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**SEASONAL CHANGES IN MICROBIAL ACTIVITY
OF BROWN SOIL FERTILISED
WITH DAIRY SEWAGE SLUDGE**

**SEZONOWE ZMIANY AKTYWNOŚCI MIKROBIOLOGICZNEJ
GLEBY BRUNATNEJ NAWOŻONEJ
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Abstract: Respiration activity and numbers of chosen group of microorganisms were determined in brown soil fertilised with dairy sewage sludge. Research were carried out in field experiment. The study comparing the effect of fertilisation with dairy sewage sludge and of mineral fertilisation on the populations of selected microbial groups and on soil respiratory activity were made. The study showed that dairy sewage sludge introduced in the soil caused similar effect on microbiological characteristics as mineral fertilization.

Keywords: brown soil, dairy sewage sludge, field experiment, mineral fertilisation

In Poland, the problem of sewage treatment, including also industrial sewage, is growing steadily, as with the increasing number of new sewage treatment plants proper wastes management is becoming an increasingly important issue, and the final utilisation of sewage sludge in particular. Due to its properties, sewage sludge is a waste that is difficult to process and utilize in a manner friendly to the environment [1, 2]. Organic and mineral compounds introduced in soil together with sewage sludge significantly affect the numbers of microorganisms whose activity in soils subjected to the effect of such wastes changes [3, 4]. Determination of the numbers of microbial groups and of the intensity of processes related with cycles with fundamental importance for soil fertility, ie carbon and nitrogen circulation, is an element included in the monitoring of the environment [5, 6]. Such tests are used in the estimation of soil

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fertility and productivity, and permit the acquisition of comprehensive knowledge on changes taking place in the soil environment [7–10].

One of the methods of determination of microbial activity of soil and changes that take place in it under the effect of natural and anthropogenic environmental factors, is the measurement of respiratory intensity [11]. Microbial activity in soil involves decomposition of organic matter and its oxidation, accompanied by emission of CO₂ [12]. The degree of microbial growth in soil depends on its physical and chemical properties, fertilisation, climatic conditions, as well as on tillage and cultivation factors, and especially on soil content of organic matter which is a source of energy and nutrients for microorganisms [10, 12]. Quantitative-qualitative studies on microorganisms are among the basic indices for the estimation of the degree of soil degradation or for improvement of its quality, and are also used for the monitoring of soils fertilised with sewage sludge [9].

The objective of the study reported herein was to make a comparison of the effect of dairy sewage sludge and mineral fertilisation on the populations of selected microbial groups and on the respiratory activity of a brown soil.

Material and method

The study comparing the effect of fertilisation with dairy sewage sludge and of mineral fertilisation on the populations of selected microbial groups and on soil respiratory activity were conducted on the basis of a field experiment. The experiment was set up on a brown soil, developed from a silty clay formation, that 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 washable particles (< 0.02 mm). The sludge used in the experiment originated from the sewage treatment plant of the Regional Dairy Cooperative in Krasnystaw. The basic characteristics of the soil and the sludge are given in Table 1.

Table 1

Characteristics of soil and dairy sewage sludge

Measurements	Unit	Brown soil	Dairy sewage sludge
pH	[-]	5.95	8.4
C	[g · kg ⁻¹ d.m.]	8.2	397
N	[g · kg ⁻¹ d.m.]	1.2	33.1
C/N	[-]	6.8	12.0
P	[g · kg ⁻¹ d.m.]	17.9	11.4
K	[g · kg ⁻¹ d.m.]	25.8	2.3

The design of the experiment included two objects – soil amended with dairy sewage sludge (1) and soil with mineral fertilisation (2). In autumn of 2004, object 1 was fertilised with dairy sewage sludge at the dose of 35 Mg · ha⁻¹ (10.5 Mg d.m. · ha⁻¹), and in spring ammonium nitrate fertilisation was applied, at the dose of 200 kg · ha⁻¹, to complement of level of potassium in the soil. Mineral fertilisation in object 2 was

applied as follows: in autumn – $300 \text{ kg} \cdot \text{ha}^{-1}$ of “polifoska” (multicomponent NPK fertiliser – 6 % N, 20 % P_2O_5 , 30 % K_2O), and in spring – $150 \text{ kg} \cdot \text{ha}^{-1}$ of ammonium nitrate (34 % N). Both objects were sown with winter wheat. Soil samples for analyses were taken at 3 phases of plant vegetation, ie in the phases of heading, milk ripeness, and full ripeness.

Microbiological analyses included determinations of respiratory activity [13], of so-called total microbial number [14] and filamentous fungi [15], of the numbers of cellulolytic bacteria, “proteolytic” bacteria and fungi, and of ammonifying and nitrifying bacteria [22]. To investigate the effect of the experimental treatments and the times of analyses on the values of the studied microbiological features of the soils, two-factor analyses of variance were performed. Mean values of the features under analysis, for the experimental treatments and for the times of analyses, were compared by means of 95 % Tukey intervals of confidence, at the level of significance $\alpha = 0.05$. Statistical processing of results was made using the Statistica 7.1 software package. No analysis of variance was performed for the numbers of cellulolytic, ammonifying and nitrifying bacteria, as the numbers of those microbial groups were determined using McCrady tables, based on the principles of mathematical statistics.

Results

The study did not reveal any significant differences between the experimental objects in terms of respiratory activity of the soil. Significant differences were found only between the particular times of analyses. The highest respiratory activity was noted, in both experimental objects, in the phase of wheat heading, and the lowest in milk ripeness (Fig. 1).

The seasonal changes of bacteria number in both experimental objects are presented in Fig. 1. Both in the soil with the dairy sewage sludge and in that with mineral fertilisation seasonal variations were observed in the populations of the studied microbial groups. In the soil with mineral fertilisation an increasing trend was observable in the total bacterial numbers, while in the soil with the sludge an increase was noted in the numbers of those microorganisms in the milk ripeness phase, and a decrease in the full ripeness phase of winter wheat.

The presented results (Fig. 1) indicate that the numbers of fungi, throughout the period of the study, were significantly higher in the soil with the dairy sewage sludge than in that with mineral fertilisation, and displayed seasonal variations. The highest number of fungi was observed in the phases of wheat heading and full ripeness, both in the soil with the sludge and in that with mineral fertilisation. The lowest numbers of fungi, in both experimental objects, were recorded for the 2nd date of analyses, ie in the milk ripeness phase of winter wheat.

Analysis of the results presented in Fig. 1 indicates notable differences in the numbers of cellulolytic bacteria in the experimental objects. Analysis of the mean numbers of cellulolytic bacteria shows that application of dairy sewage sludge caused stronger stimulation of growth of the microbial group in question compared to the soil with mineral fertilisation.

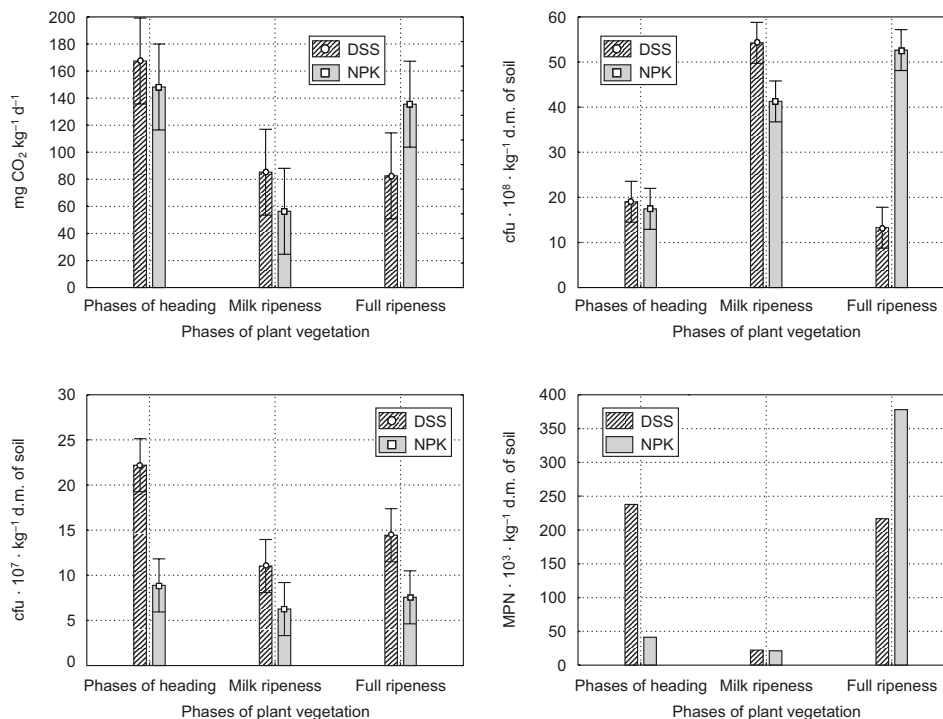


Fig. 1. Respiratory activity, total numbers of bacteria, total numbers of cellulolytic fungi and bacteria in soil; DSS – dairy sewage sludge, NPK – mineral fertilisation

Data concerning the numbers of proteolytic bacteria in the experimental objects are given in Fig. 2. The study shows that in the soil with mineral fertilisation there was a tendency towards increase in the number of proteolytic bacteria during the period of the study. On the other hand, in the object with dairy sewage sludge a decrease was observed in the numbers of those microorganisms with the progressing duration of the experiment.

Throughout the whole period of the study the numbers of the microbial groups under examination were on a somewhat higher level in the soil amended with dairy sewage sludge than in the soil with mineral fertilisation. However, seasonal changes were observed in the numbers of those microbial groups (Fig. 2). Analysis of variance did not reveal any significant differences in the numbers of the studied microbial groups, the only such differences being those between the dates of successive analyses.

Data presented in Fig. 2 indicate that on the 1st and 2nd dates of analyses (phases of heading and of milk ripeness) the number of ammonifiers was notably greater in the soil amended with dairy sewage sludge than in that with mineral fertilisation. On the other hand, on the 3rd date of analyses, ie in the phase of full ripeness of winter wheat, a greater number of the studied microorganisms was recorded in the object with mineral fertilisation. Noteworthy is the considerable drop in the numbers of the studied

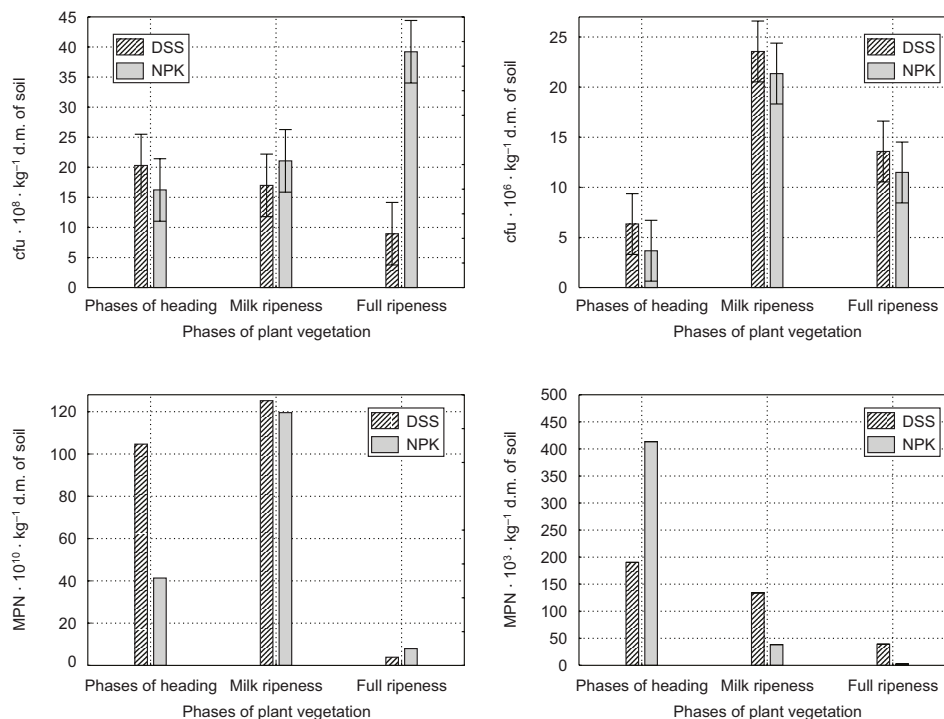


Fig. 2. Numbers of proteolytic bacteria, numbers of "proteolytic" fungi, numbers of ammonifying bacteria and numbers of nitrifying bacteria in soil; DSS – dairy sewage sludge, NPK – mineral fertilisation

microbial groups on the 3rd date of analyses, in both experimental objects. Analysis of mean numbers of the studied microorganisms for the experimental objects indicates that the number of ammonifiers in the soil amended with dairy sewage sludge was on a notably higher level compared to that in the object with mineral fertilisation.

The number of nitrifying bacteria in both experimental treatments decreased considerably with the progressing duration of the experiment, reaching the lowest level in the final stage of the study, ie in full ripeness phase of winter wheat (Fig. 2). Analysis of mean numbers of nitrifiers for the experimental objects indicates that the soil with mineral fertilisation was characterised by a greater number of nitrifying bacteria compared to the soil with the sludge. That result was largely affected by the significantly greater number of the studied microorganisms in the soil with mineral fertilisation than in that with the sludge on the 1st date of analyses, ie in the heading phase of winter wheat.

Discussion

Under conditions of improper management, organic wastes, included sewage sludge, contribute to degradation of the environment [1, 16]. Therefore, it is necessary to

develop methods of utilisation of such wastes that would be safe for the environment, eg through their application in agriculture [16, 17]. From the viewpoint of ecology, an important issue is to acquire knowledge on the effect of dairy sewage sludge on microorganisms and on their activity in soils. Therefore, in the study presented herein, an attempt was undertaken at determining the effect of such wastes on the microbiological parameters of soils.

Our own studies have shown that the respiratory activity and the numbers of microbial groups in the studied soils were significantly dependent on the experimental factors applied, ie on the fertilisation and the time of its effect in the soil. The results of studies indicate stimulating effects of dairy sewage sludge with relation to soil microorganisms. That effect should be attributed to soil enrichment with organic matter, total nitrogen and mineral components, which has been indicated by numerous authors [10, 18–21] who observed stimulation of microbial growth in soils amended with sewage sludge. The significant increase in the numbers of bacteria in the treatment with sewage sludge could have been a result of selection of bacterial populations utilising the available nutrients occurring in the sludge. Stimulation of so-called total bacterial population by sewage sludge has been observed by Furczak and Joniec [12] in a laboratory experiment. Increase of bacterial and fungal populations in soil under the effect of sewage sludge has been recorded in a study by Gostkowska et al [23] under field conditions. In the course of discussed studies was also demonstrated statistically confirmed increase of populations of the microbial groups under study in soil amended with dairy sewage sludge. However, the acquired data indicate that the changes in the bacterial and fungal populations were also affected by the development phase of the crop plant. The obtained results in the study indicate that the introduction of dairy sewage sludge had a positive effect on the populations of soil bacteria and fungi in comparison with the mineral fertilisation applied.

A distinctly stimulating effect of dairy sewage sludge on populations of cellulolytic bacteria in spring, and weakening in subsequent months of analyses, was observed in a study by Jezierska-Tys et al [24]. Comparing the numbers of cellulolytic bacteria in a soil amended with dairy sewage sludge at the dose of $22 \text{ Mg} \cdot \text{ha}^{-1}$ to those in a treatment with mineral fertilisation, they found that the growth of those microorganisms was stronger in the treatment with dairy sewage sludge. The results obtained in our study indicate participation of cellulolytic bacteria in breaking up carbon complexes occurring in dairy sewage sludge introduced in the soil.

Our own studies indicate that dairy sewage sludge had a stimulating effect on the growth of “proteolytic” microorganisms. Dairy sewage sludge is a rich source of organic nitrogen compounds [25], hence the stimulation of growth of “proteolytic” bacteria and fungi, observed in this study, was likely due to soil enrichment in organic nitrogen compounds of sludge origin, constituting a source of nutrients for that microbial group. The acquired data indicate that dairy sewage sludge had more favourable effect on the growth of “proteolytic” bacteria and fungi compared with mineral fertilisation. The weakening effect of the applied waste on the numbers of soil microorganisms with progressing duration of the experiment should be probably attributed to exhaustion of nutrient substrates available to those microbial groups.

Another microbial group whose population increased following the introduction of dairy sewage sludge was that of nitrifying bacteria. It was demonstrated that the sludge introduced in the soil had more favourable effect on the population of nitrifying bacteria compared with mineral fertilisation. In the course of the vegetation season a decrease was observed in the numbers of those microorganisms in the soil amended with dairy sewage sludge, which was probably related with depletion of substrates for that microbial group. Mazur [26] emphasizes that the presence of nitrifier activity in itself is an obvious indicator of soil properties favourable to crop plants, due to the high requirements of that microbial group with respect to available nutrients, its sensitivity to soil acidity and insufficient aeration. It can also be accepted that the mobility of nitrates(V), uninhibited by processes of sorption, is conducive to the uptake of that form of nitrogen by plants as compared with the ammonium form. It appears, therefore, that stimulation of the growth of nitrifying bacteria as a result of soil amendment with dairy sewage sludge indicates also a favourable effect of that waste on soil properties.

Conclusions

Dairy sewage sludge effected the seasonal changes of microbial activity of brown soil. The presented study showed that dairy sewage sludge had a favourable effect on the microbiological properties of brown soil. The study showed that soil amendment with dairy sewage sludge had a favourable effect on the microbiological equilibrium of the soil compared with the soil with mineral fertilisation, which is evidenced by more intensive growth of most of the analysed microbial groups in the soil with sludge than in the soil with mineral fertilisation. The microbiological and biochemical tests applied proved to be sensitive indicators of the biological properties of soil amended with dairy sewage sludge.

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SEZONOWE ZMIANY AKTYWNOŚCI MIKROBIOLOGICZNEJ GLEBY BRUNATNEJ NAWOŻONEJ OSADAM ŚCIEKÓW MLECZARSKICH

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Abstrakt: Celem przeprowadzonych badań było porównanie oddziaływania osadu ścieków mleczarskich i nawożenia mineralnego na liczebność wybranych grup mikroorganizmów i aktywność respiracyjną gleby brunatnej. Wzbogacenie gleby osadem ścieków mleczarskich wywarło korzystny wpływ na jej równowagę mikrobiologiczną w porównaniu z glebą nawożoną mineralnie, o czym świadczy bardziej intensywny rozwój większości analizowanych grup drobnoustrojów w glebie z osadem niż nawożonej mineralnie.

Słowa kluczowe: doświadczenie polowe, gleba brunatna, nawożenie mineralne, osad ścieków mleczarskich