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Original Research

Size of Selected Groups of Microorganisms and Soil Respiration Activity Fertilized by Municipal Sewage Sludge

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Abstract

The authors investigated the impact of different sewage sludge doses on the developmental dynamics of soil microorganisms as well as on soil respiration activity.

The following soil combinations were employed: control (soil + NPK), 2 t d.m. of sewage sludge·ha^{-1}·year^{-1} + NPK, 4 t d.m. sewage sludge·ha^{-1}·year^{-1} + NPK and 8 t d.m. sewage sludge·ha^{-1}·year^{-1} + NPK.

The experiments were carried out in field conditions. During the first year of analyses, winter rye was used as the test plant, while in the second year rye was replaced by potatoes as test plants. Soil samples for analyses were collected during the consecutive plant developmental stages and, using the plate method, the total numbers of bacteria, fungi as well as pathogenic bacteria from the *Salmonella, Clostridium perfringens* and *Escherichia coli* genera were determined. In addition, using the absorption method, the amount of CO_2 released from the soil was also ascertained.

On the basis of the obtained results it was found that the introduction into the soil of organic matter in the form of sewage sludge failed to cause any statistically significant changes in the total counts of bacteria and fungi. Moreover, pathogenic bacteria were found in the examined soils (*Salmonella* sp., *Clostridium perfringens* and *Escherichia coli*) and they persisted in the soils even after 66 (*Salmonella* sp.) and 94 days (*Clostridium perfringens* and *Escherichia coli*) of analyses.

The analysis of the CO_2 released on the control plot and on other soil treatments showed that the fertilization of soils with sewage sludge resulted in a slight increase of their respiration activities in relation to the control.

Keywords: microorganisms, plants, respiration activity, sewage sludge

Intoduction

One of the many anthropogenic factors exerting a strong influence on soil microorganisms is organic fertilization. The type of the applied fertilizer or household waste may have a considerable influence on the numbers of microorganisms, qualitative selection of entire complexes of soil microbes and yields of crop plants. Incorrect agrotechnical treatments as well as unreasonable application of fertilizers can result in serious disturbances in the functioning of entire agroecosystems as well as in epidemiological contamination of the environment. The application of sew-

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age sludge as an organic fertilizer may introduce into soils pathogenic bacteria and their toxins [1-6].

The amount of pathogenic bacteria in the municipal sewage sludge may vary significantly and, in the case of *Escherichia coli*, may range from $2.0·10^7$ to $9.5·10^7$ cfu·g^{-1} d.m. of the sewage sludge, while in the case of *Salmonella* – about $0.9·10^2$ cfu·g^{-1} d.m. of the sludge [7]. Despite the commonly known sanitary function of the soil, it would not be wise to rule out completely the possibility of epidemiological hazards when sewage sludge is used as an organic fertilizer.

Steadily improving living standards and urban development of Poland both contribute to continuously increasing amounts of sewage sludge, resulting in problems with its disposal [8]. Appropriate standards connected with the correct handling of sewage sludge, its management and protection against its harmful effects on the natural environment can all be found in the Directive of the Minister of Agriculture and Rural Development of October 19th, 2004 [9].

In accordance with the regulations currently in force, sludge can be used in agriculture when no bacteria of the *Salmonella* genus are found in it. Sewage sludge microbiological composition depends on the origin of sewage but, generally speaking, apart from pathogenic microbes it contains large quantities of organic matter as well as macro- and microelements [10, 11]. Bearing in mind the fact that there is shortage of humus compounds in Polish soils, the use of appropriately stabilised sewage sludge could certainly improve soil fertility.

The performed two-year long experiment aimed at determining the impact of different doses of sewage sludge and two crop plants: winter rye and potatoes at various developmental stages on the dynamics of changes in the numbers of selected groups of microorganisms in the soil.

Experimental Procedures

The discussed experiments were carried out in 2003-04 on plots established at the Experimental-Didactic Stations in Złotniki which belongs to the Agricultural University in Poznań.

The experiments were established in random block design on 42 m^2 plots. Two test crop plants were used in the study: winter rye in 2003 and potatoes in 2004. The experiment was conducted on grey-brown podzolic soil of the lVa and lVb class of the following chemical parameters: pH_{KCL}– 5.60, C– 6.68 g · kg^{-1} d.m., N– 0.60 g · kg^{-1} d.m., C/N- 11.1.

The following soil combinations were employed in the discussed experiment: control (soil + NPK), 2 tons d.m. sewage sludge·ha^{-1}·year^{-1} + NPK, 4 tons d.m. sewage sludge ·ha^{-1}·year^{-1} + NPK and 8 tons d.m. sewage sludge·ha^{-1}·year^{-1} + NPK. All treatments were established in three repetitions.

Nitrogen was applied in the form of ammonium saltpeter, phosphorus – in the form of triple superphosphate and potassium – in the form of 60% potassium salt. The applied quantities were as follows: (2003) 60 kg N · ha^{-1}, 35 kg P · ha^{-1}, 90 kg K · ha^{-1}; (2004) 120 kg N · ha^{-1}, 83 kg K · ha^{-1}, 22 kg P · 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 with the second as top-dressing.

The sewage sludge used in the experiment was characterised by acceptable concentrations of heavy metals and the following chemical composition: (2003) pH_{H2O} – 6.59, dry matter – 18.85%, MO -75.33%, C_{org} – 31.02%, N_{org} – 6.18% in d.m., C/N – 4.95; (2004) pH_{H2O} – 7.18, dry matter –18.44%, MO – 75.45%, C_{org} – 34.17%, N_{org} _ 5.55% in d.m., C/N – 6.16.

Soil samples for analyses were collected in ten replications at five dates associated with the developmental stages of the growing crop plants. In the case of winter rye (2003), these dates included: the stage of the first jointing, full heading, end of flowering, full maturity and soil samples 2 weeks after harvest, whereas in the case of potatoes (2004) – soil samples were collected: at the closing of inter-row spaces, end of flowering, development of fruits, final picking and two weeks after picking.

In the collected soil samples the total bacterial, the number of fungi and *Salmonella, Clostridium perfringens* and *Escherichia coli* were determined. The examined groups of microorganisms were determined by the plate method (in five replications) on solid media employing appropriate dilutions of soil suspensions and expressed in cfu·g^{-1} d.m. of soil.

The total bacteria count was determined on the medium with the soil extract [12] after 14 days of incubation at 27°C. Fungi were determined on the Martin medium [13] at 28°C after 5 days of incubation. Bacteria from the *Salmonella* genus were determined by the plate method on the Merck medium [14] at 37°C for 24 hours. In order to make sure that the obtained bacteria were those of the *Salmonella*, procedures recommended by Polish Standard PN-Z-19000-1 were applied [15]. In order to determine numbers of the *Escherichia coli* bacteria, a selective medium of the Merck Company was used. Plates were incubated at 37°C for 24 hours [16]. *Clostridium perfringens* bacteria were determined on the TSC agar medium with tryptose, sulfate and cycloserin incubating the plates in a thermostat with 22% CO_2 atmosphere at 44°C for 24 hours [17].

In additions, using the absorption method, the amount of CO_2 released from the soil was determined [18].

In order to estimate interrelations between the total bacterial count and the amount of CO_2 released from the soil, linear correlation coefficients were calculated.

The obtained research results were subjected to statistical analysis [19] using Statistica 7.1 software.

Results

See Table 1 and Figs. 1-10

Discussion of Results

The obtained research results (Fig. 1) showed that the introduction into the soil of different doses of sewage sludge increased the proliferation of the total numbers of bacteria but the performed statistical analysis revealed that these differences were not significant. Some departures were observed in 2003 on the date of collection at the end of winter rye flowering when the numbers of bacterial cells in soil objects fertilized organically were lower than in the control soil and these differences were statistically highly significant.

In 2003, in the case of the soil under winter rye cultivation, a positive impact of the inclusion of sewage sludge on the propagation of bacterial cells was recorded after 52 days, i.e. from the full maturity of winter rye and this condition remained unchanged until the final date of collection (66 days). The numbers of the bacteria determined at the remaining dates of sample collection were lower.

Table 1. Microbiological composition of sewage sludge used in the experiment.

King of microorganisms	Year 2003	Year 2004
	Mean (cfu·g^{-1} d.m. of sludge)	
Total bacterial number	212.26·10^{5}	851.01·10^{5}
Fungi	127.04·10^{4}	88.62·10^{4}
Salmonella sp.	2136.60	0
Escherichia coli	1.31·10^{4}	211.23·10^{4}
Clostridium perfringens	1100.49	490.77

It also is evident from the performed studies (Fig. 1) that the total bacterial number was 53% higher in the soil under potatoes (2004) than in the soil under winter rye (2003). Those observed large differences could have been caused by: a greater number of bacteria introduced into the soil with the sludge in 2004 (Tab. 1), climatic conditions or the type of the cultivated crop plant. Wielgosz et al. [20] reported that the type of the applied plant can act selectively on the bacteria present in the soil, both with regard to their quality and quantity.

The comparison of the numbers of bacteria in the control soil and the remaining soil treatments during the two years of the trial revealed that the addition of the experimental sewage sludge did not always stimulate the proliferation of bacteria. Likewise, the sludge dose level failed to exert a significant impact. This observation is corroborated by Giller et al. [21], who reported a negative impact of sewage sludge on both numbers and species composition of soil microflora. On the other hand, Joniec, Furczak [11]; Jezierska-Tys, Świca [10]; Gostkowska et al. [22]; Wolna-Maruwka, Sawicka [23] maintain that sewage sludge introduced into the soil exhibits a stimulatory effect on the total bacterial number. Nguyen Thi and Greinert [24] reported that numbers of soil microorganisms increased proportionally to increasing doses of the applied sewage sludge. Also, Martyn et al. [25] found a positive correlation between the amount of bacteria in soil and the content of organic matter.

Martyniuk et al. [26] maintain that, in relation to soils fertilized only with mineral fertilizers, soils which are fertilized with organic fertilizers are characterized by con-

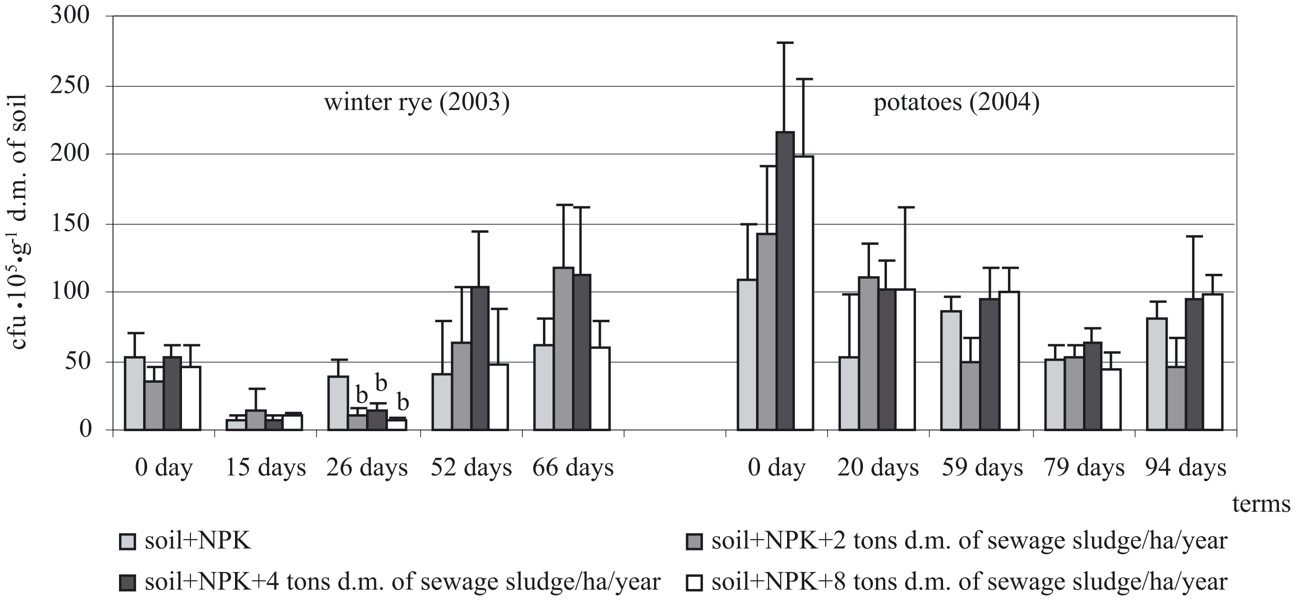

Fig. 1. Developmental dynamics of total bacterial number in the soil with different levels of fertilization with sewage sludge.
Explanations: Year 2003: 0 day – stage of first jointing, 15 days – full heading, 26 days- end of flowering, 52 days – full maturity, 66 days – two weeks after harvest; Year 2004: 0 day – closing of inter-row spaces, 20 days – end of flowering of potatoes, 59 days – development of fruits, 79 days – final picking, 94 days – two weeks after picking; b-highly significant difference between the number of cells in the control soil and their numbers in a given soil combination.

siderably improved microbiological (number of bacteria, respiration activity) and biochemical properties.

It is evident from investigations carried out by Brabasz et al. [27] that mineral fertilization, in the case of excessive doses of nitrogen fertilizers, may result in the decrease of the bacterial count as well as the activity of bacteria. The above phenomenon may be attributed to the accumulation of carcinogenic nitrosamines.

Analyzing the number of fungi in the selected soil combinations (Fig. 2), it was found that their propaga-

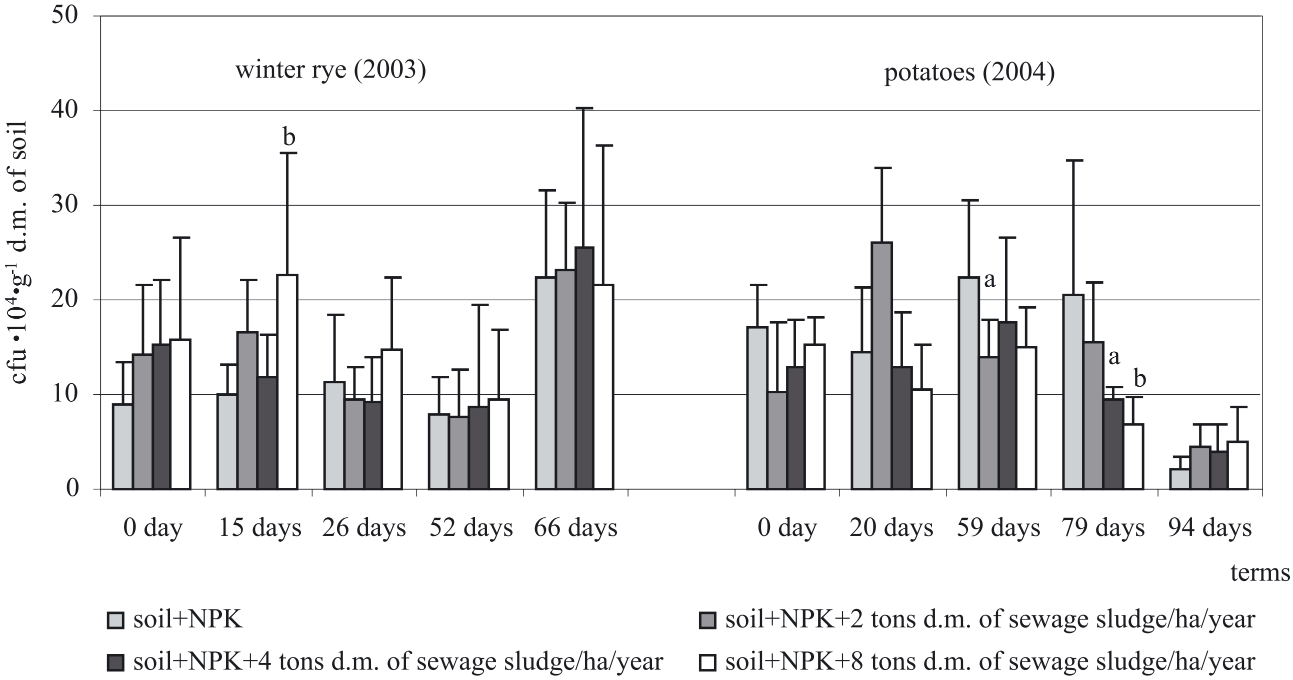

Fig. 2. Developmental dynamics of fungi in the soil with different levels of fertilization with sewage sludge.
Explanations as in Fig. 1; a-significant difference between the number of cells in the control soil and their numbers in a given soil combination, b-highly significant difference between the number of cells in the control soil and their numbers in a given soil combination.

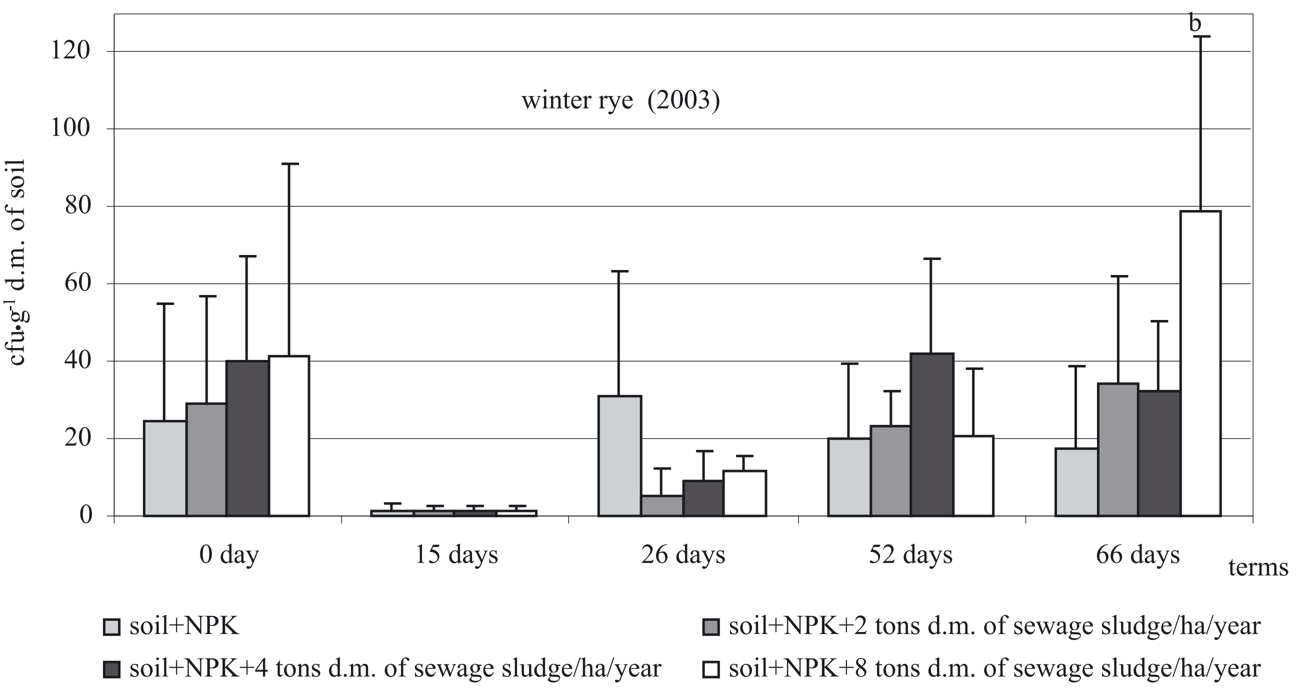

Fig. 3. Dynamics of *Salmonella* sp. development in the soil with different levels of fertilization with sewage sludge under winter rye cultivation.
Explanations as in Fig. 1; b-highly significant difference between the number of cells in the control soil and their numbers in a given soil combination.

tion depended on the crop plant developmental stage and the applied sewage sludge. The greatest numbers of fungi were determined in 2003 under the winter rye cultivation in the treatment with the highest dose of sewage sludge, while the lowest – in all analysed soil combinations at the date of final potato picking and two weeks after picking (2004). According to the literature, reasons for the better or worse proliferation of fungi can vary. Breza-Boruta et al. [28] reported that the number of fungi in the soil in which potatoes were grown depended on the method

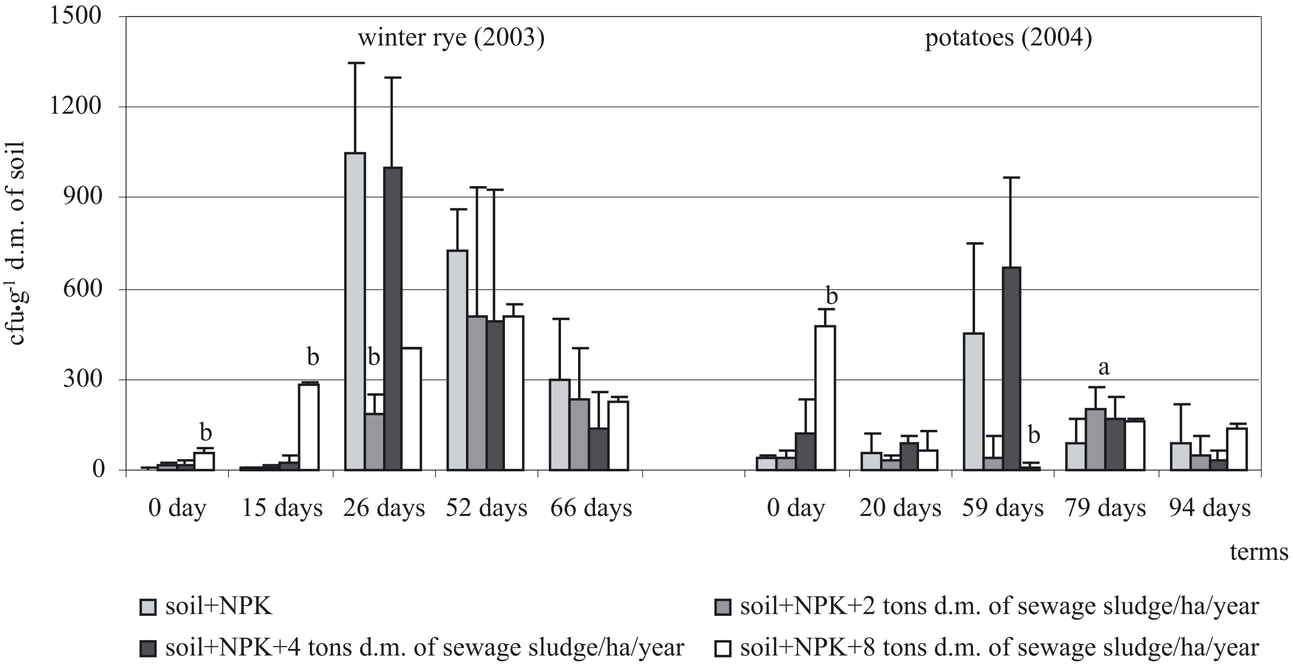

Fig. 4. Dynamics of *Escherichia coli* development in the soil with different levels of fertilization with sewage sludge.
Explanations as in Fig. 1; a-significant difference between the number of cells in the control soil and their numbers in a given soil combination; b-highly significant difference between the number of cells in the control soil and their numbers in a given soil combination.

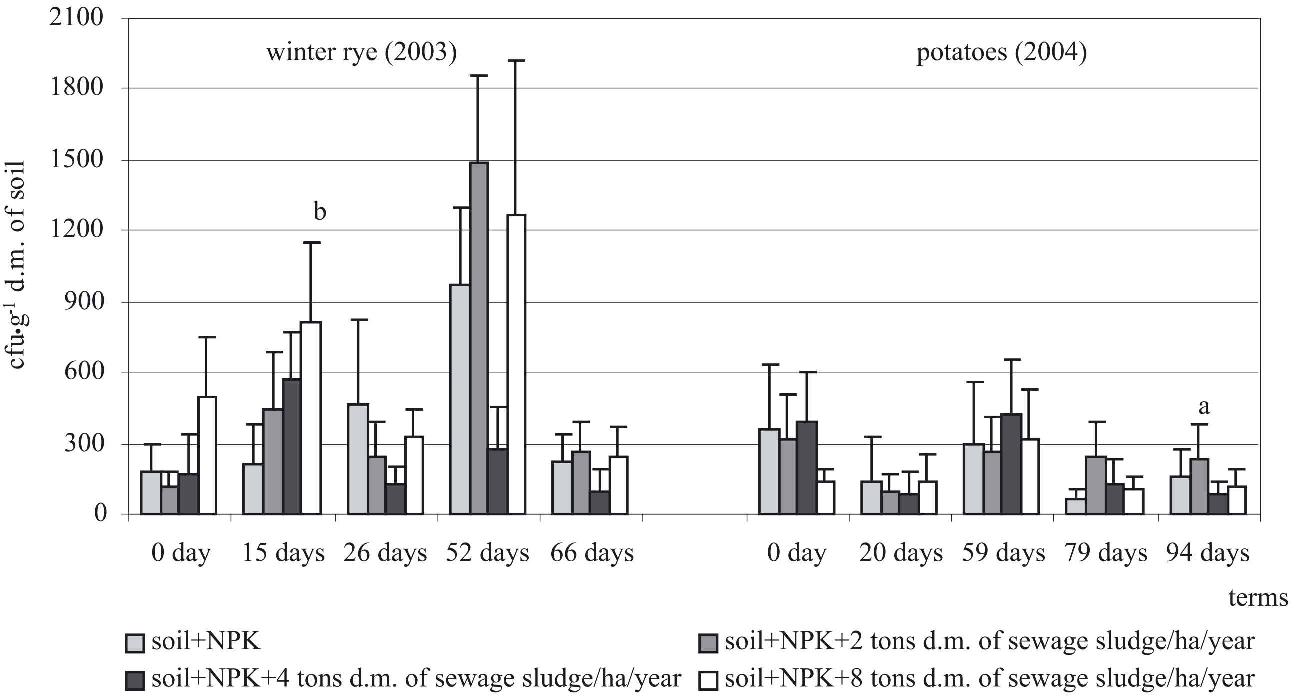

Fig. 5. Dynamics of *Clostridium perfringens* development in the soil with different levels of fertilization with sewage sludge.
Explanations as in Fig. 1; a-significant difference between the number of cells in the control soil and their numbers in a given soil combination; b-highly significant difference between the number of cells in the control soil and their numbers in a given soil combination.

of its cultivation. They maintain that *Fusarium* sp. proliferated better in the soil managed ecologically, whereas *Pythium* sp. showed stronger growth following the traditional system of tillage. Wyszkowska et al. [29] claim that the amount of fungi may depend on the origin of the applied sludge. From among 13 analyzed sludges derived from different sewage treatment plants, the numbers of these microorganisms in the soil increased only following the application of seven of them. When analyzing the numbers of fungi in this experiment, it can be assumed that their high levels found in the applied sludge (Tab. 1) may have affected their further proliferation in the soil. Although the applied sludges were obtained from the same sewage treatment plant, they were delivered at 1-year time intervals. Therefore, analyzing the number of fungi in the combination: soil + NPK + 8 tons d.m. of sludge·ha^{-1}·year^{-1}, it was observed that their numbers in 2003 were 37% higher than in 2004.

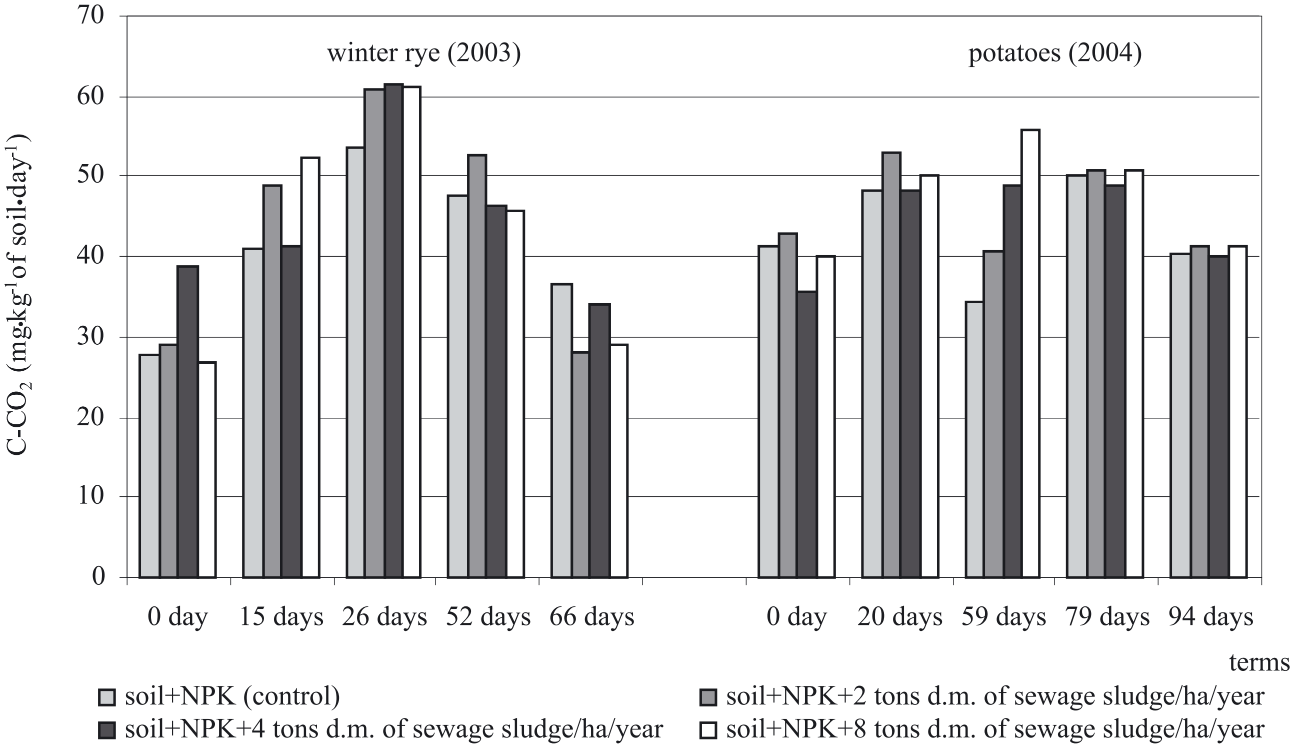

Fig. 6. Dynamics of changes of the soil respiration activity fertilized by different levels of sewage sludge. Explanations as in Fig. 1

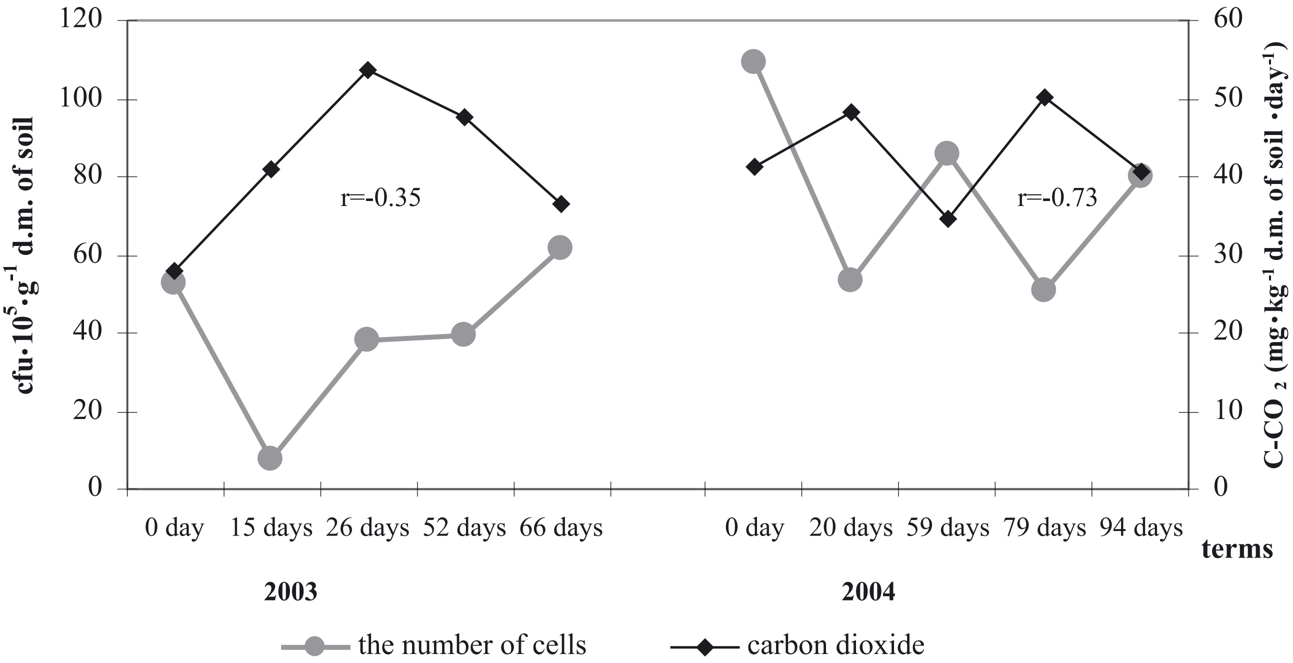

Fig. 7. Correlations between total bacterial number and the amount of CO_2 released from the control soil. Explanations as in Fig. 1.

Analyzing the number of *Salmonella* sp. bacteria (Fig. 3) in the soil under rye cultivation, it was observed that the introduced sludge stimulated strongly the proliferation of these bacteria at the first date. This was particularly evident in the combination fertilized with the highest sludge dose. However, this effect decreased significantly already after 15 days and intensified again later on in successive dates of sampling reaching the highest value in the treatment of NPK + 8 tons d.m. of sludge·ha^{-1}·$year^{-1}$ after 66 days of experiment.

Salmonella sp. bacteria were absent in the analyzed soil treatments in the next year of the performed experiment (2004), which was due to their absence in the applied sludge (Table 1). Also Budzińska et al. [1] reported

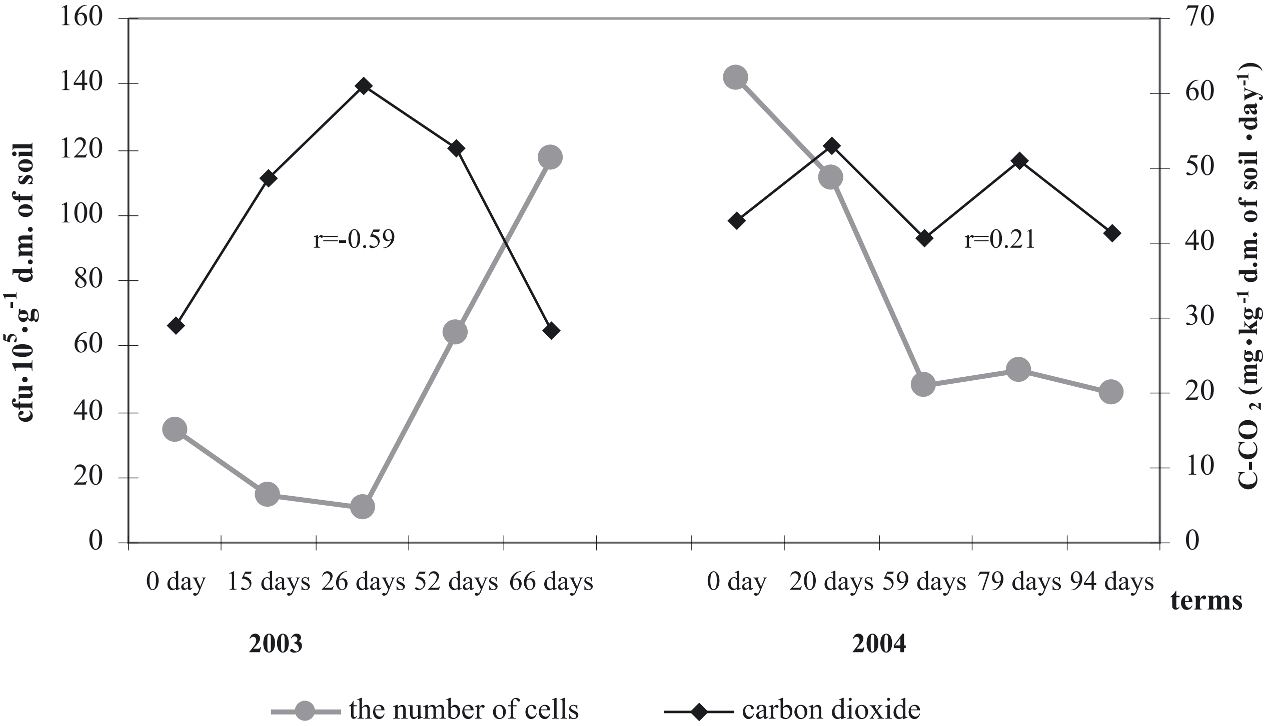

Fig. 8. Correlation between the total bacterial number and the amount of CO_2 released from the soil fertilized with 2 tons d.m. of sewage sludge. Explanations as in Fig. 1.

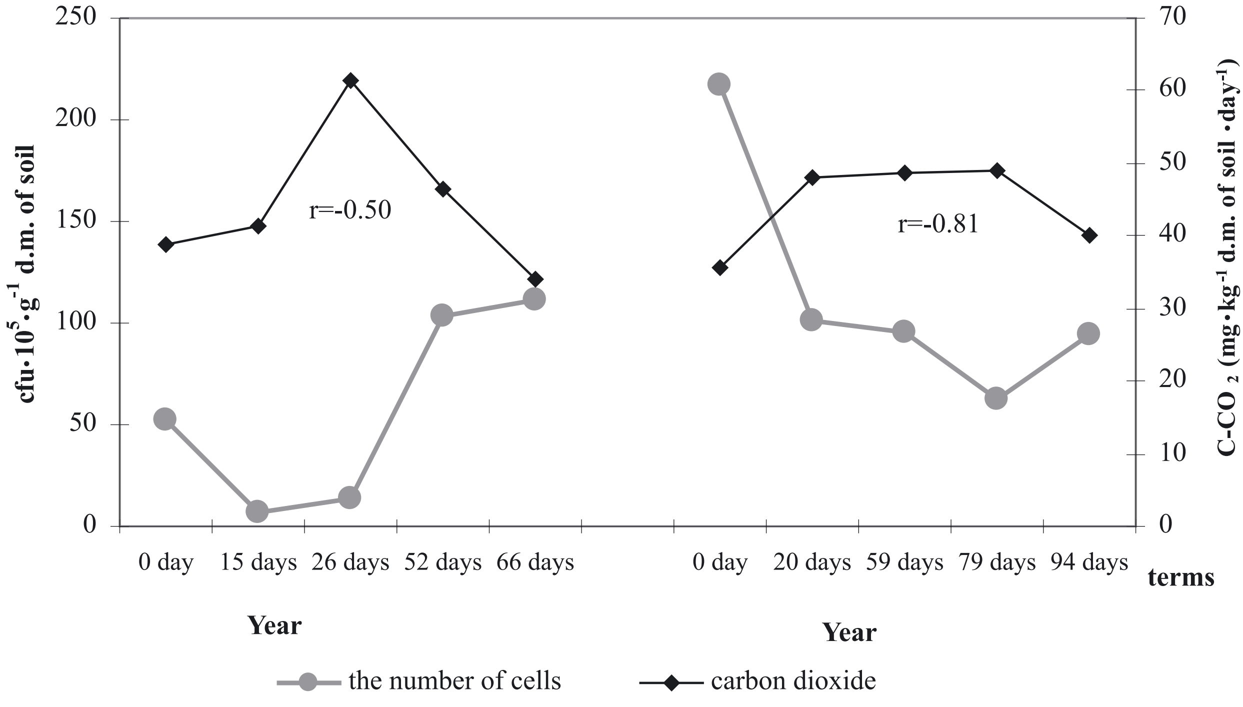

Fig. 9. Correlation between the total bacterial number and the amount of CO_2 released from the soil fertilized with 4 tons d.m. of sewage sludge. Explanations as in Fig. 1.

that numbers of *Salmonella* sp. bacteria in the sewage sludge may depend even on the way of its fermentation. The period of survivability of pathogenic bacteria in soils may be affected by climatic factors [30], soil physicochemical properties or the type of the cultivated plant as confirmed by investigations carried out by Kuczewski and Nowak [31]. Wolna-Maruwka and Sawicka [23], for instance, maintains that *Salmonella* sp. may survive in the soil for 20 days. On the other hand, Nicholson et al. [32] claim that this pathogen can survive in the soil one month.

Apart from the *Salmonella* genus bacteria, the applied sewage sludge also contained bacteria from the *Escherichia coli* and *Clostridium perfingens* genera (Table 1).

When analyzing the numbers of *Escherichia coli* (Fig. 4) and *Clostridium perfingens* (Fig. 5) bacteria in individual soil combinations, they were found present in all soil samples throughout the experiment under both crop plants. However, potatoes turned out to have a stronger phyto-sanitary influence on both of these bacterial species in comparison with winter rye. According to the literature on the subject, the period of survival of these bacteria can vary. Islam et al. [33] reported that *Escherichia coli* bacteria survived in the collected soil samples up to 196 days. On the other hand, experiments carried out by Kaźmierczuk and Kalisz [34] showed *Escherichia coli* and *Clostridium perfingens* bacteria present in sewage sludge even after three years' storage.

The survivability of pathogens in the soil is determined by soil physicochemical properties, among others, by the content of humus, moisture content, presence of nutrients and oxygen concentration. It is evident from experiments carried out by Niewolak et al. [35] that following the introduction with sewage into the soil of the above-mentioned bacteria, their greatest numbers were found to occur in the surface soil layers.

The presence of pathogenic bacteria (*Salmonella* sp., *Escherichia coli*, *Clostrdium perfringens*) in the control combination is somewhat surprising (Figs. 3-5). However, it can be presumed that they found their way there accidentally from other soil combinations as the experimental plots were not separated from one another in any way. Niewolak and Tucholski (36) reported a possibility of transfer of pathogens in a given area by, among other things, animals.

The analysis of the CO_2 released on the control plot and on other soil treatments (Fig. 6) showed that the fertilization of soils with sewage sludge resulted in a slight increase of their respiration activities in relation to the control. Similar results were reported by Dobosz et al. [37]. However, Quemada and Mencho [38], Joniec and Furczak [39] reported a strong stimulation of the rate of mineralization of organic carbon following the introduction of sewage sludge into experimental soils. On the other hand, Persson et al. [40] maintain that soil respiration activity depends on, among other things, the C/N ratio. In our own studies, it was found that differences in the respiration activities between the analyzed objects may have been additionally caused by the release of CO_2 by plants.

In the case of the soil treatments under the cultivation of winter rye (Figs. 7-10), the analysis of the obtained results showed that the respiration activity of the examined objects increased systematically together with the development of plants reaching its peak after 26 days of experiment, i.e. at the stage of the end of rye flowering. On the other hand, in the case of objects under potato cultivation (Figs. 7-10), the release of CO_2 from the soil increased after 20 days of the experiment. Later on, from day 59

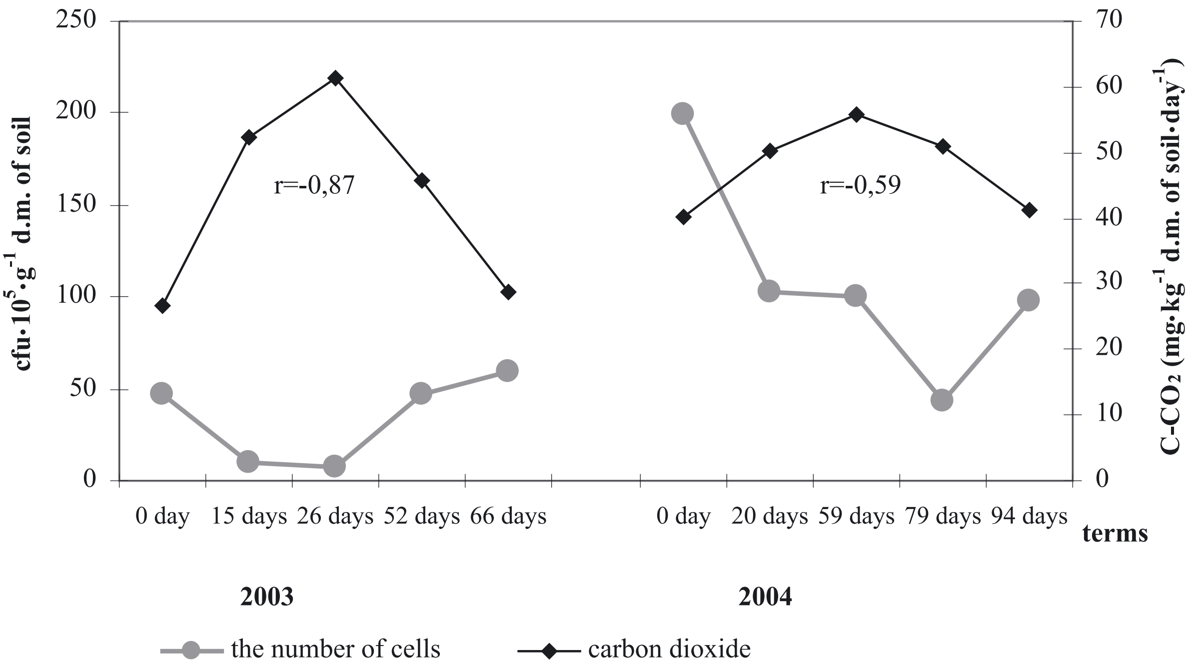

Fig. 10. Correlation between the total bacterial number and the amount of CO_2 released from soil fertilized with 8 tons d.m. of sewage sludge.

(development of potato fruits), the level of soil respiration fluctuated depending on the applied level of organic fertilization. The performed investigations showed that the mean amounts of the CO_2 released from the soil in 2003 and in 2004 differed only by 0.4%.

Analyzing the dynamics of the CO_2 release from the soil combinations in the course of the entire experiment (Figs. 7-10), it was found that in both years the soil respiration activity decreased at the last date of sample collection for analyses, i.e. two weeks after harvest. This was true about all soil treatments, including the one with the highest dose of sewage sludge. A decline in the soil respiration activity fertilized organically with the passage of time was also reported by Selivanovskaya et al. [41]. However, the above findings were not confirmed by investigations carried out by Gostkowska et al. [42] and Hattory [43].

It is also evident from the results presented in the diagrams that a positive correlation between the numbers of the examined groups of microorganisms and the amount of the CO_2 released from the employed soil objects occurred only in the treatment of NPK + 2 tons d.m. of sludge $\cdot ha^{-1} \cdot year^{-1}$ in the soil on which potatoes were grown (Fig. 8).

Conclusion

On the basis of the obtained research results, it was possible to draw the following conclusions:

1. The introduction into the soil of sewage sludge failed to intensify the proliferation of any of the examined groups of microorganisms and did not result in increased respiration processes of the microbes.
2. The performed sanitary assessment of the sewage sludge applied in 2003 revealed its significant contamination with pathogenic microorganisms; therefore, this sludge should not be used in agriculture.
3. Long periods of survivability of pathogenic bacteria in soils (*Salmonella* sp., *Clostridium perfringens*, *Escherichia coli*) makes it essential to seek more effective ways to utilize sewage sludge.

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