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EFFECT OF MANURE APPLICATION ON THE DEVELOPMENT DYNAMICS OF PROTEOLYTIC AND AMMONIFICATION BACTERIA UNDER MAIZE (*Zea mays* L.) CROPPING

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Abstract. The objective of this study was to investigate the developmental dynamics of proteolytic and ammonification bacteria at different variants of manure application under maize (*Zea mays* L.). The trials were conducted at the Swadzim Experimental-Didactic Station (52°26' N; 16°44' E) which belongs to the University of Life Sciences in Poznań. The experiment was established in accordance with the random block design method and comprised 14 soil objects, each in four replications. The investigations were single-factorial and the factor levels comprised 8 methods of manure application in the case of maize grown for silage and 6 methods, when maize was grown for seeds. Proteolytic and ammonification bacteria numbers depended on the method of manuring and the date of the performed analyses. The highest numbers of proteolytic microorganisms under maize cultivated for silage were observed during the phase of emergence in the combination of winter catch crop (rye + vetch). In the case of maize cultivated for seeds, the highest number of protein-degrading microorganisms was recorded during the phase of emergence in the combination of crop rotation with wheat.

Key words: ammonification bacteria, manure application, proteolytic bacteria

INTRODUCTION

Bearing in mind the observed shortage of humus compounds in Polish soils, it appears very important to select a suitable manure and its appropriate management, as it can have a considerable relevance for the protection of natural environment, maintenance of the biodiversity of agroecosystems and the improvement of soil fertility.

There is little doubt that the choice of a suitable manure plays an important role in the improvement of soil fertility, especially in the intensification of the humification

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process. Apart from the congeneric plant composition, soil biological equilibrium can be affected by the introduced manures [Wyczółkowski and Dąbek-Szreniawska 2002].

The test plant in the described experiment was maize. This plant species gives the highest net yield per hectare and it is also a crop plant important for Polish economy. However, maize responds strongly to fertilization and there are two ways the plant can react to the applied fertilizers. This bidirectional response of maize to fertilization results, among others, from high requirements of this plant regarding climatic and soil conditions.

It is important to determine which of the introduced manures is more effective for plant development and more suitable to maintain soil stability.

Manure application is an important agrotechnical operation and, if applied at suitable time, quantity and composition, it provides – apart from appropriate nutrients for plants – an effective catalyst for biochemical transformations in the soil leading to the improvement of its fertility. Supplied organic substance undergoes transformations caused by the activity of microorganisms of which the most important ones breaking down the organic matter are bacteria and fungi. Organic matter provides a source of energy and building material for soil microbes which utilize their powerful and complex enzymatic apparatus in the presence of water in order to degrade it [Marszewska--Ziemięcka 1982].

Alongside proper bacteria, actinomycetes and fungi which carry out important transformations of complex carbon compounds, numerous species of microorganisms can also be found in the soil, which are capable of utilizing proteins and amino acids as nutritive and energetic substrates. The process of ammonification, which is the ultimate phase of protein degradation, is of significant importance for agriculture and for the development of microbiological relationships in the soil [Szember 2001].

The objective of this study was to investigate the developmental dynamics of proteolytic and ammonification bacteria at different variants of manure application under maize (*Zea mays* L.).

MATERIAL AND METHODS

The performed investigations comprised a field experiment conducted in 2005 and microbiological analyses carried out in the laboratory.

The total area of the experimental plot was 1600 m^2. The trials were conducted at the Swadzim Experimental-Didactic Station (52°26' N; 16°44' E) which belongs to the Poznań University of Life Sciences and the experimental plants [maize (*Zea mays* L.) cultivar Felicia] were cultivated on 28 m^2 plots in four rows and inter-row spacings of 70 cm. The experimental plots were situated on a typical grey-brown podzolic soil developed from post-glacial sediments from light loamy sands on shallow deposits of light loam. The soil is classified as the quality class IVa and belongs to the 4th land capability complex (very good rye complex).

The soil had neutral reaction and was characterized by a proper content of potassium, phosphorus and magnesium (Table 1).

During the experimental period, moderate suitability of soil-climatic conditions for the field cultivation of the experimental plant was observed. The distribution of meteorological conditions during successive months of plant growth is presented in Table 2.

Table 1. Soil characterization
Tabela 1. Charakterystyka gleby

Soil level Poziom glebowy cm	pH	C $g \cdot kg^{-1}$	N $g \cdot kg^{-1}$	C : N	Magnesium Magnez (MgO) $mg \cdot 100g^{-1}$	Phosphorus Fosfor (P_2O_5) $mg \cdot 100g^{-1}$	Potassium Potas (K_2O) $mg \cdot 100g^{-1}$
0-30	6.5	8.7	0.811	10.7	8.8	16.2	16.9

Table 2. Meteorological conditions during the period of maize growing
Tab. 2. Warunki meteorologiczne w okresie wegetacji kukurydzy

Month Miesiąc	Mean temperature, °C Średnia temperatura				Total precipitation, mm Opady atmosferyczne			
	Ten-day period – Dekada			Average Średnia	Ten-day period – Dekada			Sum Suma
	1	2	3		1	2	3	
April – kwiecień	8.9	10.7	8.7	9.4	7.1	0.0	7.4	14.5
May – maj	11.3	10.4	18.2	13.5	31.1	27.3	15.9	74.3
June – czerwiec	13.0	17.3	19.2	16.5	15.2	3.3	0.6	19.1
July – lipiec	20.0	20.9	18.8	19.9	14.1	8.9	74.4	97.4
August – sierpień	15.5	17.8	18.7	17.3	35.0	11.8	13.9	60.7
September – wrzesień	19.8	14.8	13.5	16.0	0.0	25.3	9.1	34.4
October – październik	12.7	8.7	10.0	10.0	0.0	0.4	4.6	5.0

The experiment was established in accordance with the random block design method and comprised 14 soil treatments, each in four replications so that the entire trial included 56 experimental plots. The investigations were single-factorial and the factor levels comprised 8 methods of fertilization in the case of maize grown for silage and 6 levels, when maize was grown for seeds.

In the experiment, in the case of maize grown for silage, the following fertilization combinations were applied: control (mineral NPK); farmyard manure 30 $t \cdot ha^{-1}$ of fresh matter; farmyard manure 15 $t \cdot ha^{-1}$ of fresh matter; 5 t d.m. of wheat straw + 15 kg nitrogen per 1 t of wheat straw$\cdot ha^{-1}$; 5 t d.m. of wheat straw + pig slurry 40 $m^3 \cdot ha^{-1}$; winter catch crop (rye + vetch); crop rotation with wheat; pig slurry 40 m^3.

In the case of maize grown for seeds, the following fertilization combinations were applied: control (mineral NPK); farmyard manure 30 $t \cdot ha^{-1}$ of fresh matter; farmyard manure 15 $t \cdot ha^{-1}$ of fresh matter; 5 t d.m. of maize straw + 15 kg nitrogen per 1 t of wheat straw$\cdot ha^{-1}$; 5 t d.m. of maize straw + pig slurry 40 $m^3 \cdot ha^{-1}$; crop rotation with wheat.

In the applied fertilizers, the NPK content was determined and the rate of these components was balanced by mineral fertilization to the levels of: nitrogen (N) – 130 $kg \cdot ha^{-1}$; phosphorus (P) – 15.3 $kg \cdot ha^{-1}$; potassium (K) – 96.3 $kg \cdot ha^{-1}$. Nitrogen fertilization was applied in the form of ammonium saltpeter, phosphorus in the form of triple superphosphate and potassium in the form of 60% potassium chloride salt. Manures were covered by autumn ploughing, while slurry was covered by spring ploughing.

Maize was cultivated with all the cropping treatments carried out in accordance with the principles of correct agrotechnology for this crop plant and direction of utilization.

Soil samples for microbiological and physico-chemical analyses were collected at seven different dates associated with the following consecutive phases of maize development:

– date 1 – before sowing (BBCH 0),
– date 2 – phase of emergence (BBCH 09),
– date 3 – phase of 2-3 leaves (BBCH 12-13),
– date 4 – phase of the 2nd node (BBCH 16-17),
– date 5 – phase of tasseling (BBCH 65),
– date 6 – phase of cob setting (BBCH 70),
– date 7 – phase of milk maturity (in the case of maize grown for seeds) (BBCH 75).

Soil samples for analyses were collected in ten replications according to the Polish standard. Soil samples collected in inter-rows from under plants from a depth of 5-10 cm were used to determine numbers of microorganisms by means of the Koch plate method, using the appropriate agar media (in 5 replications). The mean number of colonies was converted into soil dry weight.

Proteolytic bacteria counts ($CFU \cdot g^{-1}$ soil d.m.) were determined on the medium according to Rodina [1968]. The bacteria were incubated at a temperature of 22°C for 48h. In addition, when determining the number of the developed colonies, the authors used Frazier's reagent in order to increase the contrast of the substrate in relation to proteolytic bacteria (white colonies forming with lighter patches).

Ammonification bacteria were determined on the medium according to Rodina [1968] after 6 days of incubation at a temperature of 28°C.

All the collected results were subjected to the analysis of variance appropriate for the experimental design. The results of the field trial were evaluated using the analysis of variance for multiple experiments established according to the design of completely randomized blocks. When preparing the synthetic elaboration of the results of the field experiments, the authors employed the full testing procedure of inter-object variability in relation to the mean experimental error and to the environmental interaction. All general F tests and specific t tests were performed at the level of significance – $p = 0.05$.

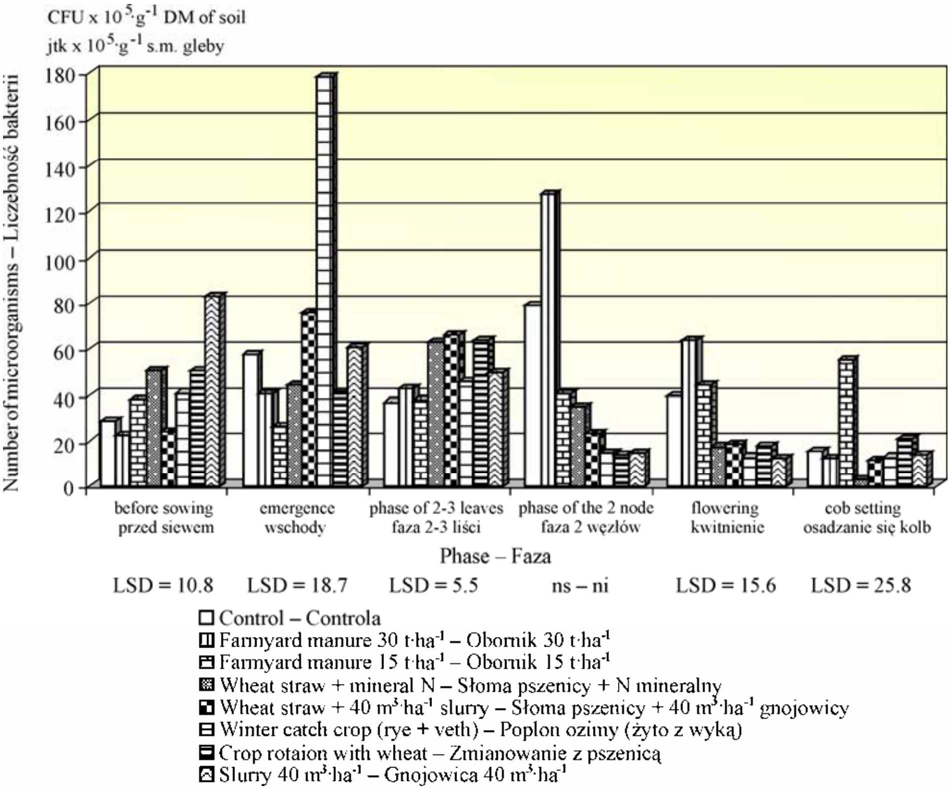
RESULTS AND DISCUSSION

Analysis of the impact of manure application on the growth of proteolytic bacteria

Several studies have confirmed a significant differentiating effect of fertilization on the counts of soil microorganisms as well as their species composition [Doran et al. 1996, Barabasz and Smyk 1997, Gawrońska 1997].

It is evident from the review of literature on the subject that there is little data concerning the effect of manure application into the soil on the developmental dynamics of proteolytic microorganisms found in it. That is why in the experiments we focused on the determination of the numbers of the above-mentioned group of microorganisms in the soil following its enrichment with different rates of the manures.

It was demonstrated, on the basis of the conducted microbiological investigations, that the proteolytic bacteria numbers were determined by the method of fertilization and the date of the performed analyses. The performed statistical analysis revealed significant differences at all developmental phases with the exception of the phase of the 2nd node, where non-significant differences were determined in the cell counts between the control soil and combinations with the manure application (Fig. 1).



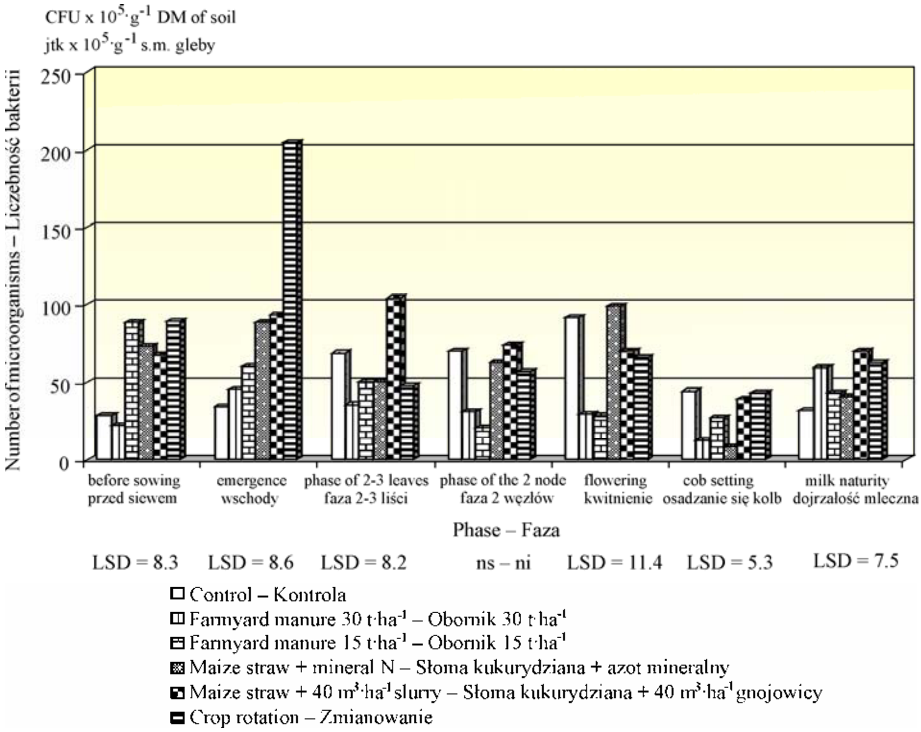
ns – ni – non significant differences – różnice nieistotne

Fig. 1. Effect of fertilization on the numbers of proteolytic bacteria under maize cropping for silage ($LSD_{0.05}$)

Fig. 1. Wpływ nawożenia na liczebność bakterii proteolitycznych pod uprawą kukurydzy na kiszonkę

The highest quantities of the proteolytic microorganisms under maize grown for silage were observed during the phase of emergence in the combination of the winter catch crop (rye + wetch) and their numbers were in the order of 178 x 10^5 CFU·g^{-1} d.m. of soil. The smallest amounts of microbes were recorded during the phase of cob setting in the combination of wheat straw + mineral N, when the cell counts of colony forming units were found to be at a level of 3 x 10^5.

In the case of maize grown for seeds, the highest number of protein-degrading microorganisms was recorded during the phase of emergence in the combination of the crop rotation with wheat and their quantity was determined at 205.12 x 10^5 CFU·g^{-1} d.m. of soil, whereas the smallest amounts of bacteria were found during the phase of cob setting in the combination when soil was fertilized with maize straw + mineral N (Fig. 2).



ns – ni – non significant differences – różnice nieistotne

Fig. 2. Effect of fertilization on numbers of proteolytic bacteria under maize cropping for grain $LSD_{0.05}$

Fig. 2. Wpływ nawożenia na liczebność bakterii proteolitycznych pod uprawą kukurydzy na ziarno

The proteolysis process and numbers of proteolytic bacteria are closely connected with the type and amount of the manure introduced into the soil [Gostkowska et al. 1997].

Green manures which include leguminous plants (also called protein plants) contain more organic nitrogen than other green manures (such as wheat or rye). Experiments carried out, among others, by Waksmann (after Marszewska-Ziemięcka [1982]) showed that the percentage content of crude protein in wheat straw is 2.1%, in maize straw – 2.4%, whereas in alfalfa shoots – 12.8%. This may explain the better development of proteolytic bacteria observed in the experiment when maize was cultivated on the soil fertilized with the winter catch crop (rye + wetch) (after Marszewska-Ziemięcka [1982]). In addition, the protein found in the above-mentioned manures belongs to the so-called easily available proteins [Kucharski and Wałdowska 2001].

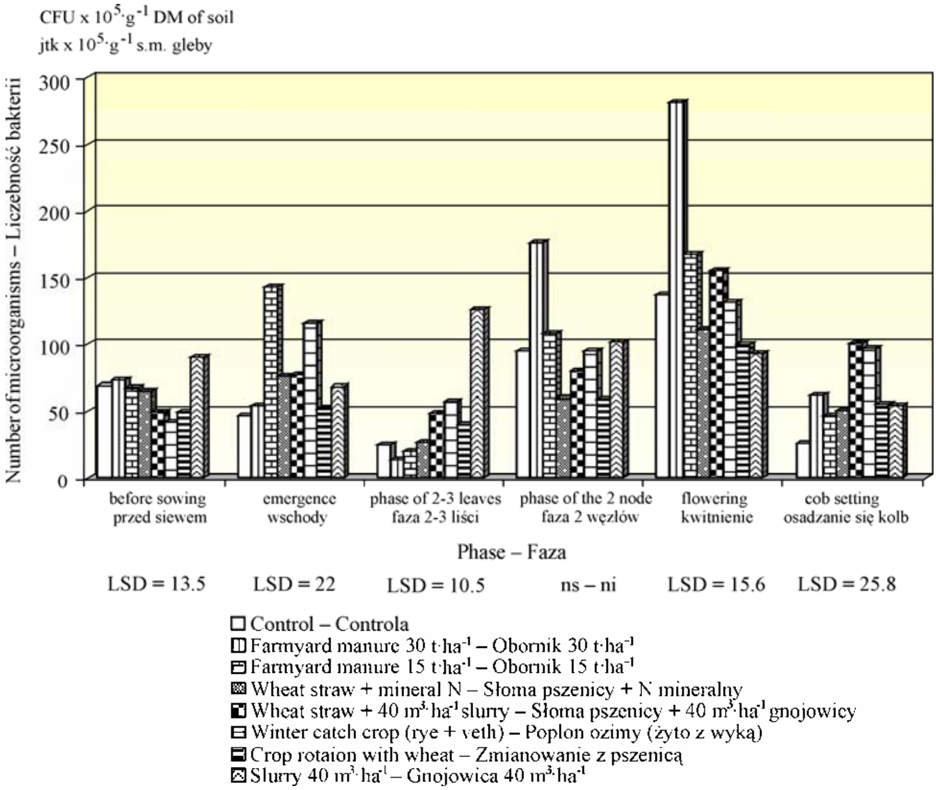
Farmyard manure, similarly to green manures, enriches the soil in organic matter and improves its physico-chemical and biological properties. This type of manure supplies very large quantities of protein contained in animal excreta but this type of protein is poorly available [Kucharski and Wałdowska 2001]. This explains the observed low quantities of proteolytic microorganisms in the combinations fertilized with the farmyard manure, especially in the case of maize grown for seeds (Fig. 2).

Apart from this, changes in the numbers of proteolytic bacteria in analyzed fertilization combinations in the soil in successive developmental phases of maize might have been caused by changes in the quantitative and qualitative composition of root exudates, which is confirmed by experiments carried out by Wielgosz [1999, 2001]. Crop plants, especially their root exudates, change physico-chemical soil properties and by doing so, exert influence on microorganisms [Pietr 1990, Barabasz and Smyk 1997].

Analysis of the effect of manure application on the growth of ammonification bacteria

The performed laboratory analyses revealed that the quantities of ammonification bacteria under maize cropping, similarly to the numbers of proteolytic bacteria, underwent considerable fluctuations depending on the type of the soil combination and kind of fertilization.

Statistical analyses showed that differences in the numbers of bacteria between the control soil and the remaining combinations were statistically significant in all the examined maize developmental phases with the exception of the phase of the 2nd node (Fig. 3).



ns – ni – non significant differences – różnice nieistotne

Fig. 3. Effect of fertilization on numbers of ammonification bacteria under maize cropping for silage $LSD_{0.05}$

Fig. 3. Wpływ nawożenia na liczebność bakterii amonifikacyjnych pod uprawą kukurydzy na kiszonkę

In the case of maize cropping for silage, the worst proliferation of ammonification bacteria was recorded during the phase of 2-3 leaves in the fertilization treatment of the full rate of farmyard manure, where the number of colonies was 13.8 x 10^5 CFU·g^{-1} d.m. of soil. On the other hand, the highest numbers of the discussed bacteria were determined in the same combination during the phase of flowering, when their numbers reached 281.4 10^5 CFU·g^{-1} d.m. of soil.

In the case of maize cropping for seeds, the lowest numbers of bacteria were recorded during the phase of 2-3 leaves, when the soil was fertilized with the full dose of farmyard manure, while their highest quantities were found at the first date of analyses (before sowing), when the soil was fertilized by maize straw + 40 m^3 slurry (Fig. 4).

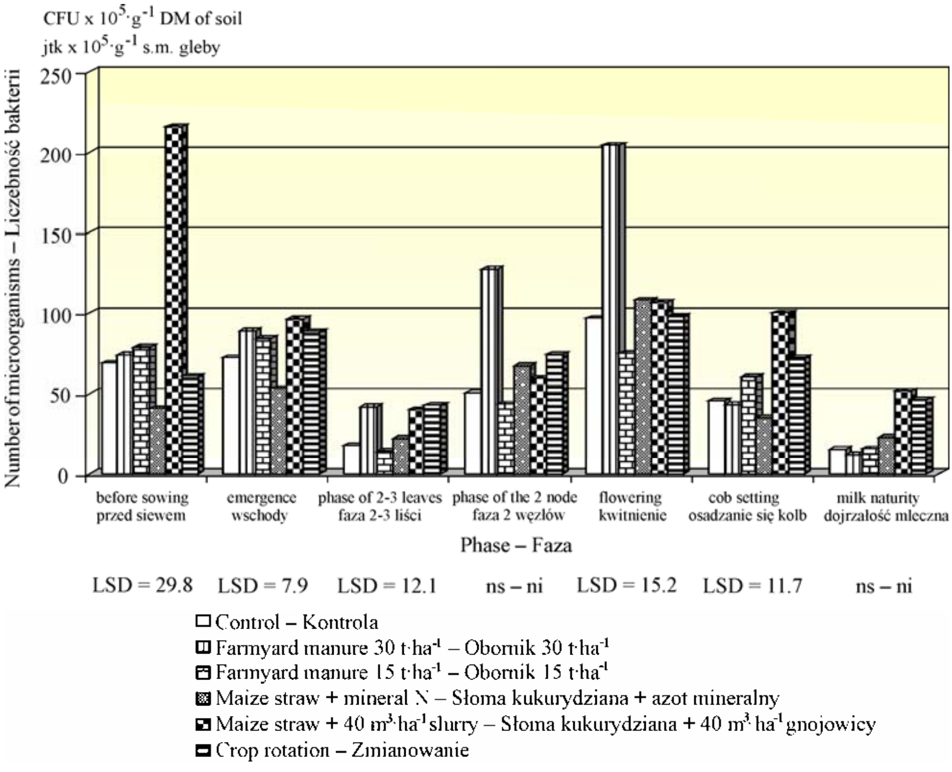


Fig. 4. Effect of fertilization on numbers of ammonification bacteria under maize cropping for grain $LSD_{0.05}$

Fig. 4. Wpływ nawożenia na liczebność bakterii amonifikacyjnych pod uprawą kukurydzy na ziarno

The intensity of the ammonification process, also referred to as dezamination, was considered as an important indicator of soil biological activity.

Data presented in Figures 3 and 4 indicate a significantly stimulating impact of different ways of manure application employed in the experiments on the quantitative increase of ammonification microorganisms recorded at all the dates of analyses.

Similar results were reported in experiments conducted by Mazur [1999] and Wyczółkowski et al. [1998], in which increased quantities of ammonification microorganisms remained in the soil supplemented with different doses of farmyard manure.

The number of ammonification bacteria can be stimulated not only by fertilization but also by the root secretions of the cultivated crop plant – maize. It is well known that these secretions, apart from large quantities of sugars, vitamins and organic acids, also contain easily available amino acids, especially during the period of flowering and cob setting and, later on, quantities of these compounds decrease.

CONCLUSION

1. Higher numbers of proteolytic bacteria were recorded in combinations in which plant rather than animal manures were introduced. This was associated with the fact that plant protein was better available for microorganisms than protein derived from animal excreta.
2. The incorporation of manures into the soil resulted in a slight increase in the counts of ammonification bacteria.

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WPŁYW NAWOŻENIA ORGANICZNEGO NA DYNAMIKĘ ROZWOJU BAKTERII PROTEOLITYCZNYCH I AMONIFIKACYJNYCH POD UPRAWĄ KUKURYDZY (*Zea mays* L.)

Streszczenie. Celem badań było zbadanie dynamiki wzrostu bakterii proteolitycznych i amonifikacyjnych przy różnych wariantach nawożenia naturalnego pod kukurydzą (*Zea mays* L.). Badania opierały się na doświadczeniu polowym prowadzonym w Zakładzie Doświadczalno-Dydaktycznym w Swadzimiu (52°26' N; 16°44' E), należącym do Uniwersytetu Przyrodniczego w Poznaniu, oraz analizach mikrobiologicznych wykonywanych w laboratorium. Doświadczenie założono metodą bloków losowanych. Obejmowało ono 14 obiektów glebowych; każdy z nich występował w czterech powtórzeniach. Cały schemat obejmował 56 poletek. Doświadczenie prowadzono jako jednoczynnikowe, a poziomami czynnika było 8 sposobów nawożenia naturalnego przy uprawie kukurydzy na kiszonkę i 6 sposobów przy uprawie kukurydzy na ziarno. Na podstawie uzyskanych wyników badań mikrobiologicznych wykazano, że liczebność bakterii proteolitycznych i amonifikacyjnych determinowana była sposobem nawożenia oraz terminem analiz. Najwięcej mikroorganizmów proteolitycznych pod uprawą kukurydzy uprawianej na kiszonkę odnotowano w fazie wschodów po zastosowaniu poplonu żyta ozimego z wyką, natomiast w przypadku kukurydzy uprawianej na ziarno najwięcej mikroorganizmów rozkładających białko wystąpiło w fazie wschodów po zastosowaniu zmianowania z pszenicą. Największą ilość bakterii amonifikacyjnych zanotowano w fazie kwitnienia po nawożeniu kukurydzy pełną dawką obornika.

Słowa kluczowe: bakterie amonifikacyjne, bakterie proteolityczne, nawożenie

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