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NUMBERS AND ACTIVITY OF SELECTED MICROBIAL GROUPS INVOLVED IN CARBON TRANSFORMATIONS IN PODZOLIC SOIL AMENDED WITH SEWAGE SLUDGE

LICZEBNOŚĆ I AKTYWNOŚĆ WYBRANYCH GRUP MIKROORGANIZMÓW, CZYNNYCH W PRZEMIANACH WĘGLA W GLEBIE BIELICOWEJ WZBOGACONEJ OSADEM ŚCIEKOWYM

Abstract: Laboratory experiments were conducted in two variants, on a podzolic soil amended with the following doses of municipal-industrial sewage sludge: 30 Mg · ha^{-1} (1 %), 75 Mg · ha^{-1} (2.5 %), 150 Mg · ha^{-1} (5 %), 300 Mg · ha^{-1} (10 %) and 600 Mg · ha^{-1} (20 %). In one of the variants non-sterile sludge was applied, and in the other variant the sludge applied had been subjected to the process of sterilisation in order to determine the contribution of the sludge microorganisms in the transformation of organic matter. After 0.5, 1, 2, 3, 4 and 5 months from the application of the sludge, analyses were performed to determine the following parameters in the soils of the two variants: so-called total number of bacteria with low nutritional requirements, so-called total number of bacteria with high nutritional requirements, total number of filamentous fungi, number of cellulolytic fungi, respiratory activity, intensification of the process of cellulose mineralisation, and dehydrogenases activity. The analyses revealed that the non-sterile sewage sludge caused a stimulation of both the growth and the level of activity of the bacterial and fungal groups under study. That effect was usually the strongest at the beginning of the experiment and increased with increase in the dose of sludge applied. The non-sterile sludge had a stronger effect only on mineralisation of cellulose and on dehydrogenases activity, which may indicate participation of sludge microorganisms in those processes.

Keywords: soil, sterile and non-sterile sewage sludge, bacteria, fungi, respiration, cellulose mineralisation, dehydrogenases.

The extensive literature on the effect of sewage sludge on microbiological activity, and especially on biochemical activity related with carbon transformations in soils

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under laboratory conditions, demonstrates that the subject matter has frequently been the object of research [1–8]. The studies reported demonstrated a positive effect of sewage sludge both on the growth of microorganisms and on the processes that take place in soil with their participation. It should be emphasised, however, that the authors of those studies focused on a limited number of parameters, and primarily on the respiratory and dehydrogenases activity of soil [1, 6–8]. Therefore, it was considered worthwhile to conduct a broader study on the subject, using a larger number of tests, which would permit a more comprehensive assessment of the effect of sewage sludge on the biology of soil. Moreover, the study presented herein was also to provide an answer to the question to what extent microorganisms introduced in the soil with the sludge cooperate with the soil microorganisms in carbon transformation of organic matter and how long their activity persists. This issue has not been devoted much attention so far. Only Bonmati et al [9] addressed the problem, but their study was concentrated on microbiological transformations of nitrogen and phosphorus in soil amended with sewage sludge.

Materials and methods

The experiments were performed with a podzolic soil developed from weakly loamy sand, taken from the Ap horizon. Selected physical, physicochemical and chemical properties of the soil and of the sewage sludge are presented in Table 1 after Baran et al [10], Baran et al [11] and Oleszczuk and Baran [12]. In conformance with procedures commonly applied in studies of this type, prior to its application air-dry sewage sludge was crushed and screened through a sieve with 0.75 mm mesh. The laboratory experiment was set up in two variants. In one of them non-sterile sewage sludge from the Mechanical-Biological Sewage Treatment Plant in Konskie was added to 1 kg weighed portions of soil screened through a sieve with 2 mm mesh, at the following doses: 30 $Mg \cdot ha^{-1}$ (1 %), 75 $Mg \cdot ha^{-1}$ (2.5 %), 150 $Mg \cdot ha^{-1}$ (5 %), 300 $Mg \cdot ha^{-1}$ (10 %) and 600 $Mg \cdot ha^{-1}$ (20 %) of dry matter. In the second variant, the same doses of the sludge were applied, but prior to the application the sludge was thermally sterilised. The sterilisation of the sludge was done in an autoclave (30 min. 0.1 HPa), three times at 24 hour intervals [9].

After the addition of the sludge, all soil samples were wetted to about 60 % t.w.c. and incubated at room temperature for a period of 5 months, maintaining soil moisture at a more or less constant level. The control treatment in the experiment was soil with no sewage sludge addition.

Microbiological and biochemical analyses and determinations of the soil reaction were made after 0.5, 1, 2, 3, 4 and 5 months of the experiment duration, while those for the sludge were performed only once, prior to the start of the experiment. At the beginning of the experiment and after 5 months from the introduction of the non-sterile sludge in the soil determinations of the total carbon content in the soil were made as well.

Within the scope of the experiment, determinations were made of the so-called total number of bacteria with low nutritional requirements, on a medium with soil or sludge extract (350 cm · dm^{-3}) and K_2HPO_4, of so-called total number of bacteria with high nutritional requirements, on Bunt–Rovira medium [13], of so-called total number of filamentous fungi, on Martin medium [14], of the numbers of cellulolytic fungi, on mineral agar covered with a circle of Whatman paper with an addition of antibiotic sin accordance with Martin's recommendations [14], of the respiratory activity, with the method of Rühling et al [15], of the rate of cellulose mineralisation, in 25-gram weight portions of the soil enriched with 0.5 % of powdered Whatman celulose, and the amount of CO_2 emitted from them during 20 days was determined with the method of Ruhling et al [15], of dehydrogenases activity, with the Thalmann method [16], of reaction, potentiometrically in 1mol · dm^{-3} KCl, and of organic carbon content, with the method of Tiurin as modified by Simakov.

Table 1

Properties of the soil and sewage sludge used in the field experiment

Properties	Unit		Soil	Sludge
Granulometric composition	% of fraction [mm]	1–01 0.1–0.02 < 0.02	86 7 7	
pH	1 mol · dm^{-3} KCl		6.0	6.4
T	mmol (+) · kg^{-1}		71.3	607.7
$C_{organic}$ (C_{org})	g · kg^{-1}		11.2	210.0
N_{total} (N_t)			1.4	17.8
C_{org}: N_t	[-]		7.9	11.8
Cd content	mg · kg^{-1}		0.5	6.0
Cu content			7.0	216.0
Pb content			18.6	125.0
Sum of 16 PAHs	μg· kg^{-1}		43.0	3894.0

All determinations of microbiological and biochemical parameters were made in three replications. The results were processed statistically with the method of analysis of variance. The significance of differences was determined with the Tukey test at p = 0.05. Moreover, means for the period of the experiment were plotted on graphs as percentages of increase or decrease, adopting mean values obtained for the control soil as 100 %.

Results and discussion

The sterile or non-sterile sludge introduced in the soil caused a significant stimulation of the number of bacteria with low nutritional requirements (Table 2). Comparing the effect of the two types of sludge it should be emphasised that the sterile sludge exerted a stronger impact in this respect, as in treatments with that sludge applied the numbers of those bacteria were notably greater. This constitutes a basis for the formulation of a thesis that there exists a negative interaction between microorganisms introduced with the sludge and oligotrophic soil bacteria. In both variants of the experiment the stimulation was more pronounced in treatments with the higher doses of the sludge.

Table 2

Selected properties of microbiological, biochemical and chemical sewage sludge

Oligotrophic bacteria, cfu · 10^9 · kg^{-1}dm of sludge	28.7
Macrotrophic bacteria, cfu · 10^9 · kg^{-1}dm of sludge	77.1
Filamentous fungi, cfu · 10^6 kg^{-1} dm of sludge	1050.5
Cellulolytic fungi, cfu · 10^6 · kg^{-1} dm of sludge	0.0
Respiratory activity, mg C-CO_2 · kg^{-1} dm of sludge · d^{-1}	1590.0
Cellulose mineralization, mg C-CO_2 · kg^{-1} dm of sluge · 20 d^{-1}	74653.3
Dehydrogenases activity, mg TPF · kg^{-1} dm of sludge · d^{-1}	95.0
Cellulose, % air dm of sludge	6.6

The observed stimulation of the growth of those bacteria was characterised, in both experimental variants, by a certain dynamics of changes over the period studied (Table 2). In the initial phase of the experiment (after 0.5 month) the stimulating effect of the sludge was the weakest. Subsequently, the effect of the sludge on the numbers of those bacteria gradually intensified, attaining the highest level after 2 and 3 months. In the final phase of the experiment (4 and 5 months) the effect of the sludge was notably reduced, which was most likely related with deterioration of the living conditions of the bacteria.

Stimulating effect of non-sterile sewage sludge on the growth of the bacterial group in question was also observed in laboratory studies by Furczak and Joniec [2] and by Kobus et al [3]. Those authors, as in the study presented here, observed a relation of those changes to the sludge dose applied and to the duration of the sludge effect on the soil. Another factor that appears to cause intensified multiplication of that bacterial group in soil amended with sewage sludge is increase in the soil reaction (Table 3).

Table 3

Reaction of soil (pH_{KCl})

Treatments		Terms of analyses, months					
		0.5	1	2	3	4	5
Control soil		6.1	6.0	6.0	5.9	6.0	6.0
Soil + 1 % of sludge	Series with non-sterile sludge	6.0	6.0	5.7	5.6	5.7	5.8
Soil + 2.5 % of sludge		6.6	6.7	6.3	6.1	5.9	6.1
Soil + 5 % of sludge		6.8	6.6	6.6	6.3	6.1	6.2
Soil + 10 % of sludge		6.9	6.8	6.5	6.6	6.5	6.5
Soil + 20 % of sludge		6.9	6.5	6.5	6.6	6.5	6.6
Soil + 1 % of sludge	Series with sterile sludge	7.0	6.6	5.8	6.5	6.4	6.5
Soil + 2.5 % of sludge		7.0	6.8	6.7	6.5	6.3	6.4
Soil + 5 % of sludge		7.1	6.8	6.6	6.5	6.5	6.5
Soil + 10 % of sludge		7.1	6.7	6.7	6.6	6.6	6.7
Soil + 20 % of sludge		7.0	6.6	6.5	6.6	6.7	6.8

These observations are supported by earlier studies by Hattori and Hattori [17] which showed that some oligotrophic bacteria, in spite of their low nutritional requirements, have a capacity to live in environments with a high concentration of nutrients. Also Wielgosz [18] noted an abundant occurrence of bacteria with low nutritional requirements in sewage sludge, which could partially support the results of our study. Data in Table 4 indicate that also the sludge used in this experiment introduced a certain pool of those bacteria to the soil. However, weaker growth of bacteria with low nutritional requirements in the soil with non-sterile sludge than in than with sterile sludge indicates that bacteria from the sludge rather did not colonise the soil environment. It is to be supposed that there may have appeared the phenomenon of competition between the microbial groups introduced with the sludge and the soil microorganisms, which led to an inhibition of the growth of the bacterial group under study.

Table 4

C-organic content (C_{org}) [g · kg^{-1}]

Treatments	Terms of analyses, months	
	0.5	5
Control soil	7.32	6.48
Soil + 1 % of sludge	8.16	5.60
Soil + 2.5 % of sludge	11.04	10.24
Soil + 5 % of sludge	14.88	12.40
Soil + 10 % of sludge	22.08	20.16
Soil + 20 % of sludge	32.40	29.52

The data in Table 5 indicate that both types of sewage sludge also had a stimulating effect on the growth of bacteria with high nutritional requirements. That effect was generally more distinct and more significant in the presence of higher concentrations of the sludge. The observed stimulation became apparent at the beginning of the experiment (after 0.5 month) more strongly than in the case of oligotrophic bacteria. In both variants of the experiment the most intensive growth of that group of bacteria occurred during the first two months of the experiment. Subsequently the effect of the sludge became slightly weaker and was statistically significant only in treatments with the higher doses of the sludge.

A positive effect of non-sterile sewage sludge on the growth of macrotrophic bacteria in soil under laboratory conditions was observed also by Lima et al [5].

The stimulation observed in this experiment was probably induced by the supply, with the sludge, of a large amount of nutrients necessary for the growth of those bacteria. This is supported by the results presented in Table 6 that indicate an increase in organic carbon content in the soil following the application of the sludge. Another factor that appears to cause, to a degree, intensified multiplication of that bacterial group in soil in treatments with higher doses of the sewage sludge was surely an increase in the soil reaction (Table 3).

It appears that, as in the case of oligotrophic bacteria, also sludge bacteria with high nutritional requirements did not colonise the soil environment, and even the microorganisms brought in with the sludge could have contributed to a reduction in the

Table 5

Total number of oligotrophic bacteria, cfu · $10^9 \cdot kg^{-1}$ dm of soil

Treatments		Terms of analyses, months						Mean for treatments	Mean for dose	Mean for kind of sludge
		0.5	1	2	3	4	5			
Control soil		20.0	1.7	1.0	1.3	2.2	3.9	5.0	5.0	16.0
Soil + 1 % of sludge	Series withnon-sterile sludge	26.4	9.4	6.6	13.4	7.7	17.3	13.7	14.8	
Soil + 2.5 % of sludge		29.5	14.0	18.8	19.0	13.3	23.7	20.0	26.2	
Soil + 5 % of sludge		26.7	22.3	15.9	17.7	5.8	20.0	18.0	25.3	
Soil + 10 % of sludge		49.0	14.2	16.9	28.6	9.5	18.0	22.8	37.4	
Soil + 20 % of sludge		8.9	7.1	13.3	22.0	13.4	33.9	16.4	30.7	
Soil + 1 % of sludge	Series with sterile sludge	8.0	18.3	20.3	16.3	12.2	20.7	16.0		30.5
Soil + 2.5 % of sludge		21.3	29.5	34.4	48.0	34.2	27.8	32.4		
Soil + 5 % osadu		42.7	29.6	35.6	32.4	35.9	21.7	32.7		
Soil + 10 % of sludge		70.4	21.7	58.0	38.6	53.7	63.8	52.1		
Soil + 20 % of sludge		64.2	52.7	32.3	33.0	21.3	64.5	44.9		
Mean for term		32.0	18.5	21.1	22.6	18.6	26.6			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): term (T) – 2.1; sterility (S) – 0.8; dose (D) – 2.1
Interactions: T x S – 3.5; T x D – 6.5; S x D – 3.5; T x S x D – 9.2

Table 6

Total number of macrotrophic bacteria, cfu · 10^9 · kg^{-1} dm of soil

Treatments		Terms of analyses, months						Mean for treatments	Mean for dose	Mean for kind of sludge
		0.5	1	2	3	4	5			
Control soil		3.8	2.9	3.1	7.2	5.0	3.9	4.3	4.3	
Soil + 1 % of sludge	Series with non-sterile sludge	22.3	12.7	29.1	5.9	18.0	15.9	17.3	19.8	21.1
Soil + 2.5 % of sludge		20.6	12.5	22.5	15.2	20.3	13.6	17.5	27.9	
Soil + 5 % of sludge		39.3	19.0	33.0	13.2	27.2	30.5	27.0	37.6	
Soil + 10 % of sludge		49.4	24.5	10.0	28.2	26.4	28.6	27.8	51.4	
Soil + 20 % of sludge		34.9	19.5	40.3	28.4	37.7	33.9	32.4	63.4	
Soil + 1 % of sludge	Series with sterile sludge	26.3	21.2	31.7	14.1	26.3	13.7	22.2		47.0
Soil + 2.5 % of sludge		45.8	35.3	44.5	47.2	29.8	27.8	38.4		
Soil + 5 % of sludge		65.2	50.3	41.7	32.4	38.8	59.9	48.1		
Soil + 10 % of sludge		114.1	12.7	78.5	68.7	98.4	77.2	74.9		
Soil + 20 % of sludge		155.6	69.7	83.4	47.6	121.9	87.9	94.3		
Mean for term		48.1	23.6	35.1	26.3	37.9	33.1			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): T – 3.4; S – 1.4; D – 3.4
Interactions: T x S – 5.6; T x D – 10.4; S x D – 5.6; T x S x D – 14.8
Explanations as in Table 5.

number of soil bacteria of this type. This is indicated by the higher number of the bacteria in question in the soil with the sterile sludge (Table 5). The stronger stimulation of the growth of that bacterial group in the soil with sterile sludge could have been caused by the provision of an additional source of organic matter, in the form of microorganisms decayed through the process of sterilisation of the sludge. This supposition is also supported by earlier studies conducted by Sastre et al [19].

Amendment of the soil with the non-sterile and sterile sludge caused also an increase in the total number of filamentous fungi (Table 7). That effect, however, was statistically proven only in treatments with the higher doses of the waste.

The positive effect of the sludge on the growth of the fungi was the most apparent after 2 weeks from the time of sludge application, then it grew weaker and until the end of the experiment remained at a lower level (Table 7).

This study supports the observations of, among others, Kobus et al [3] and Lima et al [5], indicating increased numbers of fungi in soil amended with sewage sludge.

In the opinion of many authors, among others Kobus et al [3] and Sastre et al [19], it is common knowledge that microbiological activity of soils depends on their content of organic matter. As sewage sludge abounds in various organic substances, it appears that the primary cause of the stimulation of fungal growth observed in this study was, as in the case of bacteria, enrichment of the soil in nutrients. This is indicated by the increase in organic carbon content in the soil, caused by the introduction of the sludge (Table 6). The fundamental effect of organic matter on the stimulation of fungal growth is supported by the fact that the effect appeared in spite of the soil reaction increase (Table 3) to a level close to the neutral. Data in Table 4 indicate that filamentous fungi are present in the sludge applied only at a slight level. This accounts for the lack of notable differences in the growth of those microorganisms between the analysed series of the experiment (Table 7).

The data given in Table 8 inform that the higher doses of the sterile and non-sterile sewage sludge (10 and 20 %) caused a visible, statistically proven, stimulation of the growth of cellulolytic fungi. In treatments with lower concentrations of the sludge, however, only a slight tendency towards an increase in the numbers of the microbial group in question was noted. The positive effect of the sludge on the microbiological parameter under consideration was the most apparent after 0.5, 3, 4 and 5 months (Table 8). Whereas, on the other dates of analyses it was insignificant and oscillated around a level similar to that of the control treatment.

The somewhat stronger growth of cellulose-decomposing fungi in the soil with sterile sludge than in that with non-sterile sludge may indicate a certain unfavourable effect of the sludge microorganisms (Table 8). Apparently it was primarily the cellulose and other nutrients for that microbial group, introduced with the sludge, that contributed to the growth of the number of cellulolytic fungi observed in this study. The results obtained support an earlier laboratory study by Furczak and Joniec [2], concerning the effect of the degree of fragmentation of sewage sludge on microbiological and biochemical properties of soil, that revealed an increase in the numbers of cellulose-decomposing fungi in soil amended with sewage sludge.

Table 7

Total number of filamentous fungi, cfu · 10^6 · kg^{-1} dm of soil

Treatments		Terms of analyses, months: 0.5	1	2	3	4	5	Mean for treatments	Mean for dose	Mean for kind of sludge
Control soil		19.6	28.2	30.0	17.7	8.4	44.9	24.8	24.8	
Soil + 1 % of sludge	Series with non-sterile sludge	42.7	49.0	36.6	24.1	22.4	52.0	37.8	32.8	46.6
Soil + 2.5 % of sludge		37.0	29.1	39.8	25.5	3.0	58.1	32.1	38.6	
Soil + 5 % of sludge		93.0	38.0	76.1	30.7	8.6	87.1	55.6	46.5	
Soil + 10 % of sludge		77.4	81.5	99.6	52.8	26.8	38.1	62.7	60.4	
Soil + 20 % of sludge		126.3	51.9	114.4	41.1	16.6	51.0	66.9	67.5	
Soil + 1 % of sludge	Series with sterile sludge	33.7	28.8	31.0	21.1	1.1	51.0	27.8		43.5
Soil + 2.5 % of sludge		71.6	47.2	51.3	14.6	11.8	73.7	45.0		
Soil + 5 % of sludge		59.1	60.4	31.0	32.0	8.6	33.6	37.5		
Soil + 10 % of sludge		107.9	64.8	54.8	53.8	11.3	55.4	58.0		
Soil + 20 % of sludge		128.9	102.1	46.7	54.3	6.7	70.1	68.1		
Mean for term		68.1	50.8	53.5	32.1	11.1	55.0			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): T – 4.1; S – 1.6; D – 4.1
Interactions: T x S – 6.7; T x D – 12.5; S x D – 6.7; T x S x D – 17.7
Explanations as in Table 5.

Table 8

Population of cellulolytic fungi, cfu $\cdot$ 10^6 $\cdot$ kg^{-1} dm of soil

Treatments		Terms of analyses, months						Mean for treatments	Mean for dose	Mean for kind of sludge
		0.5	1	2	3	4	5			
Control soil		2.1	2.3	2.9	2.2	2.2	2.4	2.3	2.3	
Soil + 1 % of sludge	Series with non-sterile sludge	3.9	2.7	1.7	2.2	5.9	3.6	3.3	2.8	
Soil + 2.5 % of sludge		3.4	3.8	1.9	4.0	2.7	4.3	3.3	4.1	12.8
Soil + 5 % of sludge		19.4	3.4	1.6	6.1	2.3	4.8	6.3	6.2	
Soil + 10 % of sludge		34.2	3.4	5.4	60.8	55.9	7.5	27.9	28.9	
Soil + 20 % of sludge		58.2	5.5	5.4	68.6	48.2	17.1	33.8	34.9	
Soil + 1 % of sludge	Series with sterile sludge	2.4	3.6	1.1	2.7	2.0	2.1	2.3		
Soil + 2.5 % of sludge		3.2	2.4	1.9	5.9	13.2	2.8	4.9		
Soil + 5 % osadu		4.1	3.4	2.7	21.7	1.9	2.8	6.1		13.6
Soil + 10 % of sludge		40.6	5.4	2.6	28.5	97.2	4.9	29.9		
Soil + 20 % of sludge		63.0	5.4	3.6	96.5	40.1	7.4	36.0		
Mean for term		19.7	3.6	2.8	25.1	22.8	5.2			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): T – 2.0; S – no significant differences; D – 2.0
Interactions: T x S – 3.2; T x D – 6.1; S x D – no significant differences; T x S x D – 8.6
Explanations as in Table 5.

The data presented in Table 9 show that both the sterile and the non-sterile sewage sludge caused a generally significant increase in the respiratory activity of the soil. The stimulation usually increased with increase in the sludge dose, assuming the highest values in the treatments with 10 and 20 % of the waste. The sterile sludge caused a significantly stronger stimulation of carbon mineralisation than the non-sterile sludge. The stronger stimulation of the respiratory processes by the sterile sludge was most likely a result of increase in the amount of respiration substrata, brought into the soil with the sludge microorganisms killed in the process of sludge sterilisation, and of the lack of competition.

During the 5 months of the experiment, the effect of the sludge on the emission of CO_2 varied in intensity (Table 9). A significant stimulating effect of all the doses of the sludge was observed only at the beginning of the experiment, *ie* after 0.5 and 1 month. The stimulation of respiratory activity noted after 2 months was the strongest, though it was statistically substantiated only from the second dose of the sludge, *ie* 2.5 %, upwards. On the successive dates of analyses (3 and 4 months) the stimulating effect of the sludge was generally reduced, and in the final phase of the experiment it was almost non-observable. The weakening of the effect of the sludge on the process analysed with the passage of time was probably due to the exhaustion of the more readily available respiration substrates. Similar conclusions were proposed by Debosz et al in their study [1].

Stimulation of the respiratory activity of soil by sewage sludge is indicated by numerous laboratory studies [1, 2, 6, 8]. Moreover, Saviozzi et al [8] observed, as we did in this study, a dependence of the intensity of that effect on the level of sludge dose applied, while Debosz et al [1] observed a weakening of the effect with the passage of time, that even turned to inhibition in the final phase of the experiment.

The amount of CO_2 emitted from soils primarily depends on the activity of microorganisms inhabiting it [20]. In the opinion of Hattori and Mukai [21], intensification of the processes of mineralisation of organic carbon depends primarily on the level and quality of organic matter. Therefore, it seems that the observed stimulation of respiration was caused first of all by the supply of a large amount of carbon organic matter to the soil with the sewage sludge, that organic matter being a source of respiratory substrates for microorganisms. This is indicated by the increase in the content of organic carbon in the soil amended with the waste (Table 6). Another factor that contributed to the stimulation of CO_2 emission could also be an increase in the soil reaction (Table 3) that was conducive to the growth of bacteria. As it is known, the role of bacteria in the mineralisation of organic matter is greater than that of fungi. The results given in Table 4 inform that numerous microorganisms get in the soil together with sewage sludge. However, in the experimental series with the non-sterile sludge there may have taken place an unfavourable interaction between the indigenous soil microorganisms and those introduced with the sludge. This mainly related to bacteria (Tables 2, 5), the result of which was lower stimulation of respiratory processes under those conditions (Table 9).

The sterile and non-sterile sewage sludge introduced in the soil caused also a significant intensification of cellulose mineralisation (Table 10). That stimulation corresponded with the dose of the waste, attaining, as in the case of respiratory activity,

Table 9

Respiratory activity, mg $C\text{-}CO_2 \cdot kg^{-1}$ dm of soil $\cdot\ d^{-1}$

Treatments		Terms of analyses, months						Mean for treatments	Mean for dose	Mean for kind of sludge
		0.5	1	2	3	4	5			
Control soil		159.0	294.0	31.0	65.5	23.0	102.0	112.5	112.5	
Soil + 1 % of sludge	Series with non-sterile sludge	402.5	517.0	115.5	95.5	35.5	83.0	208.0	201.0	260.0
Soil + 2.5 % of sludge		454.5	547.0	126.0	148.5	72.0	67.0	235.0	280.0	
Soil + 5 % of sludge		505.5	556.0	210.5	312.5	62.0	118.0	277.5	331.5	
Soil + 10 % of sludge		621.5	593.0	254.5	243.0	211.0	95.5	336.5	414.0	
Soil + 20 % of sludge		655.5	678.0	339.5	353.5	154.5	158.0	390.0	437.5	
Soil + 1 % of sludge	Series with sterile sludge	342.0	469.5	136.0	139.0	25.5	52.0	194.0		332.0
Soil + 2.5 % of sludge		536.0	585.5	244.0	268.0	166.0	148.0	324.5		
Soil + 5 % osadu		636.0	576.0	364.5	360.0	244.0	134.5	386.0		
Soil + 10 % of sludge		811.5	682.0	452.5	455.5	324.0	223.0	491.5		
Soil + 20 % of sludge		956.0	740.0	382.5	374.5	298.5	159.5	485.5		
Mean for term		520.0	544.5	224.0	231.5	136.5	120.0			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): T –21.5; S –8.5; D –21.5
Interactions: T x S –35.0; T x D –65.5; S x D –35.0; T x S x D – 92.5
Explanations as in Table 5.

Table 10

Cellulose mineralization, mg C-CO_2 · kg^{-1} dm of soil · 20 d^{-1}

Treatments		Terms of analyses, months						Mean for treatments	Mean for dose	Mean for kind of sludge
		0.5	1	2	3	4	5			
Control soil		147.2	1550.4	1667.6	1872.8	1370.0	1442.4	1562.4	1562.4	2952.8
Soil + 1 % of sludge	Series with non-sterile sludge	2986.0	2341.2	2193.6	2022.4	1426.8	950.4	1986.8	2088.0	
Soil + 2.5 % of sludge		4649.0	3674.0	2757.6	2657.2	1646.8	1958.4	2735.6	2399.2	
Soil + 5 % of sludge		3876.8	4112.0	3990.8	2440.0	1778.0	1118.4	2886.0	2681.6	
Soil + 10 % of sludge		4846.4	5420.0	5508.4	3824.4	3524.8	1360.0	4080.8	3870.8	
Soil + 20 % of sludge		6133.2	5956.4	6474.8	4698.4	2162.4	1362.8	4464.8	4336.4	
Soil + 1 % of sludge	Series with sterile sludge	3432.8	2208.4	2423.2	1972.8	1716.0	1379.6	2189.2		2693.6
Soil + 2.5 % of sludge		3893.6	1502.4	2126.0	2129.6	1550.0	1176.8	2063.2		
Soil + 5 % osadu		4746.4	2267.2	2954.0	1999.2	1459.2	1427.6	2477.2		
Soil + 10 % of sludge		4693.6	3860.4	5222.0	3430.0	3344.0	1412.8	3660.4		
Soil + 20 % of sludge		6992.0	4482.4	3394.4	6071.6	3641.2	1665.2	4207.6		
Mean for term		4022.0	3243.6	3372.0	2916.0	2000.0	1391.6			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): T –126.0; S–50.0; D– 126.0
Interactions: T x S –204.4; T x D –384.0; S x D –204.4; T x S x D –543.2
Explanations as in Table 5.

the highest level in treatments with 10 and 20 % sludge content. The relation of the rate of cellulose mineralisation to the concentration of sewage sludge in the soil was also noted in laboratory conditions by Furczak and Joniec [2], studying the effect of the degree of sludge fragmentation on that process. As in this study, that effect became weaker with the passage of time. The data given in Table 10 indicate that the stimulating effect of sewage sludge on the process of cellulose mineralisation was the strongest on the first three dates of analyses, ie after 0.5, 1 and 2 months. The observed stimulation grew weaker with the passage of time, that phenomenon being more rapid in soil with the lower doses of the sludge (1, 2.5 %). Therefore, on subsequent dates of analyses (3 and 4 months) the stimulation of the process of cellulose mineralisation was still clearly observable only in treatments with 10 and 20 % of the waste. Whereas, after 5 months no significant stimulating effect of the sludge on the parameter in question was observed any longer. In certain treatments there even appeared a slight, though not substantiated statistically, inhibition of the process of cellulose mineralisation.

Studies by Debosz et al [1] and by Hattori and Mukai [21] as well as the data given in Table 4 indicate that sewage sludge is a source of cellulose, among other things. Therefore, it should be supposed that the stimulation of the rate of cellulose mineralisation observed in our study was caused by the introduction of certain amounts of that polysaccharide, and other nutrients for that microbial group, into the soil together with the sewage sludge. Whereas, the decrease of the stimulation observed with the passage of time was most likely related with the exhaustion of the substrate due to the activity of cellulolytic microorganisms (Table 8). The observed higher intensity of the process of cellulose mineralisation in the soil with the non-sterile sludge (Table 10) could be attributed in part to the activity of cellulases of cells of cellulolytic bacteria brought in with the sludge (Table 4). A certain role in that complex process could also have been played by other heterotrophic microorganisms participating in the process of mineralisation of excess products of cellulose decomposition.

The results given in Table 11 indicate that the introduction of both the sterile and the non-sterile sewage sludge in the soil caused a stimulation of the soil dehydrogenase activity, generally increasing with increase in the dosage. That effect was statistically significant in almost all treatments, with the exception of that with the lowest dose of the waste (1 %).

The stimulation observed in this study was the most pronounced in the initial phase of the experiment, ie after 0.5 a month (Table 11), while on the second date of analyses (after 1 month) it weakened notably and remained on a similar level until the end of the experiment. The results of studies conducted so far in this area are not equivocal. Studies by numerous authors, conducted under laboratory conditions [2, 4, 6–8], indicate that in most cases sewage sludge has a positive effect on dehydrogenase activity of soils. Whereas, Kucharski et al [4], depending on the kind of sewage sludge applied, apart from the stimulation also observed an inhibition of the activity of the enzymes studied.

As in our study, usually a weakening of the stimulation of dehydrogenase activity with the passage of time was observed [6–8].

Table 11

Dehydrogenases activity, mg TPF · kg^{-1} dm of soil · d^{-1}

Treatments		Terms of analyses, months						Mean for treatments	Mean for dose	Mean for kind of sludge
		0.5	1	2	3	4	5			
Control soil		1.67	2.49	2.19	2.26	1.76	3.00	2.23	2.23	12.44
Soil + 1 % of sludge	Series with non-sterile sludge	4.67	5.23	2.46	3.67	3.31	5.63	4.16	4.02	
Soil + 2.5 % of sludge		7.28	8.42	5.49	5.15	3.70	8.04	6.35	6.09	
Soil + 5 % of sludge		13.89	11.48	7.29	13.01	3.85	10.18	9.95	10.19	
Soil + 10 % of sludge		30.23	28.10	17.18	14.52	10.46	24.54	20.84	18.89	
Soil + 20 % of sludge		50.13	40.13	7.83	35.35	18.28	35.05	31.13	27.28	
Soil + 1 % of sludge	Series with sterile sludge	3.94	2.98	2.90	2.82	3.30	7.39	3.89		10.46
Soil + 2.5 % of sludge		5.17	5.30	3.41	4.71	2.53	13.87	5.83		
Soil + 5 % osadu		11.40	10.36	22.26	7.05	4.01	7.46	10.42		
Soil + 10 % of sludge		25.90	20.26	12.98	9.65	6.15	26.75	16.95		
Soil + 20 % of sludge		48.42	18.08	17.80	16.62	11.44	28.26	23.44		
Mean for term		17.03	12.94	8.66	9.75	5.88	14.43			

$LSD_{(0.05)}$ ($NIR_{(0.05)}$): T – 0.91; S – 0.36; D – 0.91
Interactions: T x S – 1.48; T x D – 2.78; S x D – 1.48; T x S x D – 3.94
Explanations as in Table 5.

The activity of enzymes in soil is mostly the result of microbial activity [22]. Therefore, the stimulation of dehydrogenases activity (Table 11) was a reflection of the growth of the analysed bacterial and fungal groups that was observed in this study (Tables 2,5,7,8). In the opinion of many authors, dehydrogenases activity is strongly correlated with, among other things, the content of organic carbon in soil [23]. That observation is supported by this study which demonstrated an increase in the level of that biochemical parameter (Table 6). Another factor contributing to the stimulation of the activity of the enzymes in question could also be increase of pH in the soil with the sludge (Table 3) because**,** as reported by Chazijew [24] and by Quilchano and Maranon [25], there is a positive correlation between those parameters. In the opinion of Pascual et al [7], sludge organic matter additionally alleviates the negative effect of contaminants (eg heavy metals or toxic compounds of organic character), introduced with sewage sludge, on dehydrogenases activity. Sensitivity of dehydrogenases to heavy metals was reported by Moreno et al [26], among others.

With relation to that, the weakening of the stimulation of the activity of those enzymes, observed beginning with the second date of analyses, was probably caused by the depletion, with the passage of time, of the amount of available organic matter through its mineralisation, and therefore by an intensification of the negative effect of contaminants present in the sludge (Table 1). A progressing decrease in the level of organic matter is indicated by the results presented in Table 6.

The stimulating effect of the sterile sludge on dehydrogenases activity was, within the period studied, notably weaker than that of the non-sterile sludge (Table 11), which suggests that a certain role in the variation of that parameter could have been played by microorganisms coming from the sludge itself.

Summary and conclusions

1. In the soil with the non-sterile sludge almost all the sludge doses applied caused a stimulation of the growth of bacteria with low and high nutritional requirements and of filamentous fungi. That effect was stronger in treatments with the higher doses of the sludge (5, 10 and 20 %). The highest doses of the sludge caused also an increase in the number of cellulolytic fungi. The most intensive growth of bacteria with high nutritional requirements and of filamentous fungi occurred at the beginning of the experiment, of oligotrophic bacteria in the 2^{nd} and 3^{rd} months, and of cellulolytic fungi – at the beginning and in the 3^{rd} and 4^{th} months of the experiment. The experiments conducted in parallel with the sterile sludge suggest that bacteria from the sludge rather did not colonise the soil, and even there may have appeared unfavourable interactions between the soil bacteria and the microorganisms introduced with the sludge.

2. All doses of the non-sterile sewage sludge caused an intensification of the process of respiration, cellulose mineralisation, and of the dehydrogenases activity of the soil. That effect usually intensified with increase in the concentration of the waste in the soil. Respiration attained the highest values in the 2^{nd}, 3^{rd} and 4^{th} months of the experiment, cellulose mineralisation – in the first two months, and dehydrogenases activity only at the beginning of the experiment, ie during the initial two weeks. The higher levels of

cellulose mineralisation and dehydrogenases activity in the soil amended with the non-sterile sludge may indicate a certain significant role of other microorganisms than those analysed in this study, ie those coming from the sludge itself, in those processes in the soil.

3. The observed relatively rapid decrease of the effect of the sewage sludge, under laboratory conditions as compared with field conditions [27], on the microbiological and biochemical parameters of soil under study, indicates a lower credibility and usefulness of laboratory experiments in the assessment of ecological effects of the introduction of the waste in soil.

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LICZEBNOŚĆ I AKTYWNOŚĆ WYBRANYCH GRUP MIKROORGANIZMÓW, CZYNNYCH W PRZEMIANACH WĘGLA W GLEBIE BIELICOWEJ WZBOGACONEJ OSADEM ŚCIEKOWYM

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Abstrakt: Badania laboratoryjne przeprowadzono w dwóch wariantach na glebie bielicowej, do której dodano następujące dawki osadu ścieków komunalno-przemysłowych: 30 Mg · ha^{-1} (1 %), 75 Mg · ha^{-1} (2,5 %), 150 Mg · ha^{-1} (5 %), 300 Mg · ha^{-1} (10 %) i 600 Mg · ha^{-1} (20 %). W jednym wariancie zastosowano osad niesterylny, a w drugim osad poddany procesowi sterylizacji, w celu poznania udziału mikroorganizmów osadowych w transformacji wymienionej materii organicznej Po upływie 0,5, 1, 2, 3, 4 i 5 miesięcy oznaczano w gleby obu wariantów: tzw. ogólną liczbę bakterii o małych wymaganiach pokarmowych, tzw. ogólną liczbę bakterii o dużych wymaganiach pokarmowych, tzw. ogólną liczbę grzybów nitkowatych, liczebność grzybów celulolitycznych, aktywność oddechową, nasilenie procesu mineralizacji celulozy i aktywność dehydrogenaz. Przeprowadzone analizy wykazały, że niesterylny osad ściekowy spowodował zarówno stymulację rozwoju, jak i aktywności badanych grup bakterii i grzybów. Efekt ten na ogół najsilniej wystąpił na początku trwania doświadczenia i nasilał się wraz ze wzrostem dawki odpadu. Osad niesterylny wywarł silniejszy wpływ jedynie na mineralizację celulozy oraz aktywność dehydrogenazową, co może wskazywać na udział w nich drobnoustrojów osadowych.

Słowa kluczowe: gleba, osad sterylny i niesterylny, bakterie, grzyby, oddychanie, mineralizacja celulozy, dehydrogenazy.