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Changes in biochemical activity of podzolic soil under willow culture in the second year of treatment with municipal-industrial sewage sludge

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Abstract. The objective of the study was to investigate the effect of various doses of municipal-industrial sewage sludge (1, 2.5, 5, 10, and 20%) on selected biochemical parameters of a podzolic soil under willow culture in the second year of the experiment. The effect of the sediment was observable both in the surface horizon and in deeper layers of the soil, though only in the case of some of the tests (cellulose mineralization, nitrification) it was somewhat more pronounced in the Ap horizon. The recorded changes usually intensified with increasing doses of the treatment. The study showed a stimulating effect in the soil of all applied doses of sludge on respiration, cellulose mineralization rate, nitrification and proteolytic activity, and of the higher doses – on dehydrogenases activity. In the case of ammonification, on the other hand, an inhibiting effect of the sludge was observed in almost all of the treatment objects. The observed effect of the sludge on most of the analysed biochemical parameters was relatively consistent in both the surface horizon (0-20 cm) and in the deeper layers of the soil (20-40 cm). Only the processes of cellulose mineralization (in treatment objects with higher doses of sludge) and those of nitrification were subject to stronger stimulation in the Ap horizon.

Positive correlations were recorded between almost all of the analysed biochemical tests. An exception was ammonification which showed no correlation with cellulose mineralization and dehydrogenases activity, and negative correlations with the remaining parameters.

Keywords: biochemical activity, soil, sewage sludge

INTRODUCTION

Sewage sludge has valuable fertilization and humus-forming properties as it contains considerable amounts of organic matter and of micro- and macroelements (Baran and Turski, 1999; Czekała, 2002; Flis-Bujak *et al.*, 1996; Rosik-Dulewska, 2002). Therefore, the application of such wastes in agriculture constitutes the most favourable form of recycling (Butarewicz, 2003; Rosik-Dulewska, 2002). One of the methods of agricultural utilization of sewage sludge is the cultivation of so-called alternative crops *ie* crops grown for purposes other than food production, on sludge or on substrates with sewage sludge admixture (Baran and Turski, 1999; Baran *et al.*, 2001). Among such crops is the fast-growing basket willow (*Salix viminalis*) (Michałowski and Gołaś, 2001; Szczukowski *et al.*, 1998). It is characterized by considerable capability of absorbing nitrogen, phosphorus, water and, most importantly, heavy metals, displaying phyto-amending properties (Baran *et al.*, 2001; Michałowski and Gołaś, 2001).

It has been demonstrated repeatedly that sewage sludge introduced in soil affects not only plants, the physical, physicochemical and chemical properties, but also the biochemical activity of the soil. Studies focused on that subject matter have mainly been short-term and conducted under laboratory conditions, while research on the effect of sewage sludge on the biochemical activity of soil in multi-year field experiments has been scarce and only fragmentary in scope (Baran and Bielińska, 1998; Baran *et al.*, 1996; 1999; 2000; Bielińska and Żukowska, 2002; Debosz *et al.*, 2002; Furczak and Joniec, 2007; Kelly *et al.*, 1999; Quemada and Menacho, 2001). Due to the complexity of the processes related to the transformation of sludge organic matter in soil, a more extensive research effort was undertaken to investigate the direction and intensity of selected biochemical properties of soil in the second year of treatment with municipal-industrial sewage sludge. The processes have a determining influence on the physical, physicochemical and chemical properties of soil, affecting its fertility and productivity.

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MATERIAL AND METHODS

The study was performed on a soil originating from 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 the podzolic soil developed from weakly loamy sand was fertilized with fermented sludge of municipal (70%) and industrial (30%) sewage from the Mechanical-Biological Sewage Treatment Plant in Końskie. The sewage sludge was introduced to the Ap horizon of the soil at the following doses of dry mass: 1, 2.5, 5, 10 and 20%. After 4 weeks from the sludge application the soil was 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 Baran *et al*. (2001) and by Żukowska *et al*. (2002). In the second year of the experiment a number of determinations were performed in the soil three times (31st May, 29th July, 27th October; 1999), of the following parameters:

- respiratory activity, with the method of Rühling and Tyler (1973);
- cellulose mineralization, in 25 g weighed portions of soil enriched with 0.5% of powdered cellulose from Whatman Co. The amount of released CO_2 was determined with the method of Rühling and Tyler (1973);
- intensity of ammonification, 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 rate, in 25 g weighed portions of soil containing 0.1% of monobasic ammonium phosphate. After 7 days of incubation nitrate ions were extracted and their content was determined with the brucine method (Nowosielski, 1974);
- dehydrogenases activity, with the Thalmann method (1968);
- protease activity, according to the method of Ladd and Butler (1972);
- soil reaction, potentiometrically in 1 mol dm^{-3} KCl.

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 characterization of multivariable samples.

RESULTS AND DISCUSSION

In the second year of soil treatment with sewage sludge a significant increase was observed in the respiratory activity both in the surface horizon (0-20 cm) and in the deeper layers of the soil (20-40 cm) (Table 1, Fig .1). The continuing positive effect of sludge on the process intensified, in both horizons of the soil, with increasing concentration. However, in most of the treatment objects it was weaker than after 6 months of the experiment (Furczak and Joniec, 2007). Results of experiments in this area obtained by other authors are not free of some ambiguity (Debosz *et al*., 2002; Quemada and Menacho, 2001). Stimulation of the rate of mineralization of organic carbon under the effect of sewage sludge was observed by Quemada and Menacho (2001), while Debosz *et al*. (2002) obtained results indicating a decrease in that rate.

The rate of organic carbon mineralization depends on the quantity and quality of organic matter in the soil (Gostkowska *et al*., 2000; Hattori and Mukai, 1986). Therefore, the observed stimulation of respiratory activity was likely caused by sizeable input of organic matter together with the sludge, providing a source of respiratory substrates for soil microorganisms. This supposition is supported by studies by Żukowska *et al*. (2002), indicating increased level of organic carbon content in soil with sludge, continuing also in the second year. Our own studies show that the sludge introduced in the soil contained also a large number of microorganisms that, dying, could have become an additional source of organic matter (Sastre *et al*., 1996). Moreover, enrichment of the soil with that waste resulted in an increase in the number of microorganisms, continuing in the second year of the experiment (Joniec and Furczak, 2007). An additional cause of stimulation of the respiratory activity could have been the increase in soil moisture and reaction.

Application of sewage sludge resulted also in an increase in the rate of cellulose mineralization, though significant differences were recorded only in treatment objects with higher sludge concentration levels (5, 10, and 20%) (Table 1, Fig. 1). In those variants of the experiment the stimulation of the process in question was more pronounced in soil from the Ap horizon than in the deeper layers (20-40 cm), the effect being notably weaker than in the preceding year (Furczak and Joniec, 2007). The observed stimulation of cellulose mineralization was accompanied by an increase in the numbers of bacteria and cellulolytic fungi, and in the total number of microorganisms (Joniec and Furczak, 2007).

Studies by Debosz *et al.* (2002) and by Hattori and Mukai (1986) showed that sewage sludge organic matter includes cellulose, among other component substances. This is also confirmed by our own studies which show that the sewage sludge used in the experiments contained 6.61% of that polysaccharide. The observed increase in the rate of cellulose mineralization was, therefore, most likely caused

Table 1. Activity of selected processes related carbon transformations in soil in the second year of the experiment

Treatments	Depth (cm)	Respiration C-CO$_2$ kg^{-1} d.m. of soil d^{-1}			Mean	Cellulose mineralization C-CO$_2$ kg^{-1} d.m. of soil 20d^{-1}			Mean
		31.05.99	29.07.99	27.10.99		31.05.99	29.07.99	27.10.99	
Control soil	0-20	343.00	361.00	175.50	293.17	2635.50	2279.50	2464.50	2459.80
Soil + 1% of sludge		415.50	425.00	238.00	359.50	2544.50	2740.50	2748.00	2677.67
Soil + 2.5% of sludge		576.50	441.00	257.50	425.00	3141.50	3039.00	2975.00	3051.83
Soil + 5% of sludge		642.00	548.50	271.00	487.17	3502.50	4261.00	2539.00	3434.17
Soil + 10% of sludge		821.50	468.00	360.00	549.83	6849.50	3878.00	6205.00	5644.17
Soil + 20% of sludge		947.00	929.50	376.00	750.83	9169.00	5866.50	7466.50	7500.67
Control soil	20-40	254.50	212.00	62.50	176.33	1490.00	2375.50	2315.00	2060.17
Soil + 1% of sludge		351.50	327.50	92.50	257.17	2768.50	3058.50	1896.50	2574.50
Soil + 2.5% of sludge		402.00	382.50	171.00	318.50	3081.00	2501.00	3026.00	2869.33
Soil + 5% of sludge		415.00	359.60	247.00	340.53	3206.50	2827.00	2610.50	2881.33
Soil + 10% of sludge		417.50	404.00	326.50	382.67	3739.00	2444.50	3082.00	3088.50
Soil + 20% of sludge		416.00	545.00	334.50	431.83	3982.00	2434.00	3345.50	3253.83
Mean		500.13	450.30	242.67	397.70	3842.46	3142.08	3389.46	3458.00
Mean for horizon		0-20 cm – 477.58; 20-40 cm – 317.84				0-20 cm – 4128.05; 20-40 cm – 2787.94			
LSD									
Date		14.50				219.50			
Horizon		10.00				149.00			
Horizon x dose		41.50				620.50			
Interactions		78.00				1171.00			

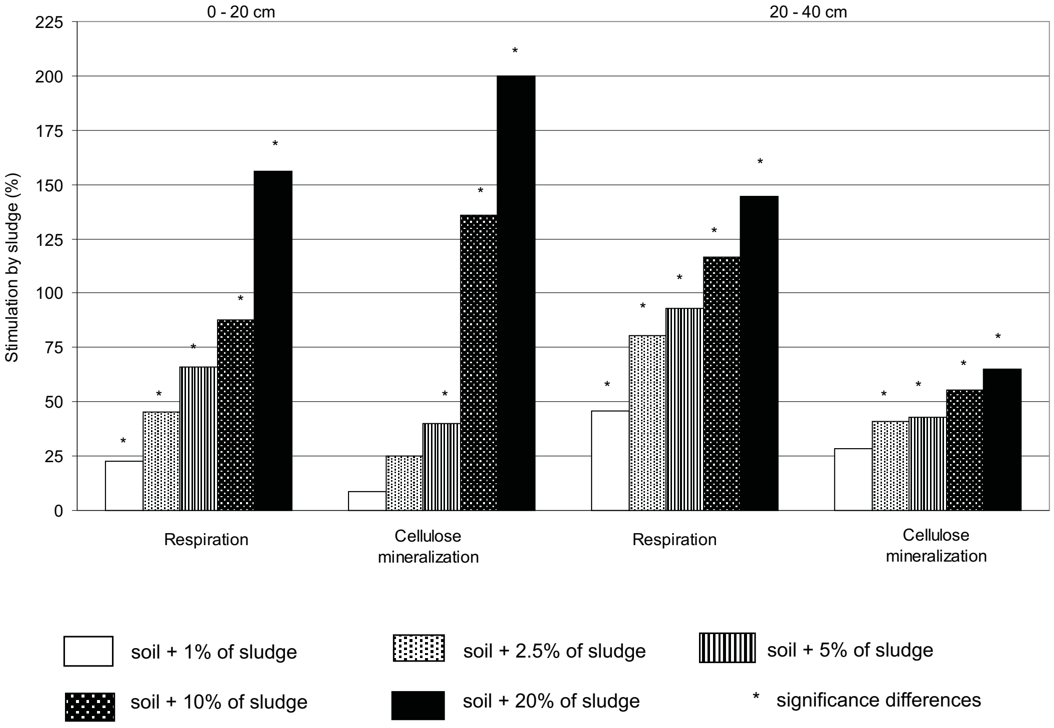

Fig. 1. Selected processes related with carbon transformations in soil in the second year of the experiment, annual mean.

by the input of a certain amount of substrate together with the sludge, among others. Another factor intensifying the process could have been the introduction in the soil, together with the sludge, of cellulases and cellulolytic microorganisms. This supposition is supported by unpublished data indicating the occurrence of cellulose decomposing microorganisms in sewage sludge. A certain role in the stimulation of cellulose mineralization by the sludge could have also been played by the resultant improvement in living conditions of those microbial populations in the soil.

Data presented in Table 2 and Fig. 2 indicate an inhibiting effect of the sewage sludge on the process of ammonification, statistically confirmed in almost all of the treatment objects. As opposed to the preceding year of the experiment (Furczak and Joniec, 2007), the effect occurred not only in the surface horizon but also in the deeper layer of the soil and was not appreciably dependent on the dosage of the sewage sludge.

According to the opinion of Babich and Stozky (1985), among others, the process of ammonification may be inhibited by heavy metals. As the studies by Baran *et al*. (2000, 2001) indicate that the sewage sludge used in the experiment had a content of such contaminants, it is to be supposed that the observed inhibition of ammonification was the result of accumulation of those elements in the soil. The lower content of ammonium ions detected in the soil with the sludge could have also been caused by simultaneous intensification of nitrification (Table 2).

As opposed to ammonification, nitrification was subject to significant stimulation in soil from the depth of 0-40 cm (Table 2, Fig. 2). However, in the Ap horizon the effect was stronger and increased with increasing dosage of the sludge. Comparison of the results obtained with data from the preceding year (Furczak and Joniec, 2007) revealed a positive effect of the sludge on the process of nitrification, intensifying with the passage of time. The stimulating effect of the sludge on nitrification was most likely caused by the introduction to the soil, together with the sludge, of a certain amount of substrate used in the nitrification process, as well as the nitrifiers as such. These assumptions are supported by studies of Wielgosz (2000), concerned with the number of nitrifying bacteria and the content of ammonium ions in sewage sludge. A certain role could have also been played by improvement in the moisture content and reaction of the soil fertilized with the sludge.

The results given in Table 3 and Fig. 3 indicate significant stimulation of dehydrogenases activity by the higher doses of the sludge (10 and 20%), both in the surface horizon and in the deeper layer of the soil. In the deeper layer of the soil an increase in that activity was observed also in treatment objects with lower sludge content, but it was not confirmed statistically. Comparison of results obtained in the first (Furczak and Joniec, 2007) and the second year of the experiment indicates that with the passage of time the effect of the sludge on dehydrogenases activity decreased in most of the treatment objects (except for the 20% sludge dose). The results of studies carried out so far in this field are somewhat ambivalent (Baran and Bielińska, 1998; Baran *et al*., 1996; 1999; 2000; Kelly *et al*., 1999). Certain authors (Baran and Bielińska, 1998; Baran *et al*., 1996; 1999; 2000) observed stimulation of dehydrogenases activity by sewage sludge in the second year of its effect on soil, while Kelly *et al.* (1999) noted a decrease in the intensity of that enzyme activity.

It is common knowledge that dehydrogenases activity in soil depends on the content of organic carbon. Increase in the content of organic carbon in the analysed soil continuing also in the second year of the experiment (Żukowska *et al*., 2002) could have been, therefore, the main cause of stimulation of the activity of those enzymes. According to Furczak *et al*. (2000), positive influence of sludge on dehydrogenases activity may also result from the input of a certain amount of microorganisms to the soil, together with the sewage sludge. This is supported by our own studies, indicating numerous microbial presence in the sludge applied in the experiment. Studies by Joniec and Furczak (2007) show that stimulation of dehydrogenases activity was accompanied by an increase in the number of the microbial groups under study. The observed stimulation of dehydrogenases activity could have also been assisted by improvement in other living conditions of those microorganisms.

Proteolytic activity of both soil horizons also increased in almost all treatments with sludge (Table 3, Fig. 3). The effect was the strongest in treatments with 20% sludge content. Positive effect of the sludge in the Ap horizon was weaker, and in the deeper layer more pronounced than in the first year of the experiment (Furczak and Joniec, 2007). Also other authors observed, under field conditions, increased proteolytic activity of soil fertilized with sewage sludge (Baran and Bielińska, 1998; Baran *et al*., 1996; 1999; Bielińska and Żukowska, 2002).

Proteolytic activity of soil depends largely on carbon and nitrogen organic matter content (Bielińska and Żukowska, 2002; Hattori and Mukai, 1986). It is widely known that sewage sludge abounds in such organic matter, so its introduction into soil together with sludge appears to be the primary cause of stimulation of protease activity. Studies by Furczak *et al.* (2000) and by Sastre *et al*. (1996) indicate that increase in proteolytic activity in a soil fertilized with sewage sludge may result from the input of a certain amount of proteases and their microbial producers together with the sludge, which could have also taken place under the conditions of the experiment discussed herein. This supposition is supported by our own studies which indicate that the sewage sludge studied contained numerous ‘proteolytic’ bacteria and fungi and displayed high proteolytic activity. Stimulation of protease activity in the 2nd year of the experiment was accompanied also by an increase in the number of bacteria and fungi decomposing proteins (Joniec and Furczak, 2007). Another cause of increase of that activity could have been improvement of moisture and reaction in the soil with sludge (Table 4).

Table 2. Activity of selected processes related with nitrogen transformations in soil in the second year of the experiment

Treatments	Depth (cm)	Ammonification			Mean	Nitrification			Mean
		$mg\ N-NH_4^+\ kg^{-1}$ d.m. of soil $3d^{-1}$				$mg\ N-NO_3^-\ kg^{-1}$ d.m. of soil $7d^{-1}$			
		31.05.99	29.07.99	27.10.99		31.05.99	29.07.99	27.10.99	
Control soil	0-20	254.18	217.74	270.32	247.41	40.17	36.74	34.81	37.24
Soil + 1% of sludge		284.26	234.79	273.95	264.33	114.21	90.91	53.16	86.09
Soil + 2.5% of sludge		189.72	106.72	263.63	186.69	275.68	155.12	180.45	203.75
Soil + 5% of sludge		107.08	108.74	231.72	149.18	333.37	168.33	187.46	229.72
Soil + 10% of sludge		100.02	208.34	156.41	154.93	361.06	140.06	381.45	294.19
Soil + 20% of sludge		263.06	119.05	246.17	209.43	262.59	202.27	505.35	323.40
Control soil	20-40	397.82	198.81	299.89	298.84	78.24	7.21	6.04	30.50
Soil + 1% of sludge		275.63	222.68	284.78	261.03	93.02	1.75	34.83	43.20
Soil + 2.5% of sludge		325.43	247.94	267.63	280.33	97.24	12.71	25.55	45.17
Soil + 5% of sludge		268.23	219.17	248.00	245.13	157.69	70.68	124.91	117.76
Soil + 10% of sludge		235.88	131.05	145.05	170.66	175.24	79.46	154.90	136.53
Soil + 20% of sludge		255.97	104.74	373.55	244.75	130.02	103.42	146.54	126.66
Mean		246.44	176.65	255.04	226.04	176.53	89.06	152.96	139.52
Mean for horizon		0-20 cm – 201.99; 20-40 cm – 250.09				0-20 cm – 195.72; 20-40 cm – 83.30			
LSD									
Date		9.14				3.26			
Horizon		6.21				2.22			
Horizon x dose		25.85				9.23			
Treatments		48.78				17.42			

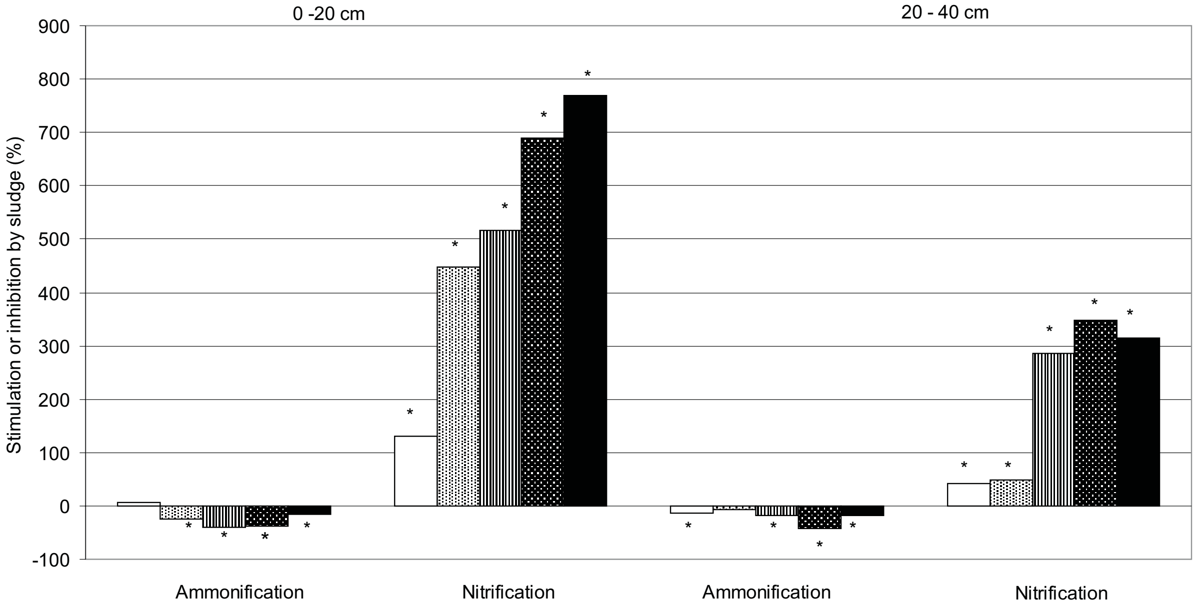

Fig. 2. Selected processes related with nitrogen transformations in soil in the second year of the experiment, annual mean. Explanations as on Fig. 1.

Table 3. Activity of selected enzymes in the soil in the second year of the experiment

Treatments	Depth (cm)	Dehydrogenases mg TPF* kg^{-1} d.m. of soil d^{-1}			Mean	Protease mg of tyrosine kg^{-1} d.m. of soil h^{-1}			Mean
		31.05.99	29.07.99	27.10.99		31.05.99	29.07.99	27.10.99	
Control soil	0-20	4.46	3.10	11.86	6.48	21.02	24.06	5.92	17.00
Soil + 1% of sludge		4.70	2.94	9.39	5.68	15.79	20.31	10.89	15.66
Soil + 2.5% of sludge		7.54	5.74	6.65	6.64	31.52	19.63	7.22	19.46
Soil + 5% of sludge		9.56	8.09	6.87	8.17	23.41	50.15	14.55	29.37
Soil + 10% of sludge		19.44	5.50	12.82	12.59	38.44	18.70	27.17	28.10
Soil + 20% of sludge		24.55	30.09	64.00	39.54	40.22	58.64	53.89	50.91
Control soil	20-40	3.17	0.59	2.59	2.11	10.94	11.10	1.79	7.94
Soil + 1% of sludge		3.89	3.29	5.58	4.26	9.40	9.92	11.25	10.19
Soil + 2.5% of sludge		4.88	1.96	4.39	3.74	14.07	19.27	2.04	11.80
Soil + 5% of sludge		4.19	3.54	5.57	4.43	9.41	18.42	5.35	11.06
Soil + 10% of sludge		6.31	3.77	6.12	5.40	16.21	12.75	11.05	13.33
Soil + 20% of sludge		7.04	7.96	19.21	11.40	29.80	24.27	6.13	20.07
Mean		8.31	6.38	12.92	9.20	21.69	23.93	13.10	19.57
Mean for horizon		0-20 cm – 13.18; 20-40 cm – 5.22				0-20 cm – 26.75; 20-40 cm – 12.40			
LSD									
Date		0.86				0.57			
Horizon		0.59				0.38			
Horizon x dose		2.45				1.56			
Treatments		4.62				3.02			

*TPF – triphenyloformazane.

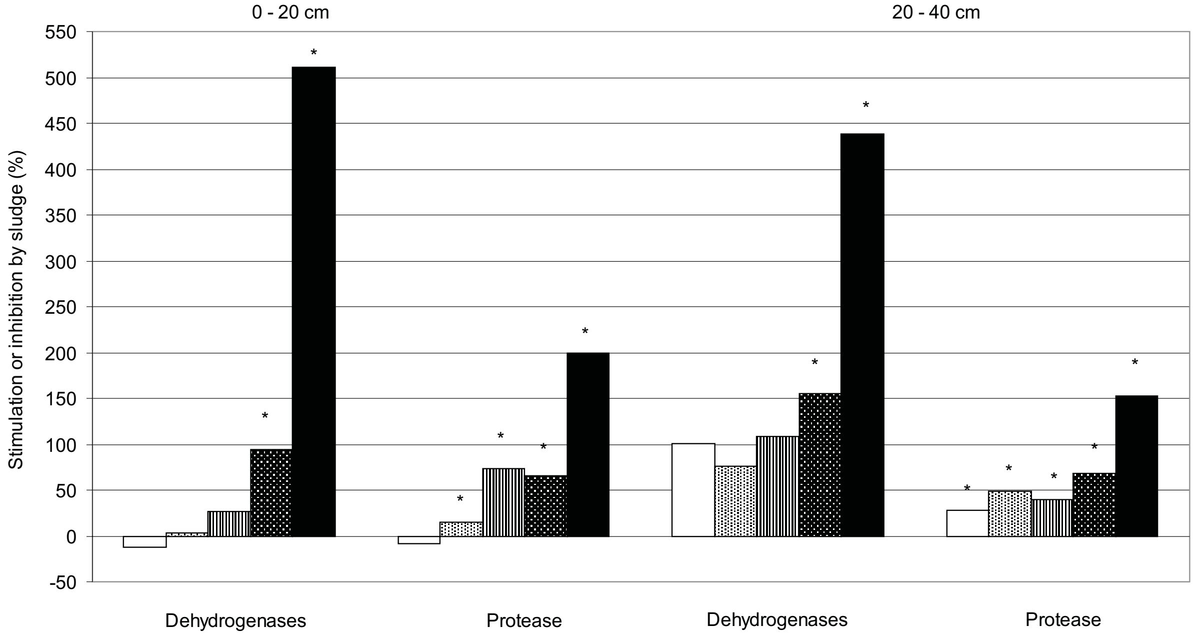

Fig. 3. Selected soil enzymes in the second year of the experiment, annual mean. Explanations as on Fig. 1.

Table 4. Soil moisture and reaction (results of the second year of the experiment)

Treatments	Depth (cm)	Moisture (%)			Mean	Reaction pH$_{KCl}$		
		31.05.99	29.07.99	27.10.99		31.05.99	29.07.99	27.10.99
Control soil	0-20	11.9	11.0	12.2	11.5	7.3	6.4	7.2
Soil + 1% of sludge		13.0	13.9	14.4	13. 8	7.6	6.8	7.2
Soil + 2.5% of sludge		13.2	12.0	15.9	13.7	7.5	6.9	7.2
Soil + 5% of sludge		14.6	12.8	11.5	13.0	7.6	7.0	7.1
Soil + 10% of sludge		24.4	12.9	23.3	20.2	7.2	6.9	7.0
Soil + 20% of sludge		27.0	31.6	25.9	28.2	7.1	6.7	7.0
Control soil	20-40	10.5	9.1	11.2	10.3	6.9	6.3	6.1
Soil + 1% of sludge		13.2	12.1	13.7	13.0	7.0	6.5	6.3
Soil + 2.5% of sludge		14.5	14.1	12.3	13.6	7.0	6.3	6.3
Soil + 5% of sludge		14.2	9.7	15.2	13.0	7.3	6.6	6.9
Soil + 10% of sludge		16.6	15.7	16.1	16.1	7.1	6.8	7.0
Soil + 20% of sludge		14.9	13.3	22.1	16.8	7.0	6.7	7.1

Table 5. Coefficients of correlation (results of the second year of the experiment)

	Respiration	Cellulose mineralization	Ammonifiation	Nitrification	Dehydrogenases	Protease
Respiration		0.692**	- 0.514**	0.541**	0.383*	0.760**
Cellulose mineralization			–	0.754**	0.742**	0.726**
Ammonification				-0.356*	–	- 0.496**
Nitrification					0.718**	0.636**
Dehydrogenases						0.659**
Protease						

Significance level: *p=0.05, **p=0.01, – no correlation.

As follows from data in Table 5, the biochemical tests analysed in this study were positively correlated between one another in almost all cases. The exception was ammonification which did not display any relations with cellulose mineralization and dehydrogenases actcivity, and was negatively correlated with the remaining parameters.

Positive correlations between dehydrogenases activity and the remaining parameters, continuing in the 2nd year of the experiment, indicate that irrespective of the passage of time microorganisms continue to conduct processes related with transformations of organic complexes of carbon and nitrogen brought into the soil with the sludge.

CONCLUSIONS

1. In the second year of the sludge effect on the soil significant stimulation of respiration and cellulose mineralization rate was observed. In most of the treatment objects the effect intensified with increasing sludge concentration and was observable both in the surface horizon (0-20 cm) and in deeper layer of the soil (20-40 cm). In objects with higher content of sludge the effect was more pronounced in the Ap horizon.

2. The process of ammonification was significantly inhibited in almost all variants of the experiment, while the intensity of the process of nitrification clearly increased under the effect of all the doses of sludge, both in the Ap horizon and in the deeper layer of the soil. However, in the soil of the surface horizon the stimulation of the parameter was decidedly stronger.

3. Soil fertilization with sewage sludge resulted in intensification of dehydrogenases and protease activity in the surface and deeper horizons of the soil. In the case of dehydrogenases, however, the effect of the sludge was significant only in treatments with the higher doses (10 and 20%), while the stimulation of proteolytic activity of the soil remained at significant levels in almost all variants of the experiment.

4. Generally, the analysed activities were positively correlated with one another. The exception was ammonification which did not display any relation with cellulose mineralization and dehydrogenases activity, and was negatively correlated with the remaining parameters.

5. The results obtained indicate that the effect of sewage sludge on all of the studied biochemical processes continued in the second year of the experiment, both in the surface horizon and in the deeper layer of the soil.

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