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Utjecaj primjene mikrobioloških preparata na odabrana svojstva kiselih tala

Jović, J., Ivezić, V., Popović, B., Guberac, V., Rastija, M., Dujmović, L., Orkić, V., Kristek, S.

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Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

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THE INFLUENCE OF MICROBIAL BIOAGENTS' APPLICATION ON THE SELECTED SOIL PROPERTIES IN ACIDIC SOILS

Jović, J.(1), Ivezić, V.(1), Popović, B.(1), Guberac, V.(1), Rastija, M.(1), Dujmović, L.(2), Orkić, V.(1), Kristek, S.(1)

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SUMMARY

The factors limiting a successful agricultural production worldwide are certainly the availability of phosphorus (P), the soil's organic matter, and a pH reaction in the agricultural soils. The significant amounts of phosphorus are found in the soils, but approximately 95–99% is present as the insoluble phosphates and cannot be utilized by the plants. Therefore, the increasing efforts are invested to activate the large amounts of plant-unavailable phosphorus by improving a crop's P use efficiency through the use of phosphorus-solubilizing microorganisms (PSM). The two primary research objectives were stated, as follows: first, to assess whether the applied microbial bioagent impacts the observed soil properties (pH, organic matter, and the available P level), and second, to assess whether a microbial bioagent applied enhances the microbial biomass phosphorus (MBP). The three independent field trials were set up as a randomized blok design, with the following treatments in four replicates: a control, "C"; a control with a microbial bioagent, "CMA"; a reduced P fertilizer with a microbial bioagent, "PMA"; and a recommended fertilization, "RF." The soil samples were analyzed concerning a pH reaction, plant-available phosphorus, soil organic matter, and the microbial biomass P. A visible increase from 3.45 to 5.02 mg P and from 0.53 to 3.42 mg P due to an applied mineral fertilizer was noticed at two locations. No negative impact from applied bioagents was recorded regarding the analyzed soil properties. Very strong relationships (r = 0.9213 in 2016 and r = 0.9508 in 2017) between the available P and the microbial biomass P were stated. The results indicate a fact that the P input and the application of microbial bioagent together with the content of the plant-available phosphorus in the soil influenced the MBP pool.

Keywords: soil pH value, organic matter, phosphorus, phosphorus-solubilizing microorganisms

INTRODUCTION

Certainly, the availability of phosphorus, soil organic matter (SOM), and a pH reaction in the agricultural soils are among the most important factors limiting a successful agricultural production worldwide. Phosphorus plays a vital role in the maintenance of soil fertility and the security of global food supply by being of a very high importance in plant, human, and animal life (Adhya et al., 2015). Although the significant amounts of phosphorus (200–800 mg kg^{-1}) are found in the soils (Blume et al., 2010), approximately 95–99% is present as the insoluble phosphates and cannot be utilized by the plants (Pradhan and Sukla, 2005). As a result, most of the mined P is continuosly added as fertilizers to the agricultural soils to meet the critical crop needs in agronomic production. However, these amounts of the P added to the soil are mostly higher then the P consumption through the plants, which leads to an accumulation of soil-bound P due to its chemical high-affinity reactions and occlusion with the soil minerals and organic matter (Gatiboni et al., 2020).

This P surplus in the soils represents the legacy P, which can be detected within the soils across the different chemical fractions, typically categorized as a labile, moderately labile, and non-labile phosphorus (Doydora et al.,2020; Mezeli et al., 2020). On the other hand, the SOM represents a complex mixture of plant and animal

(1) Assist. Prof. Jurica Jović (jjovic@fazos.hr), Assoc. Prof. Vladimir Ivezić, Prof. Dr. Brigita Popović, Prof. Dr. Vlado Guberac, Prof. Dr. Mirta Rastija, Vedran Orkić, PhD, Prof. Dr. Suzana Kristek — Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia, (2) Assoc. Prof. Lidija Dujmović — Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31000 Osijek, Croatia

materials in various stages of decomposition, along with the microbes and the substances they synthesize in the soil (Lehmann and Kleber, 2015). Also, it plays a key role in numerous soil functions, contributing to the soil structure, nutrient cycling, and water retention (Janzen, 2006). A soil pH value can be considered as a key link between the numerous soil processes and is an important measure of the soil's capacity to fulfil the environmental and economic functions. The changes in a soil pH value can indirectly impact a myriad of biogeochemical and physical processes occurring in a soil-water-microbe-plant continuum across the native and managed terrestrial and aquatic ecosystems, depending on a soil type and the environmental factors (Rengel, 2011).

The issues mentioned above are the current challenges in agriculture and are expected to become even greater problems in the future. This is especially of a concern as the world is running out of natural phosphorus resources, while a food demand is increasing due to a growing population and climate change. In order to slow down the exploitation of strategic phosphorus resources and reduce the costs of crop production by lowering the phosphorus fertilizer input, increasing efforts are invested to activate the large amounts of plant-unavailable phosphorus in the soils. This is being achieved by improving a crop's P-use efficiency through the use of PSM. The phosphate-solubilizing microorganisms, a large microflora that mediate the bioavailable soil P, play a critical role in the soil by mineralizing the organic P, solubilizing the inorganic P minerals, and storing the large amounts of P in biomass (Tian et al., 2021). It has been reported that the highest number of phosphorus-solubilizing microorganisms (PSM) depend on the different physical and chemical soil properties, including the SOM and the soil P (Seshachala and Tallapragada, 2012). Verma et al. (2024) mentioned how the plant growth-promoting microorganisms (PGPMs) possitively contribute to the nutrient cycling by solubilizing the phosphates and to the soil health by enhancing the soil structure and increasing an organic-matter content. Furthermore, Lopes et al. (2021) stated that the PGPMs are able to increase a pH value in the acid soils by the production of protons during the assimilation of ammonium (NH_4^+) while solubilizing the $AlPO_4$ and the $FePO_4$. According to Wei et al. (2024), functional microorganisms could improve the availability of residual soil P by secreting the phosphatase enzymes and utilizing other various mechanisms. In the literature and research, a greater attention is devoted to how the soil properties affect the microorganisms than how and whether the microbial bioagents affect some agricultural production's crucial limiting factors like the SOM content, P availability, and the soil pH value.

Therefore, two main research objectives were stated: first, to determine whether the application of a microbial bioagent would negatively impact the observed soil properties (pH, organic matter, and the available P level), as any detrimental effects would make its use in agriculture impractical; and second, to assess whether the microbial bioagent could enhance the microbial biomass-phosphorus (MBP) content in the soils with a varying organic-matter content, available P levels, and the pH ranges.

MATERIALS AND METHODS

Field Trial

Three independent field trials were set up in the autumn of 2015 at one location in Croatia (Harkanovci, 45.588901N, 18.309741E, luvisol according to the WRB classification) and two locations in Bosnia and Herzegowina, Osječak (45.090761N, 18.359977E, a stagnic gleysol according to the WRB classification) and Gornja Dubica (45.065222N, 18.383029E, stagnosol). The experiment was set up as a randomized blok design with the following treatments in four replicates: a control without the NP fertilizers and without a microbial bioagent, ; a control without a NP fertilizer but with a microbial bioagent, "CMA"; a reduced P fertilizer with the recommended doses of NK fertilizers containing a microbial bioagent, "PMA"; and a recommended fertilization with the NPK fertilizers, RF." According to a chemical analysis, a recommended fertilization for the winter wheat amounted to 90 kg ha^{-1} K_2O and 72 kg ha^{-1} N for all locations, while the P fertilizer rate was 52 kg ha^{-1} P_2O_5 for Harkanovci and 104 kg ha^{-1} P_2O_5 for the locations in Bosnia and Herzegowina, with the very low rates of plant-available phosphorus. A potassium fertilizer (60% KCl) was added in equal quantities. A source of phosphorus was the monoammonium phosphate (MAP), while the MAP and urea served as a source of nitrogen. In the reduced phosphorus treatments, the quantities of phosphorus fertilizer were reduced by 50% according to a recommended fertilization. The fertilization was performed in the equal amounts in both testing years. A marketed microbial bioagent containing thew lactic-acid bacteria, photosynthetic bacteria, and yeasts was used as a carrier, into which the three *Pseudomonas* strains (*Pseudomonas* rhizosphaerae—DSM 16299, *Pseudomonas* putida—ATCC 12633, and *Pseudomonas* flourescens—ATCC 13525) were added prior to being applied to the ground. The application was executed prior to sowing with a regular sprayer and introduced by a rotary harrow into the upper layer.

Soil Sampling and Chemical Analysis

The soil samples (0–30 cm) for further analysis were taken from each basic plot twice a year (in April and September) during 2016 and 2017. The samples were analyzed concerning the chemical properties such as a pH reaction (ISO 10390), plant-available phosphorus and potassium (by an ammonium-lactate extraction, Egner et al., 1960), and the soil's organic matter (by a sulfochromic oxidation, ISO 14235). The microbial biomass P in the soil was determined via chloroform ($CHCl_3$) fumigation technique (Hedley et al., 1982), wherby a concentration of P in the extracts was determined colorimetrically by Murphy and Riley's phosphomolybdate method (1962). Biomass P in the extracts (i.e., the P fixed by the microorganisms) was calculated from a difference between total labile P ($CHCl_3$) and total labile P.

Total labile P in the extracts was measured subsequent to an aliquot digestion with 2.5 M H_2SO_4 and potassium persulfate ($K_2S_2O_8$), according Bowman's method (1989) , as modified by Thien and Myers (1992).

Weather Charachteristics

The weather conditions of the nearby meteorological stations (Osijek and Gradačac) indicate an increasing trend in the average air temperature and a lack of precipitation in the estival months when compared with the long-term average (1961–90). During 2016, an above-average precipitation distribution was observable in July, which significantly alleviated a lack of moisture in the soil and prevented the occurrence of a long-term drought in the Osijek region and devided the drought period in the Gradačac region into two milder drought occurrences. The high air temperatures and a lack of precipitation marked a long-term drought during the estival months in 2017 in both regions (Table 1).

Table 1. Weather characteristics during the vernal and estival months of the observed period

Tablica 1. Vremenske prilike u proljetnim i ljetnim mjesecima promatranoga razdoblja

Month / *Mjesec*	Location / *Lokalitet Osijek*						Location / *Lokalitet Gradačac*					
	2016		2017		Long-term mean / *Višegodišnji prosjek* (1961 – 1990)		2016		2017		Long-term mean / *Višegodišnji prosjek* (1961 – 1990)	
	T (^oC)	P (mm)	T (^oC)	P (mm)	T (^oC)	P (mm)	T (^oC)	P (mm)	T (^oC)	P (mm)	T (^oC)	P (mm)
May / *svibanj*	16.5	63.1	11.3	50.6	16.5	58.5	16.3	106.9	17.5	96.8	15.9	92.1
June / *lipanj*	21.0	99.5	17.5	45.4	19.5	88.0	21.2	52.3	22.9	50.3	19.0	98.8
July / *srpanj*	22.8	110.8	22.4	64.0	21.0	64.8	22.9	129.7	24.2	34.0	20.9	85.8
August / *kolovoz*	20.6	72.1	23.5	30.0	20.3	58.5	20.8	59.7	25.3	29.4	20.3	73.7
September / *rujan*	18.1	43.0	23.7	80.3	16.6	44.8	19.0	73.2	16.7	88.3	16.9	65.0
Mean (°C) / Total (mm) / *Prosjek (°C) / Ukupno (mm)*	19.8	388.5	19.7	270.3	18.8	314.6	20.0	421.8	21.3	298.8	18.6	415.4

Statistical Data Analysis

The data were statistically processed by the analysis of variance (ANOVA) using the *Microsoft Excel* and *SAS Enterprise Guide 7.1* software, while the correlations were calculated using *Microsoft Excel*. The statistically significant differences between the treatments were determined by the F-test using the LSD values at the level of 0.05.

RESULTS AND DISCUSSION

Soil Organic Matter

The determined values of the organic-matter content at the Gornja Dubica location ranged from 1.64% to 3.58%, at the Harkanovci location from 1.10% to 2.06%, and at the Osječak location from 3.67% to 4.32%. Based on the r esults obtained, the statistically significant differences in the average organic-matter content between the individual locations were established (Table 2).

Locationally, no statistically significant differences were established regarding the organic-matter content between the individual treatments and the sampling period, except at the Harkanovci location, where the statistically significant differences were recorded between the PMA variant and the CMA and RF variants in the first sampling period and between the CMA and RF variants in the third sampling period.

However, a drastic drop in the organic-matter content was recorded between the first and the second soil-sampling period at all three locations. This decrease in the organic-matter content is most probably the result of an analytical laboratory error. It is indicated by a fact that this substantial decrease in the organic-matter content was recorded in all variants between the sampling period I and II, including the control variant in which neither mineral fertilizers nor microbiological bioagents were applied. Therefore, excluding the sampling period I and observing the sampling periods II, III, and IV, it is evident that the applied microbial bioagent and mineral fertilization did not negatively affect the organic-matter content in the soil and that the deviations were minimal (Table 2). Moreover, Verma et al. (2024) listed numerous positive effects of microbiological preparations, among which was a contribution of the plant-growth promoting microorganisms (PGPMs) to an increase in the organic-matter content in the soil.

Table 2. The organic-matter content in the soil samples during 2016 and 2017

Tablica 2. Sadržaj organske tvari u uzorcima tla u 2016. i 2017. godini

Location / *Lokalitet*	**Variant** / *Varijanta*	**Organic-matter content (%) in the soil samples during 2016 (I, II) and 2017 (III, IV)** / *Organska tvar (%) tla tijekom uzorkovanja 2016. (I., II.) i 2017. (III., IV.)*									
		Soil sampling / *Uzorkovanje tla*									
		n	I	n	II	n	III	n	IV	n	Average
Gornja Dubica	C	4	$2.97^{a\,A}$	4	$1.88^{a\,B}$	4	$1.80^{a\,B}$	4	$1.86^{a\,B}$	16	2.13
	CMA	4	$3.06^{a\,A}$	4	$1.94^{a\,B}$	4	$1.72^{a\,B}$	4	$1.73^{a\,B}$	16	2.11
	PMA	4	$3.58^{a\,A}$	4	$1.84^{a\,B}$	4	$1.65^{a\,B}$	4	$1.64^{a\,B}$	16	2.18
	RF	4	$2.81^{a\,A}$	4	$1.88^{a\,B}$	4	$1.69^{a\,B}$	4	$1.83^{a\,B}$	16	2.05
	Average	16	3.10	16	1.88	16	1.72	16	1.77	64	2.12^{β}
Harkanovci	C	4	$1.78^{ab\,A}$	4	$1.29^{a\,B}$	4	$1.16^{ab\,B}$	4	$1.31^{a\,B}$	16	1.39
	CMA	4	$2.06^{a\,A}$	4	$1.28^{a\,B}$	4	$1.10^{b\,B}$	4	$1.13^{a\,B}$	16	1.39
	PMA	4	$1.28^{b\,A}$	4	$1.21^{a\,A}$	4	$1.28^{ab\,A}$	4	$1.19^{a\,A}$	16	1.24
	RF	4	$1.96^{a\,A}$	4	$1.16^{a\,B}$	4	$1.29^{a\,B}$	4	$1.14^{a\,B}$	16	1.39
	Average	16	1.77	16	1.23	16	1.21	16	1.19	64	1.35^{γ}
Osječak	C	4	$4.22^{a\,A}$	4	$3.75^{a\,B}$	4	$3.74^{a\,B}$	4	$3.69^{a\,B}$	16	3.85
	CMA	4	$4.32^{a\,A}$	4	$3.86^{a\,B}$	4	$3.81^{a\,B}$	4	$3.81^{a\,B}$	16	3.95
	PMA	4	$4.28^{a\,A}$	4	3.96^{aAB}	4	$3.79^{a\,B}$	4	$3.90^{a\,AB}$	16	3.98
	RF	4	$4.32^{a\,A}$	4	$3.89^{a\,B}$	4	$3.72^{a\,B}$	4	$3.67^{a\,B}$	16	3.90
	Average	16	4.28	16	3.86	16	3.77	16	3.77	64	3.92^{α}

The lowercase letters indicate the significant differences within the columns, the capital letters indicate the significant differences within the rows, and the Greek letters indicate the significant differences between the locations ($LSD_{0.05}$). / *Mala slova označuju značajne razlike unutar stupaca, velika slova označuju značajne razlike unutar redaka, a grčka slova označuju značajne razlike između lokacija (LSD0.05).*

Plant-Available Phosphorus Content

The available phosphorus values ranged from 0.53 to 20.95 mg P in 100 g soil, depending on a location and treatment. Based on the results obtained, the statistically significant differences in the average available-phosphorus content were determined between the individual locations, where the highest average value was 18.29 mg P in 100 g of soil (Harkanovci location), and the lowest average value was 1.43 mg P in 100 g of soil (I Osječak location), while the average value of available phosphorus at the Gornja Dubica location was 3.50 mg P in 100 g of soil (Table 3).

At the Gornja Dubica location, no statistically significant differences between the individual variants were detected within the sampling periods I, II, and III. Nevertheless, there was a noticeable increase in the available P content from the initial to the final sampling in all observed variants, C (17.8%), CMA (23.5%), PMA (18.6%), and RF (45.5%). Also, the highest increase in the available phosphorus if compared with the control variant was oserved in the RF variant (43.4%) during the sampling period IV. This was most likely due to the use of mineral fertilization in the RF variant.

At the Harkanovci location, the initial contents of plant-available phosphorus were noticeably higher in the variants C and CMA when compared with the variants in which the microbial bioagents and mineral fertilization were applied. This was probably a result of the more suitable pH values with respect to phosphorus availability in these variants (Table 3 and 4). This coincides with the statements of how the availability of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as of the micronutrients, is heavily dependent on the soil pH value and that phosphorus availability peaks at a soil pH value of around 6.5, when it is most soluble and accessible to the plants (O'Kennedy, 2022; Hinsinger, 2001). However, no statistically significant differences were detected at the Harkanovci location regarding the amount of available phosphorus, even though there was an observable increase in all variants between the initial and final sampling, espacially in the variant's RF (20.4%) and PMB (5.7%).

At the Osječak location, no statistically significant differences between the individual variants were detected within the individual sampling periods. However, there was a noticeable increase in the amount of available phosphorus in all variants throughout the observed period. The highest increase in the available phosphorus if compared with the control variant was observed at the sampling period IV in the variant's RF (98.8%) and PMA (68%).

Table 3. Available phosphorus (AL method) in the soil samples during 2016 and 2017

Tablica 3. Pristupačna količina fosfora (AL metoda) u uzorcima tla u 2016. i 2017. godini

Location / *Lokalitet*	**Variant** / *Varijanta*	**Available phosphorus, AL method (mg P in 100 g soil) during 2016 (I, II) and 2017 (III, IV)** / *Pristupačan fosfor, AL metoda (mg P u 100 g tla) tijekom 2016. (I., II.) i 2017. (III., IV.)*									
		Soil sampling / ***Uzorkovanje tla***									
		n	**I**	**n**	**II**	**n**	**III**	**n**	**IV**	**n**	**Average**
Gornja Dubica	**C**	4	$2.97^{a\ A}$	4	$3.16^{a\ A}$	4	$3.60^{a\ A}$	4	$3.50^{b\ A}$	16	3.31
	CMA	4	$2.51^{a\ B}$	4	$2.99^{a\ AB}$	4	$3.69^{a\ A}$	4	$3.10^{b\ AB}$	16	3.07
	PMA	4	$3.06^{a\ A}$	4	$3.60^{a\ A}$	4	$3.94^{a\ A}$	4	$3.63^{b\ A}$	16	3.56
	RF	4	$3.45^{a\ B}$	4	$3.75^{a\ B}$	4	$3.98^{a\ AB}$	4	$5.02^{a\ A}$	16	4.05
	Average / *Prosjek*	16	3.00	16	3.37	16	3.80	16	3.81	64	3.50^{β}
Harkanovci	**C**	4	$18.06^{a\ A}$	4	$18.34^{a\ A}$	4	$19.39^{a\ A}$	4	$18.85^{a\ A}$	16	18.66
	CMA	4	$18.72^{a\ A}$	4	$19.47^{a\ A}$	4	$18.68^{a\ A}$	4	$19.41^{a\ A}$	16	19.07
	PMA	4	$16.28^{a\ A}$	4	$17.90^{a\ A}$	4	$16.96^{a\ A}$	4	$17.21^{a\ A}$	16	17.09
	RF	4	$17.40^{a\ A}$	4	$17.27^{a\ A}$	4	$17.77^{a\ A}$	4	$20.95^{a\ A}$	16	18.35
	Average / *Prosjek*	16	17.61	16	18.24	16	18.20	16	19.11	64	18.29^{α}
Osječak	**C**	4	$0.78^{a\ A}$	4	$1.08^{a\ A}$	4	$1.74^{a\ A}$	4	$1.72^{a\ A}$	16	1.33
	CMA	4	$0.80^{a\ A}$	4	$1.16^{a\ A}$	4	$1.40^{a\ A}$	4	$1.74^{a\ A}$	16	1.27
	PMA	4	$0.68^{a\ B}$	4	1.18^{aAB}	4	$1.25^{a\ AB}$	4	$2.89^{a\ A}$	16	1.50
	RF	4	$0.53^{a\ B}$	4	$1.26^{a\ B}$	4	$1.27^{a\ B}$	4	$3.42^{a\ A}$	16	1.62
	Average / *Prosjek*	16	0.70	16	1.17	16	1.27	16	2.44	64	1.43^{γ}

The lowercase letters indicate the significant differences within the columns, the capital letters indicate the significant differences within the rows, and the Greek letters indicate the significant differences between the locations ($LSD_{0.05}$). / *Mala slova označuju značajne razlike unutar stupaca, velika slova označuju značajne razlike unutar redaka, a grčka slova označuju značajne razlike između lokacija ($LSD_{0.05}$).*

The obtained results from all three locations are most likely due to the applied microbial bioagent and mineral fertilization in the PMA variant and the use of mineral fertilization in the RF variant (Table 3). The statistically significant differences in the available phosphorus content between the initial and final sampling were recorded for these two variants at the Osječak location. Sundara et al. (2002) also reported that the application of phosphorus-solubilizing bacteria (PSB) without any P-fertilizer increased the PSB population and the soil-available P status at different crop stages and that a much greater effect was observed when the PSB were used together with a P fertilizer.

Soil pH Value

The soil's pH values (KCl) at the Gornja Dubica location ranged from 4.17 to 4.73, at the Harkanovci location from 4.44 to 6.05, and at the Osječak location from 4.83 to 4.97 (Table 4).

Table 4. A pH reaction (KCl) in the soil samples during 2016 and 2017

Tablica 4. pH vrijednost uzoraka tla (u KCl-u) u 2016. i 2017. godini

Location / *Lokalitet*	**Variant**/ *Varijanta*	**pH reaction (KCl) in the soil samples during 2016 (I, II) and 2017 (III, IV)** / *pH reakcija (KCl) u uzorcima tla tijekom 2016. (I., II.) i 2017. (III., IV.)*									
		Soil sampling / *Uzorkovanje tla*									
		n	I	n	II	n	III	n	IV	n	Average
Gornja Dubica	C	4	$4.73^{a\ A}$	4	$4.44^{a\ AB}$	4	$4.28^{a\ B}$	4	$4.33^{a\ B}$	16	4.45
	CMA	4	$4.46^{a\ A}$	4	$4.36^{a\ AB}$	4	$4.17^{a\ B}$	4	$4.33^{a\ AB}$	16	4.33
	PMA	4	$4.50^{a\ A}$	4	$4.33^{a\ AB}$	4	$4.20^{a\ B}$	4	$4.35^{a\ AB}$	16	4.34
	RF	4	$4.48^{a\ A}$	4	$4.50^{a\ A}$	4	$4.25^{a\ A}$	4	$4.37^{a\ A}$	16	4.40
	Average / *Prosjek*	16	4.54	16	4.41	16	4.22	16	4.34	64	4.38^{γ}
Harkanovci	C	4	$5.49^{ab\ A}$	4	$6.05^{a\ A}$	4	$5.80^{a\ A}$	4	$6.02^{a\ A}$	16	5.84
	CMA	4	$5.79^{a\ A}$	4	$5.53^{ab\ A}$	4	$5.49^{ab\ A}$	4	$6.04^{a\ A}$	16	5.71
	PMA	4	$4.93^{b\ A}$	4	$4.83^{b\ A}$	4	$4.88^{b\ A}$	4	4.44^{abA}	16	4.77
	RF	4	$5.04^{b\ A}$	4	$4.85^{b\ A}$	4	$4.87^{b\ A}$	4	$5.20^{b\ A}$	16	4.99
	Average / *Prosjek*	16	5.31	16	5.32	16	5.26	16	5.42	64	5.33^{α}
Osječak	C	4	$4.91^{a\ A}$	4	$4.88^{a\ A}$	4	$4.90^{a\ A}$	4	$4.88^{a\ A}$	16	4.89
	CMA	4	$4.95^{a\ A}$	4	$4.83^{a\ A}$	4	$4.85^{a\ A}$	4	$4.87^{a\ A}$	16	4.87
	PMA	4	$4.93^{a\ A}$	4	$4.83^{a\ A}$	4	$4.97^{a\ A}$	4	$4.85^{a\ A}$	16	4.89
	RF	4	$4.97^{a\ A}$	4	$4.86^{a\ AB}$	4	$4.91^{a\ AB}$	4	$4.83^{a\ B}$	16	4.89
	Average / *Prosjek*	16	4.94	16	4.85	16	4.90	16	4.86	64	4.89^{β}

The lowercase letters indicate the significant differences within the columns, the capital letters indicate the significant differences within the rows, and the Greek letters indicate the significant differences between locations ($LSD_{0.05}$). / *Mala slova označuju značajne razlike unutar stupaca, velika slova označuju značajne razlike unutar redaka, a grčka slova označuju značajne razlike između lokacija ($LSD_{0.05}$).*

Based on the range of the obtained pH values, it was observed that the most heterogeneous soil surface was at the Harkanovci location, while the most homogeneous one was at the Osječak location. However, the statistically significant differences in the average pH value were established between the individual locations (Table 4). At the Gornja Dubica and Osječak locations, , no statistically significant differences between the individual variants were detected within an individual sampling period. Although the statistically significant differences in the pH values in the individual variants at Gornja Dubica were obtained, it can be assumed that they most likely occurred due to the influence of weather characteristics and sampling period.

As stated before regarding the Harkanovci location, the C and CMA variants were placed randomly on the plots with a higher pH reaction if compared with the rest of the surface, which most likely affected the availability of phosphorus on these plots, as stated . However, no statistical differences were discovered between the inital and final sampling of all variants. At the Osječak location, a statistically significant drop in the pH value between the initial and final sampling was observable in the RF variant, while a slight drop in the pH values was observable in other variants . Such fluctuations in the pH values were most likely caused by different weather conditions (e.g., precipitation, temperature, sampling time, etc.) during the observed 2016 and 2017 period (Table 1). Li et al. (2017) stated that a temperature, precipitation, and location-specific terrain features can influence regional soil. Also, Oberholzer et al. (2024.) mentioned that the application of effective microorganisms (EM) at a recommended dose exhibited no consistent effect on the different soil properties, including the soil pH value.

Microbial Biomass Phosphorus

The statistically significant differences in the average of the microbial biomass-phosphorus (MBP) content were found between all three locations in both research years . Also, the higher MBP contents were registered during 2017 at all three locations if compared with the average MBP contents in 2016 at the same location (Table 5). Within the same year, the statistically significant differences regarding the MBP content between the locations were driven by the soil properties, especially by the available P content in the soil, what is indicated by a very strong relationship (r = 0.9213 in 2016 and r = 0.9508 in 2017) between these two parameters, as depicted in Figures 1A and 1B.

Table 5. The average microbial biomass-P content in the soil during 2016 and 2017

Tablica 5. Prosječan sadržaj fosfora u mikrobnoj biomasi tla u 2016. i 2017. godini

Location / *Lokalitet*	**The average microbial biomass-phosphorus content (kg ha^{-1})** / *Prosječan sadržaj fosfora u mikrobnoj biomasi tla (kg ha^{-1})*	
	Research year 2016 / *Istraživačka godina 2016.*	**Research year 2017** / *Istraživačka godina 2017.*
Gornja Dubica	38.01 b	44.51 b
Harkanovci	78.68 a	87.61 a
Osječak	24.19 c	37.83 c

The obtained differences in the MBP content between the locations and years match the statements of Peng et al. (2021) that the soil MBP was highly variable in most intensive agricultural systems, especially due to the differences in temperature, moisture, soil matrix, land-use type, crop rotations, fertilizers and soil amendments, and the tillage practices.

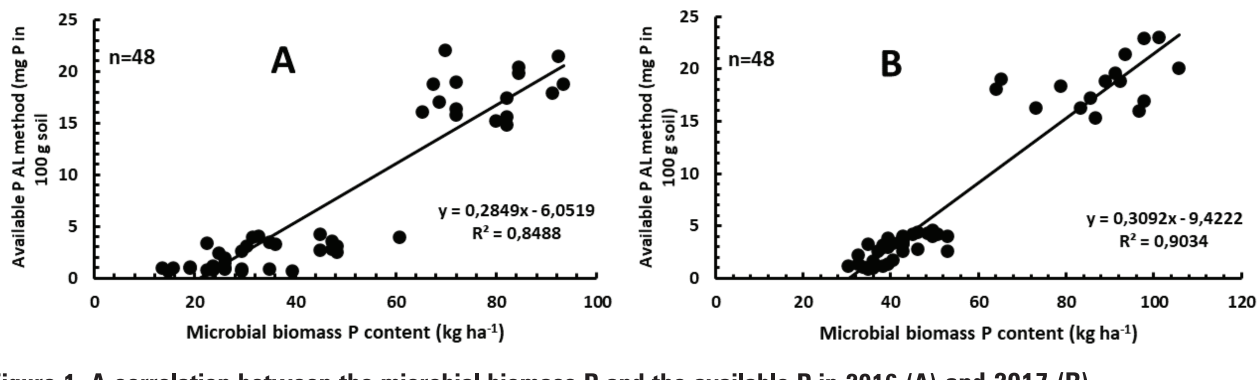

Figure 1. A correlation between the microbial biomass P and the available P in 2016 (A) and 2017 (B).

Grafikon 1. Korelacija između fosfora mikrobne biomase i pristupačnoga fosfora u 2016. i 2017.

At the Gornja Dubica location, the MBP content in 2016 and 2017 varied from 28.13 to 45.84 kg ha^{-1} and from 40.22 to 48.94 kg^{-1}, respectively. All three variants (the CMA, PMA, and the RF) exhibited an increase in the MBP content if compared with the control variant in both years. In 2016, the increase in the MBP pool of the CMA, PMA, and RF variants amounted to 38%, 63%, and 41% if compared with the control , while the increase in 2017 was 7.7%, 21.7% and 13.3%, respectively.

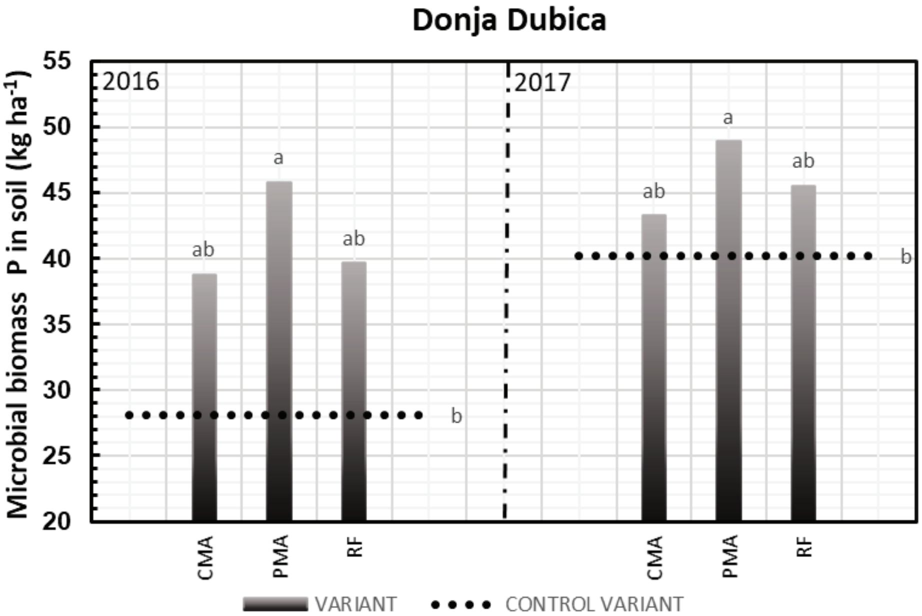

Figure 2. The microbial biomass-P content in the soil at the Gornja Dubica location.
Grafikon 2. Sadržaj fosfora u mikrobnoj biomasi tla na lokaciji Gornja Dubica.

The statistically significant differences were found between the PMA variant and the control variant in both years (Figure 2).

The highest microbial biomass-phosphorus content was achieved at Harkanovci, were the 2016 content varied from 72.28 to 82.97 kg ha^{-1}, while the 2017 content varied from 80.72 to 96.75 kg ha^{-1}. The variants CMA, PMA, and RF exhibited an increase in the MBP content when compared with the control variant in both years (Figure 3).

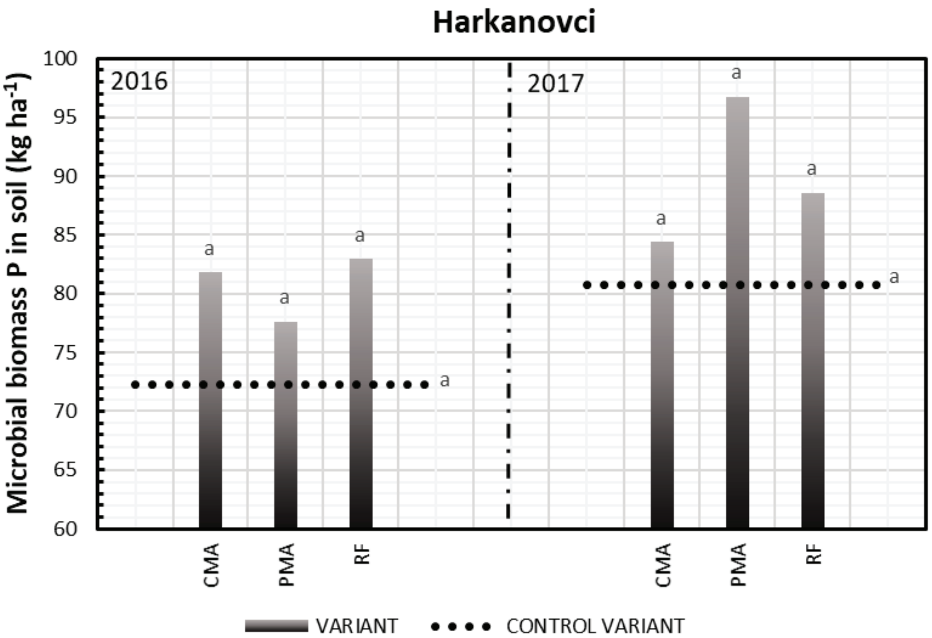

Figure 3. The microbial biomass-P content in the soil at the Harkanovci location.
Grafikon 3. Sadržaj fosfora u mikrobnoj biomasi tla na lokaciji Harkanovci.

Although those increases in the MBP content ranged from 7.4 to 14.79% in 2016 and from 4.53 to 19.86% in 2017, no statistically significant differences between the mentioned variants and the control variant were detected (Figure 3). A phosphorus surplus in the soil at the Harkanovci location (Table 3) could be a key driver of the high MBP contents at this location.

At the Osječak location, the 2016 MBP content varied from 18.84 to 29.53 kg ha^{-1}, and a statistically significant difference was established between the CMA and the RF variant (Figure 4). In 2017, the MBP content varied from 36.84 to 39.09 kg ha^{-1} and no statistically significant differences were discovered between the variants. It is interesting that the variant with a recommended mineral fertilization recorded the lowest content in the microbial biomass phosphorus in both years.

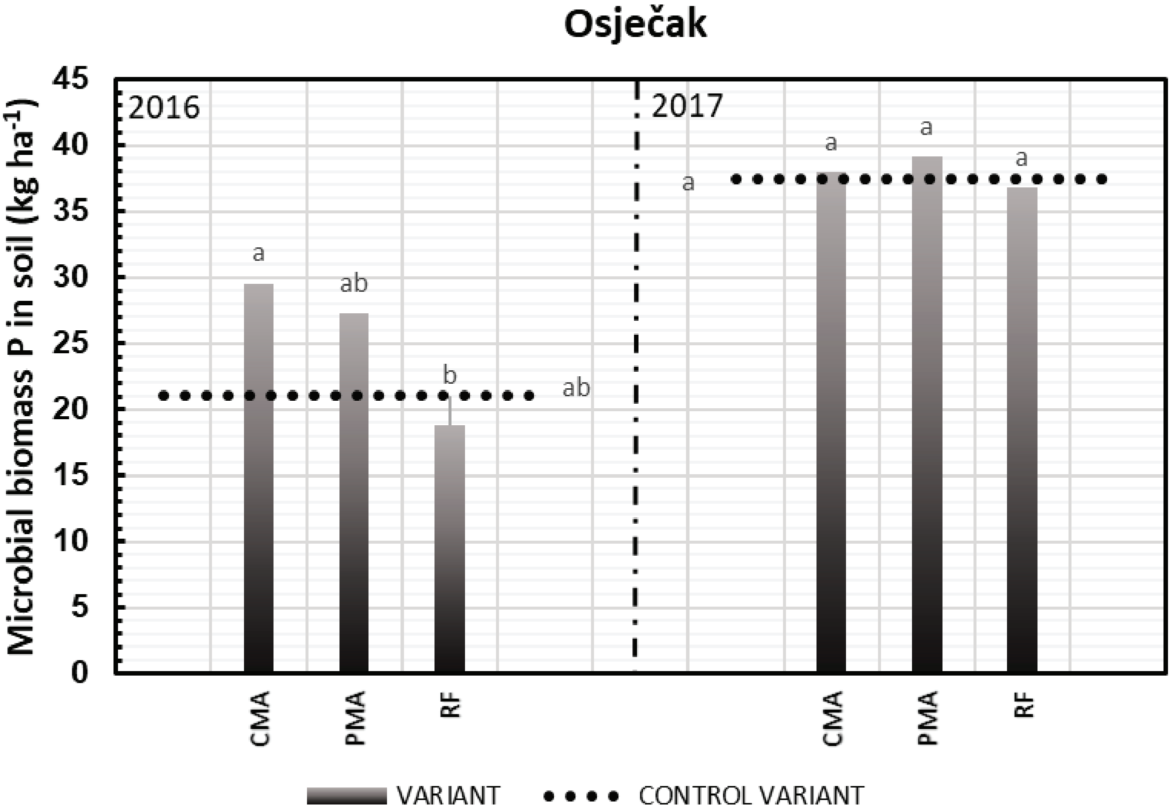

Figure 4. The microbial biomass-P content in the soil at the Osječak location.
Grafikon 4. Sadržaj fosfora u mikrobnoj biomasi tla na lokaciji Osječak.

The above results indicate a fact that the P input and the application of a microbial bioagent together with the content of the plant-available phosphorus in the soil influenced the MBP pool, except the RF variant at the Osječak location, at which the effect of P input was absent. These results were in agreement with other studies, showing that the P input (Turner and Wright, 2014), but also the P surplus in the soil (Peng et al., 2021) and soil microorganisms (Bünemann et al., 2008), can be a very important driver for the stimulation of increases in the soil MBP.

CONCLUSION

Based on the results of the conducted research and the two main research objectives, it can be stated that the application of a microbial bioagent had no negative impacts on the organic matter and the soil pH value and that the recorded deviations were the result of a sampling time, weather conditions, initial soil properties, and human error. Furthermore, a mineral fertilization had a greater effect in an increase of the amount of available phosphorus in the more acidic soils than the application of microbial bioagents. However, the application of a microbial bioagent had a visible effect on the MBP content, although its efficiency was strongly depended on the soil properties,. Thus, the MBP content in 2016 on the control variants with a microbial bioagent increased by 40% (Osječak), 37.96% (Gornja Dubica), and 13.23% (Harkanovci) when compared with the control variant, while the increases in 2017 were significantly smaller: 1.50%, 7.67%, and 4.53%, respectively. A biennial follow-up was too short to derive clear conclusions, and therefore a long-term monitoring is needed to penetrate more inside the effect of microbial bioagents on the soil properties.

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UTJECAJ PRIMJENE MIKROBIOLOŠKIH PREPARATA NA ODABRANA SVOJSTVA KISELIH TALA

SAŽETAK

Čimbenici koji znatno ograničavaju uspješnu poljoprivrednu proizvodnju u svijetu svakako su pristupačnost fosfora, organske tvari u tlu i pH reakcija tla. Iako se značajne količine fosfora nalaze u tlu, većina je prisutna u biljkama nepristupačnim oblicima. Stoga se ulažu sve veći napori u aktiviranje velikih količina biljkama nepristupačnoga fosfora upotrebom mikroorganizama koji otapaju fosfor. Navedena su dva glavna cilja istraživanja. Prvo, utječe li primjena mikrobiološkoga preparata na promatrana svojstva tla (pH, organska tvar, biljci pristupačan P), i drugo, može li primjena mikrobiološkoga preparata utjecati na povećanje razine fosfora u mikrobnoj biomasi tla. Tri neovisna poljska pokusa postavljena su prema principu slučajnoga bloknog rasporeda sa sljedećim tretmanima u četiri ponavljanja: kontrola „C", kontrola s mikrobiološkim preparatom „CMA", gnojidba sa smanjenim unosom fosfora uz mikrobiološki preparat „PMA" i preporučena gnojidba mineralnim gnojivima „RF". Uzorci tla analizirani su na pH reakciju, biljci pristupačan fosfor, organsku tvar tla i razinu fosfora u mikrobnoj biomasi tla. Vidljiva povećanja s 3,45 na 5,02 mg P u 100 g tla i s 0,53 na 3,42 mg P u 100 g tla zbog primijene mineralnoga gnojiva utvrđena su na dvije lokacije. Nije zabilježen negativan utjecaj primijenjenoga mikrobiološkog preparata na svojstva tla. Međutim, zabilježena je vrlo snažna korelacija (r = 0,9213 u 2016. i r = 0,9508 u 2017.) između biljci pristupačnoga P i razine P u mikrobnoj biomasi tla. Rezultati ukazuju na činjenicu da su unos fosfora i primjena mikrobiološkoga preparata zajedno s količinom biljci pristupačnoga fosfora u tlu utjecali na razinu fosfora u mikrobnoj biomasi tla.

Ključne riječi: pH tla, organska tvar, fosfor, mikroorganizmi koji otapaju fosfor

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