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CHANGES IN MICROBIOLOGICAL ACTIVITY OF SOILS FERTILISED WITH VARIED DOSES OF SLUDGE FROM A DAIRY SEWAGE TREATMENT PLANT

ZMIANY AKTYWNOŚCI MIKROBIOLOGICZNEJ GLEB NAWOŻONYCH ZRÓŻNICOWANYMI DAWKAMI OSADU Z OCZYSZCZALNI ŚCIEKÓW MLECZARSKICH

Abstract: The influence of dairy sewage sludge on some microbiological characteristics was studied. The experiment was carried out in laboratory conditions. Microbiological analyses were following: respiration activity and numbers of some groups of microorganisms. Different doses of dairy sewage sludge were applied into brown and grey-brown podzolic soils. The effect of the sludge on the microbial groups under study depended on the dose of the sludge, on the type of soil, and on the kind of microbiological parameter.

Keywords: dairy sewage sludge, microorganisms, respiration activity, soil

Soil is a medium in which numerous and diverse biological, chemical and physical processes take place, the run of which is affected by anthropogenic factors [1]. Microorganisms inhabiting the soil environment play a crucial role in the decomposition of soil organic matter (SOM) and in the circulation of elements, therefore the microbiological activity is used for the estimation of the ecological condition of soils [2, 3]. Microorganisms are involved in the main processes taking place in soil, ie humification, recycling or mineralisation of organic wastes, making the biogens contained in them available to plants [4–7]. The presence of certain microbial groups with known ecological requirements, eg nitrifying or cellulolytic bacteria, is considered to constitute microbiological indicators [8, 9]. Biodiversity of microorganisms in soil is important for maintaining health of the environment and for improvement of yields of crop plants [10, 11].

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In recent years a notable increase has been observed in the agricultural utilisation of sewage sludge, including sludge originating from the agricultural-food industry. Therefore, monitoring of changes in the microbiological activity of soils fertilised with such wastes appears to be important, considering the quality and health status of soils as well as the economic aspects.

Material and methods

The pot experiment was set up on two different types of soil (brown and grey-brown podzolic). The brown soil, developed from a silty clay formation, was characterised by the following grain size composition: 8 % of sand fraction (1.0–0.1 mm), 47 % of silt fraction (0.1–0.02 mm) and 45 % of fine particles (< 0.02 mm). The grey-brown podzolic soil, developed from strongly loamy sand, had the following grain size composition: 65 % of sand fraction (1–0.1 mm), 19 % of silt fraction (0.1–0.02 mm) and 16 % of fine particles (< 0.02 mm). The basic characteristics of the soils and dairy sewage sludge used in the experiments are given in Table 1. Each pot contained 4 kg of brown or grey-brown podzolic soil mixed with appropriate dose of dairy sewage sludge. The experiment comprised the following treatments 1 kg of soil: 1 – control soil, without fertilisation; 2 – 10 g of dairy sewage sludge; 3 – 20 g of dairy sewage sludge; 4 – 26.7 g of dairy sewage sludge; 5 – 40 g of dairy sewage sludge; 6 – 66.7 g of dairy sewage sludge; 7 – 100 g · kg^{-1} of dairy sewage sludge and 8 – 200 g of dairy sewage sludge.

Table 1

Characteristics of soils and dairy sewage sludge

Measurements	Unit	Brown soil	Grey-brown podzolic soil	Dairy sewage sludge
pH	[-]	6.4	4.8	8.5
C	[g · kg^{-1} d.m.]	13.5	4.5	400
N		1.6	0.4	33.2
C/N	[-]	8.3	12.5	12.0
P	[g · kg^{-1} d.m.]	18.3	5.3	11.5
K		26.8	8.7	2.5

The soil was watered to 60 % of the maximum water capacity. Incubation was conducted at room temperature, for 8 months, under conditions of controlled moisture. Microbiological and biochemical analyses were made after 14, 30, 60, 90, 120 and 240 days of the experiment. Microbiological analyses of the soil material included the following determinations: respiration activity, with the method of Rühling and Tyler [12], so-called total number of bacteria with low nutritional requirements (oligotrophic bacteria), on substrate with soil extract and K_2HPO_4 [13], so-called total number of filamenous fungi, on Martin substrate [14], number of cellulolytic bacteria, on liquid substrate [13], number of “proteolytic” bacteria and fungi, on Frazier substrate with gelatine [13], number of ammonising bacteria, on liquid substrate with peptone [13], number of nitrifying bacteria, on liquid mineral substrate [13].

To determine the effect of the experimental treatments, duration of the experiment, and types of soils used in the pot experiment on the values of the microbiological features under study, three-factor analyses of variance were performed. Mean values of the studied features for the treatments, times of incubation and soil types were compared using Tukey 95 % intervals of confidence at the level of significance $\alpha = 0.05$. Statistical processing of the results was performed by means of the Statistica 7.1 software. No analysis of variance was performed for the umbers of cellulolytic, ammonising and nitrifying bacteria, as calculation of the numbers of those microbial groups was made with the use of McCrady's Tables based on the principles of mathematical statistics.

Results

The total periodic respiratory activity in the studied soils, measured by the amount of emitted carbon dioxide, is illustrated in Fig. 1. The performed analysis of variance showed that the application of varied doses of dairy sewage sludge in the experiment had a significant effect on the average level of carbon dioxide emission from both soils; an increase in CO_2 emission was noted with increasing doses of sludge introduced in the soil. A significant decrease was observed in carbon dioxide emission from both soils with the duration of the experiment. Statistical analysis did not reveal any significant differences in carbon dioxide emission between the two soils under study.

The study showed notable periodic variation in the number of oligotrophic bacteria (Fig. 1). Analysis of variance revealed growth of the studied microorganisms that intensified with increase in the doses of sludge introduced in the soil. The number of bacteria decreased significantly with the time of the experiment. Greater numbers of the studied microbial groups were characteristic of the brown soil.

The periodic numbers of fungi in the brown and grey-brown podzolic soils with different doses of dairy sewage sludge are presented in Fig. 2. Analysis of variance showed that the highest numbers of the studied fungi were characteristic of treatments 3, 4 and 5, ie those with sludge doses of 20, 26.7 and 40 $g \cdot kg^{-1}$ of soil, respectively. As in the case of bacteria, a significant decrease in the numbers of fungi was observed with the duration of the experiment. A higher number of fungi was found in the grey-brown podzolic soil compared with the brown soil.

As a result of the performed analyses it was found that the applied doses of dairy sewage sludge had differing effect on the numbers of cellulolytic bacteria, depending on the type of soil and on the period of the experiment (Fig. 2). All the doses of the sludge, with the exception of the highest (100 and 200 $g \cdot kg^{-1}$), caused stimulation of the growth of the microbial group under study.

The number of bacteria mineralising cellulose under aerobic conditions decreased during the period of the experiment. In the grey-brown podzolic soil a greater number of those bacteria was observed than in the brown soil.

The results presented in Fig. 3 indicate that the applied doses of the sludge had a significant effect on the numbers of proteolytic bacteria in both studied soils. As in the case of the total number of bacteria, the number of proteolytic bacteria increased

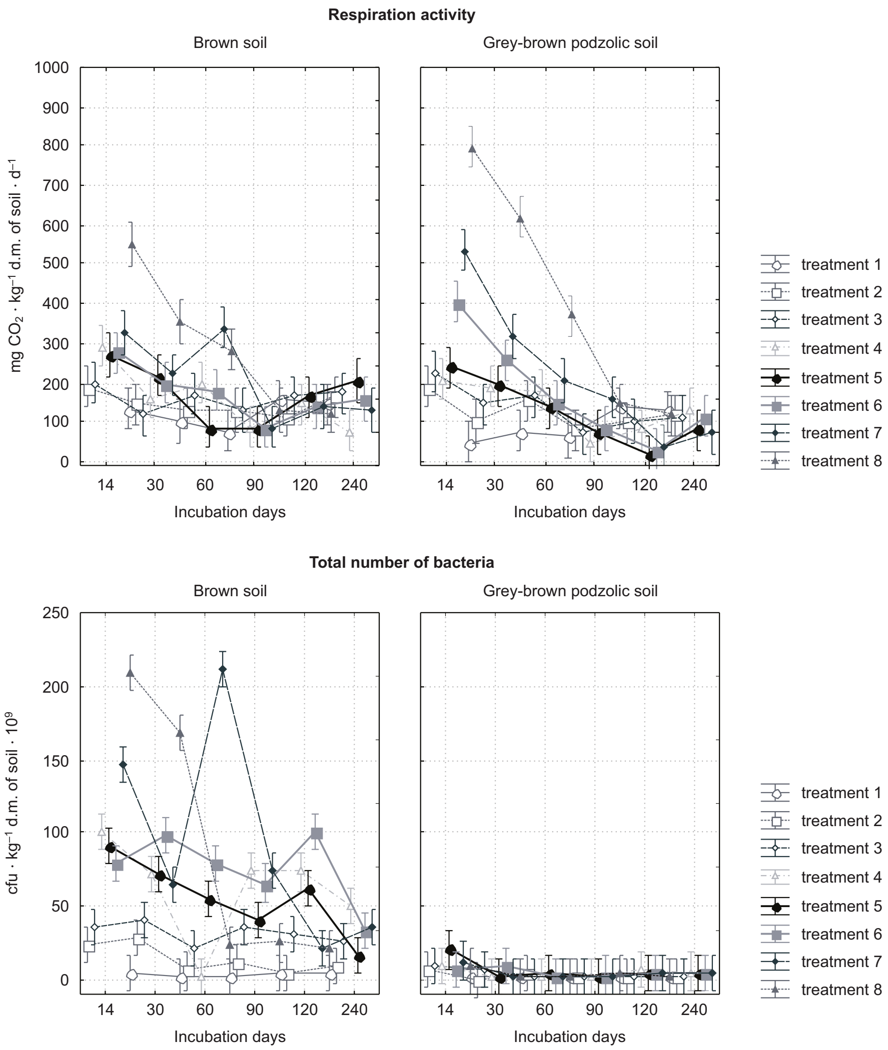

Fig. 1. Respiration activity and numbers of bacteria depending on dairy sewage sludge addition
Explanations: Treatments per 1 kg of soil: 1 – control soil, without fertilisation; 2 – 10 g of dairy sewage sludge; 3 – 20 g of dairy sewage sludge; 4 – 26.7 g of dairy sewage sludge; 5 – 40 g of dairy sewage sludge; 6 – 66.7 g of dairy sewage sludge; 7 – 100 g · kg^{-1} of dairy sewage sludge; 8 – 200 g of dairy sewage sludge

significantly with increasing doses of sludge introduced in the soil. Beginning from the 30^{th} day of incubation there occurred a rapid decrease in the numbers of the studied microorganisms, attaining the minimum level on 90ties day of the experiment, after which the numbers of that group of bacteria increased again.

Significantly higher numbers of proteolytic bacteria were found in the grey-brown podzolic soil than of the brown soil. The numbers of fungi with proteolytic capabilities

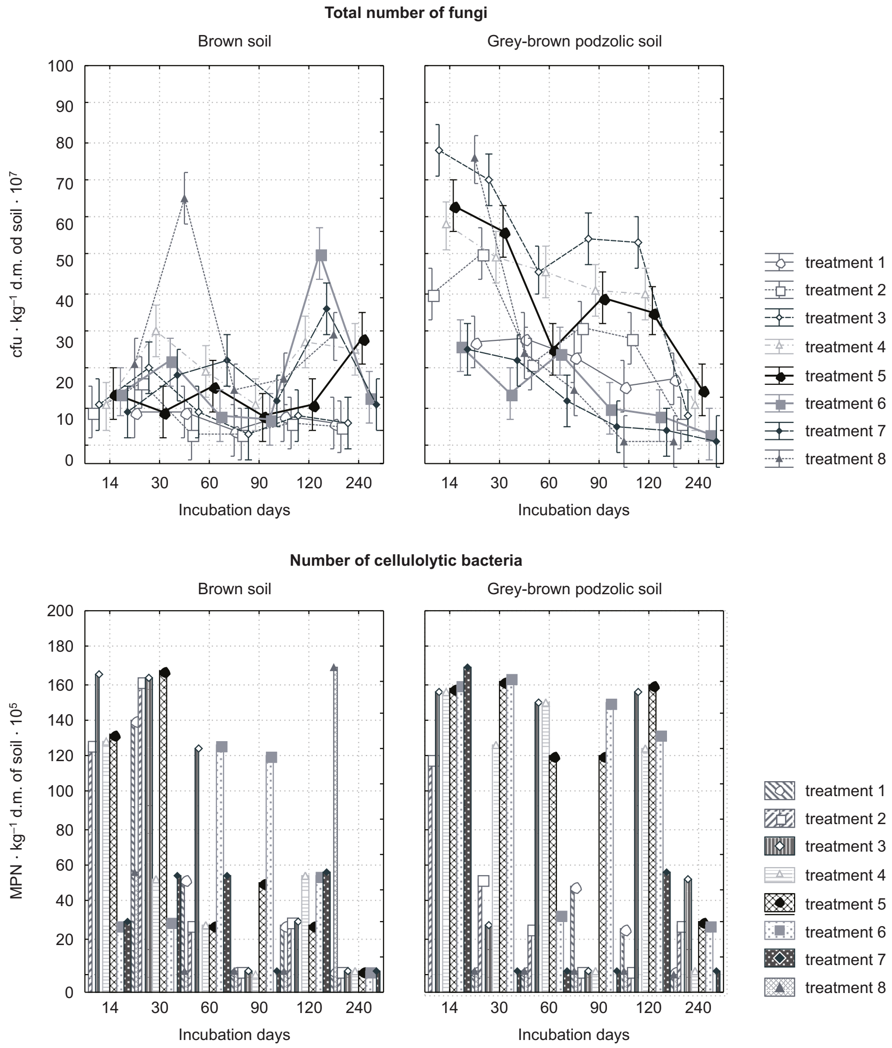

Fig. 2. Numbers of fungi and celulolytic bacteria depending on dairy sewage sludge addition
Explanations: See Fig. 1

in the soils amended with varied doses of dairy sewage sludge were subjected to periodic variation (Fig. 3). All of the applied sludge doses caused stimulation of the growth of the studied group of fungi and the highest numbers being characteristic of treatments with the highest dose of the sludge, ie 200 g · kg^{-1}. A significantly greater number of proteolytic fungi was found in the grey-brown podzolic soil compared with the brown soil.

Periodic changes in the numbers of ammonising bacteria in the individual experimental treatments for both soils studied are presented in Fig. 4. The results of the

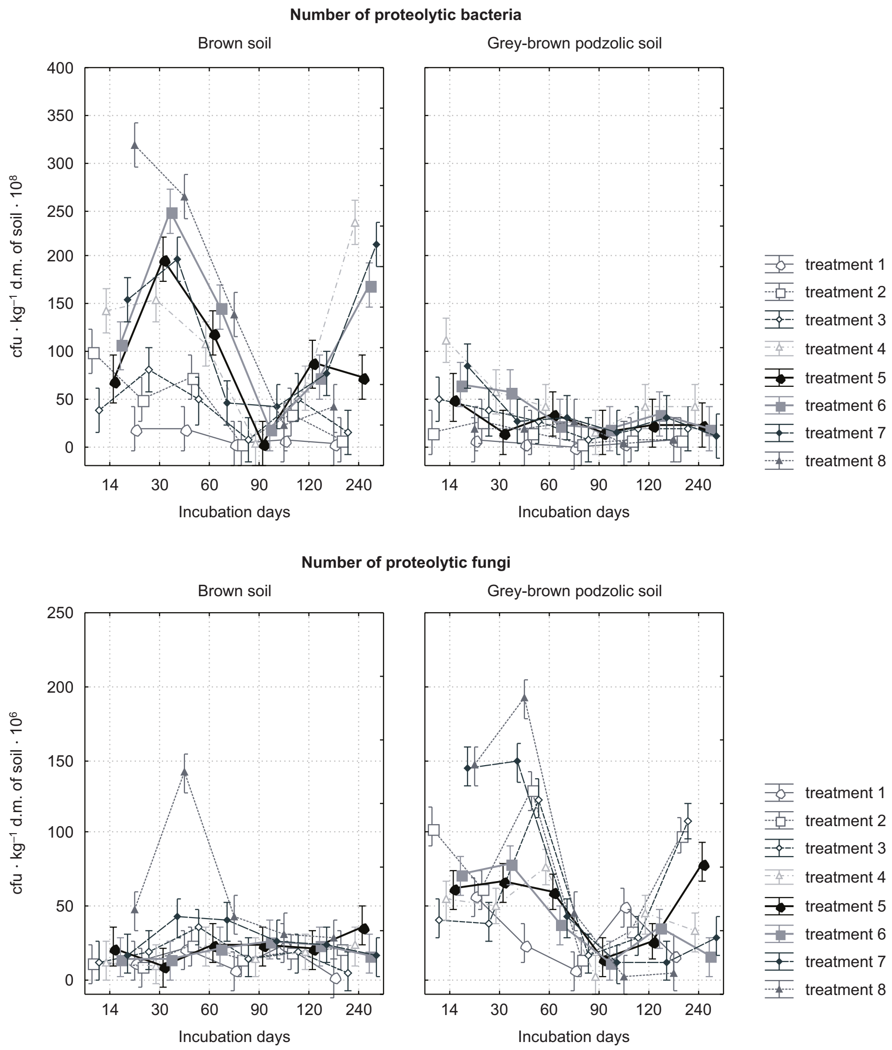

Fig. 3. Numbers of proteolytic bacteria and fungi depending on dairy sewage sludge addition
Explanations: See Fig. 1

analyses indicate that the growth of that microbial group was related to the applied dose of the sludge and increased with increasing amounts of sludge introduced into the soil.

The numbers of ammonisers, as those of most of the studied microbial groups, tended to decrease with the progressing duration of the experiment. A higher mean number of ammonising bacteria was observed in the grey-brown podzolic soil than in the brown soil. Notable periodic variation was observed in the numbers of nitrifying bacteria, as presented in Fig. 4. Analysis of results revealed an increase in the numbers of those bacteria with increasing doses of the sludge introduced in the soil, the highest

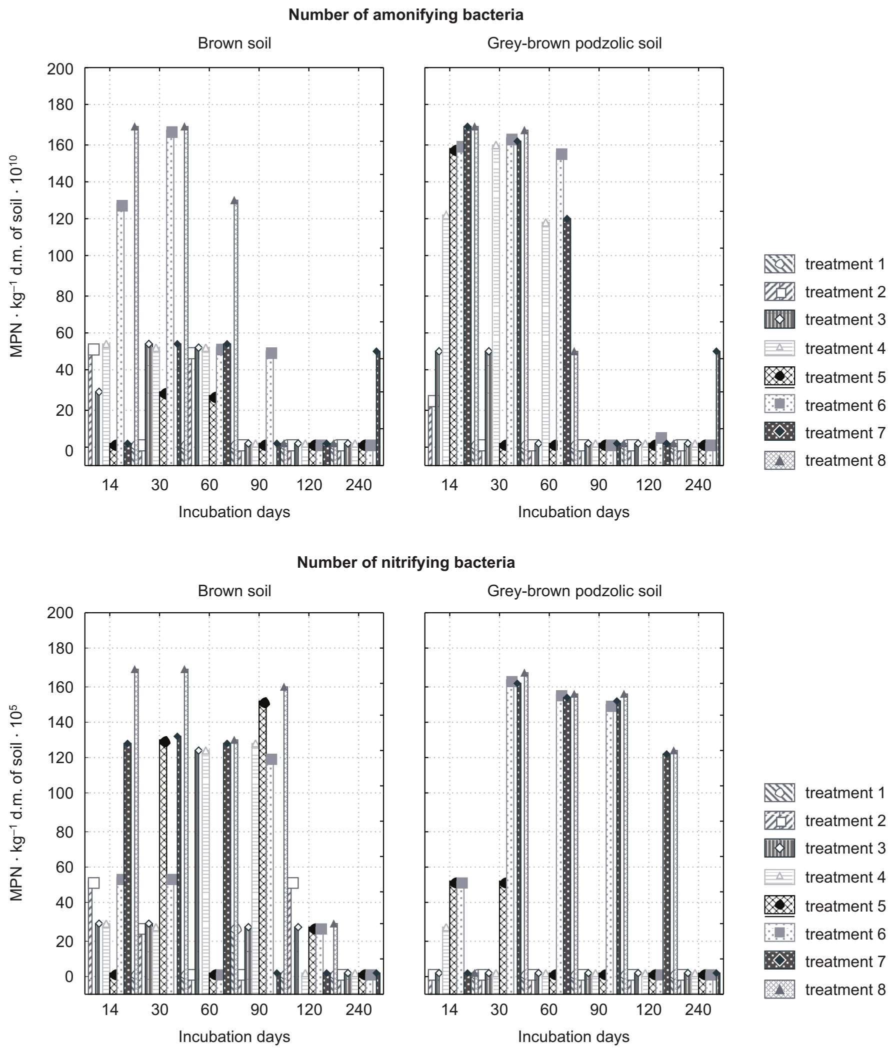

Fig. 4. Numbers of amonifying bacteria and nitryfying bacteria depending on dairy sewage sludge addition
Explanations: See Fig. 1

number of nitrifying bacteria being characteristic of the treatments with the highest dose of the sludge, ie 200 g $\cdot$ kg^{-1}. A higher number of nitrifying bacteria was found in the brown soil.

Discussion

The numbers of the particular physiological groups are employed as a microbiological parameter permitting the estimation of the quantitative composition of microorganisms participating in the decomposition of specific organic compounds [15]. For this reason determination of the number of various microbial groups is frequently used for the determination of the biological condition of the soil environment, especially in soils subjected to the effect of anthropogenic factors [16–19].

The study reported herein showed that the numbers of microorganisms in the studied soils were significantly dependent on the experimental factors applied, ie the fertiliser introduced in the soil, time of its action, as well as the type of soil. The results of the study showed a stimulating effect of the dairy sewage sludge with relation to the studied microbial groups. The effect is probably related with the introduction of organic matter and mineral components into the soil, those being nutritional substrates for the particular groups of soil microorganisms. Stimulation of microbial growth in soils fertilised with sewage sludge was also observed by other authors [16, 19–21]. This study showed that the number of bacteria and fungi increased significantly with increasing doses of the sludge introduced in the soil, which should be attributed to the accumulation of nutrients introduced into the soil with the higher doses of the waste. Lima et al [22] also observed stimulation of the growth of bacteria and fungi under the effect of fertilisation with municipal sewage sludge, which was intensified with increasing doses of sludge introduced in the soil. Stimulation of the growth of fungi should be attributed to increase in the content of organic carbon after the introduction of the sludge, and to low pH value of the grey-brown podzolic soil. Hence in this study greater number of fungi was characteristic of the grey-brown podzolic soil amended with the sludge than the brown soil. Bacterial growth became more noticeable in the brown soil, with reaction close to the neutral, as those microorganisms prefer such environments. The significantly higher numbers of bacteria and fungi at the initial stage of the experiment could have been related with the growth of microbial groups mineralising easily available organic mater. In the study the so-called total number of bacteria, and the number of "proteolytic", ammonising and nitrifying bacteria and fungi were the highest in the treatments with the sludge dose of 200 $g \cdot kg^{-1}$. A positive effect of fertilisation with dairy sewage sludge on the numbers of soil bacteria and fungi was also demonstrated in an earlier study by Jezierska-Tys and Frąc [23]. Culturing of both microbial groups under study usually involved an increase in the respiratory activity which, according to some authors [3, 15, 24], is a measure of the overall microbiological activity of soils. The study showed also stimulation of the growth of cellulolytic bacteria in soils fertilised with dairy sewage sludge. Particularly intensive growth of that microbial group was observed in the grey-brown podzolic soil. A study by Furczak and Joniec [25] also showed an increase in the number of cellulolytic bacteria in a soil fertilised with municipal sewage sludge.

Introducing into soil dairy sewage sludge which is a source of various nitrogen complexes, beginning with organic nitrogen compounds through a series of mineral forms [8], had a significant effect on the population size of microorganisms involved in nitrogen transformations in the soil. The study reported herein demonstrated a stimulating effect of the sludge on the growth of "proteolytic" bacteria and fungi. That effect was caused by organic compounds introduced in the soil with the sludge, those compounds being a substrate for those microbial groups. Another factor conducive to the growth of proteolytic bacteria in particular could have been increase in the soil reaction. Noteworthy is the fact that the numbers of the microorganisms under study increased in the soil together with increasing doses of the sludge applied. Determinations of the numbers of proteolytic bacteria and fungi show that their growth was related both to the dose of the diary sewage sludge and to the soil type. Greater numbers of proteolytic bacteria were observed in the brown soil compared with the grey-brown podzolic soil, while in the case of fungi with the capability of decomposing organic nitrogen complexes an inverse tendency was noted. The population sizes of the studied microbial groups increased with increasing doses of the sludge introduced in the soil, the effect – in the case of bacteria – being more pronounced in the brown soil, and in the case of fungi – in the grey-brown podzolic soil. This phenomenon may be attributed to the introduction in the soil of a substrate necessary for the growth of those microorganisms, in the form of organic nitrogen compounds present in the dairy sewage sludge. As reported by Fidecki [26], nitrogen occurs in such wastes mainly in the organic form, and its mineralisation takes place with participation of proteolytic microorganisms. A stimulating effect of municipal sewage sludge on the growth of proteolytic bacteria was also observed in their studies by Joniec and Furczak [27] and by Jezierska-Tys and Frac [23]. The study reported herein also demonstrated a stimulating effect of dairy sewage sludge on the number of ammonising and nitrifying bacteria, intensifying with increase in the dosage of the sludge introduced in the soil. That effect may indicate soil properties favourable for plants, as those microbial groups, especially nitrifiers, are sensitive to soil acidification and insufficient aeration [28]. The presence of those microbial groups is of key importance in making nitrogen available to plants. The decrease in the numbers of the studied microorganisms in the course of the experiment indicates depletion of the nutritional substrates for those microbial groups.

Conclusions

1. The study demonstrated that soil amendment with diary sewage sludge had a significant effect on the populations of soil microorganisms.
2. The effect of the sludge on the microbial groups under study depended on the dose of the sludge, on the type of soil, and on the kind of microbiological parameter.
3. It was demonstrated that the dairy sewage sludge stimulated the growth of microorganisms in the soil environment. The effect intensified with increasing dosage of the sludge introduced in the soil.
4. A significant relationship was demonstrated between the respiratory activity and the growth of microorganisms in soil fertilised with dairy sewage sludge.

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ZMIANY AKTYWNOŚCI MIKROBIOLOGICZNEJ GLEB NAWOŻONYCH ZRÓŻNICOWANYMI DAWKAMI OSADU Z OCZYSZCZALNI ŚCIEKÓW MLECZARSKICH

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Abstrakt: Celem przeprowadzonych badań była ocena wpływu zróżnicowanych dawek osadu ścieków mleczarskich na kształtowanie się liczebności wybranych grup drobnoustrojów i aktywności respiracyjnej w glebie brunatnej i płowej. Modelowe badania laboratoryjne przeprowadzono na dwóch różnych typach gleb (brunatnej i płowej). Analizy mikrobiologiczne wykonywane okresowo w czasie trwania doświadczenia obejmowały oznaczenie aktywności respiracyjnej i liczebności wybranych grup mikroorganizmów glebowych. Oddziaływanie osadu z oczyszczalni ścieków mleczarskich na badane grupy drobnoustrojów zależało od ilości dawki odpadu, typu gleby oraz rodzaju parametru mikrobiologicznego.

Słowa kluczowe: aktywność respiracyjna, gleba, mikroorganizmy, osad ścieków mleczarskich