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Original Research

Biological Activity of Grey-Brown Podzolic Soil Organically Fertilized for Maize Cultivation in Monoculture

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Abstract

Our studies utilize enzymatic indicators and microorganism numbers for the estimation of soil environment reaction to the applied differentiation of organic fertilizers and maize (*Zea mays* L.) cultivation.

The experiment had a field character. In different terms connected with the development of maize, the activities of, dehydrogenases and acid phosphatase were determined in the soil. The number of microorganisms (bacteria, actinomyces, fungi, oligotrophic, copiotrophic microorganisms) was determined by the plate method, on adequate agar substrates. Activity levels of the selected enzymes were defined using the spectrometrical method. The obtained results show that applied organic fertilization, as well as the cultivation of soil, had a significant effect on the activity of the analyzed soil enzymes.

It was found that dehydrogenases and acid phosphatase activity at the time of the experiment was the highest after the introduction into the soil of sewage sludge (5 tons d.m.·ha^{-1}·$year^{-1}$).

Keywords: microorganisms, enzymatic activity, soil, biowaste, maize

Introduction

Soil cultivation, fertilization, protection and soil pollution modify the physicochemical properties of soil and change its biological activity. Biological activity in soil, which includes all transformations of compounds and energy, can be measured by enzymatic activity [1]. It depends both on soil type, depth of soil profile, vegetation cover, atmospheric conditions and the method of soil cultivation and fertilization, as well as on many other factors acting on the soil [2-6].

According to Gliński et al. [7], the Koper and Siwik-Ziomek [8], soil enzymes are regarded as an objective, biological indicator of soil fertility, intensity of soil-creating processes and anthropopressure.

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Calderon et al. [9] and Drijber et al. [10] informed us that soil enzyme activity has been suggested as a suitable indicator of soil quality because enzymes are a measure of soil microbial activity and, therefore, are strictly related to nutrient cycles and transformation; they can rapidly respond to changes caused by both natural and anthropogenic factors and they are easily measured.

Moreover, as claimed by Trasar-Cepeda et al. [11], soil enzyme activity may be considered an early and sensitive indicator of the degree of soil degradation in both natural and agroecosystems, which qualifies them as a measuring factor of human impact on soil quality. The introduction into soil of natural fertilizers, including sewage slurry, may have significant influence on the growth and development of microorganism cells as well as on enzymatic activity. According to Koper et al. [12], the frequently applied agricultural practice of excessive and unilateral fertilization can lead to a disturbance in the balance of nutritive components in water solution. Actually, next to traditional organic fertilizers (manure, cattle slurry), attempts have been made to fertilize soil with sewage slurry [13]. Because of its high fertilization value, sewage sludge plays a significant role in soil fertility increases, particularly in the intensification of the humification process [14].

However, sewage sludge designed for agricultural utilization must be adequately stabilized by meeting the standards determined by the Regulation of the Polish Minister of Agriculture and Country Development of 2004 [15]. A measurable indicator of the biological life of soil, next to its enzymatic activity, is the dynamics of soil microorganism development expressed by changes in the number of the particular groups of microorganisms settled in this environment, i.e. the total number of bacteria, fungi, actinomycetes copiotrophic and oligotrophic microorganisms and many others. Soil favours their development because they fulfil all necessary conditions for their correct growth and development. Microorganisms occur in the soils in significant amounts and in a great variety.

The development and activity of soil microflora are closely related with plant life. The absence of microorganisms in soil would cause the extinction of higher plants because of mineral famine, and the absence of higher plants would lead to the disappearance of microorganisms. Microorganisms play an enormous role in the life of higher plants because they constitute the link which insures the inflow of nutritive components to plants. They perform many beneficial soil transformations, causing soil to be an adequate environment for growth and development. They participate in the transformation of the organic and mineral substances of the soil and they also decide about soil structure and pH. Many chemical compounds, being harmful to organisms living in soil environment, are detoxicated by soil microorganisms. Furthermore, thanks to microorganisms, other organisms that are pathogenic to plants and animals are eliminated from the soil [16].

Settlement and the way in which soils are settled by microorganisms depends to a high degree on the abundance of nutritive substances in the soil that are easily accessible, such as sugars, proteins, and fats. The occurrence of microorganisms depends also on the presence in the soil of allopathic compounds secreted by plant roots as well as by mutual interactions between different groups of microorganisms [17].

A great influence on the number of soil microorganisms is exerted by organic fertilization and mineral nitrogen fertilization, which is intended to supply nutritive components to the plants [18, 19].

Materials and Methods

Studies were carried out in 2006 on experimental 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. The experiment was established by the method of random block design in an area of 28 m^2. Plots were sown with maize (*Zea mays* L.) cultivar PR 39G12 designed for silage. Soil samples, on the basis of which biochemical analyses were carried out, were taken in six terms. Five sampling terms were connected with the successive developmental phases of maize:

I term – before sowing (BBCH0-Biologische Bundesanstalt, Bundessortenamt and Chemical Industry),
II term – germination (BBCH09),
III term – 2-3 leaves (BBCH12-13),
IV term – phase of 2^{nd} node (BBCH16-17),
V term – tasseling (flowering) (BBCH65),
VI term – phase of cob setting (BBCH70).

In maize cultivation, all necessary agrotechnical treatments were applied.

In the experiment, the following soil combinations were applied: s1- grey-brown podzolic soil + maize (control), s2- grey-brown podzolic soil + manure 15 ton fresh matter·ha^{-1}·$year^{-1}$ + maize, s3- grey-brown podzolic soil + 5 ton d.m. of wheat straw + cattle slurry 40 m^3 ·ha^{-1}·year + maize, s4- grey-brown podzolic soil + 5 ton d.m. of wheat straw + 15 kg nitrogen (on 1 ton wheat straw) ha^{-1}·$year^{-1}$ + maize, s5- grey-brown podzolic + sewage sludge 5 t d.m.·ha^{-1}·$year^{-1}$ + maize.

Soil samples were collected according to Polish Standard PN-ISO 1038-6 [20].

Additionally, all combinations were minerally fertilized with NPK. Nitrogen fertilization in the form of ammonium saltpeter, phosphorus in the form of triple superphosphate and potassium in the form of 60% potassium salt were applied in the amounts of 110 kg N·ha^{-1}, 80 kg P·ha^{-1}, and 120 kg·ha^{-1}.

Sewage sludge used in our studies were examined regarding 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. In consequence, in the doses of sewage sludge used in the experiments, the amounts of lead introducecd into the soil were 0.5% and copper 53%, according to Directive of the Minister of Agriculture and Rural Development. Apart from this heavy metals concentrations were determinated in plant organs (seeds, leaves and hypoctyl) and their values compared with the control.

Manure originated from a farm in Swadzim and cattle slurry was from a farm in Złotniki belonging to the University of Life Sciences in Poznań.

Experimental plots were situated on typical grey-brown podzolic soil created from postglacial formations, from light loamy sands lying shallowly on light loam which, according to soil classification, has been counted to the IVa quality class and to the 4^{th} complex of agricultural suitability (very good rye complex). The soil had a neutral reaction and it was characterized by very good potassium, phosphorus and magnesium contents (Table 1).

During the experimental period the suitability of climate conditions was estimated as moderate. The meterological conditions during the vegetation season are shown in Table 2.

Table 1. Selected proporties of soil.

Soil levels (cm)	pH	C (g · kg^{-1})	N (g · kg^{-1})	C:N	Mg (mg MgO·100g^{-1})	P (mg P_2O_5·100g^{-1})	K (mg K_2O·100g^{-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 temperature (ºC)			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 the enzymatic activity of soil fertilized with differentiated organic fertilizers were based on the determination of the activities of dehydrogenase and acid phosphatase (in four replications).

Studies on the enzymatic activity of soil fertilized with differentiated organic fertilizers were based on the determination of the activity. The activity of dehydrogenases was identified by spectrophotometric method using as substrate 1% TTC (triphenyl-tetrazole chloride), after 24-hour incubation at 30°C and wave length 485 nm. Enzyme activity was expressed in mg TPF·kg^{-1} d.m. of soil·24h^{-1} [21].

The activity of acid phospahtase was determined using as substrate p-nitrophenylophosphate sodium, after one hour incubation at 37°C with wave length 400 nm. Enzyme activity was expressed in mmol PNP·kg^{-1}·h^{-1} [22].

Microbiological Analyses

In soil samples taken from underneath the plants, from interrows and from the depth of 15-20 cm, the number of microorganisms was determined by the plate method according to Koch on adequate agar substrates (in five replications). The mean number of colonies was converted into soil dry matter:

- total number of bacteria and actinomycetes (CFU g^{-1} d.m. soil) was counted on 2% agar substrate, on soil extract after 14 days of incubation at 28°C [23].
- fungi were counted on Martin's nourishing substrate at 24°C [24].
- copiotrophic microorganisms (CFU g^{-1} d.m. soil) were determined on BO substrate (nutritive broth) at 28°C after 7 days of incubation [25].
- oligotrohpic microorganisms (CFU g^{-1} d.m. soil) were counted on diluted nutritive broth at 28°C after 21 incubation days [25].

Statistical Analysis

Statistical analyses applied in the experiment were used on the basis of Statistica 8.0 program.

All gathered results were subject to formal estimation by analyses of variance adequately to the experimental design. Results of field experiments were estimated in analyses of multiple experiments established in a completely randomized design. In the synthetic elaboration of field experiments, the full procedure of inter-object variation exact to the mean experimental error and to environmental interaction were applied. All general tests and detailed tests were carried out at the significance levels of $p < 0.01$.

Results and Discussion

Results of studies have shown that fertilization of grey-brown podzolic soil with natural fertilizers and with sewage sludge evoked changes in soil enzymatic activity. Also, the cultivation of maize contributed to changes in the enzymatic activity of soil. Intensity and the direction of the developed changes depended on the type of the applied organic matter, on the size of its doses introduced into the soil and on the developmental phase of the cultivated plant.

Phosphatase activity (Fig. 1) usually was impeded by the applied natural fertilizers and the sewage sludge. This fact was most distinctly visible in the case of soil fertilized with 5 ton d.m. of wheat straw ha^{-1}·year^{-1} + 15 kg nitrogen per 1 ton of straw. One of the reasons of the observed inhibition could have been the introduction together with the applied substances of some amounts of assimilable phosphorus that impeded the synthesis of the discussed enzymes. The above-mentioned phenomenon has found a reflection in the studies of Baran et al. [26], where the activity of phosphatases was most intensively impeded in the soil fertilized with sewage slurry; the inhibition effect was stronger the greater the dose of applied fertilizers. During the performed studies, the activity of phosphatase was subject to oscillations dependidng on the type of the applied biowastes and on the developmental phase of the plant. According to Wielgosz [27], the composition and number of soil microflora and the production of enzymes connected with it depends not only on the species of the grown plant, but also on its developmental phase.

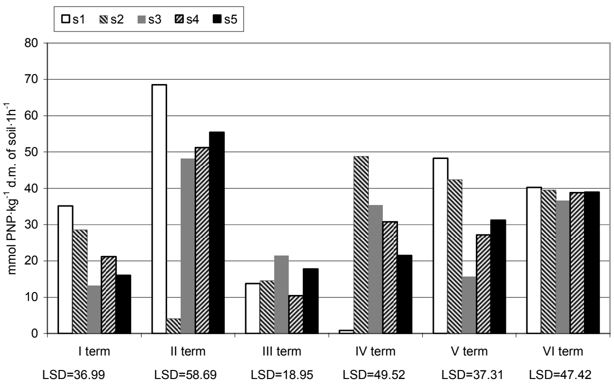

Fig. 1. Changes of acid phosphatase activity in organically fertilized soil.
Explanation:
s1-grey-brown podzolic soil + maize (control),
s2- grey-brown podzolic soil + manure 15 ton fresh matter·ha^{-1} · year^{-1} + maize,
s3- grey-brown podzolic soil + 5 ton d.m. of wheat straw + cattle slurry 40 m^3 ·ha^{-1}·year + maize,
s4- grey-brown podzolic soil + 5 ton d.m. of wheat straw + 15 kg nitrogen (on 1 ton wheat straw)·ha^{-1}·year^{-1} + maize,
s5- grey-brown podzolic + sewage sludge 5 t d.m.·ha^{-1}·year^{-1} + maize.
LSD- Least Significant Difference.

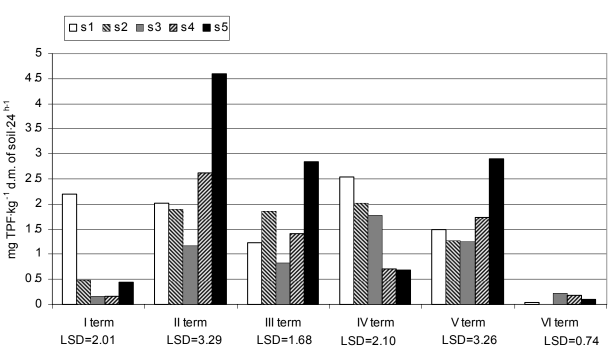

Fig. 2. Changes of dehydrogenase activity in organically fertilized soil.

We have accepted that the metabolic activity of microorganisms (mainly of bacteria) is revealed by the activity of their enzymes, and in the experiment we also took into consideration the changes in the activity of dehydrogenases in the soil (Fig. 2). It is known that the level of dehydrogenase activity determines the rate of oxidoreductive transformations in soil environment and characterizes the given soil, being a measuring indicator of its fertility [28]. On the basis of data shown in Fig. 2 it was found that only the introduction into the soil of sewage sludge caused the stimulation of dehydrogenase activity (increase by 20% in comparison to the control soil). Similar tendencies were recorded in the studies by Baran et al. [26]. The authors explained the increase of dehydrogenase activity in soil after the introduction of sewage sludge by a greater content in the slurry of organic substances that are more disposed to decomposition.

In our experiment, it was also found that the activity of dehydrogenases in combination with sewage sludge showed, in comparison with the remaining combinations, the highest positive correlation coefficient with the total number of bacteria (Fig. 7).

According to Kucharski [29], sewage sludge, after introduction into the soil, can contribute to the inhibition of soil enzymatic activity. Sewage sludge usually contains some amounts of heavy metals, PCB, PAH, pesticides and other compounds which have a negative effect on the activity of dehydrogenases and other enzymes. After a comparison of the activity of the studied enzymes between the control soil and the remaining fertilizations applied during the experiment, it was found that the used natural fertilizers inhibited dehydrogenase activity. This effect was most distinctly visible in the case of soil fertilized with 5 tons d.m. of wheat straw·ha^{-1} year^{-1}+15 kg nitrogen per one ton of straw (activity was decreased by 43% in relation to the control soil).

Statistical analysis has shown that a positive correlation (Figs. 3-7) between the activity of the studied enzymes and the number of bacteria occurred only in the control combination and in the plot fertilized with wheat straw and manure at the same time. Activity level of dehydrogenases during the experiment depended most probably on the developmental phase of maize and on the presence of easily decomposable organic matter introduced into the soil together with natural fertilizers [8].

According to Simsabaugh et al. [30], enzyme activity is connected not only with the plant species, but it also depends on the amount of plant remains from the depth of the root system. Amdor et al. [31] stressed the strong connection between the activity of enzymes and soil properties (pH, content of organic carbon and others).

According to Koper and Piotrowska [2], the level of enzymatic activity of soil depended in a high degree on the season of the year. The studies of the above-mentioned authors indicate that enzymes are comparatively active in spring, while in summer, there follows a decrease of their activity.

Among many anthropogenic factors having a high effect on soil microorganisms, the most important is fertilization including both the organic and mineral nitrogen [18].

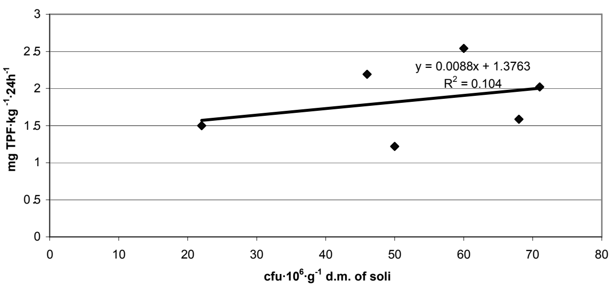

Fig. 3. Relationship between number of bacteria with dehydrogenases activity in control soil.

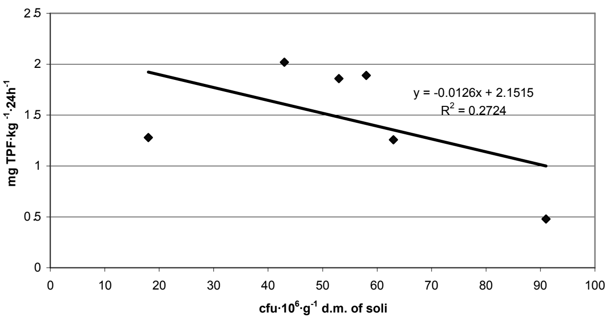

Fig. 4. Relationship between number of bacteria with dehydrogenases activity in soil fertilized with manure.

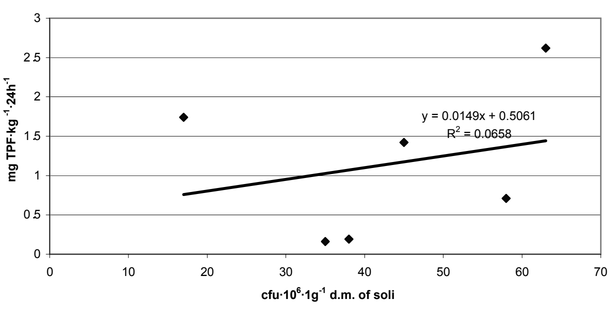

Fig. 5. Relationship between number of bacteria with dehydrogenases activity in soil fertilized with slurry and straw.

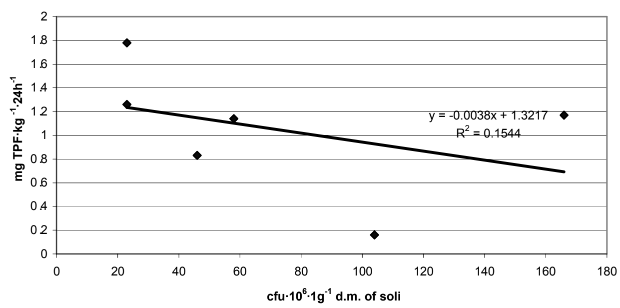

Fig. 6. Relationship between number of bacteria with dehydrogenases activity in soil fertilized with straw and nitrogen.

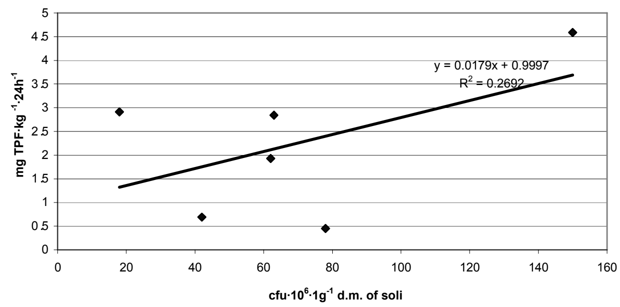

Fig. 7. Relationship between number of bacteria with dehydrogenases activity in soil fertilized with sewage sludge.

The application of differentiated fertilization and the successive stages of plant development found a reflection in the changes of the number of the studied soil microorganisms.

In the complex of the analyzed microorganisms, among others the development of dynamics of copiotrophic microorganisms was taken into consideration. It is a specific group of soil microorganisms intensively proliferating during the inflow into the soil of organic matter, mainly in the form of plant and animal remains. Therefore, their requirements are connected with a high concentration of organic components in substrates and their optimal dose is about 1,000 mg of soluble C·litre^{-1} [32].

Data shown in Fig. 8 indicate that organic fertilization had a positive effect on the development of copiotrophic microorganisms in the soil. The most intensive development of the studied group of microorgananisms was recorded in fields fertilized with 5 ton d.m. of wheat straw + cattle slurry 40 m^2·ha^{-1}·year^{-1}. Such a distinct increase of copiophytic microorganism numbers on plots where the above fertilization combination was applied was recorded throughout the whole experimental period in almost all developmental stages of maize. The above-mentioned fertilization method gave the highest increase (by 144%) of copiotrophic microorganisms in comparison with the content in the phase before sowing and in the phase of 2-3 leaves where the number in comparison with the control increased by 110%.

However, the highest number of the analyzed group was recorded in the phase of 6-7 leaves and it amounted even to 152 CFU·10^3 d.m. soil. Some reserachers stress that the use of cattle slurry for many years exerts a negative effect on the development of copiothropic microorganisms [4].

However, the applied combination of cattle slurry with the dose of 5 t d.m. of wheat straw has shown to be a treatment intensively stimulating the proliferation of copiothropic microorganisms in the soil. Studies have repeatedly confirmed the beneficial effect of the simultaneous introduction of organic fertilizer originating from two types of plant remains.

Not without importance also is the effect exerted by soil type. Kucharski [29] compared cattle slurry with manure and found that manure has a more stimulating effect on the microbiological activity of soil. Manure caused an increase of copiotrophic microflora in grey-brown podzolic soil. A higher dose of cattle slurry in brown acid soil exerted a negative effect on the proliferation of copiotrophic bacteria.

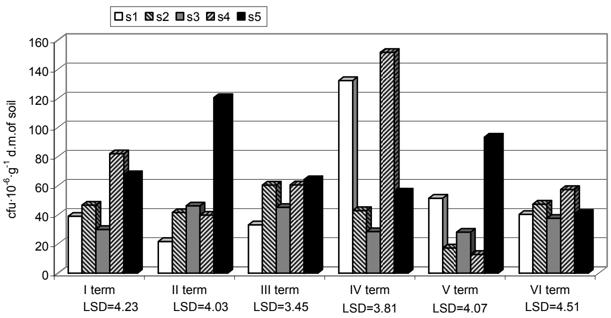

Fig. 8. The number of copiotrophic bacteria in the particular terms of analyses (developmental phases of plants) depending on organic fertilization.

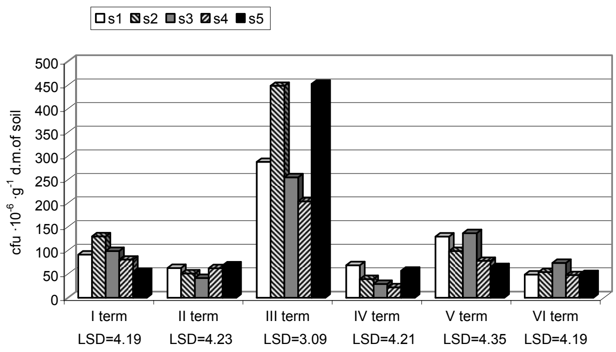

Fig. 9. The number of oligotrophic bacteria in the particular terms of analyses (developmental phases of plants) depending on organic fertilzation.

On the other hand, studies carried out by Balicka et al. [33] showed a significant increase of copiotrophic bacteria in brown soil fertilized with cattle slurry. The particularly favourable action of cattle slurry was found when it was applied in light soils. In such soils, significantly higher yields of maize dry matter fertilized with cattle slurry were obtained than with the application of the same doses of N in mineral fertilizers [34]. Cattle slurry is increasingly more frequently used for maize fertilization. It represents a fertilizer reach in nitrogen and is very well suited for plants with a high requirement for this component. Maize uptakes all nutritive components during the whole vegetation season and most intensively in the period of flowering and cob setting, which is reflected in the number of copiotrophic microorganisms.

A significant drop in the number of the studied microorganisms in the flowering phase of the plant could be explained by the peculiar type of competition between the plant and the microorganisms for the utilization of nutritive components contained in the substrate.

A good organic fertilizer for copiothropic microorganisms also has shown to be the sewage slurry from which they extracted nutritive components necessary for their development. A similar effect of sewage slurry on the development of copiothropic microorganisms in the soil was also observed by Lima et al. [35] and by Sastre et al. [36] and Wolna-Maruwka et al. [37]. The greatest dynamics of copiothropic microorganisms in soil fertilized by sewage sludge was recorded in the phase of germination in which the number of bacterial cells was even fivefold higher in comparison with the control and in the phase of flowering where the number of copiothropic microorganisms increased by about 180% in comparison with monoculture.

In the combination with the application of the dose of 5 t d.m. of wheat straw + 15 kg of nitrogen (on 1 ton wheat straw)$\cdot ha^{-1}\cdot year^{-1}$, the lowest number of the studied microorganisms was found and it was by about 50% lower than the number of copiothropic microorganisms in soil fertilized with manure in the dose of 15 tons of fresh matter$\cdot ha^{-1}\cdot year^{-1}$.

The successive group of microorganisms determined in the experiment included oligothropic microorganisms (Fig. 9). They represent microorganisms that grow in environments with a low availability of orgnic compounds. Optimal concentration of nutritive components for oligothrophs oscillates from 1 to 15 mg of solved $C\cdot litre^{-1}$. The notion of oligotrophy refers to bacteria that grow in a poor substrate with low concentration of nutritive components only at the beginning of breeding. On the other hand, in successive sowing these bacteria also grow well in a substrate rich in nutritive components (Paul, Clark 2000). These bacteria show little variability regarding the number and activity. They do not require another nourishment or energy source beyond that which usually is found in the soil. These organisms are unusually sensitive to aminoacids, organic acids, inorganic salts such as NaCl and KCl. Soil is not their only habitat, they can be found in water as well [38].

In the analysis of changes in the number of oligothropic microorganisms, among the applied fertilization combinations, manure has proven to be the best one for their development in the dose of 15 t d.m.$\cdot ha^{-1}\cdot year^{-1}$. On the other hand, an addition of wheat straw in combination with mineral nitrogen and cattle slurry decreased the number of the studied microorganisms. Hu et al. [39] reported that the high availability of carbon exerts an inhibiting effect on the presence of oligothropic microorganmisms in the soil, which may be an explanation for the obtained results. Similarly, Kobus [40] stated that the content of organic matter available to microorganisms is the main factor limiting their activity. Straw in comparison with manure contains significantly more carbon. The C:N proportion in a fermented manure is 15-20:1, while in straw the proportion is significantly wider. In the phase of 2-3 leaves, regardless of the fertilization method, a violent increase of oligothropic microorganisms was observed. This increase was greater by 2-3 times, sometimes reaching even 400% in relation to the remaining phases of development. In the phase of 6-7 leaves and in the phase of cob setting, the number of bacteria reached the lowest values. This phenomenon was probably connected with the quantitative composition of root secretions of maize. Plants, through their own root secretions as well as by the substances developing as a result of post-harvest remains act in different ways on soil microorganisms. Maize produces significant quantities of root secretions that may include aminoacids, hydrocarbons, vitamins, orgnic acids, enzymes, and ions of metals.

These substances inhibit or stimulate the growth and development of microorganisms [41]. The quantitative and qualitative comparison of root secretions depends on many factors, primarily on the species and the developmental phase of plant, soil type, temperature and moisture, nitrate nitrogen and ammonium nitrogen, as well as on assimilable phosphorus and potassium and also on the oxide-reductive potential of soil and its reaction [18]. Most probably, the intensive root secretion in the phase of 6-7 leaves shortly before plant flowering has been shown to be a factor impeding the development of oligothropic microorganisms that are sensitive to aminoacids, organic acids and vitamins of oligothropic microorganisms.

On the basis of the obtained results (Figs. 10-12) one can state that the number of other studied microorganism groups (fungi, actinomycetes, total number of bacteria) depends also on the type of the introduced fertilizer and on the developmental phase of the plant.

Statistical analysis has proven that differences between the number of microorganism cells in the control soil and their number in the remaining combinations exist.

The greatest number of bacteria (Fig. 10) in the studied experimental plots was recorded in the phase of germination at the fertilization with wheat straw + mineral nitrogen. Mineral nitrogen additions have a positive influence on the development of microorganisms in soil. This positive effect is particularly strongly visible in the presence of plant materials, e.g. straw. The smallest number of bacteria was recorded in the 5th phase of development when manure fertilization in the amount of 15 tons was applied. It is worth noting that significant growth stimulation of the total number of bacteria occurred under the influence of sewage sludge. This point of view was confirmed by Wolna-Maruwka and Sawicka [42].

According to the authors, sewage slurry introduced into the soil shows a stimulating effect on the total number of bacteria.

In the cultivation of maize for silage, a stimulating influence of sewage slurry was also noted in reference to the number of actinomycetes in the studied soils. Wolna-Maruwka et al. [43] reported in their studies that sewage sludge was characterized by a significant effect on the development of actinomycetes. These microorganisms constitute a rich source of nutritive components containing great amounts of organic substances that provide a rich environment for microorganism activity. The studies of Wolna-Maruwka and Sawicka [44] indicate that a strong stimulation of actinomycetes takes place in the presence of sewage slurry introduced into the soil as fertilizers while the stimulation is definitely less intensive in the case of manure introduced as fertilizer.

Analysis of the number of fungi in the presented experiment (Fig. 11) indicate that in the majority of soil combinations with the addition of organic matter, mould fungi showed better development than in the control soil. A proper development of fungi can be caused by competition for nutrition with other microorganisms. Kurek and Jaroszuk-Ścisel [45] wrote about the inhibiting effect of *Pseudomonas* genus on fungi development.

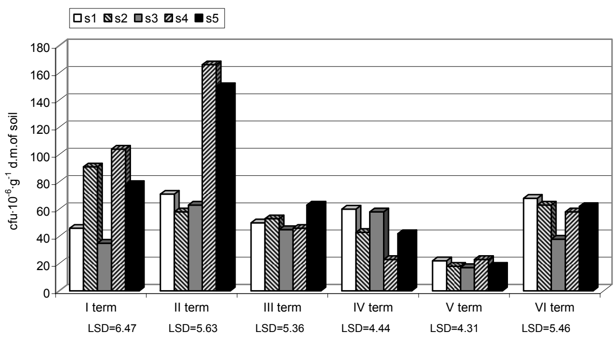

Fig. 10. Total bacteria number in the particular terms of analyses (developmental phases of plants) depending on organic fertilzation.

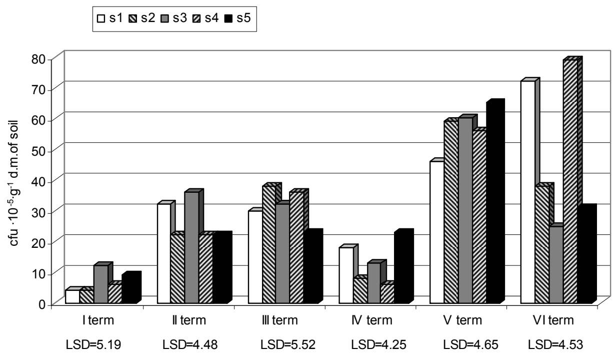

Fig. 11. The number of fungi in the particular terms of analyses (developmental phases of plants) depending on organic fertilzation.

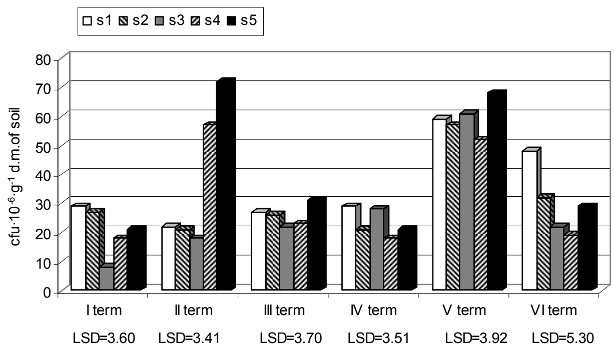

Fig. 12. The number of actinomyces in the particular terms of analyses (developmental phases of plants) depending on organic fertilzation.

A significant question in the presented experiment that should be considered is the decrease of the total number of bacteria (in the 5th term: tasseling phase). It is correlated with the increase of the number of fungi and actinomycetes (Fig. 12) in that developmental phase.

The most probable reason for this situation is the decrease of the content of easily assimilable chemical compounds and thereby the limitation of the number of bacteria to the advantage of the increase of the number of fungi and actinomycetes that are able to utilize not easily assimilable compounds. This point of view was confirmed by Wielgosz

[46], who stated that in the first place, there develop quickly growing organisms that decompose mono- and oligosacharides and aminoacids. When these compounds are depleted, there appear actinomycetes and fungi.

Conclusions

1. The applied organic fertilizers exerted a significant effect on the activity of the analyzed soil enzymes.
2. The activity of dehydrogenases and acid phosphatase was stimulated in the highest degree by the addition to the soil of sewage sludge (5 ton d.m.$\cdot$ha$^{-1}\cdot$year^{-1}).
3. Developmental phases of maize and different organic fertilization had great influence on the number of copitrophic and oligotrophic bacteria.
4. On the base of our results, it was found that the total number of bacteria, actinomyces and fungi was determined by the fertilization method and by the term of analyses.
5. Statistical analyses showed a significant effect on the applied organic fertilization on the growth and development of microorganisms in all developmental phases of maize.

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