Polish Journal of Microbiology
2013, Vol. 62, No 4, 445–452

SHORT COMMUNICATION

The Influence of the Organic Matter of Sewage Sediments on Biological Activity of Microorganisms which Carry out the Transformations of Carbon and Nitrogen Compounds

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Submitted 20 February 2012, revised 10 September 2013, accepted 18 September 2013

Abstract

Soil microorganisms play an important role in the organic matter transformation process. The soil microorganisms also are in symbiotic relationship with plants. At the same time, soil microorganisms are sensitive to both anthropogenic and natural habitat changes. Particular characteristics of organic matter (the C:N relation, pH, the content the content of assimilated nutrients, the xenobiotics etc.) modify the biotic conditions of the soils. This particularly concerns the microorganisms which carry out the changes in the mineral and organic nitrogen compounds and the transformation of the external organic matter. The first aim of this work was to assess the influence of the sewage sediments and the manure on the phytosanitary potential of the soil environment. The second aim of this article was to estimate the number and activity of microorganisms which carry out the transformation of carbon and nitrogen compounds. This work showed the stimulating effect of the external organic matter both on the number and on the activity of most of the physiological groups. The manure mainly stimulated ammonificators, amylolitic microorganisms and *Azotobacter* sp. The sewage sediments mainly stimulated ammonificators, nitrifiers of I phase and cellulolytic microorganisms. The statistically significant impact of the physio-chemical soil habitat on the biological activity of the analyzed groups of microbes was also noted.

Key words: biological activity, microorganisms, nitrogen fixation, organic matter

The content of organic matter determines the significant fertility rate and the usefulness of the soil. In scientific literature (Fernandes *et al.*, 2005; Pisarek, 2007; Pisarek *et al.*, 2012, Wilkinson *et al.*, 2003), a reduction in the amount of humic compounds was often observed. This reduction may indicate unfavorable changes in the balance of the soil carbon and nitrogen compounds. External organic matter in the form of sewage sediments added into the soil could bring many advantages to the soil environment. Those involved in the agricultural use of these materials, however, must also take into account the need to control and eventually reduce the pollution and toxic components which could be accidentally included in the trophic chain. Farm use of manure and sewage sediments is one of the most economical form of their utilization (Lee and Liu, 2002; Wang *et al.*, 2004). There is a high content of assimilated forms of nitrogen and phosphorus compounds in sewage sediments. Thus, sediments are very helpful for agricultural use an as valuable soil conditioners (Pisarek, 2007; Pisarek *et al.*, 2012; Sohaili *et al.*, 2012; Wilkinson *et al.*, 2003). Injecting sewage sediments into the soil might also cause changes in the number of microorganisms or in the intensity and direction of the physiological processes carried out by the microorganisms (Akiyama *et al.*, 2004; Jezierska-Tys *et al.*, 2004; Joniec and Furczak 2012; Li *et al.*, 2009; Piontek and Lonc, 2000). The biomass of the soil microorganisms is only a little bit more than 5% of the organic matter in the soil. Nonetheless, the microorganisms have a big impact on the quality of the soil and the productivity of the ecosystems. Microorganisms release hundreds of different kinds of enzymes into the soil environment. In the transformation of the external organic matter, the most important enzymes are the ones, which participate directly in the degradation of the lignin and cellulose degradation, and the enzymes which take part in the mineralization and the circulation of nitrogen, phosphorus and sulphur. The degradation of polymers requires multi-component enzymatic systems which are produced by various groups of organisms. Nitrogen, which is accumulated in the soil (no matter what the

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Table I
Some properties of used materials.

Material	d.m.	OM	Total forms of					pH_{H2O}	Salinity (g KCl·kg^{-1})
			N	P	K	Mg	Ca		
	%		g·kg^{-1}						
SS	24.0	27.56	11.80	11.7	1.6	1.1	29.1	6.8	6.00
CM	26.8	61.70	22.50	12.6	15.0	6.0	10.6	8.3	12.30
S	n.d.	2.42	1.07	0.94	2.3	1.0	3.4	6.4	0.17

d.m. – dissolved matter; n.d. – not determined; OM – organic matter

source is), should be used for plant biomass production and keeping the soil in the state of high fertility. Some authors (Akiyama *et al.*, 2004; Barabasz and Vorisek, 2001) emphasize that often microbiological and biochemical processes occurring in soil environments outweigh the purely chemical reactions. The microorganisms within all the ecosystems are an indispensable biochemical factor. This biochemical factor is responsible for the totality of the transformation of biogens in the soil environment. The microorganisms also form the biological activity of biogens. How these transformations in the soil environment take place depends on many ecological and physiochemical elements (pH, soil capacity, content of macro and microelements, amount of organic carbon, the presence of xenobiotics etc.).

The first aim of this article was to evaluate how sewage sediments influence the phytosanitary potential of the soil environment. The second aim was to assess the activity of microorganisms which carry out the transformations of carbon and nitrogen compounds.

The experiment was conducted on agricultural land of the plant experimental station in Glubczyce. The experiment was carried out using the randomized block design method on the Cambisol. Used Cambisol are developed from silts clay. In the Ap layer, the content of the floated parts (< 0.02 mm diameter) was 50%, and the content of clay (< 0.002 mm) was 18%.

The whole experiment consisted of 3 objects, and 4 repetitions: C – soil without organic fertilization (as the control); S+SS – soil with a dose or sewage sludge 30 Mg·ha^{-1} (7.2 Mg·ha^{-1} d.m.); S+CM– soil with a dose of manure 30 Mg·ha^{-1} (8,1 Mg·ha^{-1} d.m.). Sewage sludge and manure were used once. Mineral fertilization and the appropriate agro-techniques were used according to the requirements of peas, as peas were cultivated on these fields. One marked "S" soil sample was collected from the whole field (during autumn, before start of experiment) on which the experiment was carried out. The results of the experiment are the arithmetic mean of the four repetitions.

Sewage sediments stored on stabilization pond from the Opole Wastewater Treatment Plant were used in the research project. Cattle manure from a farm in Głubczyce was also used in this study (properties of used materials presented in Table I).

The soil samples were collected during spring (May) from the Ap level of the experimental plots. The following analyses were done on the collected soil samples:
- pH in KCl;
- salinity based on conductivity of aqueous extracts (5:1) with the conductometric method;
- the content of macronutrients:
- total nitrogen content with the method of Kjeldahl (PN-ISO-11261, 2002),
- phosphorus content by PN-R-04023 DL (1996),
- potassium content by PN-R-04022 DL (1996),
- magnesium content by PN-R-04020 DL (2004);
- the content of micronutrients in 1M HCl by AAS method (Philips PU 9100X);
- the content of organic carbon by the Tiurin's method (PN-ISO-14235, 2003).

Statistic analyses (LSD) was carried out with the Statistica program.

Microbiological analyses included the designation of the number of: ammonificators on the liquid medium with peptone (PN C-04615-18), nitrifiers of phase I (NfI) and phase II (NfII) on Winogradsky medium (PN C-04615-20), bacteria fixing the non-symbiotically nitrogen. The nitrogen is from the following: *Azotobacter* sp. on the Fenglerowa medium (Pfeiffer-Maliszewska 1974), cellulolytic bacteria (Petrycka 1993), amylolitic microorganisms on the medium which contain starch (Pochon Tardieux 1962).

The examined groups of microorganisms were determined by inoculating the appropriate media with volumes in the range 0.1–1.0 ml coming from different tenfold serial dilutions. All different microorganisms were estimated by the standard dilution-plating procedure, with the exception of ammonificators, nitrifiers of phase I and phase II and cellulolytic microorganisms, which were determined by the Most Probable Number (MPN). The MPN was calculated using McCrady's Tables, for three parallel repeats. The general number of amylolitic bacteria and bacteria fixing nitrogen non-symbiotically from the *Azotobacter* sp., was shown in cfu/g d.m of soil. The culturing process of ammonificators microorganisms took 14 days. The culturing of nitrifiers micro organisms took 21 days (readings every 2 days). Culturing cellulolytic microorganisms took 7–14 days and amylolitic microorganisms took 3–5 days. The *Azotobacter* sp. was cultured for 7 days. All microorganisms were kept at a temperature of 28 ± 2°C. Cultures were carried out in Petri dishes or in test tubes (laboratory conditions).

The potential activity of the ammonification process (Dn), and the bonding of nitrogen was checked with Nessler's reagent. The phase I of nitrification process was checked with Griess-Issolvay's reagent to find out how active the process was. The activity of the phase II nitrification process was checked using diphenylamine. The activity indicators were defined based on the intensity of the color change (2–3 degree scale). The color change was based on the reagents used and the modified Pochon method (Pfeiffer-Maliszewska 1974, Pochon Tardieux 1962).

All the readings were checked three times. The statistic calculations were subjected to variance analyses using Duncan's test.

The organic materials used in the experiment were sewage sediments and manure. These materials had different hydration levels, and different levels of basic biogenic components. The analysis of the content of these components in the manure and sewage sediments showed that manure had more organic matter and more of the analyzed micro components (except calcium) and organic matter. The micro element quantitative analysis indicated a high content of calcium and phosphorous in the sewage sediment added to the soil. The amount of nitrogen, magnesium and potassium was average (Baran and Turski 1996, Mazur 1999). pH of the sewage sediment was neutral. The salinity of the sewage sediment reached 6,00 g KCl · kg^{-1}. The used sewage sediments in the experiment was characterized with a low (low within the range of the values characteristic for sewage sediments) content of organic matter. Organic matter was 27.56%. The manure used in the experiment was described with a high (high for this kind of manure) content of phosphorus, nitrogen and magnesium and an average content of potassium and calcium (Baran and Turski 1996, Pisarek 2007). pH of the manure was alkaline. The salinity of the manure reached 12,30 g KCl · kg^{-1}. The amount of organic matter in the manure was high (61.70%).

Organic fertilization in the form of sewage sediments or manure has a relevant impact not only on the chemical or physical properties of the soil but also on the biological properties (Grata and Krzyśko-Łupicka 2005, Joniec and Furczak 2012, Pisarek 2007, Wolna-Murawka *et al.,* 2007). Various changes can affect the number and activity of the microorganisms and can also affect the direction carried out by microbes. These changes include: those of pH of the soil, changes in the relations between nitrogen and carbon, changes in the quantity and quality of the organic matter, and the change in the sediment or manure doses (Fernandes *et al.*, 2005, Kavadia *et al.*, 2007, Wolna-Murawka *et al.,* 2007). Some researchers (Singh and Agrawal 2008, Wong *et al.*, 1998) showed that the sewage sediments can significantly lower the pH level of the fertilized soil by nitrification or by producing organic acid. After the external organic matter underwent transformation for 6 months, there was no change in the reaction of the soil but the amount of pH_{KCl} showed that all the analyzed soils from the experimental plots were slightly acidic (Table II).

The assessment of the assimilated content forms of phosphorus (based on the border number PN-R-04023 DL) showed that phosphorus content in the soil remained high such soil is considered I-st class. The added organic matter had no influence on the assimilated phosphorous. Those plots of soil which had been enriched with manure showed a 118 mg · kg^{-1} increase of P. The assessment of the available potassium in the soil according to the border numbers (PN-R-04022 DL), indicated a high content of potassium (II-nd class) in all soils. The content of assimilated magnesium in the soils of the organically fertilized experimental plots showed (statistically unimportant) differentiation. The

Table II
Some chemical properties of soil in the plots.

Objects	pH_{KCl}	Macronutrient			Micronutrient			
		P	K	Mg	Fe	Mn	Cu	Zn
		mg · kg^{-1}			mg · kg^{-1}			
S	6.4	94	226	85	1900	237	7.9	18
S+SS	6.4	98	215	58	1700	257	7.8	17
S+CM	6.4	118	278	74	1900	311	7.5	18
C	6.4	94	216	59	1700	239	7.9	16
LSD	n.s.	n.s.	49	n.s.	n.s.	17	n.s.	n.s.

n.s. – not significant

Table III
The content of total forms of nitrogen and organic carbon, and the value of the C:N ratio.

Objects	C	N	C:N
	g · kg^{-1}		
S	9.80	1.07	9.1
SS	163.90	11.8	13.9
CM	356.10	22.5	15.8
S+SS	14.50	1.41	10.3
S+CM	15.20	1.43	10.6
C	11.70	1.19	9.8
LSD (between objects with soil)	4.6	0.31	0.88

soils were thus divided into two categories: soils with an average abundance (S, CM) and soils with a low abundance (S+SS, C) (PN-R-04020 DL). The number of the assimilated forms of the analyzed microelements (Mn, Cu, Zn, Fe) indicated they occur in average amounts. The experiment confirmed the significant influence of the injected sewage sediments on the level of manganese in the experimental soils.

Following the enrichment of the soil with sewage sediment and manure, there was a statistically significant increase in the content of nitrogen and organic carbon noted in some of the experimental plots (Table III). On object C, the increase of carbon and nitrogen was statistically non-significant and probably associated with microbes and with the transformation of the secretions of the cultivated plants (*Pisum sativum*). MAs it results from many researchers (Colnaght *et al.*, 1997, Wanic and Nowicki 2000) have shown that cultivation of fabaceae significantly affects the biologic life of the soil, the biochemical processes within the soil, and the sanitary state of the soil.

The value of the C:N ratio in the soils show the content of humus in the nitrogen. The organic sewage sediment and manure added to the soil had a wider range of C:N (13.9 for SS; 15.8 for CM). According to Smith (1996), this wide range in the sewage sediment and manure, points to an average and low susceptibility to mineralization processes. As far as the speed and type of the processes are concerned, Griffin *et al.* (2002) and Smith (1996) assumes that nitrogen in manure can be temporarily fixated during immobilization.

The analysis of the starting material shows that the number of ammonificators in the soil, in the sediment, and in the manure was comparable. However the intensity of this process was much higher in the manure. The manure had a smaller amount of phase I nitrifiers and the intensity of the nitrification process was also lower. However, in the manure and in the sediment, the number of phase II nitrifiers and the intensity of the nitrification process was similar but much higher than in the non-fertilized soil (Fig. 1, 2).

Sewage sediments and manure, due to the considerable amount of nitrogen they have, the presence of organic matter, and their fertilization use, can influence the microbiologic transformation of nitrogen soil, and

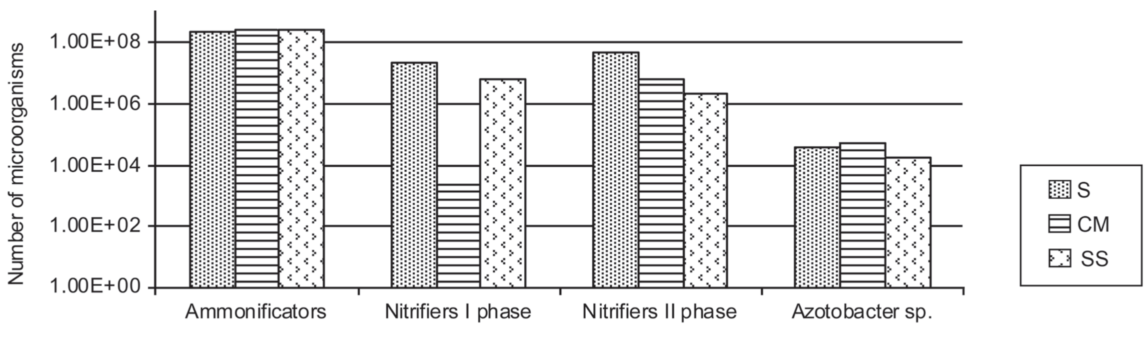

Fig. 1. Number of different groups microorganisms in the starting material expressed in MPN/ g d.m. (ammonificators, nitrifiers) and in cfu/g d.m (*Azotobacter* sp.)

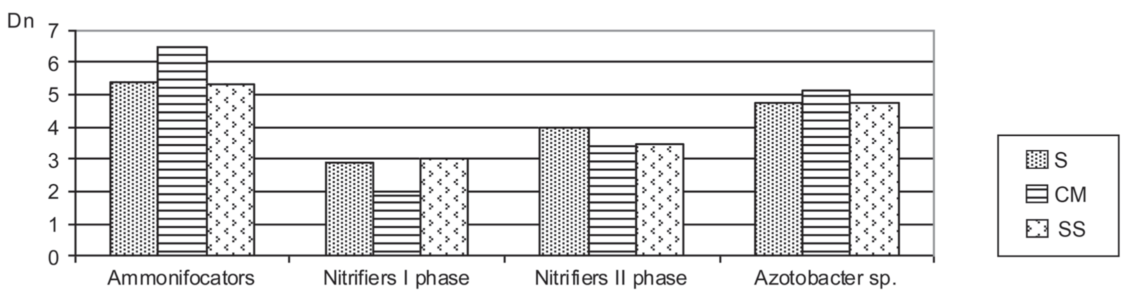

Fig. 2. Potential activity of different groups microorganisms in the starting material

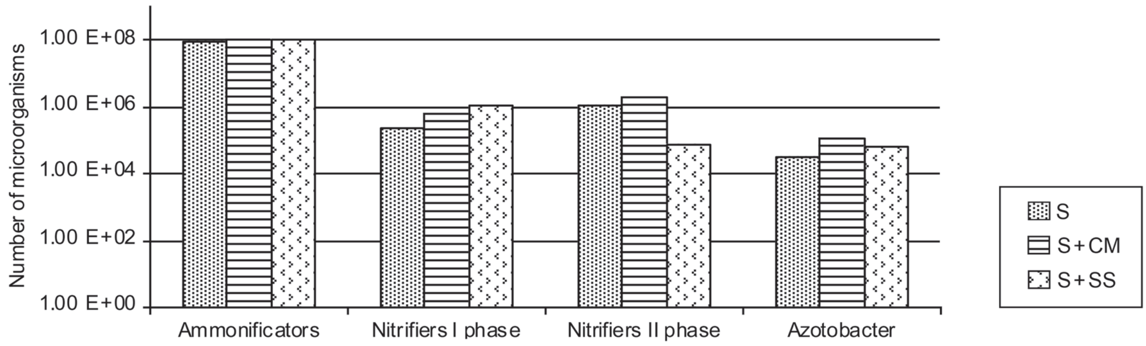

Fig. 3. Number of different groups microorganisms in the treated soil expressed in MPN/ g d.m. (ammonificators, nitrifiers) and in cfu / g d.m (*Azotobacter* sp.)

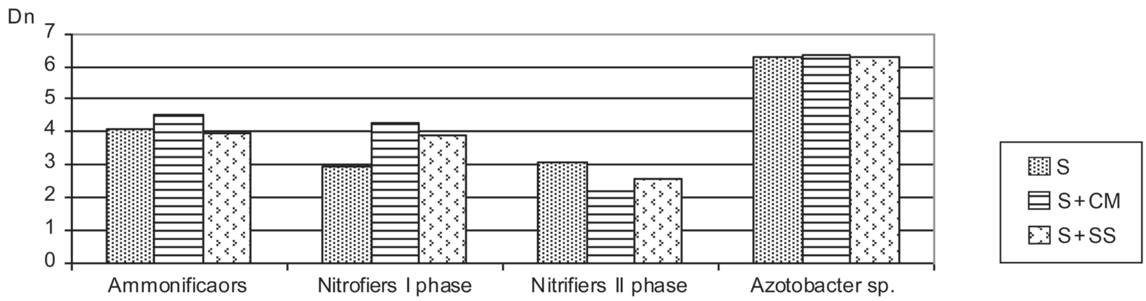

Fig. 4. Potential activity of different groups microorganisms in the treated soil

can influence the number of ammonificators, nitrifiers microorganisms or the microorganisms which fixate the nitrogen (Fernandes *et al.*, 2005, Grata and Krzyśko-Łupicka 2005, Piontek and Lonc 2000). The nitrogen present in the organic substance is transformed in the process of ammoniafication to the form NH^{4+}. This form is collected by the plants or easily undergoes changed or unchanged sorption and the NH^{4+} also remains in the soil solution. The NH^{4+}, however, is not stable. As a result of nitrogen assimilation together with the microorganisms from the "Nitroso" group, the NH^{4+} undergoes oxidation and first becomes nitrites (NO^{2-}) and then becomes nitrates (NO^{3-}). This is why finding out the amount and the activity of the mentioned microorganisms in the soil is so important (Sorensoen 2001).

Fertilizing with sewage sediment and manure slightly increased the amount of ammonificators (about 19%) compared to non-fertilized soil (Fig. 3). The amount of these microorganisms was about $1.11–1.39 \times 10^8$ cfu/g d.m. Fertilizing with only manure led to the intensification of the ammonification process compared to non-fertilized soil and soil fertilized with just sediment.

Our research also shows, that the sewage sediment stimulated the development of the nitrifiers of phase I in the soil. The manure stimulated the development of nitrifiers of phase II (Fig. 3). After adding the manure and the sewage sediment to the soil, the nitrification process of phase I was noted to be significantly higher in intensity but there was an impairment of the phase II (Fig. 4). Jezierska-Tys *et al.* (2004) noted the sediment had a stimulating impact on the amount of the nitrifiers but simultaneously a inhibitory impact on the intensity of the processes carried out by the nitrifiers. If there is an excess of organic matter, a decrease in the speed and in the efficiency of the nitrification can take place without any changes in the amount of the nitrifiers as the nitrifiers contains a lot of $N\text{-}NH^{+4}$. Such a large amount can inhibit this process (Lopez-Valdez *et al.*, 2010; Strzelec and Kobus, 1997). In an environment containing readily soluble organic substances, the nitrifiers bacteria can survive in an inactive state (Strzelec and Kobus, 1997). The intense development of the nitrifiers could be caused by the activity of the ammonificators. The reason is, that this development is the strongest in a slightly alkaline reaction and an environment which is rich with ammonium nitrogen (Johanson *et al.*, 1999; Lopez-Valdez *et al.*, 2010). When comparing the processes of ammonification and nitrification, both the amount and the intensity of the ammonification process were found to be higher than the amount of the nitrifiers in both phases and the intensity of the nitrification process. A smaller amount of the nitrifiers in relation to the ammonificators is beneficial for the of environment. The nitrate nitrogen formed in the process of nitrification is a less stable compound than ammonium nitrogen. The nitrate nitrogen can easily be washed out from the soil and find its way into ground water and surface water. Plants can also

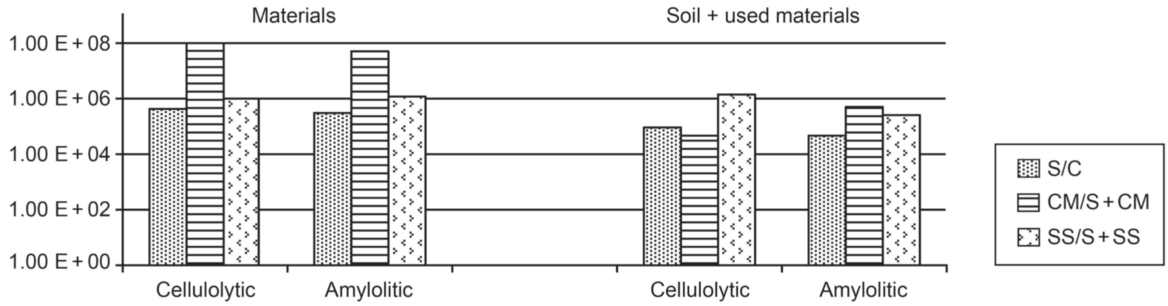

Fig. 5. Number of cellulolytic microorganisms expressed in MPN/g d.m. and amylolitic microorganisms in cfu /g d.m

accumulate nitrate nitrogen which could cause a threat to both humans and animals (Kobus 1996).

Our results did not show a significant relationship between the amount of the ammonificators and nitrifiers, and the intensity of both processes. Jezierska-Tys *et al.* (2004), and Szostak *et al.* (2005) also did not notice a dependence between the amount of the discussed microorganisms and the intensity of the processes which the microorganisms undertake.

The quantitative and qualitative composition of the organic matter, the value of the C:N ratio, the level of sorption, the content of heavy metals, and the content of ammonium nitrogen can significantly affect the microorganisms fixating the atmospheric N (Grata and Krzyśko-Łupicka 2008, Martensson and Torstensson 1996). These kinds of impacts involve the inhibition of the nitrogenase activity, the synthesis of the nitrogenase (inhibition of the formation of the glutamine synthetase), and the reduction in the diazotroph population (Colnaghi *et al.,* 1997; Kavadia *et al.*, 2007; Tsagou *et al.,* 2003).

The research on the microorganisms fixating N_2 non-symbiotically, showed that the amount of *Azotobacter* sp. was significantly higher in the soil (S) ($4{,}0 \times 10^4$ cfu/g d.m) and in the manure (CM) ($5{,}0 \times 10^4$ cfu/g d.m), than in the sediment (SS) ($1{,}66 \times 10^4$ cfu/g d.m). It is important to note, that an intense fixation process of N_2 took place only in the manure (CM) (Fig. 1, 2). After applying the manure, there was a significantly higher amount of *Azotobacter* sp. in the soil (S+CM) ($12{,}0 \times 10^4$ cfu/g d.m) compared to the soil fertilized with sewage sediment (S+SS) ($6{,}66 \times 10^4$ cfu/g d.m) and compared to non-fertilized soil (C) (3.1×10^4 cfu/g d.m) (Fig. 3). However, the fixation process of N_2 took place with the same intensity in all the variants, with only a slight, not statistically significant predominance in the soil which had been enriched with manure (S+CM) (Fig. 4). The analysis of cellulolytic microbes showed more of these microbes in the manure (CM) and in the sediment (SS) ($1{,}18 \times 10^8$ cfu/g d.m), than in the soil (S) ($5{,}49 \times 10^5$ cfu/g d.m). After fertilizing, the soil with sediment (S + SS) ($2{,}35 \times 10^6$ cfu/g d.m) showed the most microbes. The soil with manure (S + CM) ($5{,}44 \times 10^4$ cfu/d s.m) showed the least amount of microbes, as can be seen in Fig. 5.

The number and activity of this group of microorganisms may be related to content of organic matter available, the dose of fertilizer, while the coeficient of transformation of carbohydrates in the soil depends on the structure of the soil, pH, and degree of contamination of the environment. Therefore, results obtained by various investigators are differ. There was a fluctuations or an increase in their numbers, especially after high doses of organic matter (Mazur 1999, Wolna-Murawka *et al.,* 2007).

The analysis of the amylolitic microorganisms amount showed that manure (CM) and the sediment (SS) had 10–100 times more microorganisms than the soil (S) ($2{,}9 \times 10^5$ cfu/g d.m). Manure had more amylolitic microorganisms and sediment had less. Adding organic matter to the soil in the form of manure and sewage sediment, resulted in a 10 fold increase in the amount of this group of microbes, especially in the S+CM ($5{,}33 \times 10^5$ cfu/g d.m) combination, compared to non-fertilized soil (C) ($4{,}3 \times 10^4$ cfu/g d.m) (Fig. 5).

Conclusion. Microorganisms and microfauna together with the vegetation are considered to be the factors which determine the direction of the biochemical and chemical changes occurring in the soil environment. The microorganisms and microfauna also form the biological activity of cultivated soils and models the physicochemical properties of the soil (Dumontet *et al.*, 2001, Korniłłowicz-Kowalska and Bohacz 2002, Stuczyński *et al.*, 2003, Wiegand *et al.*, 2004). The microorganisms which have a metabolism ability to transform organic compounds play a significant role in the previously mentioned processes. The organic materials added to the soil are mainly used by the microorganisms as a source of carbon. Later, the microorganisms use the added organic materials injected in the soils (after the initial decomposition) as a source of nitrogen and other components. In the first stage of the transformation process of organic matter, a growth in the general number of microorganisms takes place. In the following phases, the presence of specialized strains of microbes is

Table IV
Correlation coefficients between some parameters

Feature (X)	Feature (Y)	rk
Ammonificators	P	0.923
	K	0.975
	Fe	0.610
	Mn	0.912
	Zn	0.612
	Cu	–0.911
Nitrifiers I phase	P	0.812
	K	0.601
	Fe	n.s.
	Mn	0.881
	Zn	n.s.
	Cu	–0.871
Nitrifiers II phase	P	–0.870
	K	–0.688
	Fe	n.s.
	Mn	–0.923
	Zn	n.s.
	Cu	–0.921
Azotobacter sp.	P	0.987
	K	0.961
	Fe	n.s.
	Mn	0.945
	Zn	n.s.
	Cu	–0.991

n = 9
significantly p = 0,001 (above 0.783)
significantly p = 0,05 (above 0.600)

noted. Some properties of the organic matter, especially the chemical composition, can significantly modify the biotic conditions of the soil (Golueke 1995, Lee and Liu 2002). Research shows, that adding dehydrated sewage sediment stimulates the biological activity of microorganisms, especially thermophilous (Nakano and Matsumura 2002). The results of the research showed that organic matter had different contents of basic biogenic components. Manure had the most analyzed macronutrients (except Ca) and organic matter, and manure had a higher pH. In biological terms, the sediment and the manure as organic fertilizers had more of the analyzed microorganisms, except nitrifies microbes, than were found in the soil. The manure and sediment as fertilizers had a stimulating influence on the amount and activity of almost all physiological groups. The manure mostly stimulated the ammonificators and amylolitic microbes and *Azotobacter* sp. The sewage sediment mostly stimulated the ammonificators, nitrifies of phase I, and cellulolytic microbes. The intensity of the above processes was the same or a little higher for the manure. No connection was found between the amount and the activity of the discussed groups of microbes. The marked potential activity of ammonificators, nitrifies of phase I and II, and the *Azotobacter* sp. showed differentiation. Such variation depended on the properties of the chemical surface (See Table III). The potential activity was shown by the significant value of the correlation coefficient (Table IV).

The presence of forms of copper on the surface were found to decreases the biological activity of the analyzed groups of microbes. An increase in the activity of ammonificators, nitrifies of phase I, and *Azotobacter* sp. contributes to the presence of P, K, Fe, Mn and Zn in the soil. The activity of the nitrifies of phase II is formed differently. The nitrifies of phase II show a reduced activity in the presence of the analyzed macro- and microelements.

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