Original Research

Microbial Populations and Activity of Biochemical Processes Related to Carbon and Nitrogen Transformations in Podzolic Soil under Willow Culture in Fifth Year from Treatment with Sewage Sludge

J. Furczak, J. Joniec*

Faculty of Agricultural Microbiology, University of Life Sciences in Lublin,
Leszczyńskiego 7, 20-069 Lublin, Poland

Received: 4 July 2008
Accepted: 21 April 2009

Abstract

Our study was conducted on a podzolic soil from a field experiment, fertilized with increasing doses of fermented municipal-industrial sewage sludge: 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%). Following the preparation as above, the soil was planted with basket willow (*Salix viminalis* L.). After five years of maintaining the plantation in Ap horizon of the soil, continued stimulation was observed in the growth of most of the microbial groups under study, i.e. oligo- and macro- trophic bacteria, filamentous fungi, cellulolytic bacteria and fungi, and a slight increase in the numbers of proteolytic bacteria. A certain intensification was also observed in the respiratory activity, rate of cellulose mineralization, nitrification, dehydrogenase and proteolytic activity. The process of ammonification, on the other hand, was subject to inhibition. In the deeper layer of the soil (20-40 cm) a positive effect of the sewage sludge was also observed, but it was notably weaker and related to only some of the aforementioned parameters (macrotrophic bacteria, filamentous fungi, cellulolytic bacteria, respiration, and rate of cellulose mineralization).

Keywords: podzolic soil, sewage sludge, microbial populations, biochemical activity of soil

Introduction

One of the possibilities of sewage sludge utilization is its application in agriculture for fertilization of soil for growing so-called alternative crops produced for non-food uses [1]. The fertilizing and humus-producing values of sewage sludge have already been extensively documented. Many authors have demonstrated its positive effect on numerous chemical, physicochemical and physical properties of soil, including their reaction, sorptive capacity, content of micro- and macro-elements and $C_{org.}$ and N total [2-12]. It is also a known fact that sewage sludge has a positive effect on crop yields and on the microbial activity in soil, but most frequently during the initial period of its effect on the soil environment [1, 4, 13-15]. Moreover, a majority of studies on the subject have been concerned with only a few of the microbiological and biochemical parameters [4, 14-17]. Therefore, the study presented herein was focused on comprehensive estimation of the direction, level and duration of changes in the populations and activity of microorganisms

*e-mail: jolanta.joniec@up.lublin.pl

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

Properties	Unit		Soil	Sewage sludge
Granulometric composition	% of fraction in mm	1-0.1	86	
		0.1-0.02	7	
		<0.02	7	
pH	1 mol · dm^{-3} KCl		6.0	6.4
T	mmol (+) · kg^{-1}		71.3	607.7
C-organic ($C_{org.}$)	g · kg^{-1}		11.16	210.00
N-total (N_t)			1.40	17.78
$C_{org.}$: N_t			7.9	11.8
Cd content	mg · kg^{-1}		0.5	6.0
Cu content			7.0	216
Pb content			18.6	125

active in the transformation of primary biogenic elements, i.e. carbon and nitrogen, in a soil amended with sewage sludge 5 years earlier. Also, an attempt was undertaken at verification of tests as indices reflecting long-term effects of sewage sludge and products of its transformations on the microbiological status of soil, as comprehensive microbiological monitoring may prove helpful in the prediction of ecological effects of long-term effects of sewage sludge on the functioning of soil micro-biocenosis.

Material and Methods

The study was continued on the basis of a field experiment, in the 5th year of its duration. In the preceding years of the experiment, in the soil with the sewage sludge there most frequently persisted an increase in the activity of the microbial groups under study and in the biochemical activity of the soil (except for ammonification), usually more strongly pronounced in treatments with a higher dose of the sewage sludge. The effect was more observable in the layer of 0-20cm than in that of 20-40cm, and decreased with the passage of the years of the experiment [13, 14, 18-22]. However, in the 4th year of the experiment the effect of the sewage sludge on the microbiological and biochemical parameters under study was still observable [21, 22], which justifies continuation of the study in the following year.

An experiment was set up in Końskie by the Institute of Soil Science and Management of the Environment, Lublin University of Agriculture (now University of Life Sciences in Lublin), on a podzolic soil developed from weakly loamy sand. Plots with surface area of 15m^2 had been fertilized, 5 years earlier, with increasing doses of fermented municipal-industrial sewage sludge. Sewage sludge was introduced in the surface horizon of the soil (0-20 cm) at the following doses: 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%). Next, four weeks later, on soil amended as in this way, a plantation of basket willow (*Salix viminalis* L.) was established. Control treatment in the experiment was soil from under the same crop, but without sewage sludge fertilization.

Table 1 presents the grain-sized composition and some of the physicochemical and chemical properties of the soil and of the sewage sludge applied, after Baran et al. [16] and Baran et al. [23]. In accordance with the current regulations in force in Poland, sewage sludge used in the experiment met the requirements for utilization in agriculture [24].

The study was performed on soil material, from under a 5-year old willow culture, from layers of 0-20 cm (Ap horizon) and 20-40 cm. The soil was analyzed twice, i.e. in spring (21st May) and in autumn (16th October). The analyses included determination of the total number of bacteria with low nutrition requirements, on a nutrient substrate with soil extract (350 cm^3·dm^{-3}) and K_2HPO_4, total number of macrotrophic bacteria, on the Bunt-Rovira nutrient substrate [25], total number of filamentous fungi, on Martina medium [26], number of cellulolytic bacteria, on liquid substrate acc. to Pochon and Tardieux [27], that was read from McCrady's Tables, number of cellulolytic fungi, on mineral agar covered wth a circle of Whatman paper and complemented with antibiotics acc. to Martin [26], numbers of protein-decomposing bacteria and fungi, on Frazier gelatine medium [28], complemented – in the case of fungi – with antibiotics [26]; respiratory activity with the method of Rühling et al. [29]; cellulose mineralization in 25-gram weighed portions of soil enriched with 0.5% of powdered Whatman cellulose; the amount of emitted CO_2 was studied with the method of Rühling et al. [29]; intensity of ammonification 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 with the method by Nessler [30]; intensity of nitrification in 25-gram weighed portions of soil containing 0.1% of mono-basic ammonium phosphate, from which, after 7 days of incubation, nitrate ions were extracted and their level was measured with the brucine method [30]; dehydrogenase

Table 2. Total numbers of bacteria and fungi involved in carbon transformations in the soil in the fifth year of the experiment.

Treatments	Depth, cm	Oligotrophic bacteria, cfu· 10^9 · kg^{-1} d.m.of soil				Macrotrophic bacteria, cfu· 10^9 · kg^{-1} d.m.of soil				Filamentous fungi, cfu· 10^6 · kg^{-1} d.m.of soil			
		Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%
Control soil	0-20	2.6	3.8	3.2	-	2.9	6.9	4.9	-	13.6	23.9	18.8	-
Soil+ 1% of sludge		2.3	5.0	3.6	12.5	2.6	10.8	6.7	36.7	23.5	36.6	30.1	60.1
Soil + 2.5% of sludge		2.6	3.9	3.3	3.1	2.4	8.7	5.5	12.2	14.9	46.6	30.8	63.8
Soil + 5% of sludge		7.8	2.7	5.2	62.5	8.2	11.8	10.0	104.1	27.5	44.0	35.8	90.4
Soil + 10% of sludge		5.4	9.4	7.4	131.3	5.2	13.5	9.3	89.8	22.9	50.0	36.5	94.1
Soil+ 20% of sludge		11.5	6.4	8.9	178.1	11.0	17.2	14.1	187.8	31.3	23.7	27.5	46.3
Control soil	20-40	2.4	2.5	2.5	-	3.5	7.4	5.5	-	2.3	4.9	3.6	-
Soil+ 1% of sludge		6.3	2.1	4.2	68.0	10.0	10.8	10.4	89.1	1.2	12.8	7.0	94.4
Soil + 2.5% of sludge		2.3	1.6	2.0	-20.0	2.9	4.6	3.7	-32.7	3.7	8.6	6.2	72.2
Soil + 5% of sludge		3.0	4.0	3.5	40.0	2.9	11.0	6.9	25.5	1.5	6.7	4.1	13.9
Soil +10% of sludge		2.6	2.3	2.4	-4.0	1.6	7.4	4.5	-18.2	4.3	26.8	15.5	330.6
Soil+ 20% of sludge		3.0	1.0	2.0	-20.0	2.5	2.0	2.3	-58.2	5.8	17.9	11.8	227.8
Mean for season		4.3	3.7	-	-	4.6	9.3	-	-	12.7	25.2	-	-
Mean for horizon		surface - 5.3 lower - 2.8				surface - 8.4 lower - 5.6				surface - 29.9 lower - 8.0			
LSD season		0.5				0.6				2.0			
LSD horizon		0.5				0.6				2.0			
LSD horizon x dose		2.1				2.7				8.2			
LSD horizon x dose x season		3.1				4.1				12.7			

% - stimulation or inhibition by sludge

activity – with the method of Thalmann [31]; protease activity – in accordance with the method of Ladd and Butler [32]; reaction – potentiometrically in 1 mol · dm^{-3} KCl, and moisture – with the gravimetric method. During the determinations of respiration and cellulose mineralization, as well as of the intensity of ammonification and nitrification, the moisture content of the soil samples was maintained at the level of 50-60% of total hydraulic capacity.

The results obtained were processed statistically using the method of analysis of variance. Significance of differences was determined with the Tuckey test at p = 0.05. Analysis of variance was not performed for cellulolytic bacteria, as their numbers were calculated using McCrady tables, based on the principles of mathematical statistics.

The results of the study were discussed on the basis of mean values obtained in the year for the individual experimental treatments, using the LSD of soil horizon multipled by dose. When discussing the seasonal changes, the authors took into consideration the mean value obtained from all the treatments in spring, and the corresponding value obtained in autumn, in this case using the LSD value for the season.

Results and Discussion

In the fifth year from the introduction of sewage sludge in the soil, in its upper horizon (0-20 cm) continued significant increase was still observed in the numbers of oligo- and macrotrophic bacteria and filamentous fungi (Table 2). In the case of the two bacterial groups, that effect was observed only for the higher levels of sewage sludge content (5, 10 and 20%). The growth of the fungi, on the other hand, was stimulated by all sewage sludge concentrations applied (1, 2.5, 5, 10 and 20%).

Under the conditions as above, an increase was also observed in the numbers of bacteria and fungi-decomposing cellulose (Table 3). Stimulation of the growth of those bacteria was found in all the treatments with sewage sludge, while in the case of the fungi – only under the effect of the higher doses of sewage sludge, i.e. 10 and 20%. It should be emphasized that among all the parameters under analysis, cellulolytic bacteria responded the most strongly to the 5-year effect of the sewage sludge, while in the case of proteolytic bacteria and fungi the effect of the sewage sludge on growth was notably weaker (Table 3). Only the highest dose of the sludge still caused a slight increase in the numbers of protein-decomposing bacteria.

The data obtained in the fifth year of the experiment indicate that in the soil from the depth of 0-20 cm the level of sewage sludge effect on the growth of oligotrophic bacteria, filamentous fungi, and proteolytic bacteria and fungi was similar to that observed in the fourth year of the experiment [21, 22]. The effect of sewage sludge on the growth of macrotrophic bacteria and cellulolytic bacteria and fungi was even slightly stronger than in the preceding year [21]. That effect could have been caused by, among other things, a slightly greater influx of plant residues originating from the willow grown in the experiment. As follows from our own observations, the plantation of that plant developed better in the fifth year from its establishment.

Moreover, in the soil from a depth of 0-20 cm continued significant stimulation was demonstrated for the rate of respiration, rate of cellulose mineralization, and of the process of nitrification in almost all the treatments with sewage sludge (except for 1%) (Table 4), while under the effect of all the doses of sewage sludge continued significant inhibition of the process of ammonification was observed (Table 4). Also, dehydrogenase and proteolytic activity in the studied soil horizon was stimulated, but only under the effect of the higher concentrations of the waste, i.e. 5, 10 and 20% (Table 5). Comparing the results obtained in the fifth year of the experiment with the data from the preceding year, it was noted that the processes of respiration and cellulose mineralization, as well as protease activity, were stimulated over two years to a similar degree [21, 22], whereas the effect of sewage sludge on the process of nitrification and on dehydrogenase activity was weaker in the fifth year [21, 22]. Only the inhibiting effect of sewage sludge on the process of ammonification gained a little in intensity with regard to the preceding year.

Contrary to expectations, the sewage sludge introduced in the soil five years earlier also had an effect on the studied microbiological and biochemical parameters in the soil layer of 20-40 cm. However, that effect was notably weaker than in the Ap horizon (Tables 2, 3, 4). It was observable in stimulation of the growth of cellulolytic bacteria in all treatments with sewage sludge, and of macrotrophic bacteria, filamentous fungi and proteolytic fungi, but only in individual treatments (Tables 2, 3). Stimulation of growth of protein-decomposing bacteria in the layer of 20-40cm, as distinct from cellulolytic fungi, could have been caused by the lower dependence of their growth on the content of oxygen in soil.

Also the biochemical activity of the soil under those conditions was generally less strongly stimulated than in the Ap horizon (Table 4). Positive effect of all the doses of sewage sludge was observed only in the case of respiration and cellulose mineralization, while the intensity of all remaining biochemical parameters did not undergo significant changes under the effect of sewage sludge (Tables 4, 5).

Analyzing the seasonal variations in the soil of both layers, it was demonstrated that bacteria with low nutritional requirements and cellulolytic bacteria multiplied most intensively in spring, while macrotrophic bacteria, filamentous and cellulolytic fungi, and proteolytic bacteria and fungi did so in autumn (Tables 2, 3). Respiratory processes, processes of cellulose mineralization, ammonification and nitrification, and protease activity, on the other hand, proceeded at the highest intensity in spring (Tables 4, 5). As opposed to the parameters discussed above, dehydrogenase activity was at a similar level in both seasons under study (Table 5). The higher activity (mainly biochemical) in the analyzed soil in spring was most likely due to a temperature increase after the winter period.

Table 3. Populations of cellulolytic and proteolytic bacteria and fungi in soil in the fifth year of the experiment.

Treatments	Depth, cm	Cellulolytic bacteria, $10^6 \cdot kg^{-1}$ d.m. of soil				Cellulolytic fungi, cfu $\cdot 10^6 \cdot kg^{-1}$ d.m. of soil				Proteolytic bacteria, cfu $\cdot 10^9 \cdot kg^{-1}$ d.m. of soil				Proteolytic fungi, cfu $\cdot 10^6 \cdot kg^{-1}$ d.m. of soil			
		Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%
Control soil	0-20	1.1	1.8	1.5	-	7.7	23.1	15.4	-	0.7	2.3	1.5	-	12.4	21.6	17.0	-
Soil+ 1% of sludge		1.8	5.3	3.6	140.0	22.0	32.3	27.1	76.0	0.6	1.7	1.2	-20.0	14.9	19.3	17.1	0.6
Soil + 2.5% of sludge		5.3	5.4	5.4	260.0	16.5	30.1	23.3	51.3	0.6	1.7	1.1	-26.7	11.8	16.0	13.9	-12.2
Soil + 5% of sludge		172.7	3.1	87.9	5760.0	17.3	35.9	26.6	72.7	1.8	1.7	1.7	13.3	11.1	21.2	16.2	-4.7
Soil + 10% of sludge		24.1	5.8	15.0	900.0	15.7	66.3	41.0	166.2	1.8	2.4	2.1	40.0	13.3	26.5	19.9	17.1
Soil+ 20% of sludge		24.7	69.0	46.9	3026.7	26.3	47.5	36.9	139.6	2.1	2.5	2.3	53.3	23.8	18.1	21.0	23.5
Control soil	20-40	0.5	1.1	0.8	-	7.9	4.9	6.4	-	0.5	0.8	0.6	-	3.8	4.5	4.2	-
Soil+ 1% of sludge		23.1	2.9	13.0	1525.0	8.8	5.0	6.9	7.8	0.5	0.5	0.5	-16.7	15.0	6.2	10.6	152.4
Soil + 2.5% of sludge		0.3	2.9	1.6	100.0	9.6	6.6	8.1	26.6	0.4	0.5	0.4	-33.3	11.2	4.6	7.9	88.1
Soil + 5% of sludge		0.5	3.0	1.8	125.0	9.7	4.8	7.2	12.5	0.5	1.5	1.0	66.7	8.9	3.6	6.3	50.0
Soil +10% of sludge		2.9	5.3	4.1	412.5	9.3	11.0	10.2	59.4	0.7	0.8	0.7	16.7	3.5	6.7	5.1	21.4
Soil+ 20% of sludge		23.1	0.9	12.0	1400.0	12.0	11.4	11.7	82.8	0.5	0.6	0.6	0.0	6.5	7.6	7.1	69.0
Mean for season		23.3	8.9	-	-	13.6	23.2	-	-	0.9	1.4	-	-	11.4	13.0	-	-
Mean for horizon						surface– 28.4 ; lower – 8.4				surface – 1.7; lower – 0.6				surface– 17.5; lower – 6.9			
LSD season						3.2				0.2				1.4			
LSD horizon						3.2				0.2				1.4			
LSD horizon x dose						13.2				0.8				6.0			
LSD horizon x dose x season						20.5				1.2				9.3			

% - stimulation or inhibition by sludge

Table 4. Activity of selected processes related with carbon and nitrogen transformations in soil in the fifth year of the experiment.

Treatments	Depth, cm	Respiratory activity, mg $C\text{-}CO_2 \cdot kg^{-1}$ d.m. of soil $\cdot d^{-1}$				Cellulose mineralization, mg $C\text{-}CO_2 \cdot kg^{-1}$ d.m. of soil $\cdot 20d^{-1}$				Ammonification, mg $N\text{-}NH_4 \cdot kg^{-1}$ d.m. of soil $\cdot 3d^{-1}$				Nitrification, mg $N\text{-}NO_3 \cdot kg^{-1}$ d.m. of soil $\cdot 7d^{-1}$			
		Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%
Control soil	0-20	367.12	339.62	353.37	-	1261.93	974.61	1118.27	-	274.33	255.22	264.78	-	80.37	67.73	74.20	-
Soil+ 1% of sludge		513.75	319.38	416.57	17.9	1315.48	1179.87	1247.68	11.6	214.47	146.22	180.35	-31.9	106.51	100.99	103.75	39.8
Soil + 2.5% of sludge		427.81	454.71	441.26	24.9	1317.46	1337.79	1327.63	18.7	184.87	148.00	166.43	-37.1	163.60	104.50	134.35	81.1
Soil + 5% of sludge		704.33	571.75	638.04	80.6	2609.39	1711.56	2160.48	93.2	127.33	131.60	129.47	-51.1	296.86	110.51	203.69	174.5
Soil + 10% of sludge		658.59	655.72	657.16	86.0	2033.05	1702.61	1867.83	67.0	187.54	137.21	162.37	-38.7	175.55	198.91	187.23	152.3
Soil+ 20% of sludge		711.61	537.34	624.48	76.7	2645.70	1331.90	1988.80	77.8	133.15	138.83	135.99	-48.6	218.58	137.73	178.16	140.1
Control soil	20-40	176.38	244.42	210.40	-	869.75	767.19	818.47	-	274.79	176.36	225.58	-	4.79	31.52	18.16	-
Soil+ 1% of sludge		462.75	264.46	363.60	72.8	1182.30	958.12	1070.21	30.8	147.16	177.85	162.50	-28.0	20.63	32.90	26.77	47.4
Soil + 2.5% of sludge		375.13	223.60	299.37	42.3	1067.26	1118.18	1092.72	33.5	274.68	237.82	256.25	13.6	21.75	25.49	23.62	30.1
Soil + 5% of sludge		360.11	247.90	304.00	44.5	1483.09	1211.83	1347.46	64.6	254.18	242.26	248.22	10.0	25.19	12.24	18.71	3.0
Soil +10% of sludge		301.18	290.68	295.93	40.7	1219.13	1148.54	1183.83	44.6	285.86	182.07	233.96	3.7	25.46	63.83	44.65	145.9
Soil+ 20% of sludge		288.47	334.21	311.34	48.0	1149.33	1074.18	1111.76	35.8	288.90	206.27	247.59	9.8	56.50	46.02	51.26	182.3
Mean for season		445.60	373.65	-	-	1512.83	1209.70	-	-	220.60	181.64	-	-	99.68	77.70	-	-
Mean for horizon		surface – 521.81; lower – 297.44				surface – 1618.45; lower – 1104.07				surface – 173.23; lower – 229.02				surface – 146.85; lower – 30.53			
LSD season		17.67				47.45				17.33				10.53			
LSD horizon		17.67				47.45				17.33				10.53			
LSD horizon x dose		74.14				199.12				72.72				44.19			
LSD horizon x dose x season		114.68				308.04				112.49				68.35			

% - stimulation or inhibition by sludge

Table 5. Enzymatic activity and selected physical and physicochemical properties of soil in the fifth year of the experiment.

Treatments	Depth, cm	Dehydrogenases activity, mg TPF · kg^{-1} d.m. of soil · d^{-1}				Protease activity, mg tyrosine · kg^{-1} d.m. of soil · h^{-1}				Moisture of soil, % w.w.				Reaction of soil, pHKCl	
		Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn
Control soil	0-20	3.08	10.11	6.59	-	37.79	22.61	30.20	-	16.2	17.6	16.9	-	6.6	6.5
Soil+ 1% of sludge		4.64	5.16	4.90	-25.6	46.61	15.97	31.29	3.6	17.7	18.0	17.9	6.0	6.9	6.8
Soil + 2.5% of sludge		3.20	6.94	5.07	-23.1	43.19	10.31	26.75	-11.4	17.5	20.4	19.0	12.4	7.2	6.9
Soil + 5% of sludge		11.89	8.23	10.06	52.6	97.31	21.35	59.33	96.5	23.4	22.2	22.8	34.9	7.3	6.9
Soil + 10% of sludge		7.39	13.28	10.33	56.8	73.03	46.30	59.66	97.5	20.6	28.3	24.5	45.0	7.1	6.8
Soil+ 20% of sludge		13.53	6.39	9.96	51.1	89.44	47.05	68.25	126.0	23.4	20.7	22.1	30.8	7.1	6.8
Control soil	20-40	0.96	1.62	1.29	-	12.38	8.35	10.37	-	12.7	13.7	13.2	-	6.6	6.3
Soil+ 1% of sludge		2.84	1.70	2.27	76.0	17.32	7.31	12.32	18.8	15.3	16.1	15.7	18.9	6.7	6.4
Soil + 2.5% of sludge		4.02	1.59	2.80	117.1	22.46	12.24	17.35	67.3	24.8	16.8	20.8	57.6	6.8	6.2
Soil + 5% of sludge		3.37	2.76	3.07	138.0	28.23	8.91	18.57	79.1	16.5	18.9	17.7	34.1	6.7	6.0
Soil +10% of sludge		3.92	1.31	2.61	102.3	22.04	17.01	19.53	88.3	16.5	18.2	17.4	31.8	6.7	6.5
Soil+ 20% of sludge		3.06	2.81	2.94	127.9	22.92	16.88	19.90	91.9	15.6	14.0	14.8	12.1	6.7	6.3
Mean for season		5.2	5.2	-	-	42.73	19.52	-	-						
Mean for horizon		surface – 7.8; lower– 2.5				surface-45.91; lower-16.34									
LSD season		ns				2.58									
LSD horizon		0.66				2.58									
LSD horizon x dose		2.78				10.84									
LSD horizon x dose x season		4.30				16.77									

% - stimulation or inhibition
ns- no significant differences

The increased microbial populations and biochemical activity still persisting in the soil (primarily in the Ap horizon) in the 5th year from its fertilization with sewage sludge was most likely an effect of products of transformation of sewage sludge, and of changes it caused in the physical, physicochemical and chemical properties of the soil. Although sewage sludge organic matter is composed mostly of compounds that are hard-decomposing [5, 33], it is to be assumed that over the 5-year period it should have undergone mineralization and humification. Considerable transformations of organic matter in soil over a period of 4 years from its amendment with sewage sludge are reported, among other authors, by Żukowska and Flis-Bujak [11] and by Żukowska et al. [12]. According to Czekała [5], nitrogen compounds from sewage sludge are characterized by greater solubility, and therefore greater susceptibility to decomposition, than carbon compounds. This found supporting evidence in this study, which shows that in the 5th year from the introduction of sewage sludge in the soil the activity of proteolytic bacteria and fungi was already the least stimulated from among all the microbial groups under analysis. On the other hand, still persisting stimulation of the growth of cellulolytic bacteria and fungi, and of the rate of cellulose mineralization, could have been contributed to, to a certain extent, by the supply of a certain amount of cellulose with decaying parts of the crop plant which, as follows from our own observations, continued to develop better on the soil with sewage sludge.

Data in Table 5 show that in the soil fertilized with sewage sludge there also was continued improvement of physical and physicochemical conditions, such as moisture content and soil reaction and, probably, of the water-air relations, as indicated by studies conducted by other authors [2, 3]. Studies by the referenced authors indicate that in soils fertilized with sewage sludge (especially at higher doses) there is an increase in the water retention capacity of the soil, aggregate and semi-aggregate soil structure is formed, and there occurs a decrease in soil density and an increase in its total hydraulic capacity. As is known, improvement in those properties of soil has a positive effect on microbial growth and activity which, surely, also took place in the experiment reported herein. What is intriguing here is the fact of intensification of the growth of oligotrophic bacteria in the soil with the highest doses of sewage sludge (Table 2). It is not, however, an isolated observation, as other authors have also observed numerous occurrences of bacteria with low nutrition requirements in an environment rich in nutrients [34].

In the opinion of numerous authors, among others Dick [35], Kusza [36], and Kuzyakov [37], the amount of CO_2 emitted from the soil and the enzymes present in it are mainly the result of the activity of micro-organisms inhabiting the soil. Therefore, growth in the microbial populations analyzed in this study (Tables 2, 3) was reflected in the growth of the biochemical activity of the soil (Tables 4, 5). It is to be assumed that the observed intensification of the biochemical activity of soil amended with sewage sludge, as was the case with the size of microbial populations, has been contributed to by the products of transformation of sewage sludge organic matter and by the positive changes they caused in the chemical, physical and physicochemical properties of the soil. This has been partially supported by earlier studies of other authors who demonstrated positive correlation of the enzymatic activity of soil with the content of $C_{org.}$, $N_{tot.}$, moisture, or reaction [4, 38, 39, 40]. Noteworthy is the fact that the only activity, studied in this experiment, that was subject to inhibition was the rate of ammonification (Table 4). As follows from the literature of the subject, that process may be inhibited by heavy metals [41]. Data presented in Table 1 indicate that sewage sludge used in the experiment introduced a certain amount of those elements in the soil, which could have been one of the reasons for the inhibition observed. However, it appears that the main cause of the reduction in the rate of ammonification was the simultaneous strong process of nitrification, as well as proteolysis of ammonium ions by micro-organisms. Similar conclusions have been reached by other authors, who also found a decrease in the rate of ammonification in soil amended with sewage sludge [22, 42, 43].

Notable is the fact that, in spite of the introduction of sewage sludge in the surface horizon of soil, its effect – even after 5 years – was not limited to that soil horizon (Tables 2, 3, 4). The products of transformation of sewage sludge organic matter also affected the physical and physicochemical properties of soil from the layer of 20-40 cm (Table 5). Those changes, however, were notably lower compared to those in the Ap horizon (Table 5), which was reflected also in weaker microbiological and biochemical activity of soil from that layer (Tables 2, 3, 4, 5).

The research results presented here show that, under the conditions of the experiment, the biological effects of soil amendment with sewage sludge, even at the lowest doses (in the case of certain tests), are not short-term and continue in the 5th year from sewage sludge application.

As a result of mineralization and humification of organic matter introduced with sewage sludge, there appeared and persisted positive changes in the physical, chemical and physicochemical properties of the soil [2, 3, 11, 12], thus creating more favourable conditions for the biota. These observations are not isolated, as other authors – among others Pascual et al. [44], Ros et al. [45] – demonstrated that one-time introduction of a sizeable amount of organic matter in soil results, even after many years, in increased activity of soil microorganisms, which is attributed to improved fertility of the soil.

Conclusions

1. In the 5th year of sewage sludge the effect on the soil, in the Ap horizon there still continued stimulation of biological life of the soil. This is evidenced by the stimulation of growth of almost all of the analyzed microbial groups. The effect, in the case of oligo- and macrotrophic bacteria, cellulolytic fungi and proteolytic bacteria was observable already only under the effect of higher doses (5 $Mg \cdot ha^{-1}$, 10 $Mg \cdot ha^{-1}$), or the highest dose (20 $Mg \cdot ha^{-1}$) of sewage sludge. Filamentous fungi and cel-

lulolytic bacteria developed at higher rates in all treatments with sewage sludge.

2. In the surface horizon of the soil (0-20 cm) there was also continued intensification of respiration, rate of cellulose mineralization, nitrification, and dehydrogenase and proteolytic activity. Processes of respiration and cellulose mineralization, and nitrification, were stimulated by almost all sewage sludge doses, i.e. 2.5 $Mg \cdot ha^{-1}$, 5 $Mg \cdot ha^{-1}$, 10 $Mg \cdot ha^{-1}$ and 20 $Mg \cdot ha^{-1}$, while a significant increase in dehydrogenase and protease activity was observable only in treatments with 5, 10 and 20% concentrations of sewage sludge. Moreover, in all the treatments with sewage sludge significant inhibition of the rate of ammonification was observed.
3. In the soil layer of 20-40 cm a certain continued effect of the sewage sludge applied 5 years earlier on the growth of the studied microbial groups was also observed. However, it was notably weaker than in the Ap horizon. Slight stimulation of the growth of macrotrophic bacteria and of filamentous and proteolytic fungi was now noted only in individual treatments. Only cellulolytic bacteria were stimulated by all the doses of sewage sludge.
4. Long-term effects of soil amendment with sewage sludge continued also in the biochemical activity of soil from the 20-40 cm layer. Those, however, were notably weaker than in the Ap horizon – an increase observed only in the respiratory activity and in the rate of cellulose mineralization, under the effect of all doses of sewage sludge.
5. Verification of the applicability of the tests used in the study showed that the most sensitive parameters reflecting the long-term effect of soil fertilization with sewage sludge on its microbiological properties turned out to be the number of cellulolytic bacteria and the rate of respiration and cellulose mineralization.
6. Taking into account that the effects of soil fertilization with sewage sludge and not short-term and continue in the 5th year from application, it is recommended to continue the experiment from both the cognitive and the practical points of view.

References

1. BARAN S., WÓJCIKOWSKA-KAPUSTA A., JAWORSKA B. Applicability of willow for sanitation of soil contaminated with lead and copper., Zesz. Probl. Post. Nauk Roln. **477**, 181, **2001** [In Polish].
2. BARAN S., DOMŻAŁ H., SŁAWIŃSKA-JURKIEWICZ A., KWIECIEŃ J., PRANAGAL J. Effect of sewage sludge on water-air properties of sandy soil. Zesz. Probl. Post. Nauk Roln. **437**, 53, **1996** [In Polish].
3. BARAN S., SŁAWIŃSKA-JURKIEWICZ A., PRANAGAL J. Morphological analysis of the structure of sandy soil ameliorated with sewage sludge. Zesz. Probl. Post. Nauk Roln. **437**, 61, **1996** [In Polish].
4. BIELIŃSKA E.J., ŻUKOWSKA G. Protease activity in light soil amended with sewage sludge. Acta Agrophysica **70**, 41, **2002** [In Polish].
5. CZEKAŁA J. Selected properties of sewage sludge from treatment plants of the Wielkopolska region. Part II. Carbon and nitrogen content if fractions of humus compounds. Acta Agrophysica **70**, 83, **2002** [In Polish].
6. DEBOSZ K., PETERSEN S.O., KURE L.K., AMBUS P.: Evaluating effects of sewage sludge and household compost on soil physical, chemical and microbiological properties. App. Soil Ecol. **19**, 237, **2002**.
7. KSIĘŻOPOLSKA A., ŻUKOWSKA G., FLIS-BUJAK M. Effect of fertilisation with sewage sludge on specific surface area of light soil and isolated from it humus acids. Acta Agrophysica **73**, 221, **2002** [In Polish].
8. SKORBIŁOWICZ M. Evaluation of sewage sludge from certain sewage treatment plants of Podlaskie district in terms of the content of fertilising substances. Acta Agrophysica **73**, 297, **2002** [In Polish].
9. WÓJCIKOWSKA–KAPUSTA A., BARAN S., JAWORSKA B., KWIECIEŃ J. Changes in selected properties of light soils amended with sewage sludge. Folia Univ. Agric. Stetin. 211 Agric. **84**, 533, **2000** [In Polish].
10. VEERESH H., TRIPATHY S., CHAUDHURI D., GHOSH B.C., HART B.R., POWELL M.A. Changes in physical and chemical properties of three soil types in India as a result of amendment with fly ash and sewage sludge. Environ. Geology **43**, 513, **2003**.
11. ŻUKOWSKA G., FLIS-BUJAK M. Changes in susceptibility to organic matter oxidation in light soil amended with sewage sludge. Folia Univ. Agric. Stetin. 211, Agric. **84**, 545, **2000** [In Polish].
12. ŻUKOWSKA G., FLIS-BUJAK M., BARAN S. Changes in the content of labile fractions of organic matter in light soil amended with sewage sludge with varied degree of transformation. Folia Univ. Agric. Stetin. 200, Agric. **77**, 429, **1999** [In Polish].
13. FURCZAK J., JONIEC J. Preliminary study of the effect of sewage sludge on the microbiological activity of a podzolic soil under willow culture. Int. Agrophisics **21**, 39, **2007**.
14. JONIEC J., FURCZAK J. Populations of selected microbial groups in podzolic soil under willow culture (*Salix viminalis* L.), fertilised with sewage sludge, in the second year of its effect. Ann. UMCS, sec. E **62**, 93, **2007** [In Polish].
15. WOLNA-MARUWKA A., SAWICKA A., KAYZER D. Size of selected groups of microorganisms and soil respiration activity fertilized by municipal sewage sludge. Polish J. Environ. Stud. **16**, 129, **2007**.
16. BARAN S., BIELIŃSKA E.,J., WÓJCIKOWSKA-KAPUSTA A. Effect of willow culture on dehydrogenase and phosphatase activity and on lead content in podzolic soil amended with sewage sludge. Folia Univer. Agric. Stetin. 211, Agric. **84**, 19, **2000** [In Polish].
17. GARCIA-GIL J.C., PLAZA C., SENESI N., BRUNETTI G., POLO A. Effect of sewage sludge amendment on humic acids and microbiological properties of a semiarid Mediterranean soil. Biol. Fertil. Soils **39**, 320, **2004**.
18. FURCZAK J., JONIEC J.: Changes in biochemical activity of podzolic soil under willow culture in the second year of treatment with municipal-industrial sewage sludge. Int. Agrophysics **21**, 145, **2007**.
19. JONIEC J., FURCZAK J.: Numbers of selected microbial groups in podzolic soil under willow culture (*Salix viminalis* L.) fertilized with sewage sludge, in the third year from its application. Ann. UMCS, sec. E **1**, 105, **2007** [In Polish].
20. JONIEC J., FURCZAK J.: Biochemical characterization of podzolic soil under willow culture after three years from its amendment with sewage sludge. Polish J. Soil Sci. **40**, 203, **2007**.

21. FURCZAK J., JONIEC J. Numbers of micro-organisms and activity of biochemical processes related with carbon transformation in podzolic soil under willow culture, in fourth year of the soil treatment with sewage sludge. Polish J. Environ. Stud. **16**, 587, **2007**.
22. JONIEC J., FURCZAK J. Counts and activity of microorganisms participating in nitrogen transformations in soil, four years after application of sewage sludge. J. Elementol. **13**, 545, **2008**.
23. BARAN S., WÓJCIKOWSKA-KAPUSTA A., MILCZAREK T. Sanitation of cadium contaminated soil with use of willow. Chem. Agricult. **4**, 461, **2003**.
24. JAWORSKA B. Remediation of properties of fallow soils using sewage sludge and willow (*Salix viminalis*). Doctoral Thesis, Inst. Glebozn. i Kształt. Środow. Przyrod. AR Lublin **2002** [In Polish].
25. BUNT J.B., ROVIRA A.D. Microbiological studies of some subantarctic soils. Soil Sci. **6**, 119, **1955**.
26. MARTIN J. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. **19**, 215, **1950**.
27. POCHON J., TARDIEUX O. Techniqes d'analyse en microbiologie du sol. Inst. Pasteur, Edit. De la Tourelle, Saint – Mande (Seine), Paris **1962**.
28. RODINA A. Microbiological methods for the study of waters. PWRiL, Warszawa, **1968** [In Polish].
29. RÜHLING A., TYLER G. Heavy metal pollution and decomposition of spruce needly litter. Oikos **24**, 402, **1973**.
30. NOWOSIELSKI O. Method for determination of fertilisation requirements. PWRiL, Warszawa, **1974** [In Polish].
31. THALMANN A. Zur Methodik der Bestimmung der Dehydrogenaseactivität in Boden mittels Triphenyltetrazolium chlorid (TTC). Landwirtsch. Forsch. **21**, 249, **1968**.
32. LADD J.N., BUTLER J.A.H. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. **4**, 19, **1972**.
33. HATTORI H., MUKAI S. Decomposition of sewage sludge in soil as affected by their organic matter composition. Soil Sci. Plant Nutr. **32**, 421, **1986**.
34. HATTORI R., HATTORI T. Sensitivity to salts and organic compounds of soil bacteria isolated on diluted media. J. Gen. Appl. Microbiol. **26**, 1, **1980**.
35. DICK R.P. Soil enzyme activities as indicators of soil quality. [In] Doran J.V., Coleman D.C., Bezdicek D.F., Stewart B.A. (Eds.): Defining soil quality for a sustainable environment. Soil Sci. Soc. Am., Am. Soc. Agric., Madison, pp. 107, **1994**.
36. KUSZA G. Effect of mineral fertilisation and application of sewage sludge on selected properties of post-limestone exploitation dumping grounds. Rocz. Glebozn. **48**, 124, **2006** [In Polish].
37. KUZYAKOV Y. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. **38**, 425, **2006**.
38. CHAZIJEW F. Ch. Systemic-ecological analysis of fermentation activity of soils. Nauka, Moskwa, **1982** [In Russian].
39. GOSTKOWSKA K., FURCZAK J., DOMŻAŁ H., BIELIŃSKA J. Suitability of some biochemical and microbiological tests for the degradation degree of podzolic soil on the background of it differentiated usage. Pol. J. Soil Sci. **31**, 69, **1998**.
40. QUILCHANO C., MARANON T. Dehydrogenase activity in Mediterranean forest soils. Biol. Fertil. Soils **35**, 102, **2002**.
41. GILLER K., WITTER E., MC GRATH S.P. Toxicity of heavy metals to micro-organisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. **30**, 1389, **1998**.
42. HERRERO O., CANET R., ALBIACH R., POMARES F. Enzymatic activities and content of mineral nitrogen in soil after the application of two rates of different organic products. Agrochimica **62**, 296, **1998**.
43. KOBUS J., CZABAN J., GAJDA A. Effect of sewage sludge on activity of degraded soils and on carbon, nitrogen, phosphorus, and zinc transformations in those soils. Pam. Puł. – Prace IUNG **96**, 121, **1990** [In Polish].
44. PASCUAL J.A., GARCIA C., HERNANDEZ T., MORENO J.L., ROS M. Soil microbial activity as a biomarker of degradation and remediation processes. Soil Biol. Biochem. **32**, 1877, **2000**.
45. ROS M., HERNANDEZ M.T., GARCIA C. Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biol. Biochem. **35**, 463, **2003**.