

Preliminary study of sludge effect on soil microbial activity of a podzolic soil under willow culture

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A b s t r a c t. The study was performed on a model of a field experiment in which a podzolic soil was fertilized with various doses of municipal-industrial sewage sludge (1, 2.5, 5, 10 and 20% of dry mass). Next, the soil was planted with willow (*Salix viminalis* L). After six months from the application of the sludge, determinations were made of the so-called total number of bacteria with low and high nutritional requirements, total number of fungi, number of cellulolytic and 'proteolytic' bacteria and fungi, respiratory activity, cellulose mineralization rate, intensity of ammonification, nitrification, dehydrogenases and protease activity in the soil.

In the Ap horizon of the soil higher doses of the sludge caused significant stimulation of growth of most of the studied groups of bacteria and fungi (with the exception of 'proteolytic' bacteria and fungi). Also, stimulation of almost all of the biochemical parameters studied was observed, increasing with growing concentration of sludge. Only the process of ammonification was strongly inhibited in the treatment with 20% dose of sludge.

In the deeper layer of the soil (20-40 cm) the effect of sewage sludge was weaker and less dependent on the dosage applied than in the Ap horizon. Only stimulation of growth of cellulolytic fungi was recorded and, in some treatments, of 'proteolytic' bacteria and fungi. Moreover, a slight – though in most treatments significant – increase was observed in the rate of respiration and of cellulose mineralization.

The study showed the existence of positive correlations among most of the studied microbial groups and biochemical properties of both soil horizons.

Key words: microorganisms, activity, soil, sewage sludge

INTRODUCTION

Sewage sludge is an inseparable element of operation of every liquid wastes treatment plant (Suchy, 1997). Utilization of such wastes is a problem that is still not fully solved and more and more pressing at both local and world levels.

In many European countries the primary method of solving the problem is the utilization of sewage sludge in agriculture (Butarewicz, 2003) as it is a rich source of organic matter and of such elements as nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and sodium (Baran and Turski, 1999; Czekala, 2002; Flis-Bujak *et al.*, 1995; Marschner *et al.*, 2003; Mazur, 2002; Rosik-Dulewska, 2002; Siuta, 1999; Suchy, 1997). Due to the content of these plant nutrients and the humus-forming properties, such wastes are classified among unconventional organic fertilizers (Baran *et al.*, 2002; Baran and Turski, 1999; Flis-Bujak *et al.*, 1995; Siuta, 1999). Organic matter introduced in the soil with sewage sludge gets decomposed in numerous reactions which take place primarily with microbial participation (Myśków, 1981).

Information acquired so far indicates that studies concerned with the effect of sewage sludge on soil microbial activity have been mostly conducted under laboratory conditions, little attention being devoted to the study of the problem under field conditions. Results of studies conducted so far indicate differentiated effects of sewage sludge on soil microorganisms during the initial months from its application in soil (Baran *et al.*, 1996; Bielińska *et al.*, 1999; Furczak *et al.*, 2000; Gostkowska *et al.*, 2000; Hattori and Mukai, 1986; Sastre *et al.*, 1996; Selivanovskaya *et al.*, 2001). Moreover, those authors analysed only some microbial groups and only some of the parameters of their activity. The paper presents a broader microbiological and biochemical research project aimed at the determination of the results of a six-month period of industrial-municipal sewage sludge effect on the total number and activity of microorganisms and on microbial groups and biochemical processes related with the transformations of carbon and nitrogen compounds contained in the sludge. The processes

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of mineralization and humification of the sludge organic matter that accompany the growth of microorganisms and their activity will affect the physical, physicochemical and chemical properties of soil, as well as the nutritional conditions for plants, which should be reflected in the crop yield of willow culture.

The study is an element of a multi-year experiment program aimed at the acquisition of knowledge on the direction, intensity and duration of changes – caused by the application of the sludge – on the growth of microorganisms and on processes that are important from the viewpoint of the aforementioned properties of soil.

MATERIALS AND METHODS

The study was conducted based on a field experiment set up in Końskie by the Institute of Soil Science and Natural Environment Management, University of Agriculture, Lublin, Poland. The accumulation horizon of a podzolic soil developed from weakly loamy sand was fertilized with fermented sludge of municipal (70%) and industrial (30%) sewage, taken from the Mechanical-Biological Sewage Treatment Plant in Końskie. The sewage sludge was introduced to the soil at the following doses of dry mass: 1, 2.5, 5, 10 and 20%. After 4 weeks from the sludge application, plots of 15 m² in area were planted with willow (*Salix viminalis* L.). The control treatment in the experiment was soil under the culture of the same crop but with no sewage sludge fertilization.

The determinations were performed in the soil horizon into which the sludge was introduced (0–20 cm) as well as in deeper layers of the soil (20–40 cm), assuming the possibility of down-profile migration of some compounds from the surface horizon.

The grain size distribution of the soil and certain of its physicochemical and chemical properties, as well as of the sludge introduced in the soil, are given by Wójcikowska-Kapusta *et al.* (2000) and by Żukowska *et al.* (2002). After 6 months from setting up the experiment, the following parameters were determined in the soil (in three replications):

- so-called total number of bacteria with low nutritional requirements (oligotrophic bacteria), on a substrate with soil extract (350 ml dm⁻³) and K₂HPO₄;
- so-called total number of bacteria with high nutritional requirements (macrotrophic bacteria), on Bunt-Rovira (1955) nutrient medium;
- so-called total number of filamentous fungi, on Martin (1950) nutrient medium;
- number of cellulolytic bacteria, on liquid nutrient medium acc. to Pochon and Tardieux (1962). The most probable number of the bacteria was taken from McCrady tables;
- number of cellulolytic fungi, on mineral agar covered with a circle of Whatman paper;

- number of protein-decomposing bacteria and fungi, on Frazier gelatine nutrient medium (Rodina, 1968);
- respiratory activity, with the method of Rühling and Tyler (1973);
- cellulose mineralization, in 25 g weighed portions of soil enriched with 0.5% of Whatman powdered cellulose. The amount of released CO₂ was determined with the method of Rühling and Tyler (1973);
- ammonification intensity, in 25 g 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 (Nowosielski, 1974);
- nitrification intensity, in 25 g weighed portions of soils containing 0.1% of monobasic ammonium phosphate. After 7 days of incubation nitrate ions were extracted and their content was determined with the burcine method (Nowosielski, 1974);
- dehydrogenases activity, with the Thalmann (1968) method;
- protease activity, according to the method of Ladd and Butler (1972);
- soil reaction, potentiometrically in 1 mol dm⁻³ KCl.

To the nutrient media for cultures of ‘proteolytic’ and cellulolytic fungi antibiotics were added in the same amounts as to the Martin (1950) medium.

The results obtained were processed statistically with the method of analysis of variance. Significance of differences was determined using the Tuckey test, at $p = 0.05$. Coefficients of correlation were also determined, using the CORE library software for the characterisation of multi-variable samples. Analysis of variance was not performed for cellulolytic bacteria, as the calculations of their numbers were made with the help of McCrady tables, based on the principles of mathematical statistics.

RESULTS AND DISCUSSION

Sewage sludge introduced to the soil caused, in the Ap horizon, an increase in the total number of bacteria with low and high nutritional requirements (Table 1, Fig. 1). The effect was significant in most of the treatments of the experiment and manifested the most strongly in the presence of the highest dose of sludge.

The effect of the sludge on the number of filamentous fungi was less pronounced. A significant increase in their number was recorded only in the variant with the highest content of the sludge. The observations support the results obtained by Gostkowska *et al.* (2000) which indicate stimulation of the growth of macrotrophic bacteria and filamentous fungi by sewage sludge after several months of its effect on soil. Higher doses of the sludge stimulated also the growth of cellulolytic bacteria and fungi (Table 1, Fig. 1).

Table 1. Number of selected microbial groups in the soil six months after sludge application

Treatments	Depth (cm)	Oligotrophic bacteria		Macrotrophic bacteria		Filamentous fungi		Cellulolytic bacteria		Cellulolytic fungi		Proteolytic bacteria		'Proteolytic' fungi	
		jtk 10 ⁹ kg ⁻¹ d.m. of soil		jtk 10 ⁶ kg ⁻¹ d.m. of soil		jtk 10 ⁶ kg ⁻¹ d.m. of soil		jtk 10 ⁶ kg ⁻¹ d.m. of soil		jtk 10 ⁶ kg ⁻¹ d.m. of soil		jtk 10 ⁶ kg ⁻¹ d.m. of soil		jtk 10 ⁶ kg ⁻¹ d.m. of soil	
		Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Control soil		6.4	27.3	7.7	70.0	48.9	165.1	16.7	55.18	3.4	5.0	2.0	2.1	111.5	80.3
Soil + 1% of sludge		9.2	10.2	34.9	58.4	53.2	12.9	13.1	113.7	2.0	3.0	1.9	2.4	35.6	25.4
Soil + 2.5% of sludge		23.4	21.8	39.6	80.4	80.4	113.7	113.7	30.2	5.3	5.1	2.1	2.1	60.8	116.9
Soil + 5% of sludge	0-20	14.8	87.9	43.5	284.1	95.7	144.5	144.5	11.3	11.3	1.3	1.3	1.3	131.3	131.3
Soil + 10% of sludge															
Soil + 20% of sludge															
Control soil		5.2	6.8	6.2	7.1	23.9	22.7	15.7	9.1	1.0	3.1	0.1	1.1	7.6	36.3
Soil + 1% of sludge		5.1	6.4	4.6	20.5	20.5	4.9	4.9	3.0	4.4	2.6	1.2	2.0	18.9	14.0
Soil + 2.5% of sludge		6.4	5.5	6.2	8.3	45.3	8.3	3.0	12.7	2.6	5.1	2.0	1.8	122.9	14.0
Soil + 5% of sludge	20-40	5.5	9.6	3.5	12.3	8.3	18.0	12.7	12.9	5.1	2.3	1.8	0.2	5.9	5.9
Soil + 10% of sludge		9.6	8.7	12.3	9.9	18.0	20.1	12.9	5.3	2.3	2.9	0.2	1.0	48.6	48.6
Soil + 20% of sludge															
Mean		17.1	38.6	38.6	93.9	32.1	32.1	32.1	4.0	4.0	1.6	1.6	1.6	58.3	58.3
LSD _(0.05) :															
Horizon		2.3	4.2	4.2	15.0	15.0	0.4	0.4	0.4	0.4	0.3	0.3	0.3	20.1	20.1
Dose		6.0	11.0	11.0	38.9	38.9	0.9	0.9	0.9	0.9	0.7	0.7	0.7	52.0	52.0
Interactions		9.9	18.1	18.1	64.3	64.3	1.6	1.6	1.6	1.6	1.1	1.1	1.1	85.9	85.9

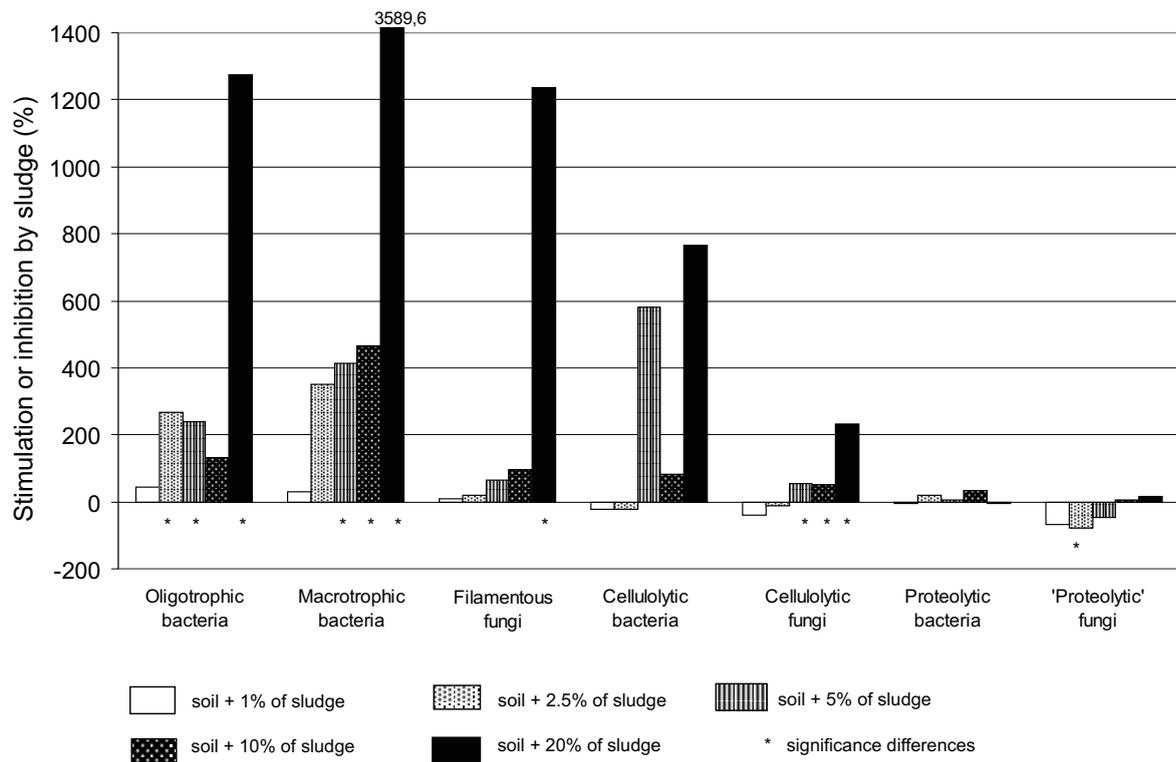


Fig. 1. Number of selected microbial groups in soil layer of 0-20 cm six months after sludge application.

The positive effect of the sludge on the growth of the mentioned microbial groups was probably caused by the introduction, together with the sludge, of a large amount of organic matter which is a source of nutrients. Increased content of organic matter in the soil under the effect of the sludge is also indicated by the results of studies by Żukowska *et al.* (2002). The observed increase in the number of bacteria and fungi could have also been the result of a certain number of them being brought into the soil with the sludge. Our own studies (unpublished data), as well as those by Hattori and Mukai (1986) and Sastre *et al.* (1996), indicate that sewage sludge is numerously inhabited by those microorganisms. Another factor conducive to the growth of those microorganisms, and especially of bacteria, was probably increase of reaction and moisture of the soil fertilized with the sludge (Table 2).

Under the conditions of the experiment, however, no greater effect of the sludge was observed on the growth of protein-decomposing bacteria and fungi (Table 1, Fig.1). Lower concentrations of the sludge (1 and 2.5%) caused even a slight decrease in the number of 'proteolytic' fungi. The lack of a clear response of 'proteolytic' bacteria and fungi to the sludge, in spite of the improvement of those properties of the soil, was probably caused by the presence in it of hard-decomposing proteins, as suggested by earlier studies by Hattori and Mukai (1986).

Sewage sludge stimulated also the biochemical activity of the soil in the Ap horizon. A significant increase, usually growing with increasing doses of sludge, was observed in the rates of respiration and of cellulose mineralization (Table 2, Fig. 2). The observed stimulation of respiratory activity by the sludge is convergent with results obtained by other authors (Selivanovskaya *et al.*, 2001) after several-month periods of sludge effect on soil.

The process of ammonification, on the other hand, was subject to slight inhibition by all the doses of sludge, the effect being statistically significant only in the variant of the experiment with 20% concentration of the waste (Table 2, Fig. 2).

Inhibition of ammonification appears to be caused by heavy metals and organic contaminants that occur commonly in sewage sludge (Baran and Turski, 1999; Rosik-Dulewska, 2002), including, as shown by studies by Baran *et al.* (2001), also the sludge used in the experiment described herein. The inhibitory effect of heavy metals on the process is also reported by Giller *et al.* (1998), among others. Decrease in the content of ammonium ions could have also been the result of simultaneous sludge-induced intensification of nitrification (Table 2, Fig. 2), as in the case of nitrification – as opposed to ammonification – significant stimulation of the process was observed, intensifying with increasing doses of sludge (Table 2, Fig. 2). The observations performed support the results obtained by Furczak *et al.* (2000) that indicate intensification of the process after several months from the moment of soil amendment with sewage sludge.

Table 2. Selected biochemical and chemical properties of the soil six months after sludge application

Treatments	Depth (cm)	Respiratory activity, mg C-CO ₂ kg ⁻¹ d.m. of soil d ⁻¹		Cellulose mineralization, mg C-CO ₂ kg ⁻¹ d.m. of soil 20d ⁻¹		Ammonification rate, mg N - NH ₄ ⁺ kg ⁻¹ d.m. of soil 3d ⁻¹		Nitrification rate, mg N - NO ₃ ⁻ kg ⁻¹ d.m. of soil 7d ⁻¹		Dehydrogenases activity, mgTPF kg ⁻¹ d.m. of soil d ⁻¹		Protease activity, mg of tyrosine kg ⁻¹ d.m. of soil h ⁻¹		Reaction Moisture (pH _{KCl}) (%)	
		Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	
Control soil		195.50	422.42	1871.00	5644.76	323.54	243.18	48.13	234.18	34.99	87.90	16.20	42.42	6.4	9.5
Soil + 1% of sludge		284.00		4126.50		237.97		119.90		63.26		16.29		7.4	13.2
Soil + 2.5% of sludge		398.50		2978.00		230.66		183.33		73.38		16.37		7.5	11.5
Soil + 5% of sludge	0-20	442.00		4711.50		223.83		194.43		101.47		32.10		7.3	14.4
Soil + 10% of sludge		615.50		6053.50		276.53		311.42		121.41		76.94		7.0	22.1
Soil + 20% of sludge		599.00		14128.00		166.52		547.89		132.87		96.63		6.8	27.4
Control soil		114.00	204.5	1668.50	2272.75	257.60	274.23	34.00	47.20	3.59	18.65	12.11	8.01	6.2	8.8
Soil + 1% of sludge		153.00		1978.50		249.37		39.20		17.67		5.62		6.3	12.1
Soil + 2.5% of sludge		173.50		2614.00		263.13		80.95		39.04		9.22		6.8	12.2
Soil + 5% of sludge	20-40	246.50		2104.00		269.33		52.14		11.10		7.81		6.3	12.7
Soil + 10% of sludge		278.50		2937.00		275.60		42.19		21.70		7.28		6.6	10.3
Soil + 20% of sludge		261.50		2334.50		330.35		34.01		18.81		6.01		6.6	11.4
Mean		313.46		3958.75		258.70		140.69		53.27		25.21			
LSD _(0.05) :															
Horizon		26.00		101.00		ns		13.73		13.69		1.56			
Dose		67.50		262.00		ns		35.59		35.49		4.05			
Interactions		111.50		432.50		150.99		58.74		58.57		6.69			

ns – no significant differences.

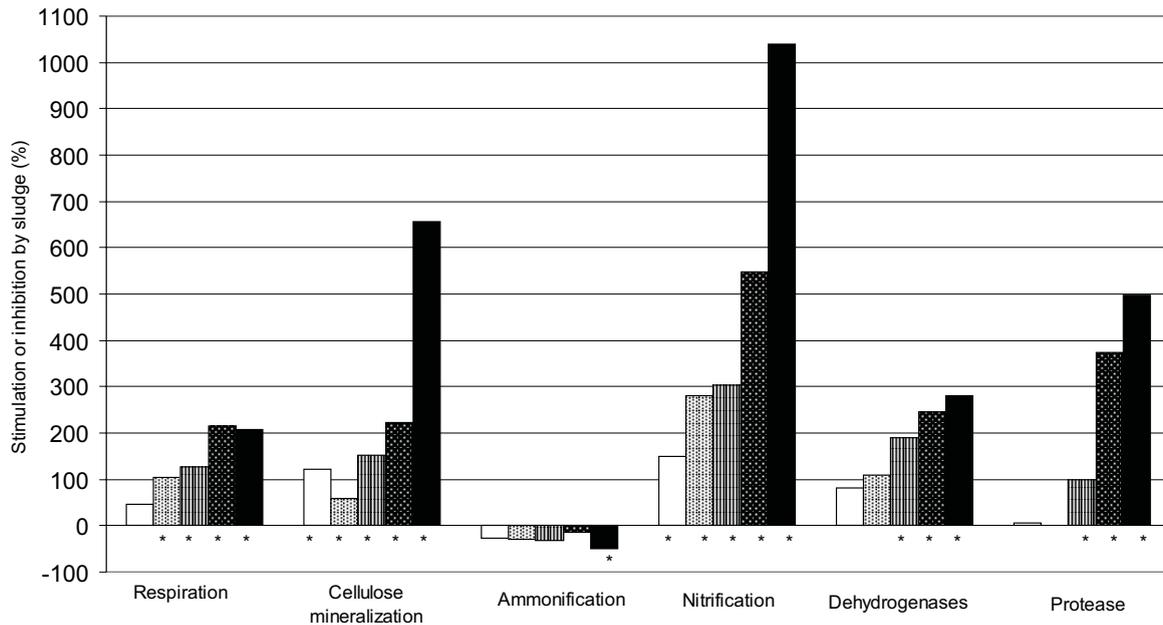


Fig. 2. Selected biochemical properties of soil in the 0-20 cm layer. Other explanations as for Fig. 1.

Also enzymatic activity *ie* dehydrogenases and protease activity in soil of the Ap horizon increased under the effect of the applied sludge (Table 2, Fig. 2). The effect, however, was significant only in treatments with higher sludge doses (5, 10 and 20%). Results of studies conducted so far in this area are not free of a certain ambivalence (Baran *et al.*, 1996; Bielińska *et al.*, 1999; Furczak *et al.*, 2000). Baran *et al.* (1996) and Bielińska *et al.* (1999) observed an increase in dehydrogenases activity in soil with sludge, while Furczak *et al.* (2000) recorded a decrease in that activity. The authors of those studies (Baran *et al.*, 1996; Bielińska *et al.*, 1999; Furczak *et al.*, 2000), like in the case of the study reported herein, observed stimulation of protease activity in soil with an admixture of sewage sludge.

Increase in the intensity of those biochemical properties was probably caused mainly by input, together with the sludge, of substrates used in the processes, and of microorganisms and enzymes responsible for their occurrence, as indicated by studies of other authors (Furczak *et al.*, 2000; Hattori and Mukai, 1986; Marschner *et al.*, 2003; Sastre *et al.*, 1996) as well as our own (unpublished data). Improvement of other living conditions of soil microorganisms *ie* of soil reaction and moisture (Table 2), could have constituted an additional factor in the increase of the intensity of those biochemical parameters.

The effect of sewage sludge on the numbers of the analysed microbial groups in the deeper layer of the soil (20-40 cm) was notably weaker than in the Ap horizon and observable only in relation to certain microbial groups (Table 1, Fig. 3). A small, though in most treatments with sludge statistically significant, increase was observed in the num-

ber of cellulolytic fungi (Table 1, Fig. 3). Sewage sludge caused also an increase in the numbers of 'proteolytic' bacteria and fungi. That effect, however, was significant only in some treatments with lower sludge doses (Table 1, Fig. 3).

All the sludge doses, on the other hand, caused a slight, though statistically proven in most treatments, stimulation of the processes of respiration and of cellulose mineralization in that horizon of the soil (Table 2, Fig. 4). The sludge applied, however, had no significant effect on the processes of ammonification and nitrification (Table 2, Fig. 4). Also, the studied enzymatic activity in the soil (dehydrogenases and protease) was not subject to any greater changes under the effect of the sludge (Table 2, Fig. 4). Although in all the treatments with sludge stimulation of dehydrogenases activity and a slight inhibition of protease activity were observed, the results were not statistically proven. The observations made are partially in support of the results obtained by Baran *et al.* (1996) and by Bielińska *et al.* (1999) which indicate an increase in dehydrogenases activity in deeper layers of soil.

The stimulation of some microbiological and biochemical parameters of soil observed in the 20-40 cm horizon was probably caused by migration of a certain amount of organic matter introduced with the sludge, and by improvement of other microbial living conditions *ie* soil reaction and moisture (Table 2).

Data in Table 3 indicate the existence of positive correlations between most of the analysed microbial groups (except for protein-decomposing bacteria and fungi) and the biochemical parameters. Only ammonification correlated negatively with the studied microbiological and biochemical tests.

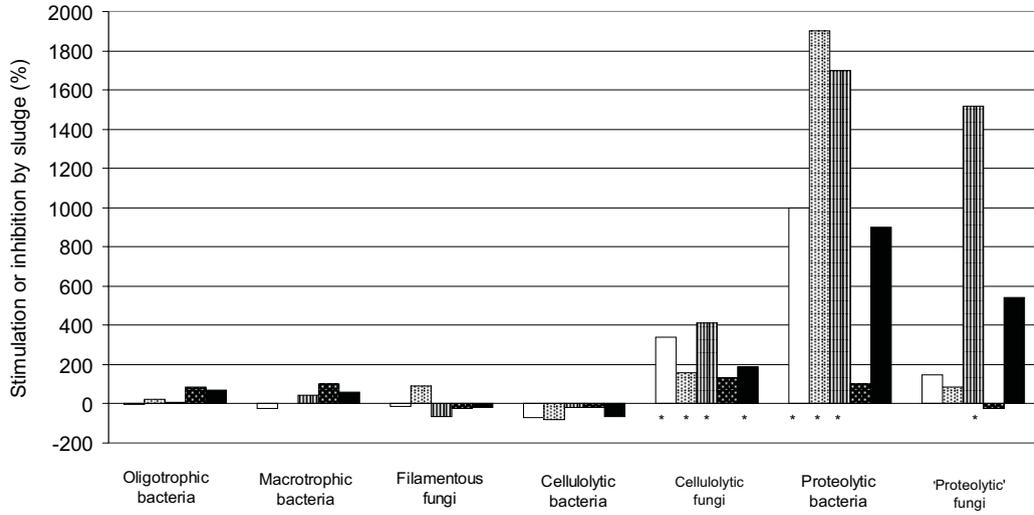


Fig. 3. Number of selected microbial groups in soil layer of 20-40 cm. Other explanations as for Fig. 1.

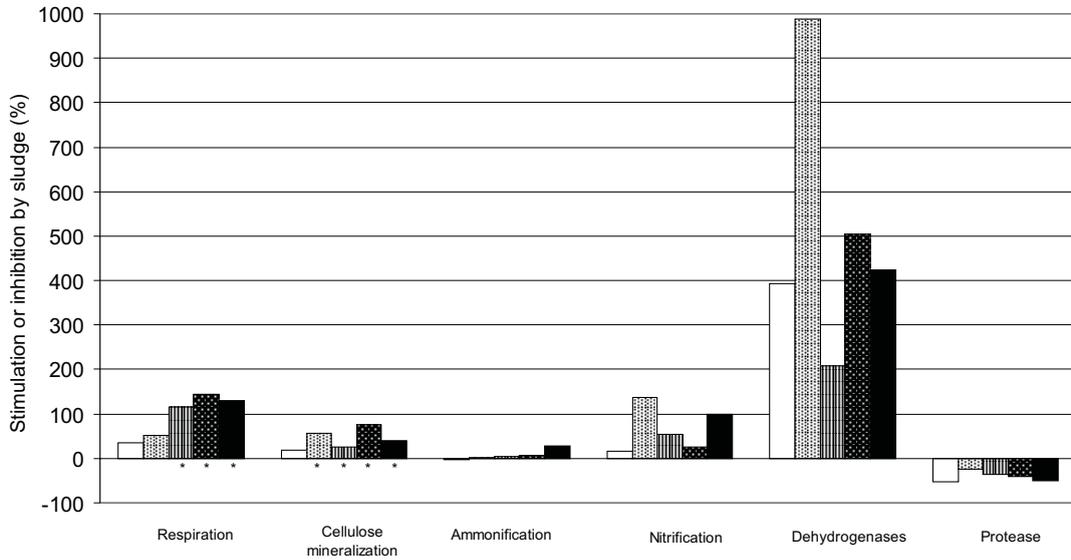


Fig. 4. Selected biochemical properties of soil in the 20-40 cm layer. Other explanations as for Fig. 1.

The observed positive correlations between the microbial groups mentioned and respiration, cellulose mineralization and dehydrogenases activity prove the participation of those microorganisms in the mineralization of organic carbon. This supposition is also supported by the high positive coefficient of correlation between respiration and the enzymatic parameter mentioned.

Positive correlation between proteolytic activity of the soil and the number of protein-decomposing fungi, and lack of such correlation in the case of proteolytic bacteria, suggest that the main role in the decomposition of proteins in the soil amended with sewage sludge was played by 'proteolytic' fungi.

Table 3. Coefficient of correlation

	Oligo- trophic bacteria	Macro- trophic bacteria	Fila- mentous fungi	Cellulo- lytic bacteria	Cellulo- lytic fungi	Proteo- lytic bacteria	'Proteo- lytic' fungi	Respiration	Cellulose minerali- zation	Ammoni- fication	Nitrifi- cation	Dehydro- genases	Protease
Oligotrophic bacteria	0.991**	0.980**	0.844**	0.871**	-	-	0.693*	0.953**	-0.735**	0.910**	0.712**	0.804**	
Macrotrophic bacteria		0.995**	0.826**	0.888**	-	-	0.674*	0.966**	-0.701*	0.910**	0.690*	0.833**	
Filamentous fungi			0.811**	0.877**	-	-	0.634*	0.963**	-0.688*	0.897**	0.673*	0.825**	
Cellulolytic bacteria				0.819**	-	-	0.682*	0.838**	-0.700*	0.807**	0.746**	0.746**	
Cellulolytic fungi					-	-	0.710**	0.877**	-0.601*	0.847**	0.679**	0.805**	
Proteolytic bacteria					-	-	-	-	-	-	-	-	
'Proteolytic' fungi							-	-	-	-	-	0.647*	
Respiration								0.789**	-	0.886**	0.932**	0.880**	
Cellulose mineralization									-0.705*	0.960**	0.809**	0.910**	
Ammonification											-0.706*	-	
Nitrification											0.910**	-0.598*	
Dehydrogenases												0.910**	
Protease													0.880**

- no correlation, significance level: *p = 0.05, **p = 0.01.

CONCLUSIONS

1. Sewage sludge introduced in the soil caused, in the Ap horizon, significant stimulation of the growth of most of the studied microbial groups (except for 'proteolytic' bacteria and fungi). That effect was more pronounced in treatments with higher doses of the sludge. Significant stimulation, increasing with growing doses of sludge, was observed also in almost all of the studied biochemical parameters. Only the process of ammonification was subject to a slight inhibition.

2. In deeper layer of the soil (20-40 cm) the effect of sewage sludge was weaker. Only in the case of cellulolytic fungi a significant increase in their number was recorded under the effect of almost all sludge doses (except for the 10% dose). Additionally, in some treatments with lower concentrations of sludge, growth stimulation was observed in relation to protein-decomposing bacteria and fungi. Among the biochemical parameters, significant intensification was noted only for the processes of respiration and cellulose mineralization, and that only in treatments with higher doses of the sludge.

3. Positive correlations were observed among almost all of the studied microbiological and biochemical parameters (except for 'proteolytic' bacteria and fungi). Only ammonification was negatively correlated with the studied microbiological and biochemical properties of the soil.

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