soil cultivated with bean and maize plants, under organic production. However, the number of *Azotobacter* was significantly higher under conventional production of both plant species, as well as the number of fungi in soils cultivated with maize plans. Number of ammonifiers and actinomycetes did not vary enough to produce a statistically significant change between the two production systems (Tables 2 and 3).

Similar results were obtained by Mrkovački *et al.* [2012], who determined a significantly higher number of microorganisms under organic production system compared with conventional. Significant differences in microbial abundance between plant species, growing systems and sampling periods were also obtained by Bjelić *et al.* [2015]. Opposite results were obtained in the research of Perez-Brandan *et al.* [2014], which can be attributed to different agrichemical soil properties, climate, and different production management. A research conducted in Brazil by Bettiol *et al.* [2002] and a research conducted in Vojvodina [Vasin *et al*., 2013] indicated no great variations in the number of microorganisms depending on the production system.

Species of the genus *Azotobacter* are one of the most significant free aerobic nitrogen fixers. Number of *Azotobacter* depends on pH reaction of the environment, organic matter and phosphorus content, and it represents an important indicator of soil fertility. Orr *et al.* [2012] indicate the possibility of higher number of *Azotobacter* under conventional production, especially during the initial phases of the vegetation period, due to higher initial concentrations of phosphorus in mineral P fertilizers. This assumption was confirmed by the agrichemical analyses of soil conducted in Đurđevo and Futog, where plots under the conventional production system had significantly higher phosphorus concentration. A higher number of some groups of microorganisms before sowing, which was exhibited in the research, as well as the increased number of *Azotobacter* sp. in the second sampling period within the conventional production system, can be explained by a higher content of humus, phosphorus, and potassium at this production plot.

Production system proved to have a significant effect on microorganism enzymatic activity in the second sampling period. Dehydrogenase activity ranged from 151 to 914 μg TPF g^{-1} soil, and similar results were obtained by Serra-Wittling *et al*. [1995], and Januszek *et al.* [2007; 2015]. At the soil surface layers, cultivated with bean and maize crops, and deeper soil layers cultivated with maize crops, a significantly higher dehydrogenase activity was detected under organic production system (Tables 2 and 3).

Dehydrogenases are enzymes which transport hydrogen between donor and acceptor in the respiration process, while their origin in soils is mainly microbial. Higher dehydrogenase activity indicates higher respiration intensity or larger microbial activity. Microbial activity in soil can be increased by adding fresh organic matter through the application of organic fertilizers (manure, compost, green manure, etc.), which ultimately leads to higher dehydrogenase activity in soils. Research conducted by Vasin *et al.* [2013] showed that soils under conventional growing system, or those undergoing conversion, have lower dehydrogenase activity compared with organic agriculture.

CONCLUSION

Differences in microbial activity between production systems were recorded for the total number of microorganisms, number of free N-fixers, *Azotobacter* sp. and dehydrogenase activity. The number of microorganisms as well as dehydrogenase activity significantly decreased with the increase of soil depth. The increase of the total number of microorganisms, number of free N-fixers and dehydrogenase activity in soil under two different crops grown within organic production confirm the positive effect of this agricultural practice on microbial activity and biological health of soil compared with conventional management.

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МИКРОБИОЛОШКА АКТИВНОСТ ЗЕМЉИШТА У КОНВЕНЦИОНАЛНОЈ И ОРГАНСКОЈ ПРОИЗВОДЊИ ПАСУЉА И КУКУРУЗА

Јелена Б. МАРИНКОВИЋ[1], Иван П. ШУШНИЦА[2], Драгана Ђ. БЈЕЛИЋ[1], Бранислава Б. ТИНТОР[1], Мирјана А. ВАСИЋ[1]

[1] Институт за ратарство и повртарство
Максима Горког 30, 21000 Нови Сад, Србија
[2] Универзитет у Новом Саду, Природно-математички факултет
Департман за биологију и екологију,
Трг Доситеја Обрадовића 3, 21000, Нови Сад, Србија

РЕЗИМЕ: Циљ ових истраживања био је да се упореде ефекти конвенционалног и органског система гајења на микробиолошку активност у земљишту које је под пасуљем и кукурузом као усевима. Оглед у Ђурђеву постављен је у систему конвенционалне пољопривредне производње, а оглед у Футогу у систему органске производње. У огледима су коришћена два хибрида кукуруза и две сорте пасуља Института за ратарство и повртарство у Новом Саду. Узорци земљишта за микробиолошке анализе узети су током 2014. године (пре сетве и у фази цветања пасуља, као и у фази 9–11 листова кукуруза), са две дубине, на оба локалитета. Микробиолошка активност праћена је на основу заступљености укупног броја микроорганизама, амонификатора, *Azotobacter* sp., слободних азотофиксатора, гљива, актиномицета и активности ензима дехидрогеназе. Резултати су показали да су укупан број микроорганизама, број слободних азотофиксатора и дехидрогеназна активност били већи у систему органске производње, док су врсте из рода *Azotobacter* sp. биле заступљеније у систему конвенционалне производње. Нису забележене разлике у бројности амонификатора, гљива и актиномицета у зависности од система гајења. Такође, значајне разлике у микробиолошкој активности утврђене су између периода и дубине узорковања.

КЉУЧНЕ РЕЧИ: пасуљ, конвенционална и органска производња, дехидрогеназна активност, кукуруз, бројност микроорганизама