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Original Research

Effect of Fertilization Using Communal Sewage Sludge on Respiration Activity and Counts of Selected Microorganisms in the Grey Brown Podzolic Soil

A. Wolna-Maruwka*, J. Klama, A. Niewiadomska

Department of Agricultural Microbiology A. Cieszkowski Agricultural University in Poznań, ul. Szydłowska 50, 60-656 Poznań, Poland

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Abstract

The objective of the performed investigation was to ascertain the impact of different doses of sewage sludge (2t, 4t and 8t d.m. sewage sludge·ha^{-1}·year^{-1}) on the developmental dynamics of oligotrophic, copiotrophic, cellulolytic and proteolytic microorganisms.

Soil samples for microbiological analyses were collected at six dates connected with the developmental phases of spring barley.

The incorporation into the soil of organic matter in the form of sewage sludge resulted in a slight increase in the counts of all the analyzed groups of microorganisms.

The performed statistical analysis showed a positive correlation between the intensity of the CO_2 release and numbers of the discussed microorganisms in all fertilization treatments.

Keywords: microorganisms, respiration activity, soil, sewage sludge, spring barley

Introduction

Municipal sewage sludge, frequently referred to as biowaste, is created during the process of treatment of municipal sewage as a waste of various composition and physico-chemical and biological properties. Organic wastes have accompanied human beings from the very beginnings of existence. In Poland, like in all other countries of the European Union, there is a serious problem of sewage management.

Depending on the quality and quantity of the produced wastes as well as local conditions, there are different methods of handling wastes. These methods include, among others, storage in waste dumps, burning, agricultural utilization or dumping them into the sea.

The share of different methods employed in the process of waste management changes. Dumping of wastes into the sea as well as their storage in landfills is increasingly restricted. Therefore, bearing in mind the fertilization value of sewage sludge, it appears quite sensible and justified to utilize it for agrotechnical purposes [1-9].

The application of sewage sludge to agricultural land does not only provide a convenient method for the disposal of a waste product, but it also has the beneficial aspect of adding valuable plant nutrients and organic matter to the soil [10, 11].

The origin of sewage sludge makes it similar to the soil humus in that it contains carbon and nitrogen organic compounds which are essential for the lives of soil micro-

*Corresponding author; e-mail: amaruwka@au.poznan.pl

organisms and fauna as well as nutrients needed by higher plants. Irrespective of the season of the year, organic substances found in sewage sludge are exceptionally rich and diverse. In the opinion of many researchers, agricultural management of sewage sludge is the most advantageous form of recycling. In addition, this method of disposal of municipal wastes appears to be the cheapest utilization method.

However, it should be remembered that sewage sludges can by utilized in agriculture if they meet the requirements specified in the directive of the Minister of Agriculture and Rural Development from October 19, 2004. [12].

There also is disagreement as to the impact of the size of the applied sewage sludge dose on the maintenance of soil equilibrium. This soil biological equilibrium depends on the ratio of oligotrophic to copiotrophic microorganisms [13]. Their quantitative relationships, referred to as O:C, provide one of the indicators of the direction of microbiological changes of organic matter [14]. The applied agrotechnical treatments, including the introduction of sewage sludge, may disturb – periodically or permanently – soil biological equilibrium [15, 16].

Moreover, carbon dioxide release from the soil is also treated as one of the biochemical indicators of soil fertility [9].

Fluxes of CO_2 between soil and the atmosphere are an important component of the global C cycle. CO_2 is produced through a variety of processes, such as biological oxidation of soil organic matter, decomposition of crop residues and root respiration [17]. Net release of soil CO_2, including that from microbial respiration and root respiration, is influenced by local biological, chemical and physical processes. Local attributes, known as proximal factors, include properties such as soil moisture, soil temperature, soil aeration, pH and carbon availability [18].

The measurement of soil respiration is one of the oldest (but still most frequently used) techniques for the quantification of microbial activities in soil. Determination of CO_2 production from soil samples can be made in the laboratory by simple and inexpensive techniques based on alkaline CO_2 traps followed by chemical titration [19].

The introduction into the soil of organic matter in the form of organic fertilizers increases the activity of microorganisms; moreover, it accelerates the rate of soil mineralization which is accompanied by CO_2 liberation from the soil. This acceleration may, sometimes, lead to the negative phenomenon of increased decomposition of the native organic matter [20].

It is evident from the investigations carried out by Quemada and Mencho [21] and Selivanovskaya et al. [22] that the introduction of sewage sludges into soil stimulates strongly the rate of organic carbon mineralization. However, this phenomenon was not corroborated by experiments conducted by Dobosz et al. [23].

According to Gołębiowska and Pędziwilk [24], the amount of CO_2 released in the soil can serve as an indicator of the rate of decomposition of organic matter or soil microbiological biomass. However, the number of soil microbiological organisms is not always correlated with soil respiration activity. Nielsen [19] reported that soil respiration is positively correlated with soil organic master content, and often with microbial biomass and microbial activity.

That is why the authors undertook in this study to assess the impact of different dose sizes of sewage sludge derived from municipal sewage treatment plants on the developmental dynamics of selected groups of soil microorganisms as well as on the respiration activity of grey brown podzolic soil.

Material and Methods

Experiments were carried out in 2002 on experimental plots of the Experimental-Didactic Station of the Department of Soil and Plant Cultivation in Złotniki, which belongs the Agricultural University in Poznań. The experiment was established using the design of random blocks of 42 m^2 which were sown with spring barley. The trial was conducted on grey-brown podzolic soil of lVa and lVb classes of the very good and good rye complexes. The selected soil properties are presented in Table 1. The sewage sludge applied in the experiment was characterized by an acceptable content of heavy metals (Table 2) and derived from a biological-mechanical sewage treatment plant in Szamotuły.

From among all fertilization combinations, this paper presents data concerning the following soil objects: control (soil + NPK), soil + NPK + 2t d.m. sewage sludge·

Table 1. Basic soil chemical and physical properties.

CHEMICAL PROPERTIES				PHYSICAL PROPERTIES				
pH_{KCL}	C	N	C:N	Percentage proportion of the fraction with diameter (mm)				Texture sub-group
	$g \cdot kg^{-1}$ d.m.			1.0-0.1	0.1-0.02	<0.02	<0.002	Pgl
				63	20	14	3	
5.60	6.68	0.60	11.1:1	sand	dust	Silt and clay	Colloidal Clay	

Table 2. Selected properties of sewage sludge.

pH_{H2O}	6.63
d.m. (%)	20.36
organic matter (OM) (%)	71.80
$C_{org.}$ (%)	29.51
C:N	4.74:1
$N_{tot.}$ (% in d.m.)	6.23
$N\text{-}NH_4$ in d.m. (%)	0.33
$N\text{-}NH_4$ in $N_{tot.}$ (%)	5.33
$g \cdot kg^{-1}$ d.m.	
Ca	12.08
K	8.05
Na	1.83
Mg	7.43
P	25.45
$g \cdot kg^{-1}$ d.m.	
Cd	3.12
Cr	39.19
Cu	169.92
Mn	210.56
Ni	42.13
Pb	35.63
Zn	936.15
Fe	11.54

$ha^{-1} \cdot year^{-1}$, soil + NPK + 4t d.m. sewage sludge $\cdot ha^{-1} \cdot year^{-1}$ and soil + NPK + 8t d.m. sewage sludge $\cdot ha^{-1} \cdot year^{-1}$.

Each of the above-mentioned soil combinations was used in two replicates. The experimental plots were fertilized with nitrogen in the form of ammonium saltpeter, phosphorus – in the form of triple superphosphate and potassium – in the form of 60% potassium salt applied at the following doses: 50 kg N ha^{-1}, 29 kg P ha^{-1}, 100 kg K ha^{-1}. Phosphorus and potassium were applied pre-sowing during plowing, and nitrogen was divided into two parts and the first of them was applied pre-sowing, while the second – as top-dressing.

Soil samples for analyses were collected at six dates (in ten repetitions) associated with the developmental stages of spring barley. These dates comprised: the stage before sowing, first jointing stage, full heading, end of flowering, barley full maturity and samples of soils two weeks after harvest.

The scope of the performed experiments comprised the determination (in five replications) of oligotrophic, copiotrophic, cellulolytic and proteolytic microorganisms. The examined groups of microorganisms were cultured by the plate method on solid substrates using the appropriate dilutions of soil solutions and expressed in the $cfu \cdot g^{-1}$ of soil dry matter. Oligotrophic microorganisms were determined on the substrate according to Hattori and Hattori [25] at 28°C and their numbers were determined after 14 days. Numbers of copiotrophic microorganisms were determined by the plate method also on the substrate according to Hattori and Hattori [25] at 28°C for 7 days. On the basis of the counts of the above-mentioned groups of microorganisms, the O (oligotrophs) to C (copiotrophs) coefficient was calculated, which is the indicator of the soil biological equilibrium preconditioning the maintenance of the stable level of humus in the soil.

In order to determine the number of proteolytic microorganisms, the authors used the selective substrate according to Rodina [26]. Plates were incubated at 22°C for 48 hours. Cellulolytic microorganisms were determined on the substrate according to Rodina [26], incubating the plates at 28°C for 8 days. The amount of the liberated CO_2 from the soil was determined by the absorption method [27].

Statistical analyses (analysis of variance, multiple comparison tests) were carried out using Statistica 7.1 software.

Analysis of variance was employed to estimate the significance of changes in the numbers of the analyzed groups of microorganisms in relation to the soil combination and date of analysis. In order to determine significant differences in the cell counts of a given group of microorganisms between the control soil and the remaining soil objects, Tukey's two-factorial procedure was used.

Pearson's linear correlation coefficient was applied to demonstrate interrelationships between the size of the given group of microorganisms in the soil and the quantity of the released CO_2.

Results and Discussion

It is evident from the review of literature on the subject that there is little data concerning the effect of sewage sludge introduced into the soil on the developmental dynamics of proteolytic microorganisms found in it. That is why, in our experiments, we focused on the determination of the numbers of the above-mentioned group of microorganisms in the soil following its enrichment with three doses of sewage sludge.

The results of microbiological analyses presented in Fig. 1 show that the type of the applied fertilization treatment modified the numbers of the proteolytic microflora in the soil statistically significant only towards the end of the flowering of the experimental spring barley. The phenomenon was not observed at the remaining dates of analyses.

It was also found on the basis of the obtained results that noticeable differences in the numbers of the examined

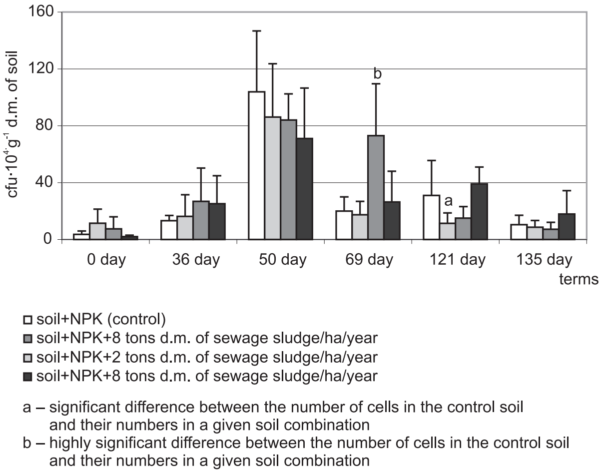

Fig. 1. The number of proteolytic microorganisms in the soil with different levels of organic fertilizations.
Explanation:
0 day - stage before sowing
36 day - stage of first jointing
50 days - full heading of rye
69 days - end of flowering
121 days - full maturity
135 days - two weeks after harvest

Wielgosz [30] and Wielgosz et al. [32] maintain that soil microflora is influenced not only by the type of crop plant, its species or variety but also their developmental stage. The effect of crop plants on soil microorganisms can be either stimulative or noxious (secretion of toxic saponins, glucosides, hydrocyanic acid [33]). Therefore, soil microorganisms are closely associated with crop plants and their root systems. They participate in the transformation of nutrients and in making them available for plants. Moreover, they improve vigour, condition and health of plants [32, 34].

As evident from the data presented in Fig. 2, numbers of the oligotrophic microorganisms in the soil were the lowest on the day of trial establishment (day 0). From day 36 of experiments onwards, numbers of oligotrophs increased gradually both in the fertilization treatments with sewage sludge as well as in the control. What is even more important, on the majority of dates of analyses it was found that the cell proliferation of the discussed microorganisms in the control soil remained on a level similar to that found in the soils fertilized with different doses of the sewage sludge or it was even higher. The performed statistical analysis microorganisms between the control soil and the remaining fertilization treatments took place primarily on the two first dates of analyses, i.e. on the day of experiment establishment (day 0) and on day 36 of the trial (i.e. at the stage of the first jointing of spring barley). However, on the above dates differences between the control treatment and the combinations with the sewage sludge were statistically non-significant.

Also Furczak and Joniec [28] as well as Joniec and Furczak [29] reported a stimulating effect of the sewage sludge introduced into the soil on the numbers of the discussed microorganisms.

Numbers of proteolytic microorganisms in the soil increased gradually throughout the entire experiment and reached their maximum values of the order of 71.04-103.89·10^5 cfu·g^{-1} of soil dry matter at the stage of earing of spring barley (day 50). On the other hand, analysis of soil microbiological condition conducted on day 69 of the trial revealed that numbers of the proteolytic microorganisms decreased and this low level remained unchanged until the end of the experiment.

Changes in the numbers of proteolytic bacteria in the analyzed fertilisation combinations in the soil in successive developmental phases of barley could have been caused by changes in the quantitative and qualitative composition of root exudates, which is confirmed by experiments carried out by Wielgosz et al. [30]. Crop plants, especially their root exudates, change physico-chemical soil properties and by doing so, exert influence on microorganisms. Plant roots release into the soil such substances as carbohydrates, amino acids, organic acids, nucleotides, and flavone substances [30, 31].

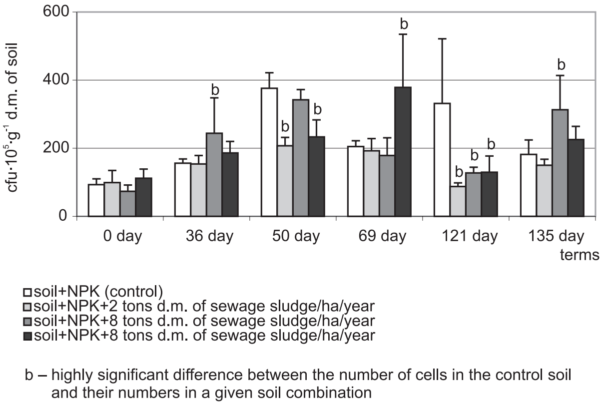

Fig. 2 The number of oligotrophic microorganisms in soil with different levels of organic fertilization.
(Explanations as in Fig. 1.)

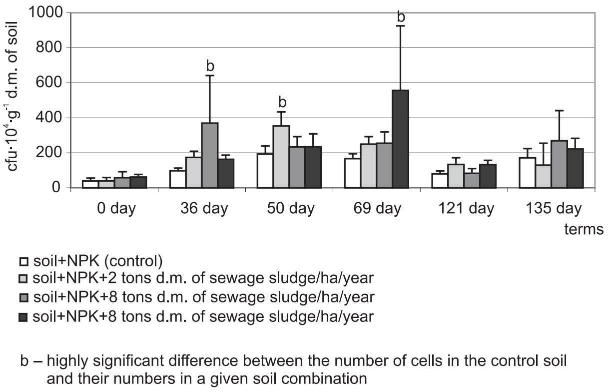

Fig. 3 The number of copiotrophic microorganisms in soil with different levels of organic fertilization.
(Explanations as in Fig. 1.)

showed that the above differences were statistically highly significant.

The quantities of copiotrophic microorganisms during the performed microbiological analyses were similar to those of oligotrophs (Fig. 3). Also, in the case of these microorganisms, their lowest proliferation was recorded on the first date of analyses, i.e. on the day of the initiation of the experiment. Numbers of copiotrophic microorganisms increased steadily during the three consecutive dates of analyses and it was only on day 121 of the trial (stage of full maturity) that the proliferation of these microorganisms was observed to drop in all the soil fertilization treatments. The obtained research results revealed that only on the last date of analyses, i.e. two weeks after harvesting the spring barley, the numbers of the determined copiotrophic microorganisms were lower in one of the treatments fertilized with the sewage sludge (soil + NPK + 2 tons d.m. sludge·ha^{-1}·year^{-1}) than in the control soil. In the case of the remaining combinations, the cell proliferation was stronger in soils fortified with organic fertilization than in the control. The analyses of microbiological results revealed that the increase in the numbers of the copiotrophic microorganisms in the soil was influenced most strongly by the introduction into the soil of sewage sludge at the dose of 8 tons d.m. sludge·ha^{-1}·year^{-1}. This amount of sewage sludge introduced into the soil caused the quantity of the discussed microorganisms throughout the experimental period to be 83% higher in comparison with the control soil. The introduction of sewage sludge at the dose of 2 tons d.m. sludge·ha^{-1}·year^{-1} turned out to exert the weakest impact on the increment in the numbers of the copiotrophic microorganisms in the soil. In comparison with the control soil, the above-mentioned quantity of sludge introduced into the soil resulted in a 44% increase of copiotrophs jointly at all dates of analyses. An increased proliferation of the above-mentioned microorganisms in the soil as a result of its fortification with organic matter was also reported by Wyczółkowski et al. [35]. These researchers maintain that, at certain dates of analyses, numbers of copiotrophs in the soil cultivated ecologically increased almost two times in comparison with the soil cultivated conventionally. This was attributed by these authors to the fact that the organic substance introduced into the soil increased pH and this, in turn, exerted a positive impact on the development of microorganisms. In addition, the increase of the weight of organic matter in the soil increased its overall porosity, resulting in the decrease of the soil-specific density. The above factors allowed microorganisms easier penetration of the environment, facilitating their access to the food base and, therefore, competition between microorganisms and plants was smaller.

Also Hu et al. [36] reported increased numbers of the copiotrophic microflora following the introduction into the soil of organic matter. Marschner et al. [37] maintain that the main factor influencing changes in the soil microflora is the composition of applied organic matter. Experiments conducted by these researchers showed that the

Table 3. Microbiological composition of sewage sludge used in the experiment.

Kind of microorganisms	Mean (cfu·g^{-1} d.m. of sewage sludge)
oligotrophic microorganisms	568.79•10^5
copiotrophic microorganisms	586.17•10^6
proteolytic microorganisms	21.12•10^5
cellulolytic microorganisms	40.11•10^5

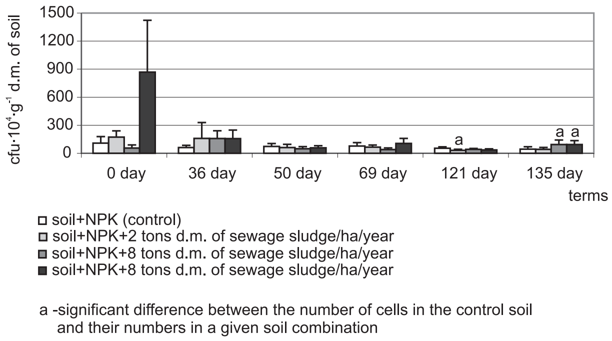

Fig. 4 The number of cellulolytic microorganisms in the soil with different levels of organic fertilizer.
(Explanations as in Fig. 1.)

Table 4. The cfu count ratio of oligotrophic to copiotrophic microorganisms (O: C).

EXPERIMENTAL TREATMENT	DATES OF ANALYSES					
	Day 0	Day 36	Day 50	Day 69	Day 121	Day 135
Control (soil+NPK)	23.83	16.08	19.48	12.33	33.82	10.62
Soil+NPK+2t d.m. sludge·ha^{-1}·year^{-1}	25.38^b	8.88^b	5.86^b	7.70^b	6.57^b	11.61
Soil+NPK+4t d.m. sludge·ha^{-1}·year^{-1}	12.90^b	6.60^b	14.68^b	7.02^b	15.35^b	11.67
Soil+NPK+8t d.m. sludge·ha^{-1}·year^{-1}	19.14^b	11.49^b	9.96^b	6.80^b	9.81^b	10.20

b – highly significant difference between the cfu count ratio of oligotrophic to copiotrophic microorganisms in the control soil and their count in a given soil combination introduction into the soil of sewage sludge in the amount of 7.6 tons d.m. sludge·ha[-1]·year[-1] resulted in over 100% increase of the soil microflora biomass in comparison with the control soil.

On the basis of the data presented in Figs. 2 and 3, the authors concluded that the introduction into the soil of organic matter in the form of sewage sludge contributed to the development of the discussed microorganisms in the soil. However, the observed increase in the numbers of oligotrophs and copiotrophs was not rapid and the performed statistical analysis revealed that it was, practically speaking, statistically non-significant in relation to the control soil. Therefore, on the basis of the above-presented data, it can be said that the biological equilibrium in the soil was not disturbed as evidenced by the narrow ratio of oligotrophs to copiotrophs (Table 4). In addition, it was observed throughout the duration of this experiment that oligotrophs prevailed in the soil microflora in all fertilization treatments (Figs. 1-4). According to Weyman-Kaczmarkowej and Pędziwilk [14], the above domination is essential for maintaining a stable level of organic soil matter.

When analysing numbers of cellulolytic microorganisms in the course of the performed trial (Fig. 4), it was found that the cell proliferation of these microorganisms was low in all fertilization combinations. The highest numbers of these microorganisms were observed on the day of trial establishment (day 0), especially in the combination with the highest dose of the sewage sludge, i.e. 8 tons d.m. sludge ·ha[-1]·year[-1]. This can probably be attributed to the microbiological composition of the sludge introduced into the soil on day 0 (Table 3). It was concluded, on the basis of the obtained results of microbiological analyses, that none of the applied sewage sludge doses resulted in an intensive and prolonged proliferation of the cellulolytic microorganisms in the soil. The results of statistical analyses (Tukey test) showed that significant differences in the cell counts occurred only on the last date of analyses, i.e. two weeks after the harvest of the spring barley and only in the case of two fertilization treatments, namely: soil + NPK + 4t d.m. sewage sludge ·ha[-1]·year[-1] and soil + NPK + 8t d.m. sewage sludge ·ha[-1]·year[-1].

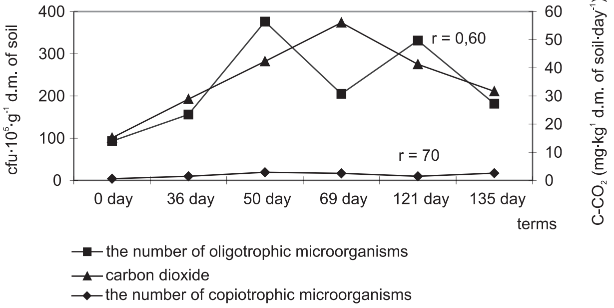

Fig. 5. Correlations between the number of oligotrophic and copiotrophic microorganisms and the amount of CO_2 released from the control soil.
(Explanations as in Fig. 1.)

It is also evident from studies conducted by Jezierska-Tys et al. [38] that the introduction of sewage sludge into the soil (at the dose of 20 tons fresh substance sludge ·ha[-1]·year[-1]) stimulated the activity of cellulolytic bacteria only slightly. Sarathchandra et al. [39] claim that the number and activity of cellulolytic microorganisms depend on numerous factors. The principal factor limiting their activ-

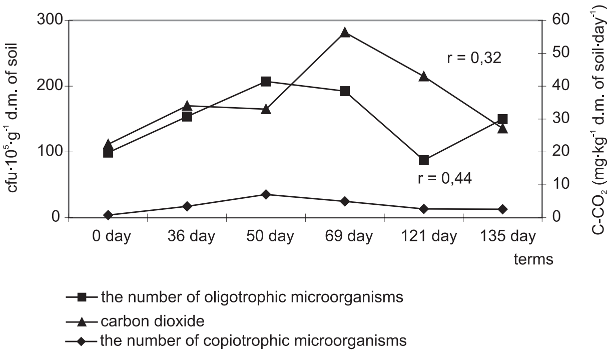

Fig. 6. Correlations between the number of oligotrophic and copiotrophic microorganisms and the amount of CO_2 released from soil fertilized with 2 tons d.m. of sewage sludge.
(Explanations as in Fig. 1.)

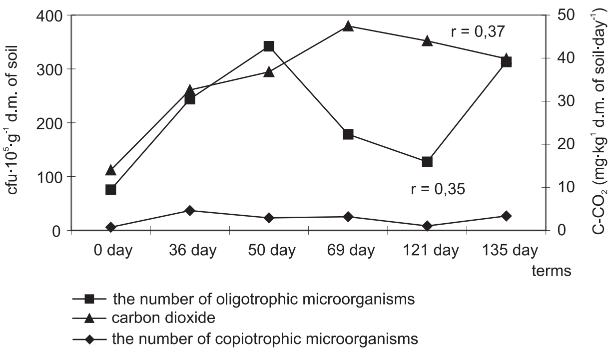

Fig. 7. Correlations between the number of oligotrophic and copiotrophic microorganisms and the amount of CO_2 released from soil fertilized with 4 tons d.m. of sewage sludge.
(Explanations as in Fig. 1.)

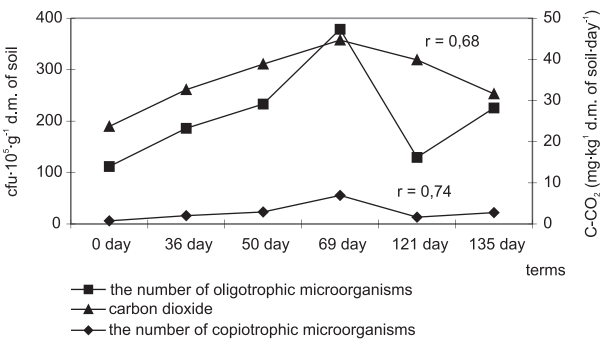

Fig. 8. Correlations between the number of oligotrophic and copiotrophic microorganisms and the amount of CO_2 released from soil fertilized with 8 tons d.m. of sewage sludge.
(Explanations as in Fig. 1.)

ity is the content of the available organic matter, while the transformation rate of carbohydrates in the soil depends, among others, on climatic conditions, soil structure and pH and the degree of environmental contamination.

One of the methods of determination of the activity of soil microflora as well as changes which occur under the influence of natural and anthropogenic environmental conditions is to carry out measurements of soil respiration activity. The main sources of CO_2 released from the soil include: mineralization of organic matter, decomposition of plant residues and respiration activity of roots [18].

Data presented in Fig. 5 show that the counts of both oligotrophic and copiotrophic microorganisms in the control soil increased up to day 50 of the trial (full earing of barley) and were accompanied by increased quantities of CO_2 released from the soil. On day 69 (i.e. end of flowering of barley), the respiration activity reached its peak, while the numbers of the examined groups of microorganisms dropped rapidly. This drop could have been caused by the stage of plant generative development as manifested by the quantitative and qualitative changes of root discharge secreted into the soil. The liberation of carbon dioxide did not always increase with the increase of oligotrophs and copiotrophs in the remaining combinations (Figs. 6-8).

Lynch and Panting [40] reported that numbers of soil microorganisms did not always increase with the increase of soil respiration activities. This conclusion was also corroborated by experiments conducted by Wolna-Maruwka and Sawicka [41]. On the successive dates of analyses, the cell proliferation of the examined microorganisms underwent fluctuations and the amount of the liberated CO_2 declined. The intensity of the CO_2 release decreased and numbers of oligotrophs and copiotrophs increased also in the remaining treatments.

On the basis of the obtained research results, it was concluded that in the control soil (Fig. 5), a stronger positive correlation between the CO_2 released from the soil and cell proliferation occurred in the case of the copiotrophic microorganisms ($r = 0.7$).

However, the highest positive linear correlation coefficients between the amount of the CO_2 released from the soil and the number of the discussed microorganisms were recorded in the soil with the addition of 8 tons of sewage sludge (Fig. 8). Also when 2 and 4 tons of sewage sludge were incorporated into the soil (Fig. 6 and 7), positive correlations between the above-mentioned factors were observed as well.

The introduction into the soil of different doses of sewage sludge failed to cause a significant increase in the intensity of the CO_2 released from the soil (Figs. 6-8). The above finding was corroborated by experiments conducted by Frąc et al. [42]. On the other hand, according to Fernandes et al. [43], Stamatiadis et al. [44] and Emmerling et al. [45], soil respiration activity increases together with the increase of the sludge dose introduced into the soil. The above researchers claim that the increase in the intensity of the CO_2 released from the soil is associated with the enhanced metabolic activity of microorganisms stimulated by organic matter introduced into the soil in the form of sludge.

Islam and Weil [46] reported that high soil respiration activity may, on the one hand, indicate biochemical disturbances occurring in the soil and, on the other, high level of productivity in the ecosystem.

Conclusions

1. The incorporation into the soil of organic matter in the form of sewage sludge resulted in a slight increase in the counts of all the analyzed groups of microorganisms.
2. Proteolytic, oligotrophic and copiotrophic microorganisms underwent the strongest proliferation in the analyzed soil combinations during the generative development of barley.
3. In the course of the performed experiments, oligotrophic microorganisms were found to prevail in the soil microflora in all the experimental fertilization combinations. This proves that the incorporated doses of sewage sludge failed to disrupt soil biological equilibrium as manifested by the decline of its fertility.
4. The performed statistical analysis showed a positive correlation between the intensity of the CO_2 release and numbers of the discussed microorganisms in all fertilization treatments.

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