

DYNAMICS OF SIZE CHANGES OF SELECTED GROUPS OF MICROORGANISMS IN THE SOIL FERTILIZED WITH MUNICIPAL SEWAGE SLUDGE

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Abstract: The object of the two-year experiments was to determine the dynamics of development of selected groups of microbes (oligotrophic, copiotrophic, proteolytic, cellulolytic and solving phosphate microorganisms) in the soil fertilized with communal sewage sludge. The aim of the performed investigations was to study the possibilities of disturbing the biological balance of soil as demonstrated by intensified and long-term development of the analyzed groups of bacteria in the soil following its fortification with different doses of organic matter in the form of sewage sludge. The following four soil treatments were applied in the trail: control – soil + NPK; 2 Mg d.m. of sludge·ha⁻¹·year⁻¹ + NPK; 4 Mg d.m. of sludge·ha⁻¹·year⁻¹ + NPK and 8 Mg d.m. of sludge·ha⁻¹·year⁻¹ + NPK. Phosphorus and potassium were applied pre-sowing during plowing and nitrogen was divided into two parts and the first of them was applied pre-sowing while the second – as top-dressing. Sewage sludge was applied pre-sowing. Experimental plots were sown with rye (variety of *Wibro*) in 2003 and planted with potatoes (variety of *Bila*) in 2004. It was demonstrated that the applied doses of sewage sludge exerted, practically speaking no statistically significant impact on the propagation of soil microorganisms. On the basis of the performed microbiological cultures, it was concluded that, during the analyzed period (2003–2004), the numbers of the examined groups of microorganisms varied depending on the date of soil samples collection. Soil samples for analyses were collected in each year of experiments at dates associated with the developmental phases of plants and in ten replications. Another factor which influenced the development dynamics of soil microorganisms was the species of the crop plant cultivated in the experiment. Rye, which was cultivated in 2003, turned out to stimulate the developmental of the majority of the examined groups of microbes (oligotrophic, copiotrophic, proteolytic, cellulolytic and solving phosphate microorganisms), while potatoes stimulated during their generative stage (16.07–28.08.2004) a stronger proliferation of cellulolytic microorganisms.

INTRODUCTION

Sewage sludges constitute an inseparable element of operation in all sewage treatment plants. Following improved effectiveness of the sewage treatment processes and the introduction of increasingly strict restrictions regarding the quality of water treatment, the amount of sewage sludge increases steadily. Simultaneously, problems associated with the utilization of these wastes continue to pose a serious and growing challenge both locally and globally [14].

Sewage sludges can be utilized in agriculture provided they do not exceed acceptable quantities of heavy metals, contain appropriate amounts of nutrients and have undergone suitable processes aiming at the reduction of pathogenic organisms [3, 16, 21].

The above-mentioned bio-wastes, apart from possible contamination, contain significant quantities of organic matter as well as macro- and micro-elements. Stabilized municipal sewage sludge introduced into the soil, alongside considerable quantities of organic matter, high quantities of nitrogen (about 6% of dry matter) and phosphorus (about 3% of d.m.). Only quantities of potassium added to the soil are low and usually do not exceed 0.5% d.m. [5, 10]. Elements introduced into the soil in this way may be utilized by soil microorganisms and crop plants and the organic matter can improve soil physical quality and increase its sorption capacity. The ratio of organic carbon to nitrogen found in the stabilized sewage sludge is similar to the C:N ratio in cultivated soils of good quality or in very ripe composts [23]. The reintroduction into the soil of components found in the sewage sludge is justified not only from the economical point of view but is essential to maintain and restore ecological balance.

Investigations carried out by Czekala [4], Wójcikowska-Kapusta *et al.* [28], Baran *et al.* [2] and Kalembasa, Kuźniemska [15] demonstrated that appropriately stabilized sewage sludge can provide a rich source of nutrients.

Organic fertilization using sewage sludges can be one of the most important agrotechnical treatments. When applied at suitable quantities and at the appropriate time, they can constitute not only a valuable source of nutritive substances for plants but, equally importantly, they can act as catalysts enhancing chemical transformations involving soil microorganisms leading, ultimately, to the improvement or at least maintenance of land fertility [25].

Soil organic substances, indigenous and supplied in fertilizers, as well as products of their biological and chemical transformations exert a decisive influence on the favorable system of the entire complex of soil properties. The organic matter introduced into the soil has a definite impact, among others, on the bio circulation of elements, exerts a positive effect on the soil water economy as well as on its phyto-sanitary condition [19]. The more abundant the supply of organic matter to the soil, the more frequent the reconstruction of humus and, consequently, the more favorable the conditions for the growth and development of crop plants [24].

MATERIAL AND METHODS

The experiment was carried out on experimental plots of the Experimental-Didactic Station of the Department of Soil and Plant Cultivation in Złotniki, which belongs to the Agricultural University of Poznań. The experiment was established using the design of random blocks of 42 m² which were sown with spring barley (2002). The trial was conducted on grey-brown podzolic soil of IVa and IVb classes characterized by the following chemical properties: $\text{pH}_{\text{KCl}} - 5.60$, $\text{C} - 6.68 \text{ g}\cdot\text{kg}^{-1} \text{ d.m.}$, $\text{N} - 0.60 \text{ g}\cdot\text{kg}^{-1} \text{ d.m.}$, $\text{C:N} - 11.1$. From among all fertilization combinations, this paper presents data concerning the following soil objects: control (soil + NPK), 2 Mg d.m. sewage sludge $\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ + NPK, 4 Mg d.m. sewage sludge $\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ + NPK and 8 Mg d.m. sewage sludge $\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ + NPK. Each of the above-mentioned soil combinations were used in two replicates. All treatments were established in three repetitions.

Nitrogen was applied in the form of ammonium saltpeter, phosphorus – in the form of triple superphosphate and potassium – in the form of 60% potassium salt. The applied quantities were as follows: (2003) 60 kg N $\cdot\text{ha}^{-1}$, 35 kg P $\cdot\text{ha}^{-1}$, 90 kg K $\cdot\text{ha}^{-1}$; (2004) 120

kg N·ha⁻¹, 83 kg K·ha⁻¹, 22 kg P·ha⁻¹. Phosphorus and potassium were applied pre-sowing during plowing and nitrogen was divided into two parts and the first of them was applied pre-sowing while the second – as top-dressing. Sewage sludge was applied pre-sowing.

The sewage sludge applied in the experiment was characterized by the acceptable content of heavy metals and the following chemical composition: pH_{H₂O} – 6.63, dry matter – 20.36 %, MO – 71.80 %, Corg. – 29.51 %, N_{tot} – 6.23 %, C:N – 4.74:1.

The chemical analyses were carried out in the Department of Soil Science, August Cieszkowski Agricultural University of Poznań.

The microbiological analyses are presented in Tables 1 and 2.

Table 1. Microbiological composition of sewage sludge used in the experiment in 2003

Kind of microorganisms	2003 year
Mean (cfu·10 ⁵ ·g ⁻¹ d.m. of sewage sludge)	
Oligotrophic microorganisms	66.92
Copiotrophic microorganisms	24.33
Proteolytic microorganisms	4.05
Cellulolytic microorganisms	49.34
Phosphate microorganisms	58.81

Table 2. Microbiological composition of sewage sludge used in the experiment in 2004

Kind of microorganisms	2004 year
Mean (cfu·10 ⁵ ·g ⁻¹ d.m. of sewage sludge)	
Oligotrophic microorganisms	1789.43
Copiotrophic microorganisms	627.63
Proteolytic microorganisms	16.02
Cellulolytic microorganisms	30.97
Phosphate microorganisms	59.48

Soil samples for analyses were collected in each year of experiment at dates associated with the developmental phases of plants and in ten replications. In the case of rye, these dates included: the phase of the first jointing – 01.05.2003, full earing – 16.05.2003, end of flowering – 27.05.2003, full maturity – 23.07.2003 and two weeks after the harvest – 06.08.2003. In the case of potatoes (2004), these dates included: the closing of inter-row spaces – 26.06.2004, end of potato flowering – 16.07.2004, development of fruits – 25.08.2004, the final harvest – 15.09.2004 and two weeks after the harvest – 29.09.2004.

Microbiological analyses comprised the determination of the numbers of oligotrophic, copiotrophic microorganisms as well as proteolytic, cellulolytic and phosphate solvolytic microorganisms. The examined groups of microorganisms were determined on solid media (in five replications) employing appropriately diluted soil suspensions and expressed in cfu·g⁻¹ of soil dry matter.

Oligotrophic microorganisms were determined on the medium according to Hattori, Hattori [12] at the temperature of 28°C and their counts were established after 14 days. Numbers of copiotrophic microbes were determined by the plate method, also on the Hattori, Hattori substrate at the temperature of 28°C and their counts were estimated after 7 days [12].

In order to establish counts of proteolytic microorganisms, the author employed a selective medium after Rodina [20]. Plates were incubated for 48 hours at the temperature of 28°C. Cellulolytic microorganisms were determined on the medium developed by Rodina by incubating plates at the temperature of 28°C for 8 days [20].

Phosphate solving microorganisms were determined on the medium according to Rodina [20] by incubating the plates at the temperature of 28°C and their counts were established after 10 days.

The obtained results were subjected to statistical analysis with the assistance of the Statistica 7.1 software.

RESULTS AND DISCUSSION

The results of microbiological analyses presented in Figure 1 reveal absence of significant differences in the counts of oligotrophic microorganisms in relation to the dose of the applied fertilization treatment. In addition, they indicate that the date of sample analyses affected changes in the numbers of oligotrophs in the soil. The obtained research results fail to confirm earlier observations by Wyszowska *et al.* [30] and Kobus [17]. The above-mentioned researchers maintained that fresh supplies of organic matter can be one of the factors influencing increased microbial numbers and activity in the soil.

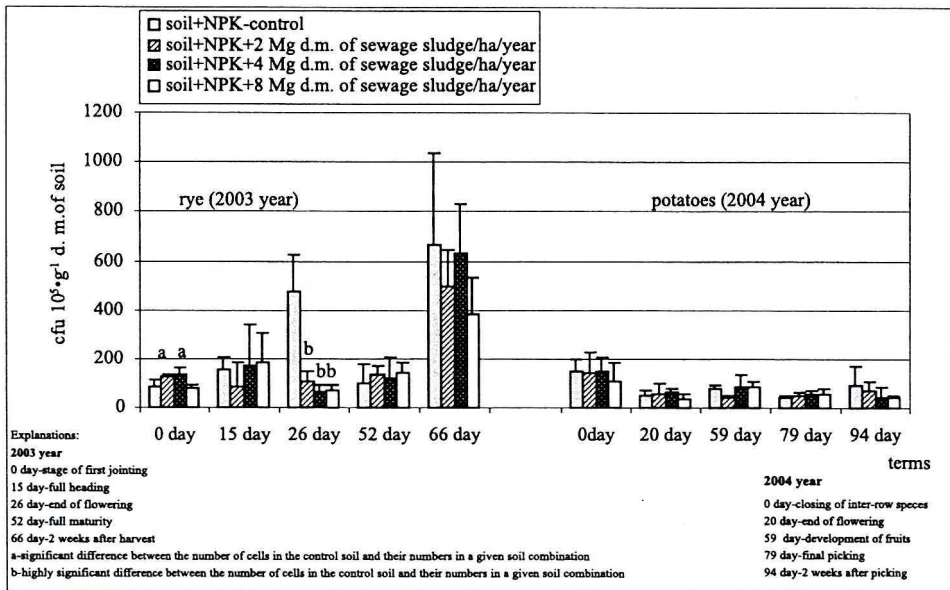


Fig. 1. The number of oligotrophic microorganisms in the soil with different levels of organic fertilizations

The performed analysis of results (Fig. 1) revealed that throughout the duration of the experiment, the numbers of oligotrophs in the examined fertilization combinations remained on a similar level. Certain deviations were observed only in the soil under rye (2003) two weeks after the harvest (Fig. 1) where strong proliferation of cells was observed. The influence of organic fertilization on the development of oligotrophic microor-

ganisms in the soil under wheat cultivation was investigated by Wyczółkowski *et al.* [29] who found that numbers of the examined microbes declined gradually in the course of the experiment reaching the lowest values during the phase after harvest.

In the described investigations, maximum cell counts occurred most frequently in the control soil fertilized only with NPK. The observed lower number of the discussed microorganisms in the soil with the addition of organic matter could have been caused by the antagonistic effect of microorganisms introduced into the soil together with the sewage sludge. However, the above interpretation is not corroborated by an experiment conducted by Furczak and Joniec [7]. These researchers claim that the increased number of oligotrophs in the soil is sometimes connected with the effect of sewage sludge.

In the case of copiotrophs (Fig. 2), their cells were found to proliferate worse in the soil under potatoes (in 2004). It was by 98% lower in comparison with their numbers recorded in 2003 (under rye). It should be mentioned here that the sewage sludge introduced into the soil in 2004 was characterized by considerably higher numbers of copiotrophic microorganisms (Tab. 1) in comparison with the sludge applied earlier.

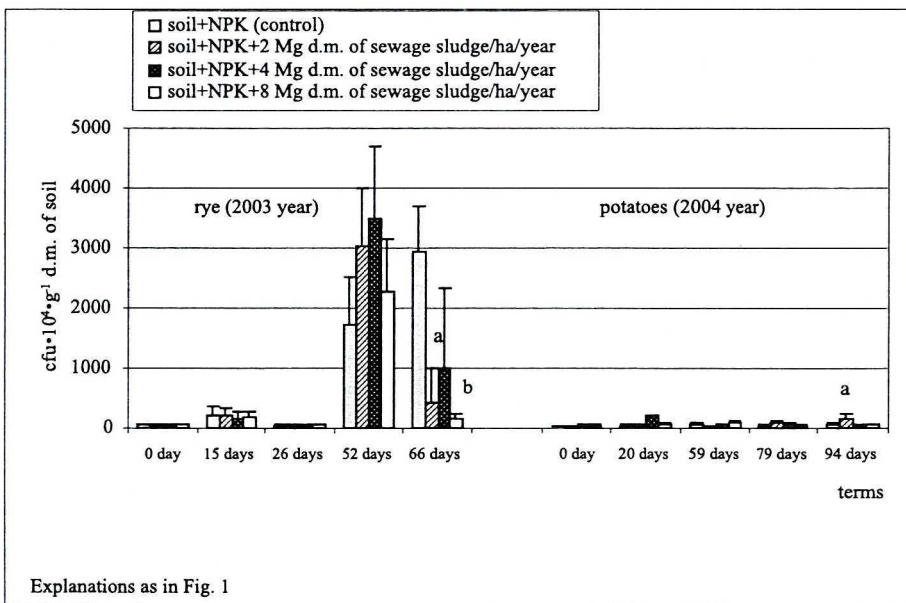


Fig. 2. The number of copiotrophic microorganisms in the soil with different levels of organic fertilizations

Initially, numbers of copiotrophic microorganisms in the soil under rye cultivation remained low and it was only during the phase of full maturity that the cells increased their proliferation and their maximum values were achieved. Generally speaking, it is assumed that the introduction of sewage sludge into the soil results in the increase in the numbers of copiotrophic microorganisms [1, 13]. However, this phenomenon was not observed in our experiments which could be attributed to a rapid depletion of the most important nutrients needed by microorganisms, the phenomenon of antibiosis, production of toxic substances (e.g. by actinomycetes) or the activity of bacterial parasites which control the numbers of microorganisms keeping them on a low level.

The results presented in Figure 3 indicate that the numbers of proteolytic bacteria were determined by the level of organic fertilization and the date of soil sample collection. However, the performed statistical analysis showed lack of significant or highly significant differences in cell counts between the control soil and treatments with the sewage sludge (Fig. 3). The results of investigations presented in this paper were not confirmed by observations of Frąć and Jezierska-Tys [6], Jezierska-Tys and Frąć [13] who reported a stimulating influence of the applied sewage sludges on the development of the above-mentioned microorganisms.

It is also evident from data presented in Figure 3 that at some experimental dates the proliferation of proteolytic bacteria in the control soil proceeded at the level similar to the treatment fertilized with organic fertilizers. Also investigations carried out by Barabasz *et al.* [1] showed a stimulating influence of mineral fertilization (NPK) on the development of proteolytic microorganisms.

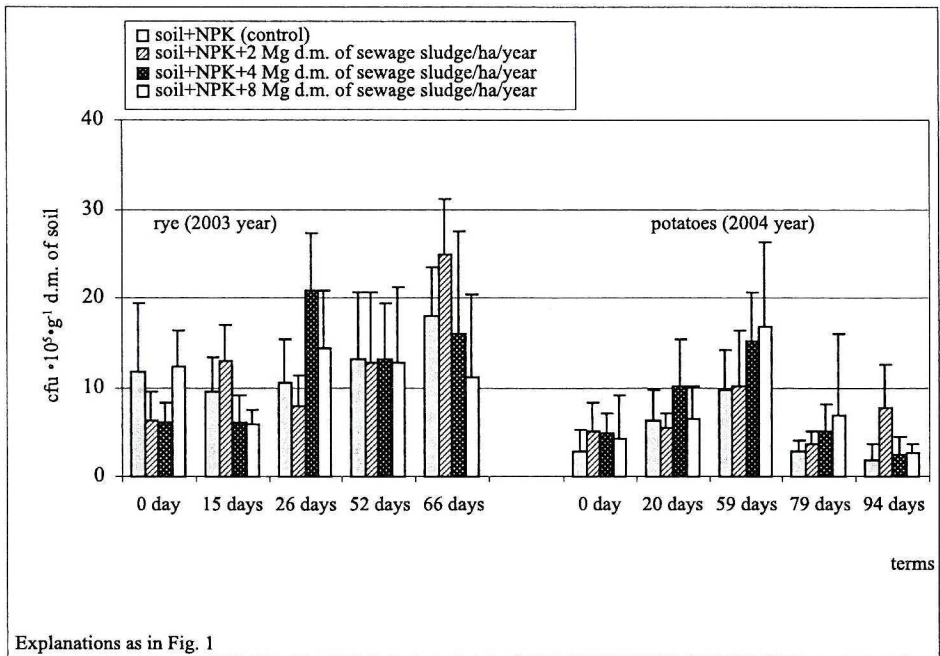


Fig. 3. The number of proteolytic microorganisms in the soil with different levels of organic fertilizations

In 2003, stronger proliferation of proteolytic microorganisms in the soil was recorded in the case of the last three dates, whereas in 2004 – during the period of development of potato tubers. Furthermore, it was found that rye enhanced the proliferation of proteolytic microorganisms more than potatoes.

The results of counts of cellulolytic microorganisms are shown in Figure 4. Data of microbiological analyses performed in 2003 showed statistically highly significant differences between numbers of microorganisms in the treatments with addition of organic matter and their numbers in the control soil. Also Górska *et al.* [11] reported that organic fertilization can affect significantly numbers of cellulolytic microorganisms in the soil. Identical conclusions were drawn by Furczak and Wielgosz [8] on the basis of their own

studies. The authors recorded increased numbers of cellulolytic bacteria in the fermented sludge together with the passage of time of its storage on experimental plots.

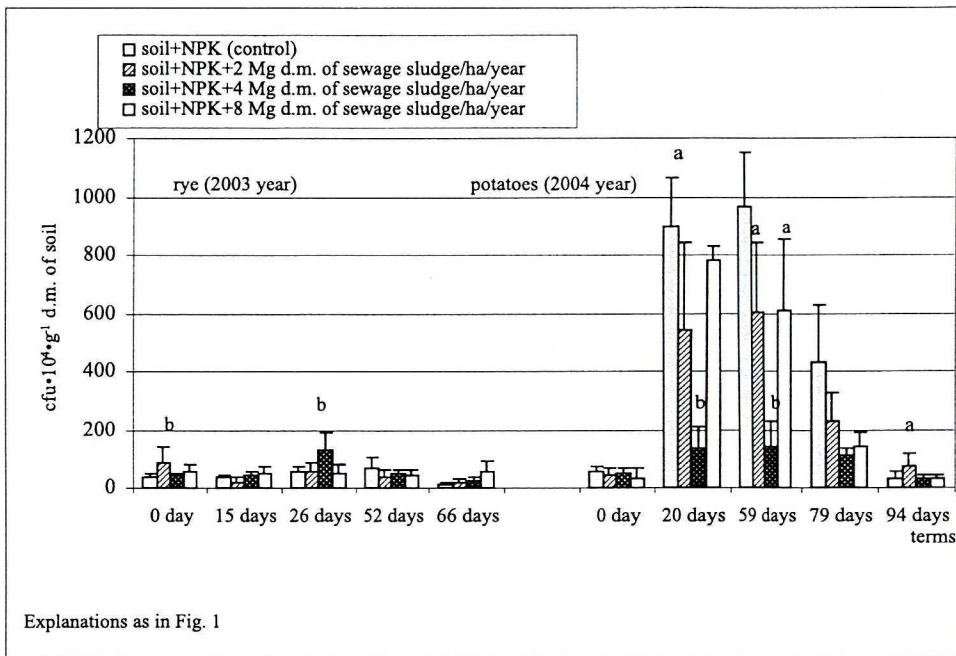


Fig. 4. The number of cellulolytic microorganisms in the soil with different levels of organic fertilizations

In 2004, cellulolytic microorganisms were not affected by the applied sewage sludge in the soil under potato cultivation irrespective of the applied dose. In the majority of dates, counts of the discussed microorganisms were higher in the control soil than in the soil objects fertilized organically. Moreover, the number of cellulolytic bacteria in all soil objects increased rapidly after 20th day of the experiment and this condition remained unchanged up to day 59. The counts of the discussed microorganisms dropped only during the stage of the final harvest of potatoes. The observed increased counts of cellulolytic microorganisms in the soil after 20 days of the experiment was most probably associated with the fact that the plants entered the phase of the generative development which manifests itself in quantitative and qualitative changes in root secretions discharged into the soil [26]. Data presented in Figure 4 demonstrate that the applied test crop plants influenced, to a different degree, the counts of the discussed microorganisms in the soil. Potatoes were found to stimulate the proliferation of cellulolytic microorganisms in the soil to a greater extent than rye. The influence of potatoes on the development of the discussed microbes was by 84% higher in comparison with rye. Also experiments conducted by Mazur [18] showed a significant increase of cellulolytic microorganisms in the soil fertilized organically under potato cultivation.

When analyzing the dynamics of changes in the numbers of phosphate solving microorganisms in the soil in the course of the three months' long experiment (Fig. 5), it was found that the applied fertilization with sewage sludges failed to exert a statistically

significant impact on the increase in the cell counts in the soil. However, these results were not confirmed by experiments carried out by Younga *et al.* [31] who reported that the proliferation of phosphate microorganisms in the soil increased following the introduction of organic matter into the soil.

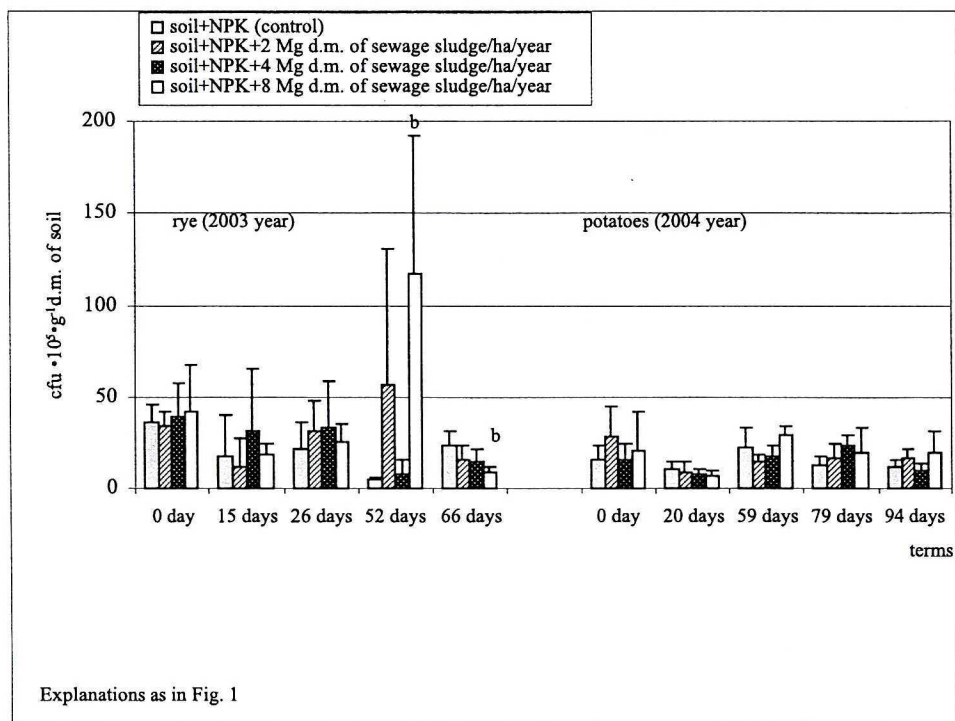


Fig. 5. The number of phosphate solving microorganisms in the soil with different levels of organic fertilizations

Literature data [9, 27] indicate that sludges from municipal sewage treatment plants as well as from various plant and animal processing enterprises are rich in nutritional components required by plants and microbes, including phosphorus. The level of this element in sludges depends on the effectiveness of the process of its precipitation from the sewage and the content of this element in the sewage sludges applied in our investigations exceeded $20 \text{ g} \cdot \text{kg}^{-1} \text{ d.m.}$ of the sludge.

Although, generally speaking, in the discussed trial no significant or highly significant changes in the cell counts between the control soil and the remaining soil objects were shown, the applied fertilization with sewage sludge resulted in a slight, nevertheless visible, increase in the amount of phosphate microorganisms in the soil (Fig. 5). According to Shekhara *et al.* [22], the proliferation and activity of phosphate solubilizing bacteria is not associated only with the supply to the soil of large quantities of organic matter but depends also on the C:N ratio, pH and temperature of the substrate.

When analyzing the counts of phosphate solving microorganisms in this study (Fig. 5), it was observed that the proliferation of these microorganisms depended on the size of the applied doses of sewage sludges as well as the developmental stage and kind of the

applied crop plants. Nevertheless, every year in the period two weeks after the harvest, numbers of these microorganisms declined in the majority of soil treatments. In addition, it is also clear from the data presented in Figure 5 that rye stimulated the proliferation of the discussed microorganisms in the soil more (by 45%) than potatoes. There are some questions concerning the phenomenon of a more intensive development of phosphate microorganisms in the control soil in comparison with the soil objects fertilized with sewage sludge. However, it is evident from experiments carried out by Barabasz *et al.* [1] that mineral fertilization (NPK) contributes to the increased counts of the discussed microorganisms in the soil.

CONCLUSIONS

1. The observed absence of distinct changes in the numbers of the analyzed groups of microorganisms (oligotrophic, copiotrophic, proteolytic, cellulolytic and phosphate solving microorganisms) in the soil fertilized with different doses of sewage sludges proves that the applied sewage sludge was not the factor which exerted a significant influence on their development.
2. Cultivation of rye enhanced the cell proliferation of oligotrophic, copiotrophic, proteolytic and phosphate solving microorganisms. The cellulolytic microbes represented the only group of microorganisms which gave to stronger development in the soil on which potatoes were cultivated.

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DYNAMIKA ZMIAN LICZEBNOŚCI WYBRANYCH GRUP MIKROORGANIZMÓW W GLEBIE NAWOŻONEJ KOMUNALNYMI OSADAMI ŚCIEKOWYMI

Przedmiotem dwuletnich badań było poznanie dynamiki rozwoju wybranych grup drobnoustrojów (mikroorganizmy oligotroficzne, kopiotroficzne, proteolityczne, celulolityczne, rozpuszczające fosforany) w glebie nawożonej komunalnymi osadami ściekowymi. Celem przeprowadzonego doświadczenia było wyjaśnienie możliwości zachwiania równowagi biologicznej gleby, przejawiającej się wzmożonym i długotrwałym rozwojem

analizowanych grup drobnoustrojów w glebie, po wprowadzeniu do niej materii organicznej w formie osadów ściekowych, w różnych dawkach. W doświadczeniu zastosowano cztery obiekty badawcze: kontrola-gleba + NPK, 2 Mg s.m. osadu·ha⁻¹·rok⁻¹ + NPK, 4 Mg s.m. osadu·ha⁻¹·rok⁻¹ + NPK oraz 8 Mg s.m. osadu·ha⁻¹·rok⁻¹ + NPK). Fosfor i potas stosowano przedsięwzięciu pod orkę, natomiast azot: część przedsięwzięciu i drugą część pogłównie. Osady ściekowe stosowano przedsięwzięciu. Poletka glebowe, na których przeprowadzono badania obsiano żytem odmiany *Wibro* (2003 r.) oraz obsadzono ziemniakami odmiany *Bila* (2004 r.). Wykazano, że zastosowane dawki osadów ściekowych nie wpływały istotnie statystycznie na namnażanie się mikroorganizmów glebowych. Na podstawie przeprowadzonych analiz mikrobiologicznych stwierdzono, że w badanym okresie (2003–2004) liczebność oznaczonych grup drobnoustrojów ulegała wahaniom, zależnym od terminu pobierania próbek glebowych. Próbkę glebowe, niezbędne do przeprowadzenia analiz, pobierane były w terminach związanych z kolejnymi fazami rozwojowymi roślin. Kolejnym czynnikiem wpływającym na dynamikę rozwoju mikroorganizmów glebowych był gatunek rośliny użytej w doświadczeniu. Żyto uprawiane w 2003 r. stymulowało rozwój większości grup drobnoustrojów (mikroorganizmów oligotroficznych, kopiotroficznych, proteolitycznych, rozpuszczających fosforany). Z kolei ziemniaki w okresie rozwoju generatywnego (16.07–28.08.2004) spowodowały silniejszy rozwój mikroorganizmów celulolitycznych.