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SIZE EVALUATION OF THE SELECTED GROUPS OF MICROORGANISMS IN THE SOIL FORTIFIED WITH COMMUNAL SEWAGE SLUDGE

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OCENA LICZEBNOŚCI WYBRANYCH GRUP DROBNOUSTROJÓW W GLEBIE WZBOGACONEJ KOMUNALNYM OSADEM ŚCIEKOWYM

Rolnicze wykorzystanie odpowiednio stabilizowanych osadów ściekowych wydaje się być najbardziej racjonalną metodą ich utylizacji. Wiele kontrowersji budzi jednakże wpływ osadów na zachowanie równowagi biologicznej gleby. Dlatego też celem badań o charakterze polowo-laboratoryjnym było określenie dynamiki rozwoju wybranych grup mikroorganizmów w glebie płowej nawożonej dopuszczalną i niedopuszczalną dawką osadu ściekowego oraz obornikiem. W różnych terminach, związanych z rozwojem jęczmienia jarego oznaczano w glebie liczebność sześciu grup drobnoustrojów – ogólnej liczebności bakterii, promieniowców, grzybów, bakterii z rodzaju *Azotobacter, Pseudomonas fluorescens*) oraz bakterii chorobotwórczych z rodzaju *Salmonella*. Wybrane grupy mikroorganizmów oznaczano na podłożach wybiórczych metodą płytkową. Uzyskane rezultaty badań wykazały, że zastosowane poziomy nawożenia organicznego w większości przypadków nie miały istotnego wpływu na kształtowanie się liczebności mikroorganizmów w glebie. Stosowanie więc zarówno dopuszczalnych, jak i niedopuszczalnych dawek osadów ściekowych w postaci nawozów, nie prowadzi do zachwiania równowagi biologicznej gleby. Na podstawie uzyskanych wyników badań stwierdzono również, że rolnicze zagospodarowanie osadów ściekowych powinno być poprzedzone końcowym procesem higienizacyjnym (np. kompostowaniem) w celu eliminacji bakterii chorobotwórczych, a w szczególności bakterii z rodzaju *Salmonella*.

Summary

Agricultural utilization of appropriately stabilized sewage sludge appears to be the most rational method of its utilization, even though there is no agreement among scientists as to the impact that these wastes can exert on the maintenance of the soil biological balance. That is why the objective of the performed field-laboratory experiments was to determine the developmental dynamics of selected groups of microorganisms in a grey-brown podzolic soil fertilized with acceptable and unacceptable doses of sewage sludge and farmyard manure. Numbers of six groups of microorganisms were determined at various dates associated with the development of spring barley (total bacterial number, number of actinomycetes, fungi, bacteria from the *Azotobacter genus* and *Pseudomonas fluorescens*) as well as pathogenic bacteria from the *Salmonella* genus. The selected groups of microorganisms were determined on selective media by the plate method. The obtained research results showed that, in the majority of cases, the applied levels of organic fertilization did not have a significant impact on the numbers of microorganisms in the soil. Therefore, it can be said that the application of both acceptable and unacceptable doses of sewage sludge in the form of fertilizers failed to disturb the biological balance of the examined soil. In addition, the results of the performed experiments indicated that the agricultural utilization of sewage sludge should be forestalled by a sanitation process (e.g. composting) in order to get rid of pathogenic bacteria, especially bacteria from the *Salmonella* genus.

INTRODUCTION

In Europe, but also in Poland, we can observe a growing interest in the protection of the environment that surrounds us. The observed increased concern to maintain good soil, water and air quality – the three basic components of the biosphere – stems, among others, from the increasing awareness of the society that the quality and stability of the survival of both the Mankind and the Earth depend on these very elements.

Organic wastes have accompanied mankind from time immemorial. For several years now, problems of sewage sludge disposal in Poland have been growing increasingly serious due to the increasing quantities of these wastes associated with improving living standards and urbanization.

With the progress of evolution, a complex system developed, whereby all wastes were subjected to secondary utilization. The delicate balance was disturbed by a rapid economical expansion [34]. An acute shortage of sufficient quantities of organic fertilizers in modern agriculture which could counteract the deficit of humus compounds, prevent soil degradation and increase yields of crop plants makes it necessary to look for new, effective ways of improving soil properties [4].

The biological origin of sewage sludge implies that, similarly to soil humus, it contains carbon and nitrogen organic compounds which are essential for the growth and development of microorganisms of the soil fauna as well as nutrients for higher plants [37]. Regardless of the season of the year, organic substances found in sewage sludge are exceptionally rich and diverse. The reported observations and experiments carried out so far in Poland by Gawlik *et al.* [12] and Kalembasa *et al.* [16] indicate that there is a possibility of utilization of sewage sludge as a valuable fertilizer.

Agricultural utilization of household wastes appears to be the cheapest method of their disposal [5, 27, 36, 40] and first articles on this subject were published already in 1970s [10, 20, 24]. However, sewage sludge can only be applied in agriculture when it contains acceptable concentrations of heavy metals, appropriate quantities of nutrients and following its suitable treatments and processes aiming to reduce pathogenic organisms [3, 6, 16]. Problems connected with microbiological soil contamination fertilized by poorly stabilized sewage sludge have been discussed in the literature on the subject for a long time [17, 20, 36].

MATERIALS AND METHODS

Experiments were carried out in 2002 on experimental plots of the Experimental-Didactic Station of the Department of Soil and Plant Cultivation in Złotniki, the Agricultural University in Poznań. The experiment was established using the design of random blocks of 42 m^2 which were sown with spring barley. The trial was conducted on grey-brown podzolic soil of IVa and IVb classes characterized by the following chemical properties: pH_{KCL} – 5.60, C – 6.68 $g \cdot kg^{-1}$ d.m., N – 0.60 $g \cdot kg^{-1}$ d.m., C:N – 11.1.

From among all fertilization combinations, this paper presents the data concerning the following soil objects: control (soil + NPK), 20 Mg farmyard manure fresh matter$\cdot ha^{-1} \cdot years^{-3}$ + NPK, 2 Mg d.m. sewage sludge$\cdot ha^{-1} \cdot year^{-1}$ + NPK and 8 Mg d.m. sewage sludge$\cdot ha^{-1} \cdot year^{-1}$

+ NPK. Each of the above-mentioned soil combinations were used in two replicates. The experimental plots were fertilized with nitrogen in the form of ammonium saltpetre, phosphorus – in the form of triple superphosphate, and potassium – in the form of 60% potassium salt applied at the following doses: 50 kg $N \cdot ha^{-1}$, 29 kg $P \cdot ha^{-1}$, 100 kg $K \cdot ha^{-1}$. 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.

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%, Nog. – 6.23%, C:N – 4.74:1.

Soil samples for analyses were collected at six dates (in ten repetitions) associated with the developmental stages of spring barley. These dates comprised: the stage before sowing, first jointing stage, full heading, end of flowering, barley full maturity and samples of soils two weeks after harvest.

The scope of the performed investigations comprised the determination (in five repetitions) of the total bacterial number, the numbers of actinomycetes, fungi, bacteria from the *Azotobacter* genus and two indices of sanitary state of soil: *Pseudomonas fluorescens* and bacteria from the *Salmonella* genus. The examined microorganisms were cultured on solid media applying appropriate dilutions of soil solutions and were expressed in $cfu \cdot g^{-1}$ d.m. of soil.

The total bacteria count was determined on the medium with the soil extract and with $CaSO_4$ and K_2HPO_4 [38] after 14 days of incubation at the temperature of 27°C. Colonies of actinomycetes were determined separately from among the developed colonies of bacteria. Fungi (only moulds) were determined on the Martins medium [23] at the temperature of 24°C after 5 days of incubation. *Azotobacter* sp. were determined flooding 1 g of fresh soil with a medium in Fanglerowa modification [9] followed by the incubation of plates at the temperature of 25°C. The numbers of the *Pseudomonas fluorescens* bacteria were determined on the medium King B according to Burbianka and Pliszka [7] following 48-hour incubation at the temperature of 28°C. In order to verify whether these are the *Pseudomonas fluorescens* bacteria, its colonies were marked under the ULTRA LUM INC. lamp which emits light in the wavelength area of 365 nm. Bacteria of the *Salmonella* genus were determined by the plate method on the Merck medium [1] at the temperature of 37°C for 24 hours. In order to verify the obtained results of the *Salmonella* test, procedures recommended by the Polish Standard PN-Z-19000-1 were applied [33]. In addition, eggs of *Ascaris* sp., *Trichuris* sp. and *Toxocara* sp. parasites were isolated from the experimental sewage sludge using the floatation method [15].

The obtained research results were subjected to statistical analysis [31] using the Statistica 7.1 software.

RESULTS AND DISCUSSION

The results presented in Figure 1 show that the total bacteria number in the examined soil underwent fluctuations depending on the level of organic fertilization and the developmental stage of spring barley. The performed statistical analysis (Fig. 1) proved that significant or highly significant differences between the number of cells in the control soil and their numbers in the remaining soil combinations occurred most frequently on the 5th date of collection, i.e. at the stage of full barley maturity.

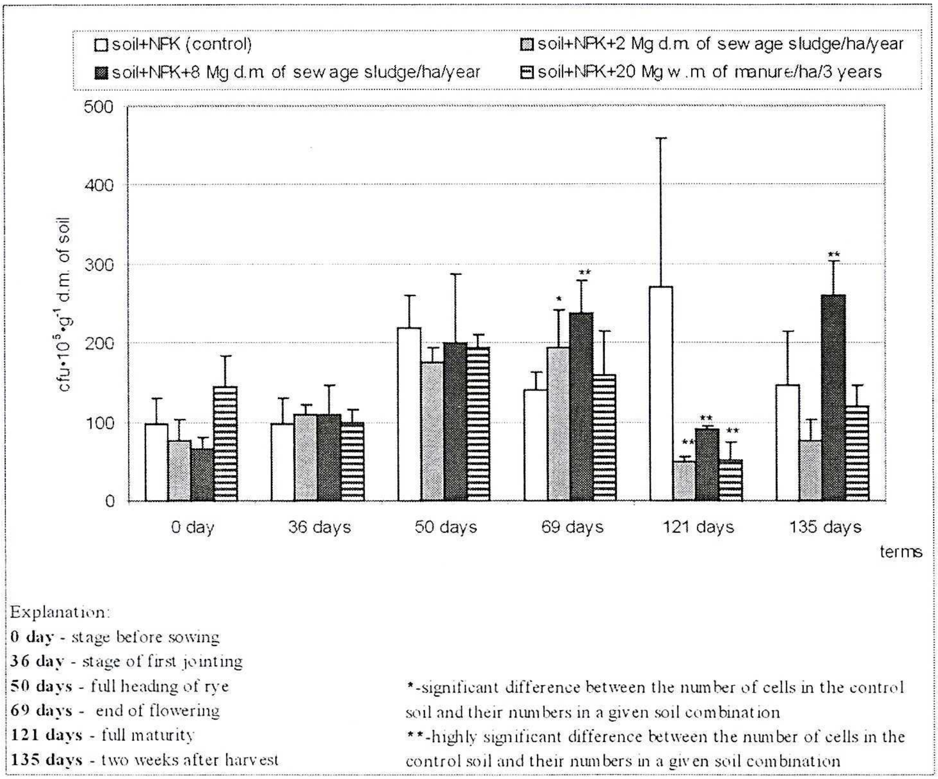

Fig. 1. The total bacteria number in the soil with different levels of organic fertilizations

The highest numbers of bacteria in the examined soil objects were recorded during the generative development of spring barley (the 3rd date – full heading and 4th date – end of flowering). When comparing bacteria counts in the control soil with their numbers in the remaining fertilizer combinations, it was observed that the addition of suitable quantity of organic matter to soil did not always result in the increase of microorganisms. Unfavorable effects of sewage sludge on the number of species composition of the soil microflora were also reported by Babich and Stotzky [2] and Giller *et al.* [13]. However, this opinion was not corroborated by investigations conducted by Furczak, Joniec [11], Gostkowska *et al.* [14]; Wolna-Maruwka, Sawicka [39]; Sawicka, Wolna [35] who maintain that sewage sludge introduced into the soil showed a stimulatory effect on the total bacteria number. Ciećko *et al.* [8] reported that the direction of changes of the soil microflora depended on the type of the applied sewage sludge as well as on the size of the applied dose.

The lack of the stimulatory effect of the applied organic fertilization on the soil bacteria number can be attributed to the small quantities of nitrogen and carbon introduced into the soil together with sewage sludge. The annual introduction of 8 Mg d.m. of the sewage sludge·ha^{-1}, after 4 years of experiments resulted in the increase of nitrogen in the soil of 2928.0 kg·ha^{-1} and of carbon – of 10178.0 kg·ha^{-1}.

On the basis of the obtained results it was found that the applied doses of sewage sludge were too small to contribute to the significant growth of the total bacteria number.

Increasing the sewage sludge dose applied to soil to size 10 or 30 Mg·ha^{-1} certainly would have let obtaining analogical results to those in Ciećko *et al.* [8].

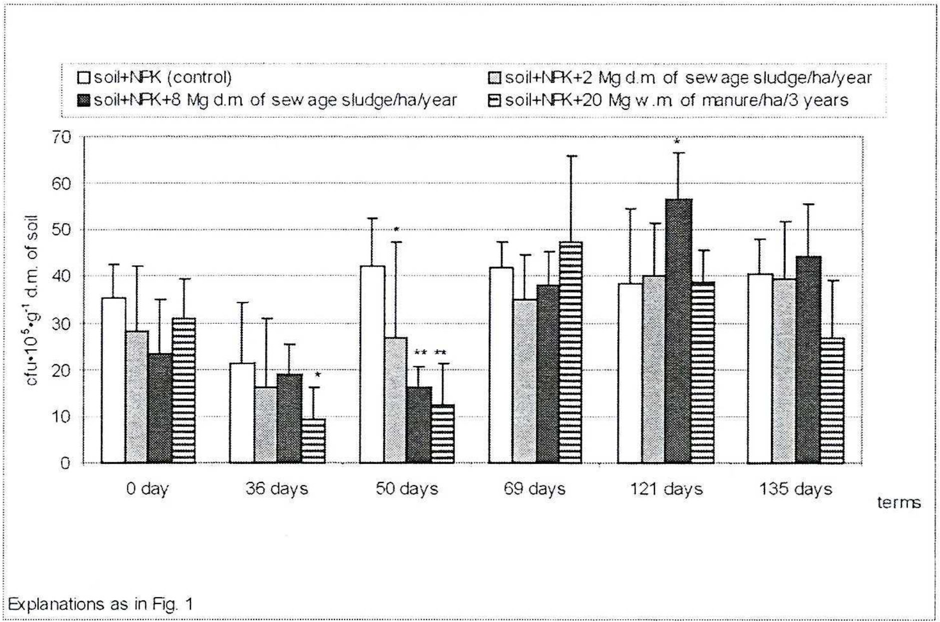

Fig. 2. The actinomyces number in the soil with different levels of organic fertilizations

Figure 2 presents numbers of actinomycetes determined in the experimental soils and it is evident that the applied doses of sewage sludge failed to cause a noticeable development of microorganisms in the soil as confirmed by the performed statistical analysis (Tukey test) which showed that, in the majority of cases, the differences were statistically non-significant. However, the above results were not confirmed by studies carried out by Nowak *et al.* [30] who claim that the introduction into the soil of composted sewage sludge caused a two- or even three-fold increase in the numbers of actinomycetes in the soil. In our experiments, during the initial stages, we observed the strongest multiplication of actinomycetes in the control soil (fertilized only with NPK).

When analyzing the numbers of actinomycetes in the soil during the entire period of experiment, it was also found that the observed increase in their numbers, in all combinations fertilized organically, occurred from the 4th date (end of barley flowering) and remained on a similar level until the 6th date (two weeks after harvest).

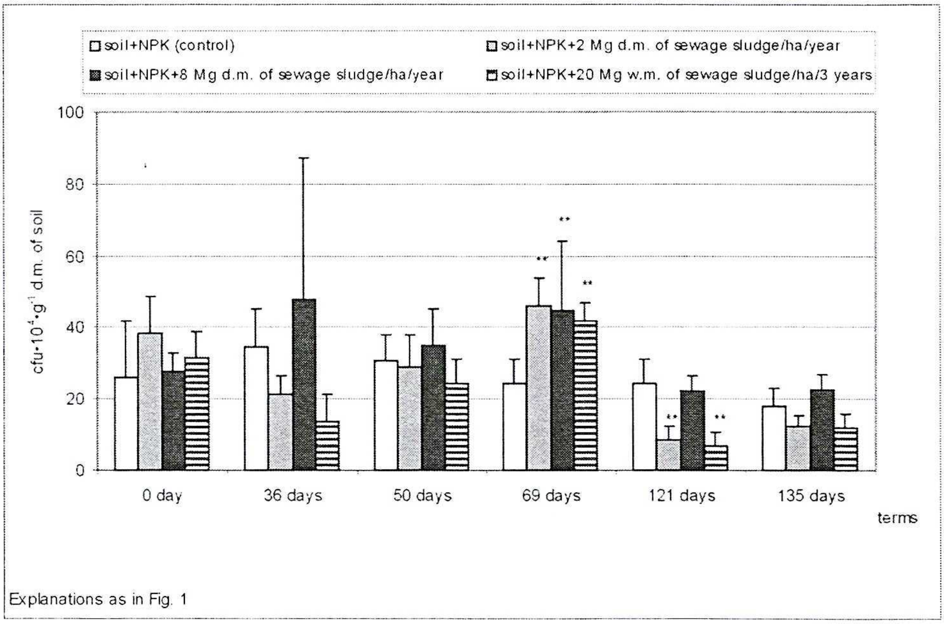

Fig. 3. The fungi number in the soil with different levels of organic fertilizations

The numbers of fungi (moulds) in the analyzed soil combinations under spring barley cultivation (Fig. 3) were found to decline gradually. The smallest numbers of fungi were recorded in the last two dates (the stages of barley full maturity and two weeks after harvest).

When comparing the numbers of fungi determined in the control soil at the first date (Fig. 3) with their numbers in the remaining fertilization combinations, it can be said that no statistically significant differences were observed at the levels of significance of $\alpha_{0.01}$ and $\alpha_{0.05}$ Similar values were observed on the 2nd, 3rd and 6th dates. On the other hand, in the case of the 5th date, the examined fungi showed a weaker development in the majority of soil combinations with the addition of organic matter than in the control soil. It may be suspected that this trend could have been caused by the competition for food among the microorganisms. Nizametdinova and Safiyazov [29] reported that the presence of *Azotobacter* sp. inhibited the development of fungi in soils. Kurek and Jaroszuk-Ściseł [19] attribute a similar inhibiting impact on these organisms to the bacteria from the *Pseudomonas* genus.

It was concluded, on the basis of the obtained results, that highly significant and significant differences in the amount of cells between the control soil and the remaining combinations occurred, primarily, on the 4th (end of flowering) and 5th (full maturity of spring barley) dates.

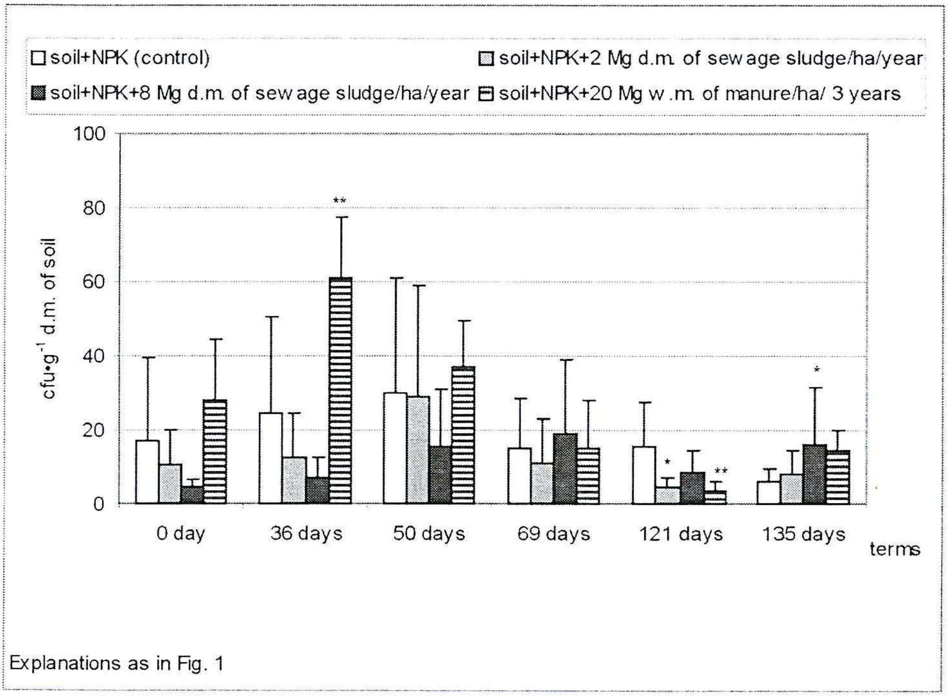

Fig. 4. The number of *Azotobacter* sp. in the soil with different levels of organic fertilizations

In the course of the performed experiments, in the majority of soil combinations fortified by organic matter in the form of farmyard manure or sewage sludge, *Azotobacter* sp. cells were not observed to proliferate intensively (Fig. 4). The numbers of these bacteria in the analyzed fertilization combinations increased initially but from the 4th date (end of flowering) onward, it decreased. Also the performed statistical analysis failed (with very few exceptions) to show significant or highly significant differences between the number of bacteria in the control soil and their numbers in the remaining fertilization objects.

The analysis of the numbers of the *Salmonella* sp. bacteria (Fig. 5) showed that their highest numbers were recorded on the 4th date (end of flowering) in the soil fertilized with 2 Mg d.m. of sewage sludge. The performed statistical analysis revealed that only in the soil fertilized every year with 2 Mg of sewage sludge, the numbers of these bacteria differed highly significantly in comparison to their numbers in the control soil.

The presence of this pathogen in the soil could have been associated with the sanitary condition of the sewage sludge applied in this experiment (Tab. 1). Kłapeć [18] reported that the percentage of isolated *Salmonella* sp. ranged from 8.8% to 100%.

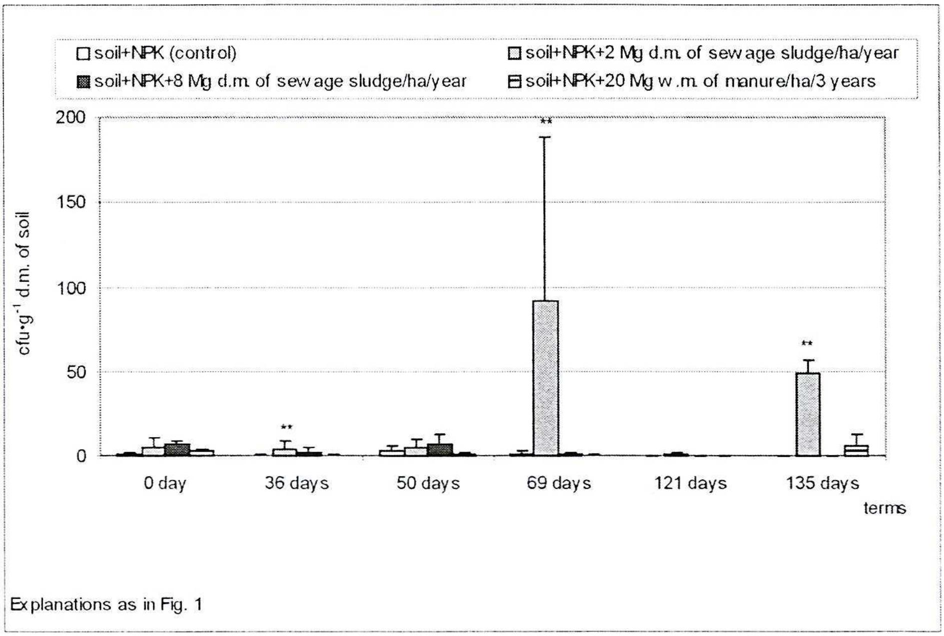

Fig. 5. The number of *Salmonella* sp. in the soil with different levels of organic fertilizations

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

Kind of microorganisms	Mean (cfu·g $^{-1}$ d.m. of sludge)
Total bacterial number	$842{,}43 \cdot 10^{5}$
Actinomyces	$97{,}19 \cdot 10^{4}$
Fungi	$1144{,}99 \cdot 10^{4}$
Azotobacter sp.	27,98
Salmonella sp.	15,32
Pseudomonas fluorescens	$12{,}32 \cdot 10^{4}$
Ascaris sp., *Trichuris* sp., *Toxocara* sp.	0

The numbers of the discussed bacteria may have been also affected by the temperature which is one of the most important factors reducing numbers of pathogenic microorganisms in the soil. Nicholson *et al.* [26] reported that numbers of *Salmonella* sp. in the farmyard manure at the temperature of 55°C declined systematically reaching the value of zero within one month, whereas Nguyen Thi [25] claims that in spring the number of bacteria from the *Salmonella* genus in the stored sewage sludge was higher than in August. However, this observation was not confirmed in our experiments in which the number of these bacteria varied considerably and these differences depended not only on the date of the performed analysis but also on the soil combination.

During the course of our trial, the lowest numbers of bacteria from the *Salmonella* genus were observed in the soil combination with the addition of farmyard manure. This was probably connected with the initial microbiological composition of the farmyard manure applied in this experiment. Unfortunately, the manure was not subjected to microbiological analyses because the experiment started in 2002, while the farmyard manure was taken out and left on experimental plots in 2001.

The results presented in Figure 5 show that it was only in the control treatment and in the combination soil + NPK + 8 Mg d.m. sewage sludge$\cdot$ha$^{-1}\cdot$year^{-1} that the number of bacteria dropped to zero after the period of four months (03.04.–16.08.2002). It may be assumed that the process of cell demise observed in the above-mentioned combinations was associated with the presence of the native soil microflora.

The presence of pathogens determined in the control soil is somewhat controversial. Presumably, it was connected with accidental transfer of pathogenic bacteria from adjacent plots as they were not isolated from one another in any way. Niewolak and Tucholski [28] also reported possibility of transfer of pathogens in a given area by, among others, animals.

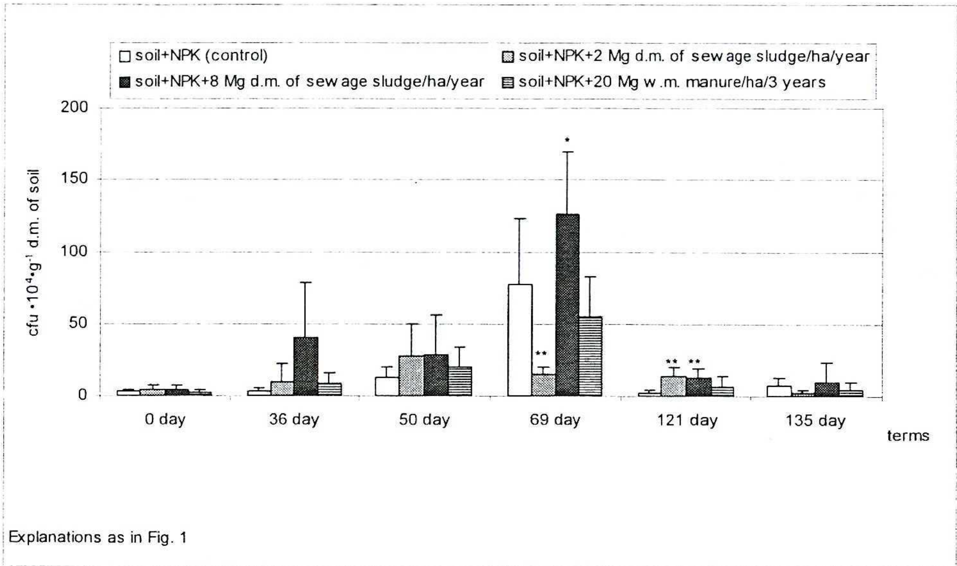

Fig. 6. The number of *Pseudomonas fluorescens* in the soil with different levels of organic fertilizations

The results of microbiological analyses presented in Figure 6 show that numbers of *Pseudomonas fluorescens* bacteria increased in all soil combinations up to the 4th date (end of barley flowering) and then declined. Identical results were reported by Paszkowski and Masiak [32]. On the other hand, Lilyeroth and Baath [22] claim that numbers of *Pseudomonas fluorescens* bacteria in the barley rhizosphere decreased with the age of plants.

The growth in number of bacteria *Pseudomonas fluoresces* and *Salmonella* sp. (Fig. 5) at the end of the spring barley blooming was probably connected with the generative development period of spring barley. For, in the blooming period, the quantity and the composition of root secretion increases. Thus, the decrease in number of the bacteria mentioned above in next phases might have been connected with some quantitative as well as qualitative changes in root secretion.

The results obtained in our trial also showed that the strongest cell proliferation of the discussed bacteria took place in the soil combination with the highest inclusion of the sewage sludge and it was twice as high as in the control treatment.

The performed statistical analyses showed that the highest number of statistically highly significant differences between the amount of cells in the control soil and the remaining soil combinations were observed on the 4th and 5th dates (end of flowering and barley full maturity). It seems interesting that at some dates, the discussed bacteria proliferated strongly in the control soil. Presumably, the number of these cells in the soil could have depended on many different biotic and abiotic factors and not only on the kind of the applied organic fertilization.

Latour *et al.* [21], for instance, reported that the survivability of *Pseudomonas fluorescens* in the soil depended, among others, on the type of soil or its water-air relationships.

CONCLUSIONS

1. The application of the unacceptable (8 Mg d.m. sewage sludge·ha^{-1}·year^{-1}) and acceptable (2 Mg d.m. sewage sludge·ha^{-1}·year^{-1}) doses of sewage sludge did not cause a rapid proliferation of the analyzed groups of microorganisms.
2. In the soil treatment with increased doses of fertilization with farmyard manure and NPK, numbers of the examined microorganisms failed to increase, too.
3. The utilization of stabilized sewage sludge as a fertilizer does not provide sufficient protection against microbiological contamination (*Salmonella* sp.); on the contrary, it can increase bacteriological and epidemiological hazard.

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