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The Effect of Mineral and Biological Nitrogen on Microbiological Traits of Smonitza and Maize Yield

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Abstract: The paper presents the study on the effect of inoculation of maize seed (NSSC-640) by asymbiotic nitrogen-fixing bacteria (*Azotobacter chroococcum*, strain 84) and increasing rates of mineral nitrogen on quantitative composition of microorganisms (total number of bacteria, number of actionomycetes and azotobacter) in smonitza under maize and on the yield of the grown crop.

The following fertilization treatments were studied: N_1PK (90:75:60 kg/ha^{-1}); N_2PK (120:75:60 kg/ha^{-1}); N_3PK (150:75:60 kg/ha^{-1}), as well as the treatment with presowing inoculation of maize seed.

Microbiological analysis included the assessment of total number of microorganisms, actinomycetes and azotobacters in the rhizosphere and edaphosphere during maize vegetation. Total numbers of microorganisms were determined by growth on the medium for the total number with an appropriate amount (0.5 ml) of 10^{-6} soil dilution, numbers of actinomycetes - by growing on synthetic agar according to Krasil'nikov with 10^{-4} of soil dilution and azotobacters - by growing on Fyodorov's agar with 10^{-2} of soil dilution.

The study results showed that the numbers of microorganism groups were affected by the type and rate of fertilizers applied, as well as by the time and sampling zone. The application of *Azotobacter chroococcum*, strain 84, resulted in an increase of the total numbers of microorganisms, actinomycetes and azotobacters, especially in the rhizosphere soil at the onset, and even in the middle of maize vegetation. Lower rates of nitrogen fertilizers (90 and 120 kg/ha^{-1}) led to a significant increase of total bacteria numbers, as well as an insignificant change in azotobacter numbers, whereas their high rate (150 kg/ha^{-1}) had a depressive effect on the mentioned microorganisms, paricularly in the edaphosphere of maize. In contrast, actinomycete numbers were not reduced even with this nitrogen treatment.

Under the studied agroecological conditions, the highest maize yield, but not economically justified one, was obtained with the highest nitrogen rate. Seed inoculation with *Azotobacter chroococcum*, strain 84, caused an insignificant rise in maize yield, which can be associated with acid reaction of the soil studied and slower release of nitrogen accumulated in their cells.

Key words: mineral fertilizers, nitrogen fixation, inoculation, maize, yield, microorganisms

Introduction

In the mid-fifthies, intensification of agriculture started worldwide, as a way of providing extended soil reproduction. This concept is focused on solving major issues in agricultural production, and primarily the provision of good and quality yields, as well as improved utilization of arable land, which is of vital importance, since increasing population numbers on the Earth relate to the concurrent decrease in sown areas. Therefore, the intensification of agriculture is both progressive and neceassry. Industrial technologies have been taking special measures for a longer time now, aiming at reduction of environmental pollution, whereas the technologies in agricultural production neglect it. Such attitude can result in serious ecological consequences, which will not only aggreviate the organization of agricultural production, but could ultimately affect adversely living conditions.

Within a series of cultural practices (conventional and modern technologies), special attention should be paid to timely and proper application of different fertilization systems, in order to achieve the full effect of those measures. Studies in this field have frequently aimed at increasing yields of agricultural crops, whereas the basis of their cumulative effect (changes in biological and chemical soil properties, content of biogenic elements and heavy metals, etc.) is often neglected (Bogdanović et al., 1997). Thus, solving the problems of rational and efficient use of different fertilization systems is possible only if a complex approach is chosen, which stresses microbiological investigations, since microorganisms represent an important step for successful functioning of agroecosystem as a whole.

As concerns this issue, a special notice should be paid to the application of different types and rates of nitrogen fertilizers, since their unsupervised usage could result in negative consequences. This implies primarily inhibitory effects on the highest number of soil microorganisms (Gomorova et al., 1986; Powlson et al., 1986; Milošević et al., 1994), as well as the direct influences on animals and man (Berestecki, 1983; Artamanov, 1989). Due to this and other issues, the importance of the biological nitrogen has recently been on the increase. Thus, it is more frequenty used as an alternative and/or supplement to mineral fertilizers. However, as the activity of the incorporated biofertilizators depends on pedochemical, agroecological and biological soil properties, as well as on the plant with which it forms association, a growing attention has been paid to these studies (Postagte, 1978; Emstev, 1994; Đukić, Mandić, 1997).

The paper aimed at studying the effect of inoculation of maize seed (NSSC-640) with asymbiotic nitrogen-fixing bacteria (*Azotobacter chroococcum*, strain 84) and increasing rates of mineral nitrogen on the quantitative composition of microorganisms (total numbers of bactera, actinomycetes and azotobacter) in smonitza under maize and on the yield of the grown crop.

Material and Methods

The trial was set up in 1996 in a randomized block design in four replications. The elementary plot was 21.25 m^2, and interspace 0.5 m, i.e. 1 m between the blocks.

The following fertilization treatments were studied: N_1PK (90:75:65 kg/ha); N_2PK (120:75:60 $kg \cdot ha^{-1}$); N_3PK (150:75:60 $kg \cdot ha^{-1}$); and the treatment with presowing inoculation of maize seed (the inoculum used, *Azotobacter chroococcum*, strain 84, derived from the collection of microorganisms of the Department for Microbiology, Faculty of Agriculture, Novi Sad).

Microbiological analysis involved determination of total numbers of microorganisms, numbers of actinomycetes and azotobacters in edaphosphere, and rhizosphere during maize vegetation (at the stage of intensive plant growth - 19 June, at the milky-waxy stage - 21 August, and the stage of full maturity - 8 October, 1996). Total number of microorgansms was determined by growth on the medium for the total number ('Torlak', Belgrade) with an appropriate amount (0.5 ml) of soil dilution (10^{-6}), of actinomycetes - by growth on synthetic agar according to Krasil'nikov with 10^{-4} of soil dilution, and those of azotobacters - by growth on Fydorov's medium with 10^{-2} of soil dilution.

Maize kernel yield was determined at the stage of full waxy maturity (with 14% humidity) and is epressed in $t \cdot ha^{-1}$.

The results obtained were processed using analysis of variance in a three-factorial trial, 5×2×3 (fertilizer × sampling zone × vegetation stage), and testing of difference significance of individual and interaction means by t-test.

Results and Discussion

Analysis of variance of the data obtained (Tables 1, 2 and 3) revealed all the factors studied (fertilizer type, sampling zone, maize vegetation stage) to have a highly significant effect on the tested parameters of biological soil productivity. On the basis of those parameters their highly expressed statistical interaction was assessed (statistical significance of interaction).

Medium nitrogen rate (120 kg/ha^{-1}) showed the most stimulating effect on the total numbers of bacteria, which is related to the stimulation of mineralizing processes in the soil (Jakovljević, 1979). However, excessive stimulation of these processes can also be harmful, since under such conditions the change in the structure of nitrogen fractions (the increase of relatively stable forms) may occur, which could directly or indirectly result in reduced values of biological component. Decline in total numbers of microorganisms with the treatment applying high nitrogen rate (150 kg/ha^{-1}) proves the mentioned effect (Tab. 1).

Presowing inoculation of maize seed with *Azotobacter chroococcum*, strain 84 led to a significant increase in total bacteria numbers, which is related to the trait of most aerobic nitrogen-fixing bacteria, may produce, besides its major function (fixation of atmospheric nitrogen), some active substances (vitamines, hormones) too, as well as to absorb certain amounts of toxic matters from the soil (Milošević et al., 1997). The increase in total numbers of microorganisms following such treatments have been also reported by other authors (Vose, 1983; Dart, 1986; Emstev, 1999; Milošević, Govedarica, 2001).

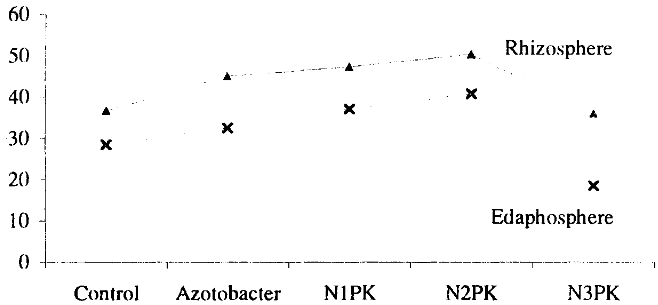
Tab.1. Total numbers of microorganisms (10^{-6}. g^{-1} of absolutelu dry soil) in the soil under maize, as affected by the applied fertilizers (A), sampling zone (B) and vegetation period (C)

A	Control		Azotobacter		N_1PK		N_2PK		N_3PK		$\overline{X}$
B	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	
Periods (C) I	22.0	24.3	30.6	38.6	35.0	40.3	40.0	44.3	24.3	26.3	**32.52**
Periods (C) II	24.0	40.0	28.3	44.6	29.3	48.0	40.3	55.0	11.3	19.3	**34.01**
Periods (C) III	39.3	46.3	38.3	38.0	47.3	54.3	42.3	52.3	20.0	47.3	**42.54**
$\overline{X}$	**32.55**		**36.44**		**42.28**		**45.67**		**24.72**		
$\overline{X}$ **Edaphosphere**	**31.47**										
$\overline{X}$ **Rhizosphere**	**42.81**										

Lsd	A	B	C	A×B	A×C	B×C	A×B×C
0.05	1.94	1.05	1.27	2.75	3.36	1.78	4.77
0.01	2.56	1.39	1.68	3.64	4.45	2.36	6.31

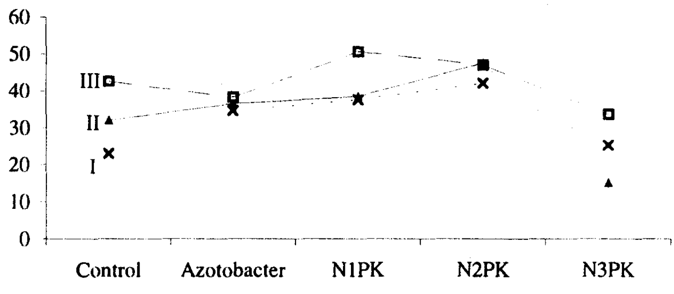
The trends stated are especially pronounced in the rhizosphere soil (Fig. 1), which resulted from more expressed root activity and the level of exuded metabolites in the immediate zone of maize root. This especially refers to the variant with the high nitrogen rate (150 kg·ha^{-1}), where the level of toxic effect of the unused nitrogen is especially predominant in the edaphosphere. It can be concluded that the organic exudates irreversibly bound free nitrogen and thus reduced its activity in relation to edaphosphere. This can account for significantly higher numbers of microorganisms in the rhizosphere soil (in many crops) fertilized with different kinds and rates of mineral fertilizers in relation to edaphosphere (Milosevic et al., 1994). Since the concentration of the introduced nitrogen fixing bacteria was highest in the root zone, the increase of microorganism numbers in this zone is somewhat more expressed in the treatment with azotobacter could be expected.

Fig. 1. Interaction (A x B) of fertilizers applied (A) and sampling zone (B) and the effect on the total numbers of microorganisms in the soil under maize (10^6 g^{-1} of absolutely dry soil)



The fertilizers studied had different effects during maize vegetation (Fig.2). Thus, the most pronounced stimulating effect was found with lower rates of nitrogenous mineral fertilizers (90 and 120 kg·ha^{-1}) and azotobacter, shown at the initial stages of maize development, whereas high nitrogen rates had a particularly depressing effect in the second stage of the study, when the ecological conditions favouring the development of the highest number of microorganisms were very adverse (high temperatures and low soil humidity). The decline in total numbers of microorganisms in the third study period with the inoculation treatment was correlated to the total numbers of azotobacters in the soil over the same period (Fig. 6).

Fig.2. Interaction (A x C) of fertilizers applied (A) and maize vegetation perid (C) and the effect on total numbers of microorganisms in the soil (10^6 g^{-1} of absolutely dry soil)



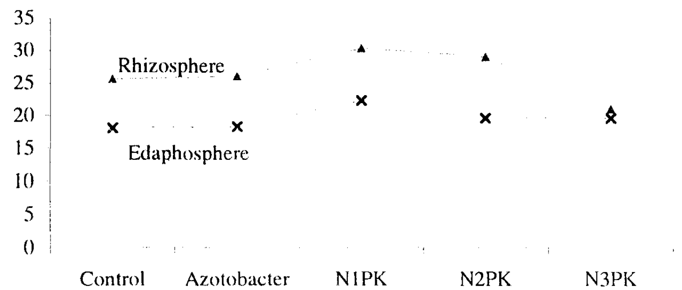
In contrast to the stated, high nitrogen rates (150 kg·ha^{-1}) did not affect significantly the decline in actinomycetes numbers, whereas lower rates (90 and 120 kg·ha^{-1}), as with the total numbers, had a stimulating effect on this microorganism group (Tab.2). The used azotoacter strain insignificantly affected actinomycete numbers, which is in accordance with results obtained by other authors (Antić, 1999; Papić-Vidaković, 2000). The trends stated were relatively more marked decline of actinomycete numbers in the rhizosphere soil in relation to edaphosphere (Fig.3). This can be accounted for by the fact that actinomycetes, as marked soil representatives, are more dominant in the edaphosphere (Saric and Ristic, 1973), and therefore the neutralization of high nitrogen rates in this zone is more pronounced.

Tab.2. Numbers of actinomycetes ($10^4 g^{-1}$ of absolutely dry soil) in the soil under maize as affected by the fertilizers applied (A), sampling zone (B) and vegetation period (C)

A		Control		Azotobacter		N_1PK		N_2PK		N_3PK		$\overline{X}$
B		Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	
Periods (C)	I	14.33	19.3	8.3	14.0	13.3	25.6	20.3	27.3	18.0	26.0	**18.64**
	II	10.0	16.6	15.0	25.3	10.6	9.3	7.6	9.3	8.3	7.3	**11.93**
	III	30.3	41.3	31.6	38.6	43.0	55.7	31.3	50.6	33.6	30.6	**38.66**
$\overline{X}$		**21.89**		**22.17**		**26.33**		**24.33**		**20.33**		
$\overline{X}$	**Edaphosphere**			**25.68**								
	Rhizosphere			**31.05**								

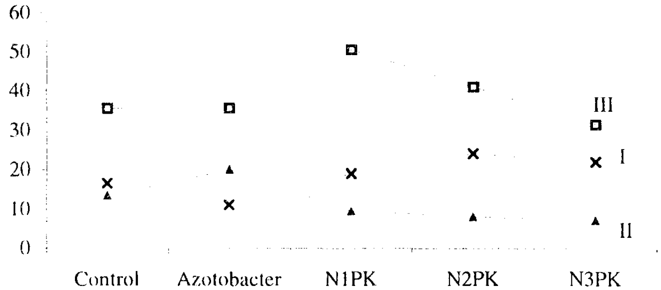
Lsd							
Lsd	A	B	C	AxB	AxC	BxC	AxBxC
0.05	2.18	1.19	1.39	3.17	3.88	2.08	5.50
0.01	2.88	1.59	1.83	4.19	5.13	2.75	7.28

Fig.3. Interaction (AxB) of fertilizers applied (A) and sampling zone (B) and the effect of actinomycetes numbers in the soil under maize ($10^4 g^{-1}$ of absolutely dry soil)



In all the studied treatments, the highest numbers of actinomycetes were obtained at the end of the maize vegetation (Fig. 4), when the physiological activity of plant was reduced and the influx of fresh organic residues into the soil was increased. In this case, the introduced azotobacter, similar to the total numbers of bacteria, did not affect the actinomycete development in the third study period either, which is not true for other vegetation periods. The most pronounced depressing effect of all nitrogen rates was recorded in the second period, when the numbers of these microorganisms were lowest. Further decline in their numbers can be partially accounted for an increase in the soil acidity after application of these fertilizers (urea), which do not favour development of this microorganism group.

Fig.4. Interaction (AxB) of fertilizers applied (A) and maize vegetation period (C) and the effect on actinomycetes numbers in the soil (10^4 g^{-1} of absolutely dry soil)



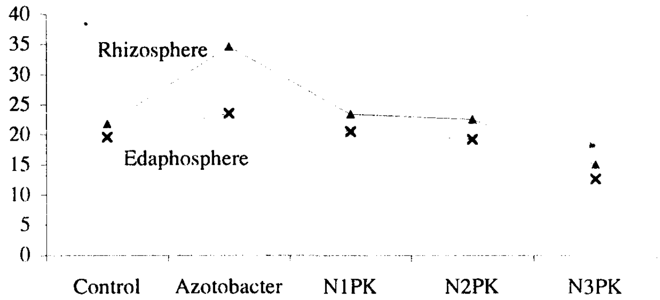
The highest numbers of azotobacters were recorded with the treatment in which it was incorporated in the form of its active strain *Azotobacter chroococcum*, strain 84 (Tab. 3). This increase was especially pronounced in the rhizosphere soil (Fig. 5), in which the influx of exudates resulting from plant activity was highest. As the root zone is distinguished by somewhat higher acid reaction (Lunch, 1983), this phenomenon, taking into account the azotobacter physiology, can be unexpected. However, the results of other authors also indicate the possibility of multyplying of these microorganisms even under these conditions, although their nitrogen fixing capacity is considerably reduced (Charyulu et al., 1979; Kravchenko, 2001), which affect the yield of the grown crops.

Tab.3. Numbers of azotobacter (10^2 g^{-1} of absolutely dry soil) in the soil under maize as affected by the fertilizers applied (A), sampling zone (B) and vegetation period (C)

A		Control		Azotobacter		N_1PK		N_2PK		N_3PK		$\overline{X}$
B		Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	Edaph.	Rhiz.	
Periods (C)	I	19.3	20.0	23.0	41.3	19.0	27.3	17.0	25.6	7.0	11.3	**21.08**
	II	16.3	18.3	20.0	28.3	14.3	12.0	16.3	12.0	11.3	11.3	**16.01**
	III	23.3	27.3	27.6	34.3	28.3	31.0	24.3	30.0	20.3	22.6	**26.90**
$\overline{X}$		**20.77**		**29.11**		**22.03**		**20.88**		**13.94**		
$\overline{X}$	**Edaphosphere**			**18.98**								
	Rizosphere			**23.21**								

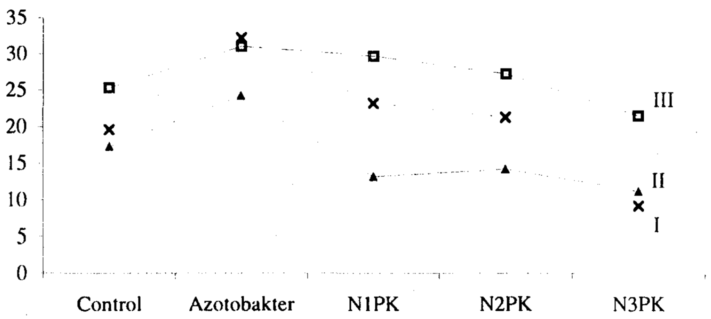
Lsd	A	B	C	AxB	AxC	BxC	AxBxC
0.05	1.35	0.71	0.87	1.91	2.34	1.25	3.31
0.01	1.78	0.94	1.15	2.52	3.09	1.65	4.38

Fig.5. Interaction (AxB) of fertilizers applied (A) and sampling zone (B) and the effect on azotobacter numbers in the soil (10^2 g^{-1} of absolutely dry soil)



High nitrogen rates had a particularly depressing effect on this group of microoorganisms, which is in accordance with results of other authors (Govedarica et al., 1999; Cvijanović et al., 2000). The values of this effect decreased with time, so that at the end of vegetation it almost disappeared (Graph 6). The similar holds true for the other two nitrogen rates. The lowest numbers of azotobacter, with all treatments, were recorded in the middle of vegetation, which is the result of its exceptional susceptibility to high temperatures and decreased soil humidity (Lindbreg et al., 1979; Reddy, Patrick, 1979).

Fig. 6. Interaction (AxC) of fertilizers applied (A) and maize vegetation period (C) and the effect on azotobacter numbers in the soil (10^2 g^{-1} of absolutely dry soil)



Higher mineral nitrogen rate resulted in higher maize yield (Tab. 4). However, considering the level of the yield obtained with the lower nitrogen rate, as well as the price of mineral nitrogen and its adverse effects on some agrochemical and ecological parameters, its higher rate cannot be economically and ecologically justified. Seed inoculation with *Azotobacter chroococcum*, strain 84, had an insignificant effect on maize yield increase, which can be related to the acid reaction of the soil studied and slower release of the nitrogen accumulated in its cells, which has also been reported by other authors (Đukić et al., 2001).

Tab.4. Maize kernel yield (t/ha) as affected by the kind and rate of fertilizers

Control	**Azotobacter**	**N_1PK**	**N_2PK**	**N_3PK**
6.69	6.94	8.19	8.697	9.272

Lsd

0.05	0.01
0.462	**0.695**

Conclusion

On the basis of the results on the effect of maize seed inoculation (NSSC-640) by asymbiotic nitrogen-fixing bacteria (*Azotobacter chroococcum*, strain 84), and increasing rates of mineral nitrogen on some biological properties of smonitza and maize yield, the following conclusions may be inferred:

- Numbers of the studied microorganism groups depend on the kind and rate of fertilizers applied, as well as on the time and zone of sampling;
- Lower rates of nitrogenous fertilizers (90 and 120 $kg \cdot ha^{-1}$) significantly increased total microorganism numbers, whereas their high rate (150 $kg \cdot ha^{-1}$) had a depressing effect, especially in the maize edaphosphere, whereas actinomycete numbers were not lowered even with such treatments;
- Maize seed inoculation with *Azotobacter chroococcum*, strain 84 had a stimulating effect on the mentioned biological parameters of the soil, excluding actinomycetes;
- The highest numbers of all the microorganisms studied were recorded at the start and at the end of maize vegetation;
- The number of the studied microorganisms is significantly higher in the rhizosphere soil as compared to edaphosphere, in which the level of the inhibitory effects of high nitrogen rates is somewhat lower;
- The inoculation of maize seed with *Azotobacter chroococcum*, strain 84 resulted in insignificantly maize yield as compared to the control, whereas increasing nitrogen rates also led to the proportional rise in maize yield. However, with regard to the level of the yield achieved with lower nitrogen rates, as well as to the price of mineral nitrogen and its negative effects on some agrochemical and ecological parameters, its higher rate may be considered as economically and ecologically unjustifiable.

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UTICAJ MINERALNOG I BIOLOŠKOG AZOTA NA MIKROBIOLOŠKE KARAKTERISTIKE SMONICE I PRINOS KUKURUZA

-originalni naučni rad-

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Rezime

U radu je ispitivan uticaj inokulacije semena kukuruza (NSSC-640) asimbioznim azotofiksatorima (*Azotobacter chroococcum*, soj 84) i rastućih doza mineralnog azota na kvantitativni sastav mikroorganizama (ukupn brojnost bakterija, brojnost aktinomiceta i azotobaktera) u smonici pod kukuruzom i prinos gajene kulture.

Ispitivane su sledeće varijante đubrenja: N_1PK (90:75:60 kg·ha^{-1}); N_2PK (120:75:60 kg·ha^{-1}); N_3PK (150:75:60 kg·ha^{-1}) i varijanta sa predsetvenom inokulacijom semena kukuruza.

Mikrobiološka analiza obuhvatila je određivanje ukupne brojnosti mikroorganizama, aktinomiceta i azotobaktera u rizo- i edafosferi tokom vegetacije kukuruza. Ukupna brojnost mikroorganizama određivana je zasejavanjem podloge za ukupan broj sa odgovarajućom količinom (0.5 ml) zemljišnog razređenja (10^{-6}), brojnost aktinomiceta - zasejavanjem sintetičkog agara po Krasiljnikovu sa 10^{-4} zemljišnog razređenja i azotobaktera - zasejavanjem agara Fjodorova sa 10^{-2} zemljišnog razređenja.

Rezultati istraživanja su pokazali da je brojnost ispitivanih grupa mikroorganizama zavisila od vrste i doze primenjenih đubriva, kao i od vremena i zone uzimanja uzoraka. Primena *Azotobacter chroococcum*, soj 84, uslovila je povećanje ukupne brojnosti mikroorganizama, aktinomiceta i azotobaktera, posebno u rizosfernom zemljištu početkom, pa i sredinom vegetacije kukuruza. Niže doze azotnih đubriva (90 i 120 kg·ha^{-1}) izazivaju značajno povećanje ukupne brojnosti bakterija, kao i neznačajnu promenu brojnosti azotobaktera, dok je njihova visoka doza (150 kg·ha^{-1}) delovala depresivno na pomenute mikroorganizme, posebno u edafosferi kukuruza. Za razliku od navedenog, brojnost aktinomiceta nije smanjena ni pri ovakom azotnom tretmanu.

U ispitivanim agroekološkim uslovima najveći, ali ne i ekonomski najopravdaniji, prinos kukuruza dala je najveća doza azota. Inokulacija semena sa *Azotobacter chroococcum*, soj 84, uticala je na neznačajno povećanje prinosa kukuruza, što se može povezati sa kiselom reakcijom ispitivanog zemljišta i sporijim otpuštanjem azota akumuliranog u njihovim ćelijama.