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THE IMPORTANCE OF SLUDGE MICROORGANISMS IN NITROGEN TRANSFORMATIONS IN PODZOLIC SOIL AMENDED WITH SEWAGE SLUDGE

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Abstract: The laboratory experiment was set up on a podzolic soil in two variants. In one of them non-sterile sewage sludge was introduced into the soil, and in the second – the same sludge but subjected previously to the process of sterilisation. In both variants the same doses of the sludge were applied: 30 (1%), 75 (2.5%), 150 (5%), 300 (10%) and 600 Mg·ha^{-1} (20%). Then, after 0.5, 1, 2, 3, 4 and 5 months, the soil of both experimental variants was analysed for the numbers of bacteria and fungi decomposing proteins, the rate of the process of ammonification, the rate of the process of nitrification, and for proteolytic activity. The results obtained revealed a stimulating effect of the sludge, both sterile and non-sterile, on the numbers of the microbial groups under study and on the rate of nitrification and protease activity. Only the process of ammonification was subject to inhibition. The observed effects of the sludge were the most pronounced in the case of the higher sludge doses. Significantly greater numbers of protein-decomposing fungi and higher activity of almost all (except for ammonifcation) analysed biochemical parameters in the soil with non-sterile sludge compared to that with sterile sludge indicate an effect of microorganisms from the sludge on the microbiological transformations of nitrogen in soil amended with sewage sludge.

INTRODUCTION

The effect of sewage sludge on the numbers of microorganisms and on the biochemical activity of soil related with nitrogen transformations under laboratory and fields conditions has been studied by many authors [3, 5, 7, 10, 12, 14, 17, 26]. However, those were fragmentary studies and, moreover, they did not address the participation of microorganisms from the sludge in those transformations. Only Bonmati *et al*. [5] made an attempt at addressing this issue, but their study was concerned solely with the processes of ammonification and nitrification. The study presented here is a part of a broader research on the directions and intensity of changes in the microbiological and biochemical activity of soil amended with sewage sludge and on the role of sludge microorganisms in that activity. In our previous report [13] we presented results related with the transformations

of carbon organic matter contained in the sludge. Whereas, this study was focused on the directions and intensity of transformations of nitrogen compounds in soil with sewage sludge, and on the role of sludge microorganisms in those transformations.

MATERIAL AND METHODS

The soil material used in the study came from the Ap horizon of a podzolic soil developed from weakly loamy sand. The sewage sludge was obtained from the Mechanical-Biological Sewage Treatment Plant in Końskie. Selected physical, physicochemical and chemical properties of the soil and the sewage sludge are compiled in Table 1 after Baran *et al.* [1], Baran *et al.* [2], and Oleszczuk and Baran [19]. Fresh soil material was screened through a sieve with 2 mm mesh, and air-dry sewage sludge (following the procedure commonly used in studies of this type) was fragmented and screened through a sieve with mesh size of 0.75 mm.

Table 1. Properties of the soil and sewage sludge used in the experiment

Properties	Unit		Soil	Sludge
Granulometric composition	% of fraction in mm	1-0.1 0.1-0.02 <0.02	86.0 7.0 7.0	
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	3 894.0

The laboratory experiment was set up in two variants: in one, non-sterile sewage sludge was added to 1 kg weighed portion of the soil, placed in glass containers with perforated covers that permitted gas exchange, at doses of 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%) of dry matter, while in the second, the same doses of thermally sterilised sludge were applied. The sludge was sterilised in an autoclave (30 min., 0.1 HPa), three times at 24-hour intervals [5]. The control treatment in the experiment was soil, where no sludge was added. Soil samples prepared in that manner were moistened to approximately 60% of total water capacity and incubated at room temperature for a period of 5 months maintaining the soil moisture at a level similar to the above.

Microbiological, biochemical and pH_{KCl} analyses were performed after 0.5, 1, 2, 3, 4 and 5 months of the experiment. Prior to the experiment the same analyses were also performed, once, for the sewage sludge.

The analyses comprised the determination of the numbers of bacteria decomposing protein, on the Frazier gelatine medium [21]; of the numbers of fungi decomposing protein, on the Frazier medium [21] with an admixture of antibiotics in amounts recommended by Martin [16]; of the intensity of ammonification, in 25-gram weighed portions of soil containing 0.1% of asparagine (after 3 days of incubation ammonium ions were extracted and their content was determined with the Nessler method [18]; of the rate of nitrification, in 25-gram weighed portions of soil containing 0.1% monobasic ammonium phosphate (after 7 days of incubation ammonium ions were extracted and their level was measured with the brucine method [18]; of protease activity, following the method of Ladd and Butler [15]; and of the soil reaction – potentiometrically in 1 $mol \cdot dm^{-3}$ KCl.

All analyses were performed in three replications. The results of the periodic microbiological and biochemical analyses were processed statistically with the method of analysis of variance. The significance of differences was determined with the Tukey test at $p = 0.05$.

RESULTS AND DISCUSSION

Soil amendment with non-sterile and sterile sewage sludge resulted in significant stimulation of the growth of proteolytic bacteria (Tab. 2).

This effect was the most pronounced with the application of the higher doses of the sludge, and displayed certain variations. The non-sterile sludge had the strongest effect after the first month, while the sterile sludge after the 1st, 2nd and 4th months. On the remaining dates of analyses the stimulation of the growth of protein-decomposing bacteria continued at a lower, generally stable level.

The stimulation of the growth of that microbial group in soil samples under the effect of non-sterile sewage sludge has so far been observed only by Furczak and Joniec [7], in a laboratory study of methodological character.

Studies by Hattori and Mukai [11] show that sewage sludge is a rich source of proteins. Therefore, the stimulation of the growth of proteolytic bacteria observed in this experiment was likely due to the enrichment of the soil with proteins from the sludge, being a source of nutrition for that microbial group. Another factor that may have contributed to the stimulation of that bacterial group in combinations with sludge was surely the increase of the soil pH (Tab. 3).

Data given in Table 4 indicate that the sewage sludge used in the experiment had large populations of proteolytic bacteria.

Therefore, a certain number of those bacteria were introduced in the soil with the application of the sludge. However, the lower numbers of proteolytic bacteria in the soil with the non-sterile sludge than in that with the sterile sludge (Tab. 2) would indicate that the sludge proteolytic bacteria did not find suitable growth conditions in the soil and gradual decay. The possibility of mortality of microbes introduced in soil with sewage sludge has been reported by Sastre *et al.* [22]. Additionally, sludge microorganisms may have had, initially, a negative effect on the analysed group of soil bacteria, weakening the positive effect of the application of the sludge. Similar observations were made with relation to oligotrophic and macrotrophic bacteria introduced in soil with sewage sludge [13].

The stronger stimulation of the growth of proteolytic bacteria in the soil with sterile sludge was probably caused in part by the supply to the soil, together with the sludge, of an

Table 2. Numbers of proteolytic bacteria in the soil, cfu 10^9 kg^{-1} d.m. 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		2.3	1.4	2.6	1.8	1.8	3.3	2.2	2.2	
Soil+ 1% of sludge	Series with non-sterile sludge	9.0	2.8	4.1	6.1	4.0	9.8	6.0	7.7	
Soil +2,5% of sludge		8.4	8.8	15.9	6.1	10.0	14.1	10.6	12.6	10.7
Soil +5% of sludge		11.4	13.4	16.7	6.5	8.2	25.3	13.6	17.2	
Soil +10% of sludge		15.7	13.8	9.2	8.7	13.0	20.4	13.5	20.6	
Soil +20% of sludge		17.9	18.3	19.3	13.1	21.5	21.0	18.5	23.8	
Soil +1% of sludge	Series with sterile sludge	8.5	10.4	17.3	3.3	8.5	8.6	9.4		
Soil+2,5% of sludge		10.7	10.8	27.6	1.1	19.9	18.1	14.7		
Soil +5% osadu		12.3	28.5	38.7	2.7	25.8	17.2	20.8		17.3
Soil +10% of sludge		11.6	6.4	57.2	15.6	47.4	28.7	27.8		
Soil +20% of sludge		15.1	48.6	39.1	17.6	31.3	22.2	29.0		
Mean for term		10.4	13.7	20.9	7.0	16.1	16.0			

$LSD_{(0.05)}$: term (T) – 2.3; sterility (S) – 0.9; dose (D) – 2.3
Interactions: T × S – 3.7; T × D – 7.0; S × D – 3.7; T × S × D – 9.9

Table 3. Reaction of soil, pH_{KCl}

Treatments		Range
Control soil		5.9 – 6.0
Soil+ 1% of sludge	Series with non-sterile sludge	5.6 – 6.0
Soil +2,5% of sludge		5.9 – 6.7
Soil +5% of sludge		6.1 – 6.6
Soil +10% of sludge		6.5 – 6.8
Soil +20% of sludge		6.5 – 6.6
Soil +1% of sludge	Series with sterile sludge	5.8 – 6.6
Soil+2,5% of sludge		6.3 – 6.8
Soil +5% osadu		6.5 – 6.8
Soil +10% of sludge		6.6 – 6.7
Soil +20% of sludge		6.5 – 6.8

Table 4. Selected properties of microbiological and biochemical sewage sludge used in the experiment

Proteolytic bacteria, cfu 10^9 kg^{-1} d.m. of sewage sludge	11.60
Proteolytic fungi, cfu 10^6 kg^{-1} d.m. of sewage sludge	308.10
Ammonification rate, mg $N\text{-}NH_4 \cdot kg^{-1}$ d.m. of sewage sludge $\cdot$ $3d^{-1}$	213.33
Nitryfication rate, mg $N\text{-}NO_3 \cdot kg^{-1}$ d.m. of sewage sludge $\cdot$ $7d^{-1}$	1617.77
Protease activity, mg tyrosine $\cdot$ kg^{-1} d.m. of soil $\cdot$ h^{-1}	131.60

additional source of nutrition in the form of microorganisms killed in the process of sterilisation. Similar conclusions were reached in an earlier study by Sastre *et al.* [22]. Another cause for that phenomenon could have been a lack of negative interactions between native proteolytic bacteria and the microbial assemblage brought in with the sludge.

As in the case of protein decomposing bacteria, both types of sludge applied in the experiment caused stimulation of the growth of proteolytic fungi (Tab. 5).

That effect was the most pronounced in the initial stage of the experiment (i.e. after 0.5 and 1 month) and it was usually observed in treatments with larger doses of the sludge. On other dates of the analyses only higher doses of the non-sterile sludge caused an increase in the numbers of that microbial group, though the growth was not always statistically significant. The stimulation generally continued at a relatively stable rate until the end of the experiment (Tab. 5).

This issue has not been given much attention so far. Only Furczak and Joniec [7], in a laboratory methodological experiment, observed a similar effect of sewage sludge on the numbers of protein-decomposing fungi.

Table 5. Numbers of proteolytic fungi in the soil, cfu 10^6 kg^{-1} d.m. 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		14.8	10.9	27.6	10.9	51.0	39.8	25.8	25.8	
Soil+ 1% of sludge	Series with non-sterile sludge	48.3	76.2	29.5	14.9	84.4	47.0	50.0	40.8	
Soil +2,5% of sludge		44.9	36.8	36.2	22.8	55.5	33.3	38.3	39.6	
Soil +5% of sludge		64.8	29.8	34.1	15.4	50.6	49.0	40.6	38.2	55.6
Soil +10% of sludge		81.7	95.7	91.6	31.8	85.1	113.8	83.3	65.0	
Soil +20% of sludge		166.2	70.6	77.1	33.9	125.5	98.7	95.3	72.9	
Soil +1% of sludge	Series with sterile sludge	22.2	10.8	48.0	11.1	37.0	60.6	31.6		
Soil+2,5% of sludge		67.1	46.8	26.4	11.2	33.1	61.4	41.0		
Soil +5% osadu		72.5	18.8	30.6	11.4	33.6	47.5	35.7		38.6
Soil +10% of sludge		58.0	45.0	15.8	35.1	46.7	80.3	46.8		
Soil +20% of sludge		83.4	81.0	31.9	16.7	50.1	39.6	50.5		
Mean for term		61.6	44.4	39.7	18.8	58.6	59.2			

$LSD_{(0,05)}$: T – 12.6; S – 5.0; D – 12.6
Interactions: T × S – 20.4; T × D – 38.3; S × D – 20.4; T × S × D – 54.1
Explanations as in Table 2.

The observed stimulating effect of sewage sludge on the numbers of proteolytic fungi, analogous to that on the numbers of bacteria, was most likely mainly due to the enrichment of the soil in proteins. It is probable that a part of the proteolytic fungi introduced with the sludge (Tab. 4), as opposed to bacteria, found favourable growth conditions in the soil and contributed to the increase in the total pool of those microorganisms. This is indicated by their higher and longer-lasting numbers in treatments amended with non-sterile sludge compared to those with the sterile sludge (Tab. 5).

Data presented in Table 6 indicate a decrease of the rate of ammonification, both by the non-sterile and the sterile sewage sludge.

The inhibition of organic nitrogen mineralisation by both types of sewage sludge (Tab. 6) was the most pronounced in the initial stage of the experiment (0.5 and 1 month), being statistically significant only in treatments with the highest doses of the sludge (10 and 20%). In the successive months the unfavourable effect of the sludge was notably weaker, and even a slight periodical stimulation of ammonification was observed, though it was not statistically proven. In the case of the sterile sludge the negative effect of its higher doses persisted until the end of the experiment (Tab. 6).

Results obtained by other authors [3, 5, 7, 10, 12, 14, 26], concerning the effect of sewage sludge on nitrogen mineralisation, are not consistent. Beltran-Hernandez *et al.* [3], Bonmati *et al.* [5], Furczak and Joniec [7], Hattori [10], Kobus *et al.* [14] and Wong *et al.* [26], as opposed to the study presented here, observed an increase in the intensity of ammonification, usually weakening with the passage of time. Whereas, Beltran-Hernandez *et al.* [3] and Wong *et al.* [26] observed in the same combinations a decrease in the activity of the process in question. Moreover, some of those authors [7, 10, 12, 14, 26] noted a positive or negative relation between ammonification and the amount of sludge introduced in the soil. The limitation of the rate of organic nitrogen mineralisation observed in this experiment could have been a result of unfavourable effect of noxious components of the sludge on the process. Data given in Table 1 indicate that the sewage sludge used in the experiment contained heavy metals and PAHs. A negative effect of chemical contamination on ammonification is reported by Giller *et al.* [9]. That effect could have also been caused by simultaneous increase in the rate of nitrification (Tab. 7), during which there was intensive oxidation of ammonium ions to nitrate ions, contributing to a reduction in the level of the product of ammonification.

The weakening of the negative effect of sludge with time observed in our study could have been a result of transformation of those contaminants and of the selection of ammonifiers with greater resistance to them.

Based on the results obtained (Tab. 6), these authors did not find any significant difference in the process of ammonification in the soils amended with the non-sterile and sterile sludge. Similar conclusions were attained also by Bonmati *et al.* [5] in their studies.

The sewage sludge introduced in the soil caused an intensification of the nitrification process, increasing together with the increase in the doses of the sludge applied (Tab. 7).

In both variants of the experiment, during the initial four months (except for the 2nd month) that effect remained on a generally constant level (Tab. 7). The strongest stimulation of nitrification was noted in the final phase of the experiment, but most frequently in treatments with the highest doses of the sludge (5, 10 and 20%).

Stimulation of ammonium nitrogen oxidation by sewage sludge under laboratory conditions was also noted by many other authors [3, 5, 7, 10, 12, 14, 26]. Beltrand-Her-

Table 6. Ammonification rate, mg $N\text{-}NH_4 \cdot kg^{-1}$ d.m. of soil · $3d^{-1}$

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		301.97	88.80	184.04	272.62	255.93	172.63	212.66	212.66	196.88
Soil+ 1% of sludge	Series with non-sterile sludge	291.30	68.06	190.51	298.53	249.68	187.48	214.26	213.25	
Soil +2,5% of sludge		270.66	60.31	206.19	312.27	226.53	216.64	215.38	214.40	
Soil +5% of sludge		263.35	62.04	181.85	280.23	221.08	181.94	198.41	198.35	
Soil +10% of sludge		198.64	37.79	158.97	312.16	196.35	134.67	173.10	176.80	
Soil +20% of sludge		107.29	30.84	170.48	310.51	236.73	148.84	167.45	149.79	
Soil +1% of sludge	Series with sterile sludge	306.14	76.85	203.80	276.71	239.32	170.65	212.24		191.54
Soil+2,5% of sludge		287.88	88.73	218.06	251.31	250.79	183.70	213.41		
Soil +5% osadu		297.12	66.58	196.10	272.01	192.05	165.80	198.28		
Soil +10% of sludge		243.27	66.51	172.14	244.14	205.99	150.94	180.50		
Soil +20% of sludge		96.71	30.47	90.25	254.34	205.79	115.25	132.14		
Mean for term		247.19	63.82	179.70	279.79	228.02	166.74			

$LSD_{(0.05)}$: T – 10.54; S – 4.18; D – 10.54
Interactions: T × S – 17.09; T × D – 32.11; S × D – 17.09; T × S × D – 45.41
Explanations as in Table 2.

Table 7. Nitrification rate, mg $N\text{-}NO_3 \cdot kg^{-1}$ d.m. of soil $\cdot 7d^{-1}$

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		156.59	148.92	455.89	135.67	167.72	99.56	194.06	194.06	
Soil+ 1% of sludge	Series with non-sterile sludge	241.68	241.85	660.66	231.90	345.78	210.52	322.06	319.58	
Soil +2,5% of sludge		669.75	366.07	1120.08	369.13	707.31	447.32	613.28	556.01	665.67
Soil +5% of sludge		765.36	571.45	1205.35	677.59	791.84	646.28	776.31	765.81	
Soil +10% of sludge		1068.19	808.18	1229.71	787.94	922.54	917.84	955.73	928.59	
Soil +20% of sludge		1216.78	942.84	1349.17	942.93	1153.62	1190.14	1132.58	1077.98	
Soil +1% of sludge	Series with sterile sludge	281.94	150.23	761.18	262.75	230.18	216.34	317.10		
Soil+2,5% of sludge		415.94	215.49	1000.92	386.58	641.16	332.34	498.74		
Soil +5% osadu		872.00	562.78	1242.12	564.24	642.67	649.30	755.31		615.01
Soil +10% of sludge		1045.35	684.33	1272.08	813.52	860.39	734.77	901.45		
Soil +20% of sludge		1177.49	762.17	1413.02	909.24	998.71	881.72	1023.38		
Mean for term		672.30	466.94	1013.84	518.10	635.39	535.47			

$LSD_{(0.05)}$: T – 21.24; S – 8.43; D – 21.24
Interactions: T × S – 34.43; T × D – 64.67; S × D – 34.43; T × S × D – 91.45
Explanations as in Table 2.

nandez *et al*. [3], apart from the stimulation, observed also inhibition of the process by the sludge in the initial stage of their experiment.

The stimulation of nitrification observed in this study was probably related with the introduction in the soil, together with the sludge, of the substrate and a certain number of microorganisms involved in the process. This thesis is supported by data given in Table 1, indicating that the rate of nitrification in the sewage sludge applied was at a high level. The occurrence of nitrifiers and of high levels of ammonium ions in sewage sludge is also reported by Wielgosz [24, 25]. The stimulation of the process of nitrification could also have been partly due to improvement in the living conditions of nitrifiers, i.e. increased pH of the soil (Tab. 3).

The significantly higher stimulation of nitrification in the soil with the non-sterile sludge suggests that nitrifiers introduced with the sludge also contribute to the oxidation of ammonium nitrogen. Whereas, Bonmati *et al.* [5] did not find any significant differences in the rates of nitrification in soils amended with non-sterile and sterile sludge.

Results concerning protease activity indicate its significant increase in soil amended with both the non-sterile and the sterile sludge (Tab. 8).

During the initial three months of the experiment the stimulating effect of the non-sterile sludge intensified with increasing levels of the waste, attaining the highest values for the 10 and 20% doses of the sludge. Analysing the changes in the intensity of the effect of the sludge over time it was noted that the strongest stimulation appeared only after 3 months of the experiment (Tab. 8). In the final stage, i.e. after 4 months, the stimulation of proteolytic activity weakened notably, to increase slightly again on the final date of analysis (after 5 months since the application of the sludge).

In the series with the sterile sludge the activity of protease was also stimulated, but it was notably weaker than in the soil with the non-sterile sludge (Tab. 8). After 4 months of the experiment there was even a slight, though statistically proven, inhibition of the activity this enzyme.

Stimulation of proteolytic activity in soil with sewage sludge under laboratory conditions was also noted by Furczak and Joniec [7], Hattori [10], Pascual *et al.* [20], and Saviozzi *et al.* [23]. Moreover, Furczak and Joniec [7], Pascual *et al.* [20] and Saviozzi *et al.* [23] demonstrated the relation of such changes with the level of sludge dose applied. Studies by those authors indicate that the increase in that activity, as in the study presented here, was the strongest at the beginning of the experiment and grew weaker with the passage of time [7, 10, 19, 23].

Literature data indicate that the proteolytic activity of soil depends on the levels of carbon and organic nitrogen and on the reaction of the environment [4, 6, 8, 10]. Studies by Żukowska *et al.* [27] indicate that the application of analogous doses of sewage sludge caused a notable increase in the content of total carbon. The results given in Table 3 show that in the soil an increase of the soil reaction was noted too. This was surely the main cause of the stimulation of the synthesis of protease. Another reason for the increased proteolytic activity of the soil appears to be the introduction of proteolytic enzymes with the non-sterile sludge (Tab. 4), and partial colonisation of the soil by sludge proteolytic fungi (Tab. 5). Whereas, the process of sterilisation of the sludge caused the destruction of proteolytic microorganisms and free proteases in the sludge, which resulted in significantly lower stimulation of that activity in the soil with that sludge.

Table 8. Protease activity, mg tyrosine · kg^{-1} d.m. of soil · h^{-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		6.76	11.14	12.54	1.42	11.46	3.37	7.78	7.78	
Soil+ 1% of sludge	Series with non-sterile sludge	14.81	17.12	22.88	4.52	10.01	6.42	12.63	10.70	
Soil +2,5% of sludge		22.96	28.53	26.75	5.62	14.68	11.20	18.29	14.73	19.83
Soil +5% of sludge		34.49	23.76	38.56	10.46	14.25	6.57	21.35	16.45	
Soil +10% of sludge		39.59	36.76	46.38	13.68	16.54	12.33	27.55	19.34	
Soil +20% of sludge		50.88	38.78	57.44	17.17	11.73	12.19	31.37	22.33	
Soil +1% of sludge	Series with sterile sludge	11.17	14.09	15.61	4.45	5.67	1.62	8.77		
Soil+2,5% of sludge		17.82	19.44	12.18	5.01	3.71	8.91	11.18		
Soil +5% osadu		19.86	16.85	19.69	6.30	2.14	4.50	11.56		10.62
Soil +10% of sludge		25.45	12.09	18.03	3.50	2.64	5.08	11.13		
Soil +20% of sludge		23.36	18.38	17.23	3.69	7.98	9.15	13.30		
Mean for term		22.86	20.67	24.99	6.44	9.36	7.06			

$LSD_{(0.05)}$: T – 0.94; S – 0.37 D – 0.94
Interactions: T × S – 1.52; T × D – 2.89; S × D – 1.52; T × S × D – 4.03
Explanations as in Table 2.

The results obtained in this study indicate that sludge microorganisms may partially participate in the processes related with nitrogen transformations in soil. Similar observations were made in relation to microbiological transformations of sludge carbon organic matter in soil [13].

CONCLUSIONS

1. Non-sterile and sterile sewage sludge introduced into the soil caused a stimulation of the growth of proteolytic bacteria and fungi. The effect was the most pronounced with higher doses of the sludge. The lower numbers of proteolytic bacteria in the variant with the non-sterile sludge than in that with the sterile one suggest that bacteria from the sludge did not rather colonise the soil, which might have been the result of antagonistic interactions. Whereas, the higher numbers of protein-decomposing fungi in the soil with the non-sterile sludge may indicate that certain sludge fungi found favourable growth conditions in the soil.
2. The process of ammonification was subject to strong inhibition that remained at a similar level in both experimental variants. This indicates a lack of effect of ammonifiers introduced with the sludge on the course of that activity in the soil.
3. Amendment of the soil with the non-sterile and sterile sludge caused statistically significant stimulation of the process of nitrification, intensifying with increasing doses of the sludge. The significantly higher level of that parameter in the soil with non-sterile sludge than in that with sterile sludge permits the assumption that nitrifiers from the sludge had also a certain role in the process of nitrification in the soil.
4. All the doses of non-sterile and sterile sewage sludge caused statistically significant stimulation of proteolytic activity of the soil. The higher protease activity in the soil with the non-sterile sludge suggests that the parameter could have been affected, to a certain extent, by the sludge proteolytic fungi and the free proteases introduced into the soil with the sludge.
5. The results indicate that most of the studied activities related to nitrogen transformations in the soil may be augmented for a period of time by sludge microorganisms and by the enzymes they contain.

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ZNACZENIE MIKROORGANIZMÓW OSADOWYCH W PRZEMIANACH AZOTU W GLEBIE BIELICOWEJ WZBOGACONEJ OSADEM ŚCIEKOWYM

Doświadczenie laboratoryjne założono na glebie bielicowej w dwóch wariantach. W jednym z nich do ww. gleby wprowadzono niesterylny osad ściekowy, a w drugim ten sam osad, ale poddany uprzednio procesowi sterylizacji. W obu wariantach wprowadzono identyczne dawki osadu: 30 (1%), 75 (2,5%), 150 (5%), 300 (10%) i 600 $Mg \cdot ha^{-1}$ (20%). Następnie po upływie 0,5, 1, 2, 3, 4 i 5 miesięcy w glebie obu wariantów analizowano: liczebność bakterii i grzybów rozkładających białko, nasilenie procesu amonifikacji, nasilenie procesu nitryfikacji oraz aktywność proteolityczną. Uzyskane wyniki wykazały stymulujący wpływ zarówno niesterylnego, jak i sterylnego osadu na liczebność badanych grup drobnoustrojów oraz nasilenie nitryfikacji i aktywność proteazy. Jedynie proces amonifikacji podlegał hamowaniu. Odnotowane oddziaływanie najwyraźniej zaznaczyło się w przypadku większych dawek osadów. Istotnie wyższa liczebność grzybów rozkładających białko oraz aktywność prawie wszystkich (wyj. amonifikacja) analizowanych parametrów biochemicznych w glebie z osadem niesterylnym niż sterylnym wskazuje na udział mikroorganizmów pochodzenia osadowego w kształtowaniu mikrobiologicznych przemian azotu w glebie wzbogaconej osadem.