THE INFLUENCE OF NITROGEN FERTILIZER ON THE TOTAL NUMBER OF MICROORGANISMS AND AMINOAUTOTROPH DYNAMICS UNDER ”UGAR” AND SOWN MAIZE

SUMMARY

Soil microorganisms have a key role in creation and maintaining of soil fertility. Anthropogenic impact on the soil microorganisms is extremely powerful. The application of fertilizer represents the most significant one.

Applying fertilizers increase biological productivity of agroecosystem and microbiological activity of soil, but, at the same time, they cause certain changes inside them through the influence on microorganisms. This research examines how microbiological activity of soil is influenced by the quantity of applied N fertilizer and "ugar" or tillage and the connection between this given activity and the maize quality. The total number of microorganisms and the number of amino-autotrophs depend on a phenophase of a plant, "ugar" or tillage and the quantity of applied N fertilizer. Less quantity of applied N fertilizer affects the increase of a number of microorganisms and components of the maize income whereas higher concentration of applied N fertilizer has an inhibitory effect.

Keywords: soil, fertilizer, total number of microorganisms, number of amino-autotrophs, maize.

INTRODUCTION

Biogeochemical cycles of matter and energy flowing are basic for obtaining life on the planet, they are constant, developing themselves in the ecosystems due to microorganisms. In the agroecosystems, which cover nearly one ninth of the whole Earth's surface (Lješević, 2005), soil microorganisms have a primary role in these processes. Through the enzymes which are being free into surrounding area, these soil microorganisms take part in almost all soil biochemical reactions which are present in C, N, P, S cycles and microelements, where organic substances of soil are produced and degraded and fertilizers again become available for plants. Decomposing of organic synthesis of carbon...
and nitrogen, dissolving of phosphate, \( \text{H}_2\text{S} \) oxidation, nitrification, de
nitrification and nitro fixation, transformations of microelements and heavy
metals are just some of the processes which continuously happen in soil thanks to
metabolic activity of microorganisms as well as to enzymes which are being
released. Enzymes are very significant protein molecules which allow chemical
reactions that are life important (Topisirović, 1998). Plants use released mineral
salts, which are the result of the enzyme catalyzed processes. At the same time,
microorganisms also compete with plants for these biogenic elements in soil
which they also include in the biosynthesis of their cells’ plasmas (Kuzyakov,
2002). Microorganisms are uncontested the most important factor in forming of
soil fertility. Having the possibility to rule microbiological processes in soil,
many perspectives for soil fertility refinement are promising. The success in the
first place depends on as better evaluation of soil biogenesis as possible whereas
the number and the enzyme activity are the most remarkable indicators. The
number and enzyme activity stand for the current state of microbiological soil
activity, so, for a better considering of biogenesis, it is preferable to observe these
parameters for several times during the vegetation of the growing plants (Jarak et
al. 2006). Apart from abiotic factors, microbiological soil activity is also affected
by biotic factors among which anthropogenic have a leading function. In agro
ecosystems, the application of fertilizers is one of the most significant
anthropogenic. Mineral fertilizers increase biological productivity of
agroecosystems (Marković et al. 1985, Nedić and Šuput 1977). Among the N, P
and K elements which are being used as fertilizers, nitrogen is considered to have
the most important part in soil fertility, its microbiological activity, growing and
development of cultivating plants (Albinska et al. 2002; Popović, 2010;
Glamočlija et. al. 2015). On the other hand, fertilizers which are being used cause
a number of changes in the given agroecosystem, they have an impact on the
physical and chemical soil features, the structure of microbiocenosis and all the
former mentioned processes which happen in a soil - microorganism - plant
system. Considering the key function of microorganisms, the appearing changes
can influence the cycles of matter and energy flowing as well as the creation and
maintaining of soil fertility (Doran and Zeiss 2000).

Examining the activities of soil microorganisms under the tillage of
cultivated plants and noticing the correlation between the given activity and
growth and development of a plant in conditions under the applying doses of the
concentration of mineral fertilizer, it is possible to determine the optimal
concentration of fertilizer by which a plant productivity is highly improved and
the costs of production are decreased in great measure. The research in this area
enters the essence of the ecosystem functioning, that is, the way of
biogeochemical cycles of matter and energy flowing as the basis of life and it
conducts us how to get back the de-arranged courses into their natural forms by
using anthropogenic influence. As it is mentioned before, the aim of this research
is establishing the influence of different concentration of nitrogen fertilizer on
microbiological soil activity under the sown maize.
MATERIAL AND METHODS

The research of the impact of nitrogen fertilizer concentration on microbiological soil activity, type "cernozem" under the sown maize is carried out in the area of Eastern Srem (The Maize Institute " Zemun Polje"). During the research field micro tests are used, conducted by the split plots method in four goes. The surface of the main plot was 403,2 squares, the subplot was 16,8 squares (6,0 x 2,8 sqrs) and the accounting plots for a grain yield was 8,4 sqrs.

Testing covered the following systems of maize fertilizing as well as the variations on "ugar", i.e. without tillage:

1. follow up (without fertilizing);
2. P_{90} K_{60} N_{30} kg/ha (basis, phon);
3. P_{90} K_{60} N_{60} kg/ha;
4. P_{90} K_{60} N_{120} kg/ha;
5. P_{90} K_{60} N_{180} kg/ha.

During the tests the standard agro technique is applied. In the both years of the research, the prior crop was winter wheat. After the wheat harvest, the ground is plowed on the depth of 10-15 cm. Before the fundamental soil cultivation, 300 kg/ha of mineral fertilizer NPK10:30:20 formulations are used.

The fundamental soil cultivation had been finished during the autumn on the depth of 25 cm. During the spring, the additional cultivation was carried out, as well as the additional nitrogen KAN fertilizer (27%N), in the concentration of 30, 90, 150 kg/ha of active matter and finally the pre- harvest preparation took place.

The seed used for sowing was maize hybrid ZPSC- 578 (FAO group of reaping 500). The harvest (gathering) was done manually in the physiological reap of the seeds. The chemical structure of the maize yield was determined on the DICKEY-JOHN NIR Analyzator machine. Soil samples were taken in the period of 2006 and 2007, before maize sowing, in the phase of a period of silking and at the end of the vegetation period on the depth of 0-30 cm, on "ugar" and under the sown maize. During the research meteorological conditions were also taken into consideration.

In the soil samples the basic chemical features were determined by the following methods:

1. reaction ( ph in H_{2}O abd KCl) of soil- by electrometric method;
2. % CaCO_{3}- by Scheibler's method;
3. % humus - by Turin's method, Simankov's modification;
4. % total percent of nitrogen-by Kieldahl's method;
5. the percent of mineral nitrogen (NO_{3} and NH_{4})- by Bremmer's method;
6. the percent of P_{2}O_{5} and K_{2}O - by AL- method (Egner and Reihm).

Microbiological analysis has determined the total number of microorganisms and aminoautotrophs. The soil samples were taken (under the
sown maize and on "ugar") on the depth of 30 cm. The number of microorganisms is detected by the standard indirect method of sowing diluted soil samples on selective nutritious substrata, which were afterwards incubated on the temperature of 28 degrees. The number is expressed in grammas of completely dry soil.

The following features were determined in soil: - the total number of microorganisms on nutritious substratum tripton- soya agar (TSA), \(10^{5}\); - the number of aminoautotrophs on the starch- ammoniac agar substratum, \(10^{-4}\).

The research results were analyzed by descriptive statistics method.- the number of aminoautotrophs on the starch- ammoniac agar substratum, \(10^{-4}\). The research results were analyzed by descriptive statistics method.

**Agroecological conditions during the experiment conducting**

The Eastern Srem is settled down in the area of medium- continental climate and it borders to the north, east and south with two big rivers, the Danube and the Sava. This area belongs to wide Pannonia lowland which has convenient climate and soil conditions for arable farming. The exception is an average rainfall and its unequal arrangement during years.

**Meteorological conditions.** Average monthly air temperatures in the research years as well as the lasting several years' average (1995-2004), during the vegetation period of maize is shown in the table 1. In the both years of the research, the average month temperatures increased from April until July after which they decreased. The average air temperatures for the maize vegetation period was lower in 2006 and it was 19,7 °C which is 1,1 °C lower than in 2007, but on the several years' level. (19,6 °C April is characteristic for higher average monthly temperature in the both years regard to the lasting several years' period. Air temperature in April 2006 was higher for 1,2 °C and in 2007 for 1,8 °C. In May 2006, the average monthly temperature was 17,6 °C. According to the lasting several years' period it was lower for 1,1 °C. In the same month of 2007, the temperature was higher for 1 °C than in the lasting several years' period. Similar tendency was confirmed in June, too. In this month, the average air temperature in 2006 was 20,3 °C and it was lower for 1,5 °C than in the several years' period (21,8 °C). June 2007 was warmer for 1,8 °C than a decade period. In the both research years, July was warmer than the several years' period (23,2 °C). In 2006, the average air temperature was higher for 1,4 °C and in 2007 for 2,5 °C.

On 24th july 2007 recordly high temperature of 44,9 degrees was registered. In august 2006, the average monthly temperature was 21,7 o c, which was a bit lower for 1,7 o c in comparison to the several years' period (23,4 o c). On the other hand, august 2007 was warmer for 1,0 o c than average and for 2,7 o c than the first month in the first year of the research. On the contrary, the
average monthly air temperature in September was lower in 2007 and it was 16.8°C which was lower for 1.0°C than the several years' period and for 2.9°C at 2006, Table 1

Table 1. Temperature and precipitation, 2006–2007, Zemun Polje, Serbia

| Month     | Temperature | Average long 1995-2007 | Precipitation | Average long 1995-2007 |
|-----------|-------------|-------------------------|---------------|------------------------|
|           | 1. year     | 2. year                 | 1. year       | 2. year                |
| April     | 14.1        | 14.7                    | 12.9          | 97.0                   | 4.0                     | 65.0 |
| May       | 17.6        | 19.7                    | 18.7          | 40.0                   | 79.0                    | 57.0 |
| June      | 20.3        | 23.6                    | 21.8          | 137.0                  | 108.0                   | 79.0 |
| July      | 24.6        | 25.7                    | 23.2          | 22.0                   | 18.0                     | 81.0 |
| August    | 21.7        | 24.4                    | 23.4          | 123.0                  | 72.0                     | 57.0 |
| September | 19.7        | 16.8                    | 17.8          | 26.0                   | 85.0                     | 82.0 |
| Average   | 19.7        | 20.8                    | 19.6          | 445.0                  | 366.0                    | 421.0 |

The facts about the amount of rainfall during the months of the vegetation period of maize in the research years as well as the annual average (1995-2004) are shown in Table 1. The years when this research took place distinguished between themselves according to the total amount of rainfall during the vegetation period of maize and its proportion in certain months. Bigger amounts of rainfall for the vegetation period of maize (iv-ix) were registered in 2006 (445 mm) than in 2007 (366 mm). Comparing this with the lasting several years, in the first year of the research the amount of rainfall was bigger for 24.0 mm and in the second year it was smaller for 55.0 mm and it affected growing and development as well as the maize productivity. In April 2006, the amount of rainfall was 97.0 mm which was for 32.0 mm more than in the lasting several years' average (65 mm). On the contrary, April 2007 characterized itself with 4.0 mm of rainfall. The situation was reversed in May. The bigger amount of rainfall in this month was registered during 2007 and it was 79.0 mm which was more for 22.0 mm than average and for 39.0 mm more than the amount in 2006. During June, in the both years of the research, there were suitable humidity conditions for growing and development of maize. In 2006 in June 137.0 mm of rainfall was registered and in 2007 this amount was 108.0 mm. In comparison with the lasting several years' average (79.0 mm) these amounts were bigger for 58.0 mm, that is 29.0 mm. In July, in contrast to June, humidity conditions were inconvenient for growing and development of maize. In 2006 in this month, the amount of rainfall was 22.0 mm and in 2007 it was 18.0 mm. In comparison with the lasting several years' period (81.0 mm) these amounts were smaller for 59.0 mm, that is, 63.0 mm. During August in 2006 the amount of rainfall was 123.0 mm which was more for 66.0 mm than a ten-year period average in Zemun Polje (57.0 mm). In the same month in 2007 the rainfall was smaller for 51.0 mm than in the first year of the
research. The amount of rainfall in September also varied. In this month in 2006 the amount was 26.0 mm which was smaller for 56.0 mm than in the lasting several years' period (82.0 mm). On the other hand, September 2007 was distinguished with the amount of rainfall on the lasting several years' level (85 mm). On the basis of these results, it can be seen that more suitable temperature and humidity conditions for microbiologic activity of soil and maize cultivation were in 2006 in comparison with 2007.

RESULTS AND DISCUSSION

The research of the impact of nitrogen fertilizer quantity on microbiological activity of soil under the sown maize was conducted in the "cernozem" soil type. According to the pedological study of the soil in Zemun Polje, carbon cernozem is characterized by humus- accumulative (Ah) horizon of 0-50 cm of depth, dark black color and powdery clay argil texture. On the 0-30 cm depth its structure consists if small lumpy and grainy particles and going more deeply by the end of Ah horizon it is more compact and its structure is lumpy and nut shaped.

Table 3. Chemical analysis of soil (Zemun Polje)

| Depth (cm) | pH | CaCO$_3$ (%) | Humus (%) | Total N (%) | Available N (ppm) | mg in 100 g soil |
|------------|----|--------------|-----------|-------------|-------------------|-----------------|
|            | H$_2$O KCl NH$_4^+$ NO$_3^-$ P$_2$O$_5$ K$_2$O |
| 0-30       | 7.71 7.34 | 4.4 | 2.86 | 0.19 | 4.9 | 17.5 | 25.4 | 22.2 |

On the basis of the chemical analysis results (Table 3), which are performed in the agrochemical laboratory of the Faculty of Agriculture in Zemun, it can be said that the reaction of the soil mixture was mild alcal. Medium humus and nitrogen concentration are present in this soil and easily accessible phosphor and potassium are highly proportioned. A micro field testing station is positioned in Zemun Polje on 88 metres altitude.

The total number of microorganisms represents a general soil biogenesis and together with enzyme activity it is a reliable fertility indicator. Using the variable analysis of it is established that the total number of microorganisms was affected in great measure by all the examined factors: a phenophase of a plant (A), ugar or tillage (B) quantity of the applied fertilizer (C), and their interactions (Table 4). The number is notably expressed in a plant reaping phenophase than in a cub blooming phenophase, which can be explained by meteorological conditions, which were prevailing. The plant reaping phenophase was more liable to hydrotermic regime, i.e. more rainfall, drought absence and more equal temperatures.
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Table 4. The total number of microorganisms in the soil on account of a phenophase of a plant, ugar or tillage and a quantity of nitrogen fertilizer (105 g-1) in the first and the second years of the research.

| Year | LSD  | A       | B       | C       | AB    | BC    |
|------|------|---------|---------|---------|-------|-------|
| 1.   | 0.5  | 7.69    | 5.13    | 12.16   | 10.88 | 17.20 |
|      | 0.1  | 10.29   | 7.03    | 16.27   | 14.55 | 23.01 |
| 2.   | 0.5  | 2.05    | 1.73    | 3.24    | 2.30  | 3.71  |
|      | 0.1  | 3.72    | 2.91    | 4.33    | 3.89  | 5.23  |

Microbe populations and their biochemical activities in the soil are subjected to the season fluctuations (Matinizadeh et al., 2008). Equal temperatures and humidity stimulate proliferation of soil microorganisms, and water availability is a crucial environmental parameter which affects the quantity and diversity of soil microflora (Anderson, 1984).

In the plant reaping phenophase the excretion of the root sugar secretes more intensely likewise the organic acid, amino acid, vitamins, hormones and other organic matters so an easier development of microorganisms is probably connected to reachable resources of carbon and energy from plants. The total number of microorganisms is more expressed on ugar than under the sown maize (table 4). Knowing the fact that "ugar" is soil without tillage, a higher number of microorganisms can be explained by the absence of competitive interactions with roots for nutrients. The variable analysis showed very significant differences in the total number of microorganisms within different quantity of the applying fertilizer (table 4). Apart from the control less quantity of fertilizer (n3o and n6o kg/ha) had a great effect on increasing of the
total number of microorganisms while higher quantity of fertilizer (n180 kg/ha) worked as inhibitors.

Mineral fertilizers intensified microbiological processes in soil and they affected an income increase at the beginning, but when they are used in less quantity, their effect was visible on increasing the total number of microorganisms and their systematic and physiologic groups while their higher quantities had a diverse effect (Epanchinov, 1975, Milošević, 1990, Đodević, 1998, Hajnal-Jafari, 2010).

Nevertheless, in a short period, mineralization of humus is quickened as well as of other nitrogen synthesis, nitrate is quickly accumulated, the part of biological nitrogen in plant is shortened which leads to worse conditions of soil features and decrease of culture income. Higher concentration of mineral fertilizers affects the total number of microorganisms and general soil biogenesis in negative manner (Arnus et al., 1995, Đukić and Mandić, 1997).

These testing also proved the pattern of influence of the applying fertilizers on the total number of microorganisms. Beside the total number of microorganisms, the number of some systematic and physiologic groups of microorganisms also becomes one of the general soil biogenesis indicators.

Aminoautotrophs stand for bacteria which are physiologically united by their capability to use mineral synthesis of nitrogen metabolically in metabolic purposes (Đukić i Jemcev, 2000). The variable analysis showed that the number of aminoautotrophs was in a great part influenced by the following factors: a phenophase of a plant (A), ugar or tillage (B), and the concentration of the applying fertilizer (C) as well as the interaction of factors AC, BC and ABC (Table 5).

In the first year of the research, interactions of AB factor were not statistically significant and in the second year B factor (ugar or tillage) didn’t affect the number of aminoautotrophs as well (Table 5).

In the both years of the research, there were more aminoautotrophs (table 5) in a phenophase of a plant reaping than in a phenophase of a cob blooming. The growth of a number of microorganisms in the plant reaping phenophase is stimulated in the first place by the higher quantity of rainfall and dry periods' absence. In the phenophase of a plant blooming, especially during 2007, extremely high temperatures were recorded and that had an adverse effect on development of microorganisms. The quantity of nutrients, supstrat availability, soil humidity, temperature, and all other ecologic conditions of importance can have a strong mutual dependance and affect, in that way, biologic and biochemical processes in soil in a complex way (seifert et al., 2001, tabaković et al. 2016). There is no a simple answer in which way these factors have an impact on microorganism populations and their activities. Ugar or tillage in the first year
The influence of nitrogen fertilizer on the total number of microorganisms and aminoautotrophs of the research had a significant influence on autotrophs' development in both plant phenophases. Although in the both years of the research the number on ugar was more explicit in the both phenophase than on tillage, the differences are statistically very important just in the phenophase of a cob blooming in 2006 (table 5). The concentration of the applied fertilizer had a notable impact on the number of autotrophs during the both years of the research. In average, the most outstanding increase of the number in comparison with the control is noticed in the test variation n120 kg/ha although the quantity n60 kg/ha also influenced in a very stimulative way the number of this group of microorganisms. The most numerous number of aminoautotrophs was established in n120 kg/ha applying variation and it is 364.3 x 10^4 g⁻¹ while the lowest one was in n180 kg/ha and it was 31.4 x 10^4 g⁻¹ (table 5).

Table 5. The number of the aminoautotrophs in soil depending on the phenophase of a plant, ugar or tillage and quantity of n fertilizer (104g⁻¹) quote in year one (5a) and in year two (5b) of the research.

| Year | LSD | A    | B    | C    | AB   | BC   | ABC  |
|------|-----|------|------|------|------|------|------|
| 1    | 0.5 | 14.80| 12.13| 23.40| 33.10| 30.53| 47.33|
|      | 0.1 | 19.80| 16.71| 31.31| 44.28| 41.02| 61.82|
| 2    | 0.5 | 12.54| 19.83| 17.74| 27.56| 20.17| 37.91|
|      | 0.1 | 16.78| 26.54| 23.73| 36.87| 25.37| 49.10|
Quantity of 180 kg/ha nitrogen decreased in great manner the number of aminoautotrophs in comparison with the control in the both years of the research. Besides the number of microorganisms, it was also examined how the quantity of nitrogen affected maize productivity within the productivity parameters, maize income and contents of proteins in grains. The results of this research showed that within the average for the tested factors, the maize income was 8.65 t/ha (table 6).

Table 6. The impact of nitrogen quantity on the grain (maize) income, t/ha

| Quantity of nitrogen (B) | Year (A) | Average | Index (%) |
|--------------------------|----------|---------|-----------|
|                          | 2006     | 2007    |           |
| 1                        | 8.05     | 7.54    | 7.80      | 100.0     |
| 2                        | 8.27     | 7.78    | 8.03      | 102.9     |
| 3                        | 8.82     | 8.48    | 8.65      | 110.9     |
| 4                        | 9.92     | 9.04    | 9.48      | 121.5     |
| 5                        | 9.78     | 8.81    | 9.30      | 119.2     |
| Average                  | 8.97     | 8.33    | 8.65      | -         |
| Index (%)                | 100.0    | 92.9    | -         | -         |

1. Control, 2. P90K60N30kg/ha; 3. P90K60N60kg/ha; 4. P90K60N120kg/ha; 5. P90K60N180kg/ha;

In 2006, the grain income was higher for 0.64 t/ha than in 2007. The income difference between these two years statistically was very significant.

In average for these years, when the quantity of the applied nitrogen fertilizer was 120 kg/ha, the grain income increased and then it decreased. The lowest grain income (7.80 t/ha) was measured on the control, without fertilizing and the highest one (9.48 t/ha) was on the variable with 120 kg/ha nitrogen. The differences in quantity of fertilizer and the grain income were significant and very significant (table 6).

The research results show that, in average, for the tested factors, the concentration of proteins in a grain was 8.58% (table 7). Higher concentration of proteins in a grain is established in the year with less rainfall and higher temperatures (2007) during the maize vegetation period. With the increasing quantity of nitrogen to 120 kg/ha, the concentration of proteins increased and then decreased. In the average for these years, the smallest concentration of proteins in grains (8.26%) was measured on the control and the highest concentrations (8.87%) was measured at 120 kg/ha use. In both years of the research, there was a tendency for increasing proteins with graduate increasing of dosis n (table 7).
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Table 7. The impact of N fertilizer quantity to concentration of proteins in grain, %

| Quantity of nitrogen (B) | Year (A) | Average | Index points |
|-------------------------|----------|---------|-------------|
|                         | 2006     | 2007    |             |
| 1                       | 8.02     | 8.49    | 8.26        | 100.0 |
| 2                       | 8.13     | 8.72    | 8.43        | 102.1 |
| 3                       | 8.34     | 8.75    | 8.55        | 103.5 |
| 4                       | 8.81     | 8.92    | 8.87        | 107.4 |
| 5                       | 8.69     | 8.88    | 8.79        | 106.4 |
| Average                 | 8.40     | 8.75    | 8.58        | -     |
| Index points            | 100.0    | 104.2   | -           | -     |

1. Control; 2. P₉₀K₆₀N₃₀ kg/ha; 3. P₉₀K₆₀N₆₀ kg/ha; 4. P₉₀K₆₀N₁₂₀ kg/ha; 5. P₉₀K₆₀N₁₈₀ kg/ha;

CONCLUSIONS

According to the results, there can be drawn the following conclusions.

Tested factors, a phenophase of a plant, ugar or tillage and quantity of the applied nitrogen fertilizer affected in a remarkable way the total number of microorganisms and aminoautotrophs.

The number of microorganisms is more expressive in a phenophase of a plant reaping on ugar.

Regard to the control the quantity of nitrate fertilizer N30, N60 and N120 kg/ha induced the total number of microorganisms and aminoautotrophs whereas the quantity of the applied nitrate fertilizer N180 kg/ha functioned as inhibitor on the tested parameters of soil biogenecy.

The applied quantity of nitrogen had a positive effect on nutritive values of grains and the maize income.

On "cernozem" and in the climate conditions of the Eastern Srem, the appropriate nourishment with nitrogen hybrid ZP 578 implies the use of 120kg/ha N, on a phon 90 kg/ha phosphate and 60 kg/ha potassium.

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