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COUNTS AND ACTIVITY OF MICROORGANISMS PARTICIPATING IN NITROGEN TRANSFORMATIONS IN SOIL, FOUR YEARS AFTER APPLICATION OF SEWAGE SLUDGE

Jolanta Joniec, Jadwiga Furczak

Department of Agricultural Microbiology
University of Life Sciences in Lublin

Abstract

The objective of the study was to determine the direction, intensity and duration of changes in abundance and activity of certain microbial groups active in nitrogen transformations in soil subjected to a 4-year fertilization treatment with municipal and industrial sewage sludge. The study was conducted on podzolic soil, whose accumulation horizon had been fertilized in 1998 with fermented sewage sludge at doses of 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%) and planted with willow (*Salix viminalis* L.). Four years after the application of sludge, microbiological and biochemical analyses were made in two soil horizons (0-20 cm and 20-40 cm). It was found that in the soil from the 0-20 cm depth significant stimulation of the growth of proteolytic fungi and bacteria continued, but only under the effect of the highest dose of sludge. Moreover, there was a notable stimulation of protease activity and nitrification process alongside a slight inhibition of ammonification. In the soil from the 20-40 cm layer stimulation of the growth of protein-decomposing fungi was observed as well as that of proteolytic and nitrification activity of soil, while ammonification was inhibited. However, the effect of sludge was generally less pronounced in the deeper soil layer than in the surface soil horizon.

Key words: municipal-industrial sewage sludge, podzolic soil, microbiological transformations of nitrogen, content $N\text{-}NH_4$ and $N\text{-}NO_3$.

dr Jolanta Joniec, Department of Agricultural Microbiology, University of Life Sciences in Lublin, ul. Leszczyńskiego 7, 20-029 Lublin, Poland, phone: 081 532-30-47, e-mail: jolanta.joniec@ar.lublin.pl

LICZEBNOŚĆ I AKTYWNOŚĆ MIKROORGANIZMÓW CZYNNYCH W PRZEMIANACH AZOTU W GLEBIE, W CZWARTYM ROKU OD WZBOGACENIA JEJ OSADEM ŚCIEKOWYM

Abstrakt

Celem pracy było określenie kierunku, natężenia i czasu utrzymywania się zmian w liczebności i aktywności niektórych grup mikroorganizmów czynnych w przemianach azotu, w glebie poddanej 4-letniemu oddziaływaniu osadu ścieków komunalno-przemysłowych. Badaniami objęto glebę bielicową, której poziom akumulacyjny nawieziono w 1998 r. przefermentowanym osadem ściekowym w ilości: 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%) i 600 $Mg \cdot ha^{-1}$(20%). Następnie glebę obsadzono wierzbą (*Salix viminalis* L.) i w 4-tym roku od wprowadzenia osadu wykonano w dwu jej warstwach (0-20 cm i 20-40 cm) analizy mikrobiologiczne i biochemiczne. Stwierdzono, że w glebie z głębokości 0-20 cm utrzymywało się nadal istotne pobudzenie rozwoju bakterii i grzybów proteolitycznych, ale tylko pod wpływem najwyższej dawki osadu. Ponadto występowała wyraźna stymulacja aktywności proteazy i procesu nitryfikacji oraz niewielkie hamowanie amonifikacji. W glebie z warstwy 20-40 cm odnotowano również stymulację rozwoju grzybów rozkładających białko, aktywności proteolitycznej i nitryfikacyjnej gleby oraz hamowanie amonifikacji. Jednak oddziaływanie osadu było w tych warunkach na ogół słabsze niż w wierzchniej warstwie gleby.

Słowa kluczowe: osad ścieków komunalno-przemysłowych, gleba bielicowa, mikrobiologiczne przemiany azotu, zawartość $N\text{-}NH_4$ i $N\text{-}NO_3$.

INTRODUCTION

Numerous studies, for example HATTORI and MUKAI (1986), CZEKAŁA (2002) and BIELIŃSKA and ŻUKOWSKA (2002), indicate that sewage sludge is a rich source of both carbon and nitrogen organic matter, and of various mineral components. Introduced to soil, it is a valuable source of nutrients for various soilborne microbial groups, including the ones taking part in nitrogen transformations, thus affecting their activity and consequently the fertility of soils (SIUTA 1998, BARAN et al. 1999, BIELIŃSKA, ŻUKOWSKA 2002, ŻUKOWSKA et al. 2002, FURCZAK, JONIEC 2007b, JONIEC, FURCZAK 2007b). Therefore, one possible utilisation method of such waste is its application in agriculture for fertilisation of soils under alternative crop cultures (SIUTA 1998), for example willow (*Salix viminalis*). This species is characterized by high capacity for absorption of nitrogen, phosphorus and water and - most importantly – also of heavy metals, thus displaying phyto-remediation properties (SIUTA 1998, BARAN et al. 2000). The crop yield of willow is used in the furniture and wicker-products industry, in power generation as a fuel which is less noxious than coal, and in the chemical industry – as a source of cellulose and raw material for production of bio-alcohol (SZCZUKOWSKI et al. 2001). Studies on the effect of sewage sludge on abundance and activity of microorganisms involved in nitrogen transformations in soil are mainly fragmentary and most often conducted under laboratory conditions (HATTORI, MUKAI 1986, KOBUS et al. 1990, PASCUAL et al. 2007), or else involve short-term field experiments with a variety of plants (BARAN et al. 1999, BIELIŃSKA, ŻUKOWSKA 2002, GARCIA-GIL et al. 2004, FURCZAK, JONIEC 2007b,c, JONIEC, FURCZAK 2007a). The long-term effects of sewage sludge application on the a.m. microbiological and biochemical parameters in soil under a culture of willow have not been performed until present. Therefore, the objective of this study has been to estimate the direction, dynamics and duration of changes in the numbers of microbial groups and in the activity of biochemical processes related to nitrogen transformations in soil from a long-term field experiment, in the 4^{th} year since soil amendment with sewage sludge.

Multi-year monitoring of microbiological effects of soil fertilisation with sewage sludge is not only of theoretical interest but has a practical application, such as estimating the possibility of utilisation of waste in a manner that is safe for the micro-biocenosis. Moreover, our study can help to assess to what extent the tests used here may be applicable to the estimation of microbiological effects of multi-year influence of sewage sludge on the soil environment.

MATERIAL AND METHODS

The study involved a field experiment set up in Końskie in 1998 by the Institute of Soil Science and Natural Environment Management, Lublin University of Agriculture. The experiment comprised plots of a surface area of 15 m^2 each. The accumulation horizon of podzolic soil developed from weakly loamy sand was fertilised with fermented sludge from municipal and industrial sewage produced at the Mechanical-Biological Sewage Treatment Plant in Końskie. The sludge, in accordance with the current standards in Poland (Rozp. MOŚ 2002), met the requirements set up for agricultural utilisation. It was applied at doses of 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%). Next, four weeks after the application, the soil was planted with willow (*Salix viminalis* L.). The control treatment in the experiment was soil under the same crop culture but without sludge amendment. The grain size composition and certain physicochemical and chemical properties of the soil and sludge used for the amendment are given by BARAN et al. (2000).

In the fourth year of the experiment, i.e. 2001, samples of soil were taken from the depths of 0-20 cm and 20-40 cm in three sampling seasons (spring, summer and autumn) to perform the following determinations (with three replications): counts of protein-decomposing bacteria and fungi on the Frazier substrate (RODINA 1968), to which – in the case of fungi – antibiotics were added in accordance with the recommendations by MARTIN (1950); ammonification intensity, in 25-gram weighed portions of soil containing 0.1%

of asparagine, from which – after 3 days of incubation – ammonium ions were extracted and their content was determined according to Nessler's method (Nowosielski 1974); nitrification intensity, in 25-gram weighed portions of soil containing 0.1% of monobasic ammonium phosphate, from which – after 7 days of incubation – nitrate ions were extracted and their level was measured using the brucine method (Nowosielski 1974); protease activity, according to the method of Ladd and Butler (Ladd, Butler 1972); content of N-NH_4 and N-NO_3 ions in the soil after extraction of 25-gram weighed portions of the soil, following Nessler's (Nowosielski 1974) and the brucine (Nowosielski 1974) methods, respectively; soil reaction, potentiometrically in 1 mol·dm^{-3} KCl, and soil moisture with the gravimetric method. During the determinations of ammonification and nitrification, the moisture content of the incubated soil was maintained at the level of 50-60% of total hydraulic capacity. Mineral forms of nitrogen were extracted from the soil with 2% KCl at the 1:5 ratio.

The results were processed statistically with the method of analysis of variance. The significance of differences was determined with Tukey's test at p = 0.05.

Table 1

Moisture of soil

Dose of sludge (Mg ha^{-1})	Depth (cm)	Moisture (% w.w.)			
		24. 05	26. 07	25. 10	Mean
0	0-20	16.10	13.07	13.96	14.38
30		19.25	12.97	15.73	15.98
75		17.81	14.61	17.04	16.49
150		22.40	15.38	22.49	20.09
300		22.39	18.93	17.97	19.76
600		28.02	22.68	20.86	23.85
0	20-40	12.97	11.37	14.28	12.87
30		15.72	10.90	14.34	13.65
75		14.76	14.29	15.96	15.00
150		14.70	12.80	18.62	15.37
300		16.92	11.17	18.32	15.47
600		24.53	19.17	21.97	21.89

RESULTS AND DISCUSSION

The study performed in the fourth year of the experiment showed that in the soil from 0-20 cm a significant stimulating effect of sewage sludge on the growth of protein-decomposing bacteria and fungi occurred only under the influence of the highest dose of sludge (Table 2). This indicates that the effect of sludge on the microbial groups examined was notably weaker than in the previous years of the experiment (FURCZAK, JONIEC 2007b, JONIEC, FURCZAK 2007a,c). Some changes in this respect were observable also in the soil from the depth of 20-40 cm (Table 2). Although by then no effect of sludge on the numbers of proteolytic bacteria was observed, the highest dose of sludge caused stimulation of the growth of "proteolytic" fungi, which was even stronger than in the soil from 0-20 cm (Table 2). While analysing periodic oscillations (Table 2) in the soil from the two horizons, it was noticed that generally both microbial groups occurred most abundantly in spring, which was probably caused by a temperature increase after the winter season.

The stimulation of the growth of protein-decomposing bacteria and fungi observed in the combination with the highest dose of sludge (Table 2) could have been caused by the persistently increased level of nutrients – under those conditions – for the microbial groups. This explanation is supported by ŻUKOWSKA et al. (2002), who showed a continuingly increasing content of organic carbon and total nitrogen in soil treated with sludge. Those compounds had certainly originated from the sludge added to soil and, additionally, were a product of microbiological transformation of organic matter introduced to soil with the sludge. It appears that also the growth of proteolytic bacteria and fungi could have been supported by improvement of other living conditions of these microbial groups, i.e. soil reaction and moisture (Tables 1, 3), and probably the water-air relations. Some authors report that in soils amended with sewage sludge (at greater doses in particular) the soil retention capacity increases, aggregate and semi-aggregate structure is formed, soil density decreases while the full water capacity of soil increases (BARAN et al. 1996a,b).

It has also been demonstrated that the proteolytic activity in the top layer of the soil (0-20 cm) was stimulated by the higher levels of the sludge, i.e. 150 $Mg \cdot ha^{-1}$, 300 $Mg \cdot ha^{-1}$ and 600 $Mg \cdot ha^{-1}$ (Table 4). Similar tendencies were observed by BARAN et al. (1999) in soil 0-20 cm deep originating from a 4-year field experiment with sewage sludge. It has been demonstrated (DICK 1994) that the primary producers of proteases in soil are protein-decomposing bacteria and fungi. Therefore, their stimulation in treatments with higher doses of sludge, and especially with the highest one (Table 2), was accompanied by an increase in the proteolytic activity (Table 4).

Table 2

Numbers of proteolytic bacteria and fungi in the soil

Dose of sludge ($Mg \cdot ha^{-1}$)	Depth (cm)	Proteolytic bacteria (cfu $10^9 kg^{-1}$ d.m. of soil)					"Proteolytic" fungi (cfu 10^6 kg^{-1} d.m. of soil)				
		24. 05	26. 07	25. 10	mean	(%)	24. 05	26. 07	25. 10	mean	(%)
0	0-20	3.2	2.3	1.7	2.4		85.2	41.5	45.6	57.4	
30		2.5	2.4	1.5	2.1	-12.5	63.6	50.1	38.6	50.7	-11.5
75		3.1	1.8	3.2	2.3	-4.2	66.8	65.7	31.2	54.6	-4.9
150		3.5	3.0	0.9	2.3	-4.2	57.1	53.8	36.7	49.2	-14.3
300		3.8	3.1	2.3	3.0	29.2	89.8	99.1	47.2	78.7	37.1
600		8.0	4.4	1.9	4.8	100.0	123.7	241.3	60.4	141.8	147.0
0	20-40	1.3	1.2	2.3	1.6		30.1	7.1	11.4	16.2	
30		2.2	1.3	2.1	1.9	18.8	16.2	9.6	15.2	13.7	-15.4
75		3.2	1.5	1.9	2.2	37.5	30.6	6.5	23.2	20.1	24.1
150		3.6	2.5	1.6	2.6	62.5	14.5	13.5	23.7	17.3	6.2
300		3.3	2.3	2.1	2.6	62.5	15.2	4.4	65.1	28.3	145.9
600		2.0	2.8	3.0	2.6	62.5	54.0	59.6	44.7	52.8	225.9
Mean		3.3	2.4	1.9	2.5		53.9	54.3	36.9	48.4	
Mean for depth		0-20 cm – 2.8; 20-40 cm – 2.2					0-20 cm – 72.1; 20-40 cm – 24.7				
LSD date		0.4					10.2				
LSD depth		0.3					6.9				
LSD depth x dose		1.1					28.8				
LSD treatments		2.1					54.4				

% – stimulation or inhibition by sludge

Table 3

Content of $N\text{-}NH_4$, $N\text{-}NO_3$ ions and reaction in the soil

Dose of sludge ($Mg \cdot ha^{-1}$)	Depth (cm)	Content of $N\text{-}NH_4$ (mg kg^{-1} d.m. of soil)					Content of $N\text{-}NO_3$ (mg kg^{-1} d.m. of soil)					Reaction (pH KCl)			
		24. 05	26. 07	25. 10	mean	(%)	24. 05	26. 07	25. 10	mean	(%)	24. 05	26. 07	25. 10	range
0	0-20	30.71	37.28	36.75	34.91		33.39	24.19	7.91	21.83		6.8	7.5	6.8	6.8 - 7.5
30		32.29	33.97	30.61	32.29	-7.5	38.85	22.72	13.77	25.11	15.03	7.4	7.5	7.7	7.4 - 7.7
75		34.58	24.09	29.34	29.34	-16.0	61.25	25.65	5.47	30.79	41.4	7.5	7.8	7.5	7.5-7.8
150		45.68	25.92	27.07	32.89	-5.8	60.51	30.07	22.56	37.71	72.6	7.6	7.9	7.7	7.6 - 7.9
300		46.12	35.77	22.65	34.75	-0.4	69.69	31.67	19.38	40.25	84.2	7.3	7.7	7.4	7.3 - 7.7
600		48.24	37.78	43.35	43.12	23.6	72.35	41.18	14.89	42.81	96.1	7.3	7.8	7.3	7.3 - 7.8
0	20-40	33.56	34.30	19.46	29.11		35.85	33.92	16.83	28.87		7.3	7.3	6.8	6.8 - 7.3
30		48.22	34.96	21.13	34.77	19.4	47.54	27.33	10.53	28.47	-1.4	7.1	7.1	7.0	7.0 - 7.1
75		55.72	18.63	28.57	34.31	17.9	45.68	27.20	10.68	27.85	-3.5	7.0	7.4	6.8	6.8 - 7.4
150		45.30	32.70	44.70	40.90	40.5	38.18	35.79	23.35	32.44	12.4	7.0	7.6	7.1	7.0 - 7.6
300		38.55	23.76	33.87	32.06	10.1	43.72	29.13	25.14	32.66	13.1	7.2	7.7	7.2	7.2 - 7.7
600		43.76	22.45	19.44	28.55	-1.9	46.57	46.93	25.57	39.69	37.5	7.1	7.3	7.2	7.1 - 7.3
Mean		41.81	30.13	29.75			49.47	31.32	16.34						
Mean for depth		0-20 cm – 34.56; 20-40 cm – 33.23					0-20 cm – 33.08; 20-40 cm – 31.66								
LSD date		1.61					2.91								
LSD depth		1.10					1.98								
LSD depth x dose		4.56					8.24								
LSD treatments		8.60					15.55					0.3			

% – stimulation or inhibition by sludge

Table 4

Activity of selected rocesses related to nitrogen transformations in the soil

Dose of sludge ($Mg \cdot ha^{-1}$)	Depth (cm)	Protease, mg of tyrosine (kg^{-1} d.m. of soil h^{-1})					Ammonification (mg $N\text{-}NH_4$ kg^{-1} d.m. of soil 3 d^{-1})					Nitrification (mg $N\text{-}NO_3$ kg^{-1} d.m. of soil 7 d^{-1})				
		24. 05	26. 07	25. 10	mean	(%)	24. 05	26. 07	25. 10	mean	(%)	24. 05	26. 07	25. 10	mean	(%)
0	0-20	9.94	10.63	15.05	11.88		246.40	185.44	295.64	242.49		22.86	41.06	46.01	36.64	
30		9.79	10.10	11.75	10.54	-11.1	198.28	112.35	310.35	206.99	-14.6	101.66	44.27	51.31	65.74	79.5
75		10.80	10.75	13.35	11.64	-2.0	232.69	130.95	284.02	215.88	-10.97	81.86	103.75	114.26	99.96	172.1
150		16.48	10.53	36.64	21.22	78.8	130.61	109.60	275.54	171.92	-29.1	187.22	183.69	298.26	223.06	524.2
300		22.87	15.35	19.43	19.22	61.9	115.74	116.85	318.45	183.68	-24.3	189.52	123.55	128.77	147.28	302.0
600		23.03	33.92	17.46	24.80	108.9	221.70	91.12	304.08	205.63	-15.2	288.40	196.45	208.74	231.20	531.0
0	20-40	6.62	6.94	3.87	5.80		207.03	227.25	358.10	264.13		7.20	21.03	37.72	21.98	
30		8.05	8.54	10.38	8.99	54.7	230.86	188.61	318.14	245.87	-6.9	2.02	73.47	44.22	39.90	81.5
75		4.02	5.04	9.71	6.26	7.8	222.00	265.64	316.98	268.21	1.5	1.95	19.75	21.03	14.24	-35.2
150		5.75	4.61	16.33	8.90	53.2	237.59	206.11	247.35	230.35	-12.8	6.17	87.75	37.30	43.74	99.0
300		3.00	5.01	12.18	6.73	15.8	218.31	135.33	294.81	216.15	-18.2	84.02	33.54	21.90	46.49	111.5
600		8.48	7.48	12.69	9.55	64.4	212.52	144.85	350.15	235.84	-10.7	95.87	74.26	29.72	66.62	203.1
Mean		10.74	10.74	14.90	12.13		206.15	159.51	306.13	223.92		89.06	83.55	86.60	86.41	
Mean for depth		0-20 cm – 16.55; 20-40 cm – 7.70					0-20 cm – 204.43; 20-40 cm – 243.43					0-20 cm – 133.98; 20-40 cm – 38.83				
LSD date		0.55					14.29					4.34				
LSD depth		0.37					9.71					2.95				
LSD depth x dose		1.55					40.42					12.28				
LSD treatments		2.92					76.27					23.17				

% – stimulation or inhibition by sludge

In some of the treatments, the activity of proteases was also observed to have increased in the soil from the deeper layer (20-40 cm), but the stimulation was weaker than in the 0-20 cm deep soil horizon (Table 4). Our comparison of the data given in Table 4 with the results obtained in the previous years (Furczak, Joniec 2007b,c, Joniec, Furczak 2007b) shows that the effect of sludge on the proteolytic activity in both soil layers weakened with time.

It is commonly known that proteases play the main role in nitrogen transformations in soil and their activity is largely dependent on the content of C org and total N (Chazijew 1982, Bielińska, Żukowska 2002). The present study confirms this relationship, similarly to Żukowska et al. (2002), who demonstrated a continuing increase of the level of a.m. fractions of organic matter in treatments with higher doses of sludge. This effect could have also been facilitated by increased numbers of various microbial groups (Table 2, Furczak, Joniec 2007a). Kobus (1995), Sastre et al. (1996) and Paul and Clark (2000) report that dead micro-organisms are an important source of carbon and mineralised nitrogen, affecting directly the growth in the proteolytic activity of soil. Positive correlation of the proteolytic activity with the content of microbial biomass carbon in soil is also reported by Wick et al. (2002). While analysing periodic oscillations of the proteolytic activity, it was noticed that, contrary to the most abundant occurrence of proteolytic microorganisms in spring (Table 2), the proteolytic activity was the most intensive in autumn (Table 4). Taking into account the protective function of soil with respect to extracellular enzymes (Kobus 1995), it is likely that this activity in autumn was a sum of the currently present proteolytic microorganisms and proteases released earlier.

The proteolytic activity of soil is also determined by such factors as soil reaction, moisture, temperature and oxygenation (Chazijew 1982, Cieśla, Koper 1990). This is supported by the present study, which showed continued improvement of living conditions of proteolytic microorganisms (especially soil moisture and, to a certain extent, reaction) – Tables 1, 3. Also the improvement of other soil properties (aggregate structure and water-air relations), as indicated by studies by Baran et al. (1996a,b), could have provided an additional contribution to the stimulation of this activity.

Moreover, a slight although significant inhibition of the process of ammonification was observed in the soil from 0-20 cm sampled from the plots fertilized with the higher rates of sludge (150 $Mg \cdot ha^{-1}$ and 300 $Mg \cdot ha^{-1}$) – Table 4. This effect was also noticed in the soil from the depth of 20-40 cm, but it was less intensive than in the surface horizon and observable only in the treatment with the 300 $Mg \cdot ha^{-1}$ dose of the waste. It should be emphasized that the effect of sludge on the a.m. parameter weakened in comparison to the preceding years (Furczak, Joniec 2007b,c, Joniec, Furczak 2007b). As in the case of the protease activity, the intensity of ammonification in samples from both horizons of the soil was the highest in autumn (Table 4).

A decline in ammonification in incubated soil under the effect of increased doses of sludge was probably attributable mainly to the simultaneous intensification of nitrification under these conditions. As a result of the latter process, rapid oxidation of ammonium ions occurred, which caused a reduction of their content in the soil samples (Table 4). Slight inhibition of ammonification in the incubated soil was not accompanied by any reduction in the level of ammonium ions under natural conditions (Table 3). In some treatments a significantly higher content of these ions was even recorded. Kobus et al. (1990) and Herrero et al. (1998) reached similar conclusions studying the effect of sewage sludge on intensification of the process of ammonification.

Since $N\text{-}NH_4$ ions are generally more preferred by micro-organisms as a source of nitrogen than $N\text{-}NO_3$ ions (Paul, Clark 2000), it appears that the depressed ammonifying power determined on the basis of the content of ammonium ions could have also been, to some extent, an effect of its more intensive incorporation by microorganisms into their own cells. The above observation, as in the study by Herrero et al. (1998), is supported by the fact that the decrease in ammonification in the 4^{th} year of the experiment was accompanied by a simultaneous increase in the activity of dehydrogenases in soil (Furczak, Joniec 2007a), as these enzymes are responsible for transformations of ammonium ions in the processes of nitrification and their immobilization via incorporation into microbial biomass (Herrero et al. 1998, Paul, Clark 2000).

During the study, the most pronounced changes caused by sludge occurred in the process of nitrification (Table 4). This is indicated by a distinct, usually increasing with the dosage, stimulation of this process. This effect persisted in both layers of the soil, but it was stronger in the soil from 0-20 cm. Also the content of nitrate ions in the 0-20 cm layer was higher in almost all treatments with sludge compared to the control soil (Table 3), while in the soil from the deeper layer it was higher only in the treatment with 600 $Mg \cdot ha^{-1}$ content of the waste.

As in the case of the other biochemical tests under analysis, stimulation of nitrification was weaker than in the preceding three years of the experiment (Furczak, Joniec 2007b,c, Joniec, Furczak 2007b).

The results obtained for particular time periods of the study indicate that in both layers of the soil the intensity of the process of nitrification and the content of $N\text{-}NO_3$ ions were the highest in spring (Tables 3, 4).

In the soil with sludge, stimulation of oxidation of ammonium nitrogen was most likely caused by the higher level of nutrient substrate for nitrifiers, which is indicated by the content of $N\text{-}NH_4$ ions in the soil (Table 3) and by the persisting favourable living conditions for those microorganisms, i.e. increased soil moisture, somewhat higher reaction (Tables 1 and 4) and, probably, improved oxygenation of the soil. Studies by Baran et al. (1996a,b) indicate that sewage sludge introduced to soil notably improves its water-air relations.

In the fourth year after the application of sludge, under the effect of the in the presence higher sludge doses, a slight increase of pH in the soil from 0-20 cm appeared (Table 3). The effect was more pronounced in spring and autumn. The effect of sludge on the reaction of the deeper layer of soil was notably weaker and observable only in certain treatments of the experiment (Table 3).

CONCLUSIONS

1. In the fourth year after the introduction of municipal and industrial sewage sludge to soil, it was only the highest sludge dose that continued to stimulate the growth of proteolytic bacteria and fungi in the soil from 0-20 cm. In the 20-40 cm layer the effect was observable only for "proteolytic" fungi.

2. The higher doses of sludge (150 $Mg \cdot ha^{-1}$, 300 $Mg \cdot ha^{-1}$ and 600 $Mg \cdot ha^{-1}$) caused an increase in the proteolytic activity of the soil from 0-20 cm. In the deeper layer of the soil (20-40 cm), stimulation of this enzymatic parameter was recorded as well, but it was notably weaker.

3. In some of the treatments, a slight inhibition of the process of ammonification continued in the soil from the depth of 0-20 cm (150 $Mg \cdot ha^{-1}$, 300 $Mg \cdot ha^{-1}$). The effect was also noticed in the deeper layer of soil, where it was distinctly weaker. The content of ammonium ions, on the other hand, was higher in certain treatments in the 0-20 cm (dose of 600 $Mg \cdot ha^{-1}$) and in the 20-40 cm layers (doses of 30 $Mg \cdot ha^{-1}$, 75 $Mg \cdot ha^{-1}$ and 150 $Mg \cdot ha^{-1}$) than in the control.

4. The most intensive changes persisted in the course of the process of nitrification. In the soil from 0-20 cm a distinct stimulation of nitrification was observed for all the doses of sewage applied, although larger at higher fertilization rates. Also the content of nitrates was notably higher in almost all the treatments with sludge. The positive effect of sludge on the process of nitrification and on the concentration of $N\text{-}NO_3$ ions was observable also in the deeper layer of the soil, but it was less pronounced. This process turned out to be the most sensitive indicator of the analysed changes persisting in the soil amended with sludge.

5. In the fourth year of the experiment, there was a continuing albeit slight increase of soil pH in the 0-20 cm layer in all the treatments, while in the 20-40 cm layer the effect of sludge on the soil reaction was nearly absent.

6. The results indicate that the products of transformations of fermented sewage sludge introduced in the soil four years before continued to produce a generally stimulating effect on the analysed studied parameters of microbiological activity of the soil environment. The study may be useful

for comprehensive assessment of long-term effects of agricultural utilisation of such waste. Therefore, it is recommended that research on this matter be continued also in the future.

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