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EFFECT OF ORGANIC FERTILIZATION ON DEVELOPMENT OF PROTEOLYTIC BACTERIA AND ACTIVITY OF PROTEASES IN THE SOIL FOR CULTIVATION OF MAIZE (*ZEA MAYS* L.)

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Abstract: The objective of this work was investigation of the growth dynamics of proteolytic bacteria and the enzymatic activity in soil for the cultivation of maize (*Zea mays* L.), as well as the maize yield under application of some selected organic fertilizers. Intensity and the direction of the developed changes in the soil depended on the type of applied organic fertilizer, the size of its dose introduced into the soil and on the developmental phase of the grown plant (maize). On the basis of obtained results it was found that all tested organic substances stimulated the activity of proteases. Yields of maize as raw material for silages obtained from soil fertilized with sewage sludge were similar to those obtained after the application of pig slurry and they exceeded maize yields harvested from other fertilization objects.

INTRODUCTION

Polish soils are deficient in humic compounds and, therefore, it seems very important to select an adequate organic fertilizer and its proper application. These practices appeared to be important in the protection of the natural environment and the preservation of the biodiversity of agroecosystems as well as soil quality (fertility). The selection of an adequate fertilizer plays a significant role in the increase of soil fertility and primarily in the intensification of the humification process. The introduced organic fertilizers can disturb the biological balance of soil and the congeneric vegetation composition [23].

Actually, beside the traditional organic fertilizers (manure, cattle slurry), attempts are made to fertilize soil with sewage sludge [11, 15]. Because of a high fertilizing value, sewage sludge plays an essential role primarily in the intensification of the humification process [4]. The introduction into the soil of natural fertilizers including sewage sludge satisfying the standard recommendations may have a significant effect on the growth and development of microorganisms as well as on their enzymatic activity of soil. According to Koper *et al.* [7], excessive and particularly unilateral fertilization, frequently used in agricultural practice, can disturb the balance of nutritive components in water solution.

Next to the proper bacteria, actinomycetes and fungi responsible for important transformations of carbon compounds, there are also many species of microorganisms able to utilize proteins and aminoacids as nutritive and energetic substrates. Organic nitrogen compounds which get into the soil in the form of plant or animal remains are subject to biochemical transformations. In all particular stages of the transformations, the participating microorganisms' specific intercellular or extracellular enzymes are secreted into the environment (soil). Different ways of decomposition by proteolytic microorganisms are known. However, this process is always carried out in such a way that from proteins develop peptones which are hydrolyzed into polypeptides and peptides and from them, due to further decomposition, aminoacids develop [24]. The measure of soil biological activity, which includes the total transformations of compounds and the energy taking place in it, can be an enzymatic activity [1]. According to many authors [2, 9, 19] the cognition of the enzymatic activity of soil gives an objective picture of the processes taking place in it. Actually, it is recognized that the enzymatic activity of soil is the measure of soil fertility and productivity in a much higher degree than other biological indicators such as e.g. the number of microelements in their biomass [6, 17].

The objective of the present work was to investigate the growth dynamics of proteolytic bacteria and the enzymatic activity in the soil for the cultivation of maize (*Zea mays* L.), as well as the maize yield under the application of some selected organic fertilizers.

MATERIALS AND METHOD

The studies were carried out in 2006 on plots of the Experimental and Didactic Farm of the Department of Soil and Plant Cultivation in Swadzim belonging to the University of Life Sciences in Poznań. Studies were carried out in a field after a 5-year maize monoculture. The experiment was established in randomized block method in four replications. The plants, maize (*Zea mays* L.) cultivar PR39G12, were cultivated on 28 m^2 plots.

Soil samples for biochemical analyses were taken six times at one-month interval. This interval matched the successive developmental phases of maize:

term 1 – before sowing (BBCH 0),
term 2 – phase of germination (BBCH 09),
term 3 – phase of 2–3 leaves (BBCH 12–13),
term 4 – phase of 6–7 leaves (BBCH 16–17),
term 5 – tasseling phase (flowering) (BBCH 65),
term 6 – phase of cob setting (BBCH 70).

Soil samples for analyses were collected in ten replications according to Polish standard [5].

The soil of experimental plots according to PTG classification [14] belongs to typical grey-brown podsolic soils created of light loamy sands shallowly lying on light loam. According to soil classification, it is counted to the IVa class and according to agricultural suitability; it belongs to the 5th complex (good rye complex). All cultivation treatments were carried out according to the principles of correct agrotechniques of maize cultivation designed for silage.

In the experiment, the following fertilization combinations were applied:
control (mineral NPK),
manure 15 Mg·ha^{-1} of fresh matter,

5 Mg d.m. of wheat straw + pig slurry 40 $m^3 \cdot ha^{-1}$,

5 Mg d.m. wheat straw + 15 kg nitrogen·Mg wheat straw·ha^{-1}.

Sewage sludge was used according to the regulation of the Minister of the Environment permitting the application of it in a dose of 10 $Mg \cdot ha^{-1}$ d.m, once in 5 years. In the used fertilizers, the NPK content was determined and the dose of these components was balanced by mineral fertilization to the levels of: nitrogen (N) – 130 $kg \cdot ha^{-1}$; phosphorus (P) – 15.274 $kg \cdot ha^{-1}$; potassium (K) – 96.28 $kg \cdot ha^{-1}$. 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. Organic (natural) fertilizers were covered by autumn ploughing, while sewage sludge was covered by spring ploughing.

Sewage sludges used in our own studies were examined in respect of their microbiological condition and heavy metals content in the sewage treatment plant in Szamotuły. Results of these analyses indicated that they were safe in reference to their sanitary condition. Also the content of heavy metals was significantly lower than the standards admitted for agricultural utilization. Consequently, in the doses of sewage sludge used in the experiments, the amounts of lead and copper introduced into the soil were respectively 0.5% and 53% of the norm, according to Directive of the Minister of Agriculture and Rural Development. Additionally, the concentration of heavy metals in the gathered plant material (seeds, leaves and stem) was determined and compared against the control value. Manure came from a farm in Swadzim and pig slurry from a farm in Złotniki. Both farms belong to the University of Life Sciences in Poznań.

Experimental plots were situated on a typical grey-brown podsolic soil created of postglacial formations of light loamy sand, shallowly lying on light loam, classified to the IVa class and to the 4th complex of agricultural suitability (very good rye complex). The soil reaction was neutral and it was characterized by a good content of potassium, phosphorus and magnesium (Tab. 1). During the experimental period, the suitability of soil – climate conditions was estimated as moderate. The meteorological conditions during the vegetation season are shown in Table 2. The additional indicator of the impact of organic fertilization applied was the yield of the cultivated plant determined by the weighing method.

Table 1. Some soil physical – chemical properties at site of study

Soil levels [cm]	pH	C [$g \cdot kg^{-1}$]	N [$g \cdot kg^{-1}$]	C:N	Mg [mg MgO·100 g^{-1}]	P [mg P_2O_5·100 g^{-1}]	K [mg K_2O·100 g^{-1}]
0–30	6.5	8.7	0.811	10.7	8.8	16.2	16.9

Table 2. Decade distribution of temperature and precipitation in the Experimental and Didactic Farm in Swadzim in 2006

Months	Mean day temperature [°C]			Mean of precipitation [mm]		
	Decade I	Decade II	Decade III	Decade I	Decade II	Decade III
April	8	9.5	11	2.1	10	32.1
May	14	13	12.5	22	8	29
June	14	21	22.5	7	0	21
July	24	23	26	8	18	1.5
August	18	19	16.5	68.1	11.5	23.5
September	17	18	17	21.1	0.1	1
October	16.5	9.5	12	16	0.2	8.1

Enzymatic studies

Studies of soil enzymatic activity were based on the determination of the activity of proteases by spectrophotometric method [10] in which sodium caseinate was used as substrate after 1-hour incubation at 50°C with 578 nm wave length. Enzyme activity was expressed in l mg tyrosine·kg^{-1}·ha^{-1}.

Microbiological analyses

In soil samples taken from underneath the plants from interrows at the depth of 15–20 cm, the number of microorganisms was determined by plate solutions according to Koch's method on adequate agar mediums (in five replications). The mean number of colonies was converted into soil dry matter [18] [CFU·g^{-1} d.m. soil] number of proteolytic bacteria [CFU·g^{-1} d.m. soil] was determined in a selective medium according to Rodina [16]. The bacteria were incubated at 22°C for 48 h. Additionally, in the determination of the number of the grown colonies, Frazier's reagent was applied in order to increase the content of the substrate in relation to the proteolytic bacteria (white colonies creating bright sites).

Statistical analysis

The accumulated results were statistically analyzed with the use of analysis of variance for factorial experiments established in random block design. The significance of the effect of the studied factors was defined by Fisher-Snedecker test at the confidence levels P = 0.95 and P = 0.99. Differences between the objects were tested according to the t-Student's procedure using the same confidence levels. In the case when the statistical effect of the factor on the value of the analyzed feature was proven, the calculus of correlation and regression was carried out.

RESULTS AND DISCUSSION

The results of this study have shown that the fertilization of grey-brown podsolic soil with natural fertilizers and sewage sludge evoked changes in soil enzymatic activity. Also the cultivation of maize benefited from the changes in soil enzymatic activity. Intensity and the direction of the developed changes depended on the type of the applied organic matter, the size of its dose introduced into the soil and on the developmental phase of maize. On the basis of data presented in a diagram (Fig. 1), it was found that all tested organic substances stimulated the activity of proteases. This phenomenon, however, was most visible in conditions of the use of 5 Mg d.m. wheat straw·ha^{-1}·year^{-1} + 15 kg nitrogen per 1 Mg of straw. Martens *et al.* [12], who studied the effect of organic matter introduced into the soil in the form of bird dung, barley straw, sewage sludge and green fertilizer, obtained results similar to our studies indicating that the activity of the evaluated enzymes increased in the highest degree after soil fertilization with straw.

The analysis of data presented in Figure 1 indicated that the highest activity of the studied enzymes, in the majority of combinations occurred in term 1 – before sowing. The above phenomenon could have been connected with the fact that biochemical determination was carried out directly after the introduction of organic matter into the soil in the form of natural fertilizers and sewage sludge which could have contributed to the increase of the biological activity of soil. Additionally, one should keep in mind the fact that soil after the application of organic fertilizers is enriched with autochthonous microflora

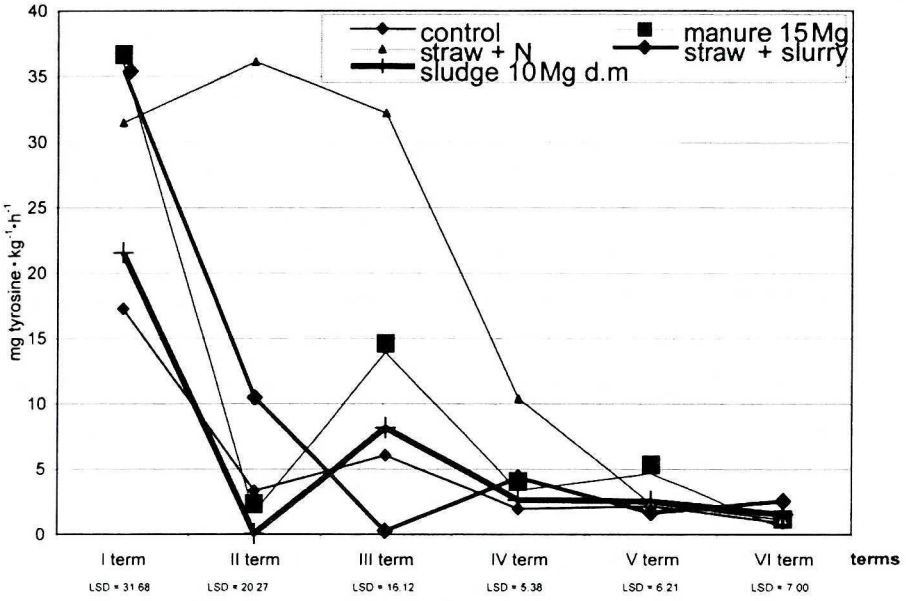

Fig. 1. The changes of protease activity in the soil organic fertilized

which can reveal strong metabolic activities. However, some controversies are evoked by the recorded increase of the activity of proteases also in the control combinations (1st term of analyses). One can suppose that the reason of such phenomenon was the absence of plants on the plots. This is because starting with the 2nd phase (plant germination), the activity of the studied enzymes showed sharp decline in the majority of combinations with the exception of plots fertilized with 5 Mg d.m.·ha^{-1} wheat straw + 15 kg of nitrogen per 1 Mg of straw. According to Wielgosz [20], some plants can secrete to the soil substances which impede the development of definite microorganisms causing in effect a decrease in the enzymatic activity of soil.

In the successive terms of analyses, the activity of proteases was subject to significant oscillations depending on the type of fertilizer combination; however, it was maintained on a low level until the end of the experiment. The studies of Koper and Piotrowska [8] indicate that the level of soil enzymatic activity depends, in a high degree, on the season of the year. According to these authors, enzymes are particularly active at the end of spring, while in summer, there follows a decrease of their activity.

Results presented in Figure 2 indicate that the number of proteolytic bacteria was determined by the fertilization method and by the developmental phase of maize. The greatest number of proteolytic bacteria was recorded in two developmental phases (terms 2 and 4), in soils where sewage sludge was used. It is reported that the number of proteolytic bacteria depends on the quality of organic fertilizer (Reference). In the experiment, it was recorded that the number of microorganisms decomposing protein, in soils fertilized with sewage sludge, significantly increased. Similar dependences were also recorded by Emmerling *et al.* [3] and Wolna-Maruwka *et al.* [21]. Sewage sludges are a very rich source of nutritive components for microorganisms, plants and soil microfauna. They

play an important role in the intensification process of the humification of organic substance [4, 22].

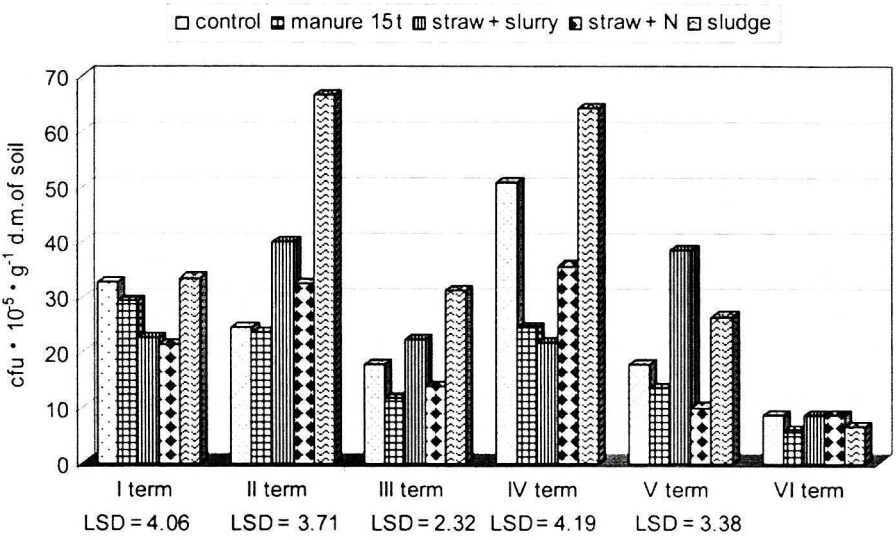

Fig. 2. Number of proteolitic bacteria in terms of analyses (developmental phases of plants) depending on organic fertilizations

On the basis of statistical analysis, it was found that in the control sample and in soils fertilized with manure at 5 Mg d.m.·ha^{-1} wheat straw + 15 kg nitrogen per 1 Mg of straw, the dynamics of proteolytic bacteria development had a similar course as the changes of proteases activity, since the Pearson's correlation coefficient was positive and it oscillated within 0.21–0.51 (Figs 3, 4 and 5). In the remaining fertilization combinations, no linear correlation was found between the number of bacteria and proteases activity in the soil (Figs 6 and 7).

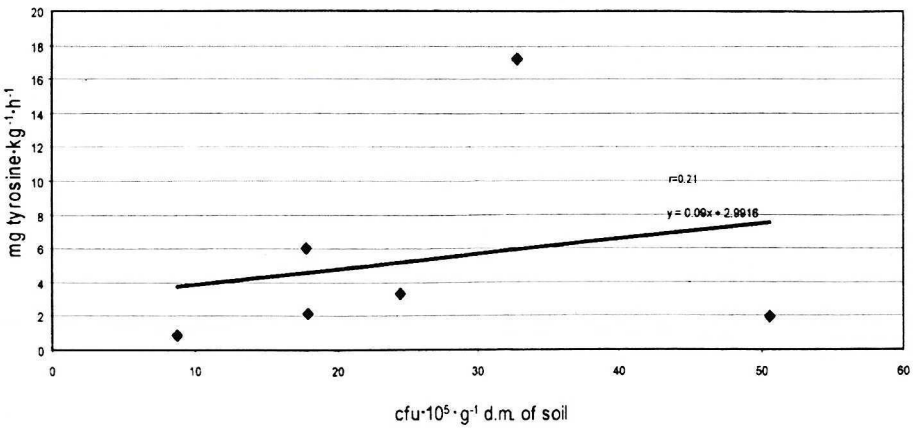

Fig. 3. Relationship between a number of proteolitic bacteria and proteases activity in control soil

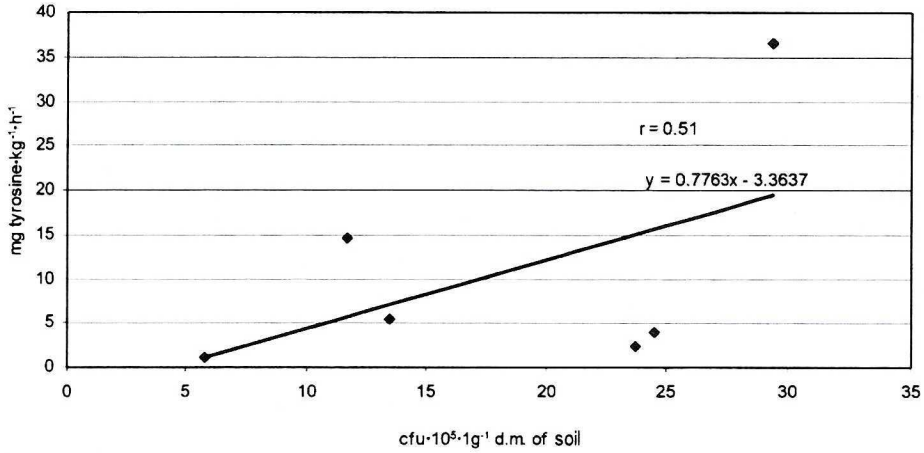

Fig. 4. Relationship between a number of proteolitic bacteria and proteases activity with manure fertilized

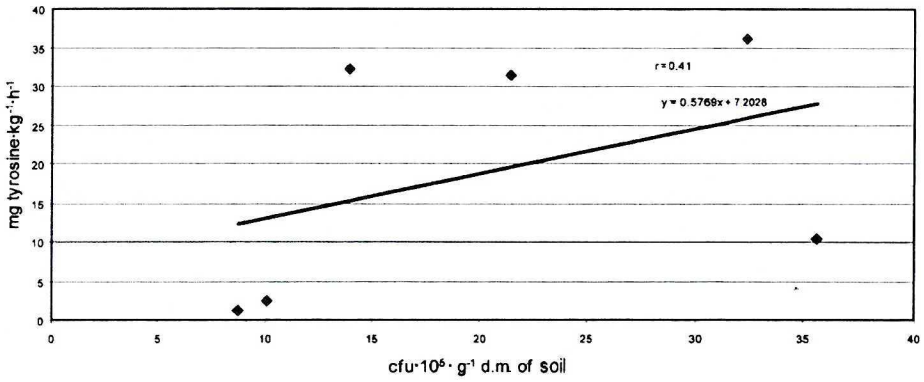

Fig. 5. Relationship between a number of proteolitic bacteria and proteases activity in straw and nitrogen fertilized

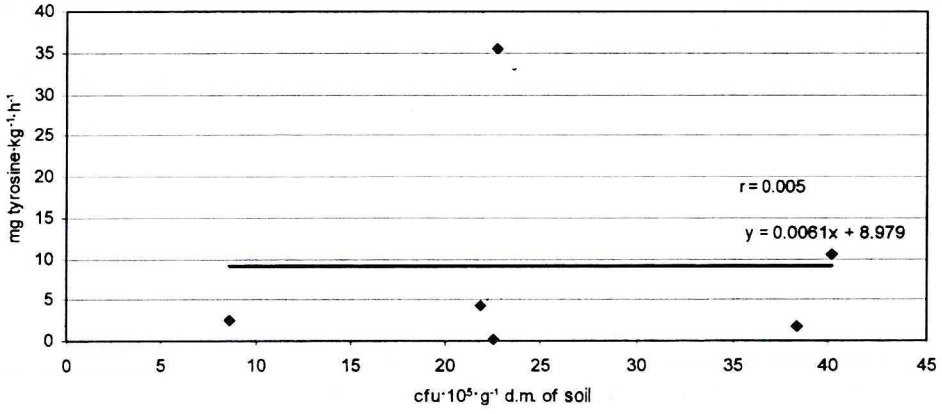

Fig. 6. Relationship between a number of proteolitic bacteria and proteases activity with straw and slurry fertilized

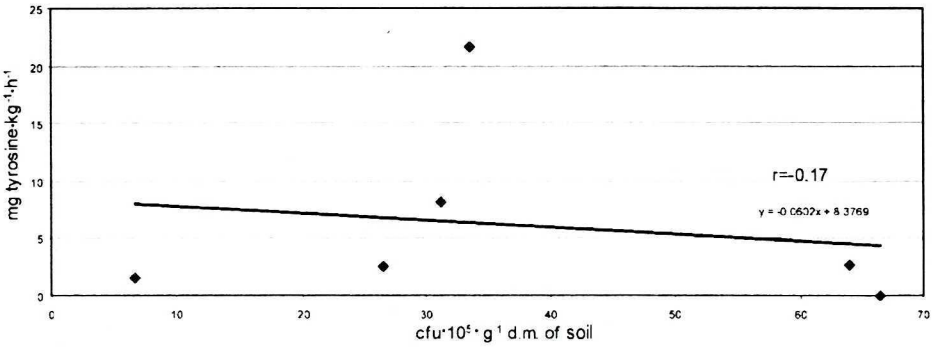

Fig. 7. Relationship between a number of proteolitic bacteria and proteases activity in soil with sewage sludge fertilized

In the growing of maize for silage, the application of each of the natural fertilizers and sewage sludge, in comparison with mineral fertilizers, caused an increase of fresh and dry matter of the whole plants, and the obtained differences were not statistically proven only for objects fertilized with straw + mineral nitrogen (Tab. 3). The highest yield of fresh matter of the whole plants was obtained using sewage sludge at 43.7 Mg·ha^{-1}.

Table 3. Yield of whole plants fresh matter [Mg·ha^{-1}] and cobs share in fresh matter of whole plants [%]

Specification	Fresh matter yield [Mg·ha^{-1}]	Cobs share in fresh matter of whole plants [%]
Control*	35.4	33.4
Manure 15 Mg	40.2	33.8
Straw + N	38.0	31.8
Straw + slurry	40.9	35.3
Sludge 10 Mg d.m	43.7	35.8
LSD = 0,05	3.93	1.98

* fertilized NPK

In the studies of Mazur and Sądej [13], a comparison of the yields of the whole plants obtained with interchangeable use of traditional natural fertilizers in the form of manure or pig slurry have shown that they were similar to those obtained with mineral fertilization while after the application of a double dose of cattle or pig slurry, they were higher. The doses of natural fertilizers in our own studies and in the quoted studies are in agreement with the Nitrogen Directive of the European Union which admits this form of fertilization up to 170 kg N·ha^{-1} annually.

Furthermore, it was found that similar results were obtained in soils fertilized with sewage sludge and pig slurry, these treatments were characterized by the highest share of cobs indicating that it was of a high quality.

Sewage sludges are characterized by a high hygroscopicity and thanks to this feature; they preserve and accumulate water improving thereby its retention in soil. Information about such properties of sewage sludge was given by Żurawski *et al.* [25] on the basis of their lysimetric studies. This may explain the good effect of sewage sludge in conditions of drought.

The above cited reports from other studies fully support the fact that agricultural utilization of communal sewage sludge is justified. Intensification of such studies is particularly recommended in regions where there is significant level of sewage sludge production for agricultural use.

CONCLUSIONS

1. The direction of changes in the soil depended on the type of the applied organic fertilizer, its dose introduced into the soil and the developmental phase of the maize. The greatest number of proteolytic bacteria was recorded in soils where sewage sludge was used.
2. All tested organic substances stimulated the activity of proteases.
3. Yields of maize as raw material for silages obtained from soil fertilized with sewage sludge were similar to use of traditional natural fertilizers in the form of manure or pig slurry.

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WPŁYW NAWOŻENIA ORGANICZNEGO NA DYNAMIKĘ ROZWOJU BAKTERII PROTEOLITYCZNYCH, AKTYWNOŚĆ PROTEAZ W GLEBIE POD UPRAWĄ KUKURYDZY (*ZEA MAYS* L.)

Celem pracy było zbadanie dynamiki wzrostu bakterii proteolitycznych oraz ich aktywności enzymatycznej przy wybranych wariantach nawożenia organicznego pod uprawą kukurydzy (*Zea mays* L.). Natężenie oraz kierunek powstałych zmian zależne były od rodzaju zastosowanej materii organicznej, wielkości jej dawki wprowadzonej do gleby oraz fazy rozwojowej uprawianej rośliny. Na podstawie uzyskanych wyników stwierdzono stymulujące działanie nawozów organicznych na aktywność proteaz. Uzyskane plony surowca do zakiszania na obiekcie nawożonym osadami ściekowymi były podobne jak po zastosowaniu gnojowicy świńskiej i przewyższały plony kukurydzy zebrane z innych obiektów nawozowych