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Original Research

Studies on Stability and Changes in Microbiological and Biochemical Activity of Podzolic Soil under Plantation of Basket Willow after Introduction of Sewage Sludge

Jolanta Joniec*, Jadwiga Furczak

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

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Abstract

Our study was conducted on a podzolic soil from a field experiment in which, 9 years earlier, municipal-industrial sewage sludge was introduced at doses of 30, 75, 150, 300, and 600 Mg·ha^{-1}. Following the application of the sludge, the soil was planted with basket willow (*Salix viminalis* L.). Soil samples for the analyses were taken twice (at the beginning and at the end of the vegetation season of the 9th year) from depths of 0-20 cm and 20-40 cm. The soil material was used for determinations of the total numbers of bacteria with low and high nutrition requirements, the numbers of filamentous fungi, proteolytic bacteria and fungi, respiratory activity, rate of cellulose mineralization, rate of ammonification and nitrification, and of dehydrogenase and protease activity.

The results obtained demonstrated that in the 9th year from the application of the sludge there was continued effect of its application on most of the microbiological properties (exception – proteolytic bacteria) and on all of the biochemical parameters. There was notable stimulation of the growth of cellulolytic bacteria and of the fungal groups under analysis. Increased activity was also displayed by almost all biochemical parameters, with the exception of ammonification (in both layers of the soil) and nitrification (in the deeper horizon of the soil), in which case significant inhibition was observed.

Keywords: soil, sewage sludge, basket willow, micro-organisms, biochemical activity

Introduction

Sewage sludge is formed as a result of the process of sewage treatment, and its properties are related to the character of the catchment area, and to the technologies of purification and processing applied [1]. The sludge, after being subjected to refinement processes, constitutes a valuable source of organic matter and nutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, or sodium [2, 3]. Due to the chemical composition as described above, and to its valuable humus-forming properties, one of the recommended methods of utilization of sewage sludge is its application in agriculture for soil fertilization [2, 4, 5]. Application of sewage sludge in nature, including agriculture, on the one hand solves the problem of its utilization, to a certain extent, while on the other it contributes to improving soil quality. Taking into account the status of the soils of Poland which, in the opinion of Sulewska and

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

Koziara [5], in ca. 74% are classified in soil quality classes IV and VI, as well as the deficit of natural fertilizers [2], more extensive application of sewage sludge in agriculture appears to be justified. Numerous studies have shown that sewage sludge introduced in soil has a favourable effect on a number of physical [6, 7] and chemical [2, 8, 9] properties of that environment. The authors of those studies have observed, in soils amended with sewage sludge, an improvement in the aggregate and semi-aggregate structure, increased water retention and total hydraulic capacity, a decrease in soil density [6, 7], an increase in the level of available forms of phosphorus, potassium, magnesium and nitrate nitrogen, and an increase in the content of $N_{tot.}$ and $C_{org.}$ [2, 8, 9]. Due to its organic character, sewage sludge also has a positive effect on soil microorganisms which are responsible for most of the processes that take place in the soil environment [10-18]. Field experiments revealed an increase in the total numbers of bacteria and fungi, and of particular or specific microbial groups, as well as stimulation of the biochemical and enzymatic activity of soils fertilized with sewage sludge [10-18]. However, those studies have mostly been concerned with short-term [2, 6-12, 15-17] or, only a few years long-term [13, 14, 18], effects of sludge on the aforementioned properties. Therefore, a study was undertaken to determine whether the effect of sewage sludge on the microbiological status of the soil environment continued also in the 9th year from its introduction in the soil, and if yes – what was the direction and intensity of such changes.

Material and Method

The study presented here is a continuation of earlier research on that model, conducted over the initial 5 years of the experiment. The earlier research showed that the soil with an admixture of sewage sludge was usually characterized by increased populations of microbial groups under study and increased biochemical activity (except for ammonification), usually more pronounced in treatments with higher doses of the sludge [11-18]. The effect was stronger in the 0-20 cm horizon of the soil than in the 20-40 cm layer, and decreased with the passage of time. As in the 5th year of the experiment, the effect of the sludge on the microbiological and biochemical parameters under study was still observable [14]. It was deemed justified to resume the study after several more years.

The analyses were performed on the basis of the model of the field experiment set up at Końskie by the Institute of Soil Science and Natural Environment Management of the University of Life Sciences in Lublin. In that experiment, nine years earlier, municipal-industrial sewage sludge was introduced in the accumulation horizon of a podzolic soil developed from weakly loamy sand, as a one-time application, in the following doses – 30, 75, 150, 300, and 600 $Mg \cdot ha^{-1}$. Four weeks after the application of the sludge, the soil was planted with basket willow *Salix viminalis* L. and the plantation was maintained for 9 years.

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

Properties	Unit		Soil	Sludge
Grain size composition	% of fraction in mm	1-0.1	86.0	–
		0.1-0.02	7.0	
		<0.02	7.0	
pH	$1 mol \cdot dm^{-3}$ KCl		6.0	6.4
T	$mmol (+) \cdot kg^{-1}$		71.3	607.7
C-organic ($C_{org.}$)	$g \cdot kg^{-1}$		11.2	210.0
N-total (N_t)			1.4	17.9
$C_{org.}$:N_t			7.9	11.8
Cd content	$mg \cdot kg^{-1}$		0.5	6.0
Cu content			7.0	216.0
Pb content			18.6	125.0
Sum of 16 PAHs	$\mu g \cdot kg^{-1}$		43.0	3,894.0

Table 1 gives the grain size composition and certain physicochemical and chemical properties of the soil and of the sludge applied, after Baran et al. [19, 20], and Oleszczuk and Baran [21]. In accordance with the relevant Polish regulations [22], the sludge used at the time of setting up the experiment met the requirements for agricultural utilization.

Samples of the soil material were taken from the layers of 0-20 cm (Ap horizon) and 20-40 cm twice, i.e. in spring (23rd May) and autumn (28th October) in the 9th year of the plantation.

The analyses comprised determinations of the total numbers of bacteria with low nutritional requirements, on a medium with soil solution ($350 cm^3 \cdot dm^{-3}$) and K_2HPO_4; total numbers of macrophytic bacteria, on the Bunt-Rovira medium [23]; total numbers of filamentous fungi, on Martin medium [24]; the most probable number of cellulolytic bacteria, on liquid medium according to Pochon and Tardieux [25], read from McCrady's Tables; the number of cellulolytic bacteria on mineral agar covered with a circle of Whatman paper and supplemented with antibiotics according to Martin [24]; populations of protein-decomposing bacteria and fungi, on Frazier gelatine medium [26], in the case of fungi supplemented with antibiotics [24]; respiratory activity with the method of Rühling and Tyler [27]; mineralization of cellulose in 25-gram weighed portions of soil enriched with 0.5% powdered Whatman cellulose; the amount of CO_2 emitted during 20 days was studied with the method of Rühling and Tyler [27]; 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 level was determined with the Nessler method [28]; nitrification rate – in 25-gram weighed portions of soil containing 0.1% of monobasic ammonium phosphate, from which – after 7 days – nitrate ions were extracted and their level was determined with the brucine method [28]; dehydrogenases activity – with the Thalmann method [29]; protease activity – in accordance with the method of Ladd and Butler [30]; soil reaction – potentiometrically in 1 mol·dm^{-3} KCl, and soil moisture – with the gravimetric method. During the determinations of respiratory activity, cellulose mineralization, ammonification and nitrification rates, soil sample moisture was maintained at 50-60% of total hydraulic capacity, and the temperature of incubation was kept at approximately 22ºC.

All biochemical and microbiological analyses were made in three replications. The results were processed statistically with the method of analysis of variance. The significance of differences was determined with the Tukey test at p=0.05. Analysis of variance was not performed for cellulolytic bacteria, as the calculations of their numbers were made using McCrady's Tables, based on the principles of mathematical statistics.

Results and Discussion

Even 9 years after its application, the introduction of sewage sludge in the soil caused changes in the growth of most of the analyzed microbial groups in the Ap horizon (Tables 2, 3). The intensity of those changes varied with relation to the microbial group under analysis. Data in Table 2 indicate that in the period under study no greater changes took place any longer in the populations of bacteria with low and high nutritional requirements. Only in individual treatments with the sludge was there a barely observable tendency toward either stimulation or inhibition of their growth. In contrast, there was a notable continued effect of the sewage sludge in the case of filamentous fungi (Table 2). The higher doses of the sludge (150, 300, 600 Mg·ha^{-1}) continued to stimulate their growth, the rate of the simulation growing with increasing content of the sludge. Under the conditions of the experiment, the strongest response to the sewage sludge was that of cellulolytic bacteria (Table 3). Stimulation of their growth was observed under the effect of the higher doses, i.e. 150, 300, and 600 Mg per hectare, while for the dose of 75 Mg of sludge per hectare a decrease in the population of those bacteria was observed. In the case of cellulolytic and proteolytic fungi (Table 3) there was a response to all doses of sludge applied. The growth of those microbial groups was subject to stimulation that, in the case of cellulose-decomposing fungi, usually intensified with increases in the sludge dose. Only proteolytic bacteria were no longer affected by the sewage sludge applied 9 years earlier (Table 3).

Analyzing the results obtained in the 9th year, we should conclude that in the Ap horizon the effect of the sludge on the bacterial groups under study was weaker, while in the case of fungi it was usually stronger than in the 5th year from the introduction of the sludge in the soil [14].

The sewage sludge also had a long-term effect on the studied parameters of biochemical activity of the soil from the Ap horizon (Tables 4, 5). In treatments with doses of 75, 150, and 600 Mg of sludge per hectare a significant stimulation of the process of respiration was observed (Table 4). That effect, however, was no longer related to the value of the dose and its intensity oscillated around a fairly constant level. Also, the process of cellulose mineralization was subject to stimulation (Table 4), and in this case the positive effect of the sludge was observable in almost all treatments with sludge (except for the dose of 30 Mg·ha^{-1}) and intensified with increase in the sludge content. The only process that was subject to significant inhibition in almost all treatments with sludge (expect for the dose of 150 Mg·ha^{-1}) was ammonification (Table 4). It should be noted, however, that the inhibition of the rate of nitrogen mineralization was no longer related with the value of the sludge dose applied and remained at a similar level in all the treatments. Among all of the biochemical parameters under study, the strongest effect of the sewage sludge was that on nitrification (Tables 3, 4). In all treatments a notable stimulation of the process was observed, but its rate was not related to the doses of the sludge applied (Table 4). The enzymatic activity of the soil was also subject to distinct stimulation, usually intensifying with increases in the level of the sewage sludge in the soil (Table 5). That effect was the most pronounced in the case of dehydrogenases. As in the case of nitrification, all applied doses of the sewage sludge caused a significant increase in the level of that parameter. On the other hand, protease activity was significantly higher only in treatments with the higher sludge doses (150, 300, and 600 Mg·ha^{-1}) and the level of its stimulation was lower than in the case of dehydrogenases.

Noteworthy is the fact that in the case of respiratory activity, mineralization of cellulose and ammonification, the effect of the sewage sludge in Ap horizon in the 9th year was weaker than in the 5th year [14], while with the passage of time the positive effect of the sludge on the process of nitrification and on dehydrogenase and protease activity of the soil intensified [14].

In the deeper layer of the soil (20-40 cm), as in the Ap horizon, certain changes were observed in the growth of the microbial groups under study (Tables 2, 3). In the case of oligotrophic and macrotrophic bacteria there was a slight tendency toward growth in their populations (Table 2). That effect, however, was observed in a greater number of treatments than was the case in the Ap horizon. The sewage sludge also had a positive effect on the growth of filamentous fungi (Table 2), though that effect was limited only to the highest dose of the sludge, i.e. 600 Mg per hectare. The strongest effect of the sludge, as in Ap horizon, was that on cellulolytic bacteria (Table 3), whose growth was distinctly stimulated by almost all of the doses applied (30, 150, 300, 600 Mg·ha^{-1}). That effect intensified with increases in the content of the sludge in the soil and was even stronger than in the Ap horizon. The growth of cellulolytic and proteolytic fungi was also subject to stimulation in most treatments with the sludge (except for the dose of 75 Mg·ha^{-1}) (Table 3). In the case of protein-decomposing fungi, like cellulolytic bacteria, that effect was even stronger than in the 0-20 cm layer of the soil, whereas the growth of proteolytic bacteria, as in the Ap horizon, was no longer affected by the sewage sludge (Table 3).

Table 2. Total numbers of bacteria and fungi involved in carbon transformations in soil in the 9th 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	2.0	2.9	2.5		6.6	7.4	7.0		20.1	17.9	19.0	
Soil+ 1% of sludge		2.1	3.3	2.7	8.0	6.0	12.4	9.2	31.4	29.7	16.8	23.3	22.6
Soil + 2.5% of sludge		1.7	2.4	2.1	-16.0	6.6	9.3	8.0	14.3	35.1	8.8	22.0	15.8
Soil + 5% of sludge		0.7	2.3	1.5	-40.0	5.1	9.1	7.1	1.4	43.7	15.4	29.6	55.8
Soil + 10% of sludge		3.0	2.1	2.6	4.0	6.2	5.4	5.8	-17.1	55.9	25.5	40.7	114.2
Soil+ 20% of sludge		3.3	3.6	3.5	40.0	4.8	5.1	5.0	-28.6	68.7	27.1	47.9	152.1
Control soil	20-40	1.2	1.8	1.5		5.0	6.7	5.9		7.7	11.9	9.8	
Soil+ 1% of sludge		0.7	4.6	2.7	80.0	4.6	11.7	8.2	39.0	6.7	8.5	7.6	-22.4
Soil + 2.5% of sludge		1.1	1.8	1.5	0.0	4.7	7.8	6.3	6.8	6.1	6.9	6.5	-33.7
Soil + 5% of sludge		0.8	4.1	2.5	66.7	7.6	8.5	8.1	37.3	8.8	8.3	8.6	-12.2
Soil +10% of sludge		1.6	4.1	2.9	93.3	8.0	4.5	6.3	6.8	9.9	13.0	11.5	17.3
Soil+ 20% of sludge		1.4	4.2	2.8	86.7	7.4	6.7	7.1	20.3	22.8	30.0	26.4	169.4
Mean for season		1.6	3.1			6.0	7.9			26.3	15.8		
Mean for horizon		Surface: 2.4 Lower: 2.3				Surface: 6.9 Lower: 6.9				Surface: 30.4 Lower: 11.8			
LSD season		0.1				0.5				2.4			
LSD horizon		0.1				ns				2.4			
LSD horizon x dose		0.6				2.1				10.4			
LSD horizon x dose x season		0.9				3.3				16.1			

% – stimulation or inhibition by sludge; ns – no significant differences.

In the 9th year in the soil horizon in question, the effect of sewage sludge on oligotrophic and macrotrophic bacteria oscillated around a level similar to that observed in the 5th year. Filamentous fungi and cellulolytic bacteria were stimulated even more strongly with the passage of time, while the effect of the sludge on the growth of cellulolytic and proteolytic fungi was usually less pronounced [14].

The introduction of sewage sludge in the soil 9 years earlier also caused changes in the biochemical activity of the 20-40 cm layer of the soil (Tables 4, 5). Respiratory activity stimulation was observed in all treatments with the sludge, even stronger than in the Ap horizon (Table 4), whereas the rate of cellulose mineralization was stimulated significantly only under the effect of the highest dose of the waste (600 $Mg \cdot ha^{-1}$). The process of ammonification, like in the Ap horizon, was subject to inhibition (Table 4), which was already stabilized at a similar level in all the treatments. As opposed to the surface horizon of the soil, nitrification in the soil layer under discussion was also inhibited in most treatments with sludge (30, 75, 150 $Mg \cdot ha^{-1}$) (Table 4). Stimulation of the process was observed only under the effect of the highest concentration of the sludge (600 $Mg \cdot ha^{-1}$). As in the Ap horizon, the enzymatic activity was also stimulated in treatments with the sludge (Table 5). Increases in dehydrogenases activity usually oscillated around a similar level in both horizons of the soil, while proteolytic activity was stimulated to a notably stronger extent in the soil from the 20-40 cm layer.

Comparing the results obtained in the 9th year with those from the 5th year [14], we must conclude that with the passage of time, in the 20-40 cm soil layer, the effect of the sludge on most of the biochemical parameters under study was generally weakened. Only in the case of the process of ammonification and of proteolytic activity did the effect of the sludge become stronger.

In the 9th year from the application of sewage sludge almost all microbiological and biochemical parameters under study (except for nitrification) were subject to seasonal variation (Tables 2-5). The strongest stimulation of growth of oligotrophic, macrotrophic and proteolytic bacteria, and of cellulolytic fungi, was observed in autumn, while in the case of filamentous and proteolytic fungi and cellulolytic bacteria the strongest stimulation of growth was recorded in spring. Moreover, intensification of the processes of respiration, mineralization of cellulose and dehydrogenases activity was observed in spring, while autumn was the season when intensification of ammonification and protease activity were noted. The seasonal variation observed during this study in the particular microbiological and biochemical activities was probably caused by changes in the temperature and precipitation distribution during the vegetation season.

In the opinion of other authors [8, 9], sewage sludge organic matter introduced in soil undergoes significant transformations already during the initial four years. Therefore, we should assume that, under the conditions of our experiment, the organic matter introduced in the soil 9 years earlier, even though it contained mainly hard-decomposing compounds [31, 32], had already undergone mineralization and humification, causing long-term changes in the physical, physicochemical and chemical properties of the soil. The intensification of bacterial and fungal growth observed in this study (Tables 1, 2) was most likely due to lasting improvement of living conditions for those microbial groups. The stimulation of their growth was probably mainly contributed to by the permanent products of transformation of sludge organic matter that were also a source of nutrients for those microbial groups. Data given in Table 5 indicate that in treatments with the sludge there was still a certain continued improvement of soil moisture and reaction. Moreover, it is to be supposed that the sewage sludge had a favourable effect on the water-air relations in the soil under analysis. Studies by other authors indicate that under the effect of sewage sludge there is an increase in the water retention capacity of soils, aggregate or semi-aggregate structure is formed, soil density decreases and there is an increase in maximum water capacity [6, 7].

The amount of CO_2 emitted from soil and the enzymatic activity of the soil are results of activity of microbial groups inhabiting the soil environment [33-35]. Therefore, an increase in the growth of bacteria and fungi (Tables 2, 3) was reflected also in the intensification of the biochemical processes and enzymatic activity under study (Tables 4, 5). The stimulation of the processes of respiration, cellulose mineralization, nitrification, and of the dehydrogenases and protease activity of the soil was most likely contributed to, as in the case of stimulation of growth of the bacterial and fungal groups under study, by the products of transformation of sludge organic mater and by the changes they caused in the soil environment. As follows from earlier studies, the enzymatic activity and the respiration of soils depend, among other things, on their content of $C_{org.}$ and $N_{tot.}$, and on soil moisture and reaction [10, 36-40].

Nine years after the introduction of sewage sludge in the soil, the only processes that were inhibited were ammonification (in both horizons of the soil) and nitrification (in the deeper horizon) (Table 4). Noteworthy is the fact that while inhibition of ammonification was observed already in the earlier years of the experiment [11, 12, 14, 17, 18], inhibition of the process of nitrification appeared only in the 9th year (Table 4). This may be attributed to, among other things, the effect of heavy metals introduced in the soil together with the sludge (Table 1). The concentration of their biologically active forms could have increased along with the decomposition of the sludge organic matter on which they had been adsorbed. The literature review elaborated by Giller et al. [41] indicates that such elements may have an unfavourable effect on the processes in question.

The results obtained in this study indicate that the effects of a one-time application of municipal-industrial sewage sludge in the soil continued in both soil horizons as long as into the 9th year from the application. The effects of the sludge were manifested in the form of intensification of almost all analyzed microbiological and biochemical activities (with the exception of proteolytic bacteria, ammonifi-

Table 3. Populations of cellulolytic and proteolytic bacteria and fungi in soil in the 9th 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	0.2	0.9	0.6		0.5	1.2	0.9		3.2	3.1	3.2		6.8	3.7	5.3	
Soil+ 1% of sludge		0.7	0.4	0.6	0.0	1.4	1.7	1.6	77.8	2.7	3.0	2.9	-9.4	21.1	12.3	16.7	215.1
Soil + 2.5% of sludge		0.4	0.0	0.2	-63.4	2.7	0.6	1.7	88.9	2.6	3.7	3.2	0.0	12.9	5.2	9.1	71.7
Soil + 5% of sludge		3.0	0.9	2.0	228.3	2.7	2.5	2.6	188.9	2.8	3.0	2.9	-9.4	18.6	4.4	11.5	117.0
Soil + 10% of sludge		11.0	0.9	6.0	895.0	0.4	2.2	1.3	44.4	3.2	2.8	3.0	-6.4	12.2	4.8	8.5	60.4
Soil+ 20% of sludge		11.0	0.9	6.0	895.0	3.8	2.4	3.1	244.4	2.2	3.0	2.6	-18.8	9.2	17.4	13.3	150.9
Control soil	20-40	0.1	0.1	0.1		0.6	0.8	0.7		1.9	2.9	2.4		3.8	2.1	3.0	
Soil+ 1% of sludge		0.1	0.4	0.3	188.9	1.1	1.1	1.1	57.1	1.6	2.7	2.2	-8.3	11.1	4.2	7.7	156.7
Soil + 2.5% of sludge		0.1	0.1	0.1	33.3	0.6	0.3	0.4	-42.9	1.6	3.1	2.4	0.0	2.9	4.7	3.8	26.7
Soil + 5% of sludge		1.5	0.2	0.9	866.7	0.6	1.4	1.0	42.9	1.9	3.2	2.6	8.3	8.6	7.6	8.1	170.0
Soil +10% of sludge		1.5	0.4	1.0	977.8	0.7	1.7	1.2	71.4	1.9	3.3	2.6	8.3	9.7	3.7	6.7	123.3
Soil+ 20% of sludge		2.0	0.4	1.2	1,255.6	1.8	2.4	2.1	200.0	2.2	2.9	2.6	8.3	13.1	16.7	14.9	396.7
Mean for season		2.6	0.5	1.5		1.4	1.5			2.3	3.1			10.8	7.2		
Mean for horizon		Surface: 2.6 Lower: 0.6				Surface: 1.8 Lower: 1.1				Surface: 2.9 Lower: 2.4				Surface: 10.7 Lower: 7.4			
LSD season						0.1				0.3				0.7			
LSD horizon						0.1				0.3				0.7			
LSD horizon x dose						0.3				ns				3.0			
LSD horizon x dose x season						0.5				ns				4.6			

% – stimulation or inhibition by sludge; ns – no significant differences.

Table 4. Activity of selected processes related with carbon and nitrogen transformations in soil in the 9th year of the experiment.

Treatments	Depth, cm	Respiratory activity, mg $C\text{-}CO_2$·kg-1 d.m. of soil·d^{-1}				Cellulose mineralization, mg $C\text{-}CO_2$·kg-1 d.m. of soil·$20d^{-1}$				Ammonification, mg $N\text{-}NH_4$·kg^{-1} d.m. of soil·$3d^{-1}$				Nitrification, mg $N\text{-}NO_3$·kg^{-1} d.m. of soil·$7d^{-1}$			
		Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%
Control soil	0-20	466.7	223.4	345.1		2,666.7	2,369.4	2,518.1		133.0	196.3	164.7		10.0	24.0	17.0	
Soil+ 1% of sludge		488.7	246.0	367.4	6.5	2,736.4	2,635.3	2,685.9	6.7	132.8	127.0	129.9	-21.1	39.2	48.9	44.1	159.4
Soil + 2.5% of sludge		664.6	227.2	445.9	29.2	3,367.7	2,753.9	3,060.8	21.6	125.4	123.5	124.5	-24.4	84.3	47.7	66.0	288.2
Soil + 5% of sludge		697.3	212.0	454.7	31.6	3,837.6	2,553.8	3,195.7	26.9	116.7	164.2	140.5	-14.7	99.4	57.5	78.5	361.8
Soil + 10% of sludge		586.5	186.2	386.4	12.0	4,220.7	2,977.0	3,598.9	42.9	131.2	127.1	129.2	-21.6	38.9	42.8	40.9	140.6
Soil+ 20% of sludge		629.0	254.2	441.6	28.0	4,698.8	2,921.0	3,810.0	51.3	120.1	135.0	127.6	-22.5	54.9	38.3	46.6	174.1
Control soil	20-40	204.7	194.5	199.6		2,046.8	2,370.7	2,208.8		139.3	235.3	187.3		28.1	19.2	23.7	
Soil+ 1% of sludge		343.1	202.2	272.7	36.6	2,183.3	2,539.3	2,361.3	6.9	141.3	240.9	191.1	2.0	6.3	14.0	10.2	-57.0
Soil + 2.5% of sludge		330.45	200.5	265.5	33.0	2,168.9	1,686.8	1,927.9	-12.7	136.3	134.9	135.6	-27.6	7.6	14.5	11.1	-53.2
Soil + 5% of sludge		377.3	245.5	311.4	56.0	2,496.0	2,487.9	2,492.0	12.8	151.2	133.0	142.1	-24.1	5.9	26.5	16.2	-31.6
Soil +10% of sludge		283.9	257.8	270.9	35.7	2,347.5	2,163.7	2,255.6	2.1	132.3	134.9	133.6	-28.7	16.0	36.5	26.3	11.0
Soil+ 20% of sludge		438.6	279.2	358.9	79.8	3,041.9	2,647.7	2,844.8	28.8	133.2	122.8	128.0	-31.7	30.0	36.6	33.3	40.5
Mean for season		459.2	227.4			2,984.4	2,508.9			132.7	156.2			35.1	33.9		
Mean for horizon		Surface: 406.8 Lower: 279.8				Surface: 3,144.8 Lower: 2,348.4				Surface: 136.0 Lower: 153.0				Surface: 48.8 Lower: 20.1			
LSD season		14.7				101.8				7.8				ns			
LSD horizon		14.7				101.8				7.8				1.4			
LSD horizon x dose		61.6				427.2				32.7				6.0			
LSD horizon x dose x season		95.2				660.9				50.5				9.3			

% – stimulation or inhibition by sludge; ns – no significant differences.

Table 5. Enzymatic activity and selected physical and physicochemical properties of soil in the 9th 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, pH_{KCl}	
		Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn	Mean for year	%	Spring	Autumn
Control soil	0-20	3.0	3.2	3.1		4.9	17.9	11.4		2.3	1.3	1.8		6.5	6.0
Soil+ 1% of sludge		5.0	5.3	5.2	67.7	9.0	15.5	12.3	7.9	2.8	1.2	2.0	11.1	6.7	6.1
Soil + 2.5% of sludge		6.6	4.4	5.5	77.4	5.7	21.7	13.7	20.2	2.6	0.9	1.8	0.0	6.8	6.9
Soil + 5% of sludge		6.1	6.6	6.4	106.5	8.3	22.1	15.2	33.3	3.0	1.6	2.3	27.8	7.0	6.8
Soil + 10% of sludge		13.1	6.6	9.9	219.4	9.1	20.1	14.6	28.1	3.4	1.1	2.3	27.8	6.8	6.8
Soil+ 20% of sludge		12.3	6.0	9.2	196.8	14.4	18.9	16.7	46.5	3.4	1.0	2.2	22.2	6.7	6.8
Control soil	20-40	1.3	1.7	1.5		2.7	9.3	6.0		1.6	1.3	1.5		6.1	6.1
Soil+ 1% of sludge		2.0	1.7	1.9	26.7	0.9	15.6	8.3	38.3	1.9	1.1	1.5	0.0	6.2	6.4
Soil + 2.5% of sludge		2.3	3.4	2.9	93.3	0.4	22.7	11.6	93.3	2.2	1.0	1.6	6.7	6.4	6.4
Soil + 5% of sludge		2.7	2.9	2.8	86.7	1.9	19.6	10.8	80.0	2.3	1.4	1.9	26.7	6.4	6.2
Soil +10% of sludge		1.8	5.0	3.4	126.7	4.0	22.9	13.5	125.0	2.3	0.9	1.6	6.7	6.5	6.7
Soil+ 20% of sludge		3.7	8.6	6.2	313.3	4.7	22.5	13.6	126.7	2.4	0.8	1.6	6.7	6.9	6.6
Mean for season		5.0	4.6			5.5	19.1			2.5	1.1				
Mean for horizon		Surface: 6.5 Lower: 3.1				Surface: 14.0 Lower: 10.2									
LSD season		0.2				0.6									
LSD horizon		0.2				0.6									
LSD horizon x dose		1.0				2.5									
LSD horizon x dose x season		1.5				3.8									

% – stimulation or inhibition; ns – no significant differences.

cation and – partially – nitrification). Increased activity of soil microorganisms after many years of soil enrichment with organic matter has also been observed by other authors [42, 43]. In the course of 9 years of the experiment, the organic matter introduced in the soil had already undergone the processes of mineralization and humification, enriching the soil in products of that transformation and improving the chemical, physical, and physicochemical properties of the soil environment. This was reflected in the continued intensification of growth and biochemical activity of soil microbial groups.

Conclusions

1. The effects of introduction, 9 years earlier, of sewage sludge to the soil were still observable in the Ap horizon. They were manifested in the form of stronger growth of most of the microbial groups studied, especially of fungi, and of stimulation of biochemical activity. In the case of bacteria the effect was clearly observable only in relation to cellulolytic bacteria, while almost all of the sludge doses applied significantly stimulated the fungal groups under analysis, i.e. filamentous, cellulolytic and proteolytic fungi. Respiration, mineralization of cellulose, nitrification, dehydrogenases and protease activity of the soil were also stimulated in almost all sludge treatments. That stimulating effect was the strongest in the case of nitrification and dehydrogenases activity. Only ammonification was still subject to inhibition.
2. In the deeper horizon of the soil there were also certain continued changes in the growth of the microbial groups under study and in biochemical activity. Among bacteria, the strongest stimulation of growth was observed in the case of cellulolytic bacteria. In the case of the total counts of oligotrophic and macrotrophic bacteria there was only a slight, though statistically significant, tendency toward stimulation of their growth. A favourable effect of sludge on the growth of filamentous fungi was found only in the treatment with the highest dose of the sludge, while the numbers of celulolytic and proteolytic fungi were greater in almost all treatments with the sludge (except for the 75 $Mg \cdot ha^{-1}$ dose). All of the sewage sludge doses applied stimulated the process of respiration and dehydrogenases and protease activity. The highest dose (600 $Mg \cdot ha^{-1}$) also had a stimulating effect on the rate of cellulose mineralization and on the process of nitrification. In almost all sludge treatments (75, 150, 300, 600 $Mg \cdot ha^{-1}$) inhibition of ammonification was observed, and in some treatments (30, 75, 150 $Mg \cdot ha^{-1}$) – also inhibition of nitrification.
3. Analyzing the long-term effects of sewage sludge on the soil, the microbiological and biochemical properties under study, it should be concluded that the count of cellulolytic bacteria, the rate of nitrification and the dehydrogenases activity of the soil proved to be the most sensitive indicators in this respect.
4. The results obtained indicate that the effects of agricultural application of sewage sludge in the production of industrial crops are not short-term and continue even in the 9th year from the introduction of the waste in soil. In the case of most of the microbiological and biochemical parameters under analysis, those effects had a favourable character. Our study indicates the necessity of conducting long-term monitoring programs for soils on which sewage sludge is applied.

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