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Full Length Research Paper

A study of dairy sewage sludge as the source of soil quality improvement or its degradation

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Biochemical indices related with the transformations of C, N and P are frequently used in the diagnosis of soil quality. Such an assessment usually includes analysis of the following enzymes – the activity of dehydrogenases and of hydrolytic enzymes such as phosphatases, urease and protease. The objective of the study presented here was evaluation of dairy sewage sludge as an agent causing an improvement of soil quality or its degradation. Estimation of soil quality was performed on the basis of a comprehensive study comprising analyses of microbiological and biochemical parameters. The results of the study indicate a stimulating effect of dairy sewage sludge on the soil microorganisms, which was apparent also as an increase in the activity of the enzymes studied. That effect should be attributed to soil enrichment in organic matter, total nitrogen and minerals.

Key words: Dairy sewage sludge, enzymatic activity, microorganisms, soil.

INTRODUCTION

The increasing level of urbanization and industrialization has a significant effect on the dramatic increase in the amount of sewage produced all over the world. That increase is also reflected in increasing numbers of sewage treatment plants that produce continually increasing amounts of sewage sludge. The vast volume of sewage sludge produced must be neutralized and utilised in an appropriate manner (Wang et al., 2008).

Sewage sludge utilization in agriculture has now become common practice not only in European countries but also all over the world. Agricultural utilization of sewage sludge is permitted when the content of heavy metals in such wastes does not exceed specific limits of those elements (Carbonell et al., 2009). Sewage sludge application to the soil is favourable due to its fertilizer properties, improving the productivity of ecosystems through the introduction of biogens (N, P, Na, K) and the soil structure through the addition of organic matter (Ngole and Ekosse, 2009). However, because of the potential content of toxic compounds (heavy metals, detergents, pesticides retardants and other), the introduction of sewage sludge into the soil environment creates the risk of a harmful effect on soil micro-organisms and on plants, as well as on the soil structure (Wang et al., 2008; Alvarenga et al., 2009). Leaching, surface runoff and erosion may lead to the migration of those contaminants from soils amended with sewage sludge to natural ground waters, which may also have an effect on aquatic organisms (Parkpian et al., 2002). Therefore, control of the quality of sewage sludge used for soil fertilisation and continuous monitoring of the effect of those wastes on the parameters of the soil environ-ment are a necessity (Devaney et al., 2008; Halasz et al., 2008). Among the numerous parameters that may be used in the monitoring, analysis and characterisation of soil subjected to the effect of sewage sludge that could be employed for the expansion of knowledge about and understanding of the processes that take place in soils

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amended with sewage sludge the enzymatic and micro-biological activity of soil deserve special note. Enzymes released by microorganisms during the degradation of sewage sludge play a key role in the biological and biochemical transformations that take place in the soil environment. Microbiological enzymes are also responsible for the decomposition of complex organic compounds (Castaldi et al., 2008). It is soil bacteria and fungi, and the enzymes they produce, that are the most effective in the processes of decomposition of organic matter in the soil, thus also affecting plants, through supplying them with nutrients, as well as the physical and chemical properties of the soil (Cenciani et al., 2008). The characteristics of sewage sludge, espe-cially in terms of its content of toxic substances, depend on the potential sources of contamination that, in turn, depend on where the sludge comes from – municipal or industrial sewage treatment plants, and on the branch of industry involved (e.g. the dairy industry, fruit and vegetable processing industry, chemical industry, etc.). The composition of sewage sludge includes organic and inorganic compounds, biogens, as well as biomass produced in the course of aerobic and anaerobic processes of degradation (Carbonell et al., 2009). There-fore, the sludge may exert an enormous effect on the physical, chemical and biological properties of soil. The overall improvement in soil quality should be assessed not only on the basis of chemical properties of the soil estimated with the use of conventional analytical tests, but also through additional measurements of the biological and enzymatic activity of soils (Alvarenga et al., 2008). As follows from studies by numerous authors (Nannipieri et al., 2003; Jezierska and Frąc, 2008, 2009; Carbonell et al., 2009), the biological parameters of soil are modified by environmental and anthropogenic factors to a greater extent, and may be a potential indicator of ecological stress (Dick and Tabatabai, 1992).

The biological activity of soil, covering the transformations of various compounds and energy in the soil environment, can be measured through the deter-mination of the enzymatic activity (Halasz et al., 2008; Alvarenga et al., 2009; Wolna-Maruwka et al., 2009). In the opinion of many authors (Castaldi et al., 2008; Jezierska-Tys and Frąc, 2008; Alvarenga et al., 2009; Antonious, 2009) soil enzymes are perceived as biological indicators of the quality, fertility and productivity of soils, as well as of the run of processes taking place in the soil and of anthropopressure. Nannipieri et al. (2002) and Trasar-Cepeda et al. (1998) are of the opinion that the enzymatic activity of soil may be an early and sensitive indicator of the degree of degradation of soils and it can be used for the estimation of anthropogenic factors affecting soil quality. The introduction of organic fertilisers and wastes into soil, including sewage sludge, has a significant effect on the growth and development of microorganisms and on the enzymatic activity of soil (Cenciani et al., 2008; Jezierska-Tys and Frąc, 2008, 2009).

Biochemical indices related with the transformations of C, N and P are frequently used in the diagnosis of soil quality. Such an assessment usually includes analysis of the following enzymes – the activity of dehydrogenases and of hydrolytic enzymes such as phosphatases, urease and protease (Alvarenga et al., 2008, 2009; Jezierska-Tys and Frąc, 2009; Tejada, 2009). The objective of the study presented here was evaluation of dairy sewage sludge as an agent causing an improvement of soil quality or its degradation. Estimation of soil quality was performed on the basis of a comprehensive study comprising analyses of microbiological and biochemical parameters.

MATERIALS AND METHODS

Sludge formation in dairy sewage treatment plants

Dairy sewage is formed during the washing of technological lines on which various dairy products are produced. The sewage is purified in a mechanical-biological treatment plant and the product of the process of purification is excess sludge. Raw sewage flows by gravity to a grating which is the first element of the mechanical purification of the sewage. After the initial mechanical purification on the grating, the sewage flows to a horizontal sand bed, then flows by gravity to the preliminary aeration reservoir, and thence to a distribution chamber and to stirring pools with an active sediment.

Sewage purification with the active sediment method consists in providing an adequate oxygen supply to the sewage and maintaining the sediment in a state of suspension by enforcing constant motion of the fluid. That function is fulfilled by vertical and horizontal aerators. Thanks to the absorption abilities of the floccules of the active sediment, organic contaminants present in the sewage are absorbed by microorganisms included in the active sediment. The supply of oxygen in an amount adequate to the load of contaminants in the sewage is necessary for the organic contaminants to be optimally utilised by the active sediment microorganisms for their own metabolic processes. During a year a dairy sewage treatment plant produces ca. 2000 tons of sludge. Excess sludge is used as a fertiliser, therefore it is necessary to monitor its value and to identify its effect on microbiological processes that naturally take place in soil, and on the soil microorganisms and their biochemical activity that can be used in the estimation of soil fertility and quality.

Description of the experiment

The study comparing the effect of mineral fertilisation and of amendment with dairy sewage sludge on the enzymatic (dehydrogenase, acid and alkaline phosphatases, protease, urease) activity of soil and on the intensity of ammonification and nitrification was conducted on the basis of a 1 ha field experiment. The field experiment was set up on a brown soil, situated in the vicinity of a dairy sewage treatment plant in Krasnystaw, East Poland, Europe (Figure 1). The experiment was designed with the method of complete randomisation, on a brown soil developed from a clay formation. Characteristics of the soil are given in Table 1. The experiment comprised of two treatments:

(1) Soil with mineral fertilisation
(2) Soil amended with dairy sewage sludge.

In treatment 1 the mineral fertilisation was applied as follows: in

Figure 1. Location of the experiment, * marks the locality at which the experiment was conducted.

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

Parameter	Brown soil	Sewage sludge
pH	6.4	7.23
C (g kg^{-1} dwt)	13.5	803.0
N (g kg^{-1} dwt)	1.6	58.2
C:N	8.3	13.8
P (g kg^{-1} dwt)	18.3	40.0
K (g kg^{-1} dwt)	26.8	4.6
Heavy metals (mg kg^{-1} dwt)		
Zn	28.7	194.0
Cd	0.16	-
Cu	7.16	18.7
Pb	10.3	5.3
Ni	10.1	21.7
Cr	18.4	14.1
Hg	0.09	-
Texture (g kg^{-1})		
Sand	80	-
Silt	470	-
Clay	450	-

autumn, 300 kg· ha^{-1} of Polifoska (N, P_2O_5, K_2O = 6, 20 and 30%), and in spring 150 kg·ha^{-1} of ammonium nitrate (N = 34%). Both treatments were sown with winter wheat. In treatment 2 the soil was amended with dairy sewage sludge at the dose of 50 t·ha^{-1}. Characterisation of the sewage sludge is given in Table 1. In spring saltpetre was applied, at the dose of 200 kg·ha^{-1} (K_2O=60%), as a measure aimed at potassium supplementation. Soil samples for analyses were taken from the depth of 0 to 20 cm in various stages of plant vegetation: heading, milk ripeness and full ripeness phases of wheat. The soil samples were screened through sieves with 2 mm mesh and then used for microbiological and biochemical analyses.

Microbiological and biochemical analyses

The study included determinations of microbiological and biochemical parameters of the soil. Dehydrogenase activity was determined spectrophotometrically, at wavelength of 485 nm, using the method of Thalmann (1968) in the modification described by Alef and Nannipieri (1995), the results being expressed in microgram TPF kg^{-1} d^{-1}. Acid and alkaline phosphatase activity was determined with the spectrophotometric method described by Alef et al. (1995), at wavelength of 400 nm. Protease activity was assayed with the spectrophotometric method after soil incubation with sodium caseinate for 1 h at 50°C (Ladd and Butler, 1972; Alef and Nannipieri, 1995). Urease activity was determined spectrophotometrically at wavelength of 410 nm, following the modified method of Zantua and Bremner (1975). The numbers of ammonifying and nitrifying bacteria were determined with the method of the most probable number (MPN). The numbers of the ammonifiers were determined on a liquid substrate with peptone and those of the nitrifiers on a liquid mineral medium with $CaCO_3$ (Alef and Nannipieri, 1995). The results obtained were processed statistically using the method of analysis of variance and Tukey's intervals of confidence.

RESULTS

The objective of the experiment was comparison of the effect of mineral fertilisation and of soil amendment with dairy sewage sludge (50 t ha^{-1}) on the activity of selected soil enzymes, on the intensity of ammonification and nitrification, and on the numbers of ammonifying and nitrifying bacteria in the brown soil Figures 5a and 5b. In both experimental treatments equal amounts of nitrogen were supplied to the soil.

The activity of dehydrogenases (Figure 2a) increased in the course of the experiment, attaining the highest values in both experimental on the 3rd date of analyses (in the phase of full ripeness of wheat). In the initial phase of the experiment, the fertilisation applied did not cause any stimulation of activity of the enzymes studied, whose activity in both treatments was at a level close to zero. In this study, the activity of acid phosphatase (Figure 2b) on the particular dates of analyses was similar in both experimental treatments. The exception was the 3rd date of analyses (full ripeness phase of wheat) on which a significantly higher activity of that enzyme was noted in the soil with the sludge. The activity of alkaline phosphatase (Figure 2c) was significantly higher in the treatment with the sludge compared to that with mineral fertilisation only on the 2nd date of analyses, that is, in the phase of milk ripeness of wheat. The average activity of phosphatases (acid and alkaline) was higher in the soil amended with the sludge than in the soil with mineral fertilisation.

The activity of protease (Figure 3a) and urease (Figure 3b), enzymes involved in the circulation of nitrogen, was subject to variation in the treatments during the run of the experiment. Significantly higher protease activity was noted in the soil amended with the sludge compared to the soil with mineral fertilisation only on the final date of analyses – in the phase of full ripeness of wheat. Also in the phase of milk ripeness of wheat the activity of protease was at a notably higher level in the soil with the sludge than in the soil with mineral fertilisation. On the 1st date of analyses proteolytic activity was at a similar level in both experimental treatments. Urease activity was higher in the soil amended with the sludge than in that with mineral fertilisation throughout the duration of the experiment. A significant effect of the sludge on urease activity in the soil studied was noted already in the initial period of analyses, that is, in the phase of heading of wheat. The same tendency was found also in phases of milk ripeness and full ripeness. However, on the 2nd date of analyses the difference in the activity of the enzyme between the treatments was not significant.

The intensity of the process of ammonification was similar in both experimental treatments (Figure 4a). Significant periodic differences were noted in the intensity of ammonification, the highest values of the process in question being recorded in both experimental treatments in the phase of milk ripeness of wheat. Changes in the rate of ammonification were accompanied by changes in the intensity of the process of nitrification. The highest intensity of nitrification was recorded on the 1st date of analyses, that is in the phase of heading of wheat (Figure 4b). Subsequently, in the successive development phases of the plants a decrease was observed in the intensity of that process. No significant differences were observed in nitrification intensity between the experimental treatments.

DISCUSSION

Dehydrogenases are enzymes from the group of oxidoreductases that are present only in living cells. For this reason those enzymes are used as indicators of the average microbiological activity of a given environment (Izquierdo et al., 2005; Alvarenga et al., 2009). The lack of any stimulation after dairy sewage sludge fertilisation in initial part of the experiment may indicate a lack of easily available sources on carbon in the material introduced in the soil environment. Non-easily available carbon compounds, such as cellulose or lignins, present in the sludge are used as a source of by most soil microorganisms only after their mineralisation.

Acid and alkaline phosphomonoesterases (phosphatases)

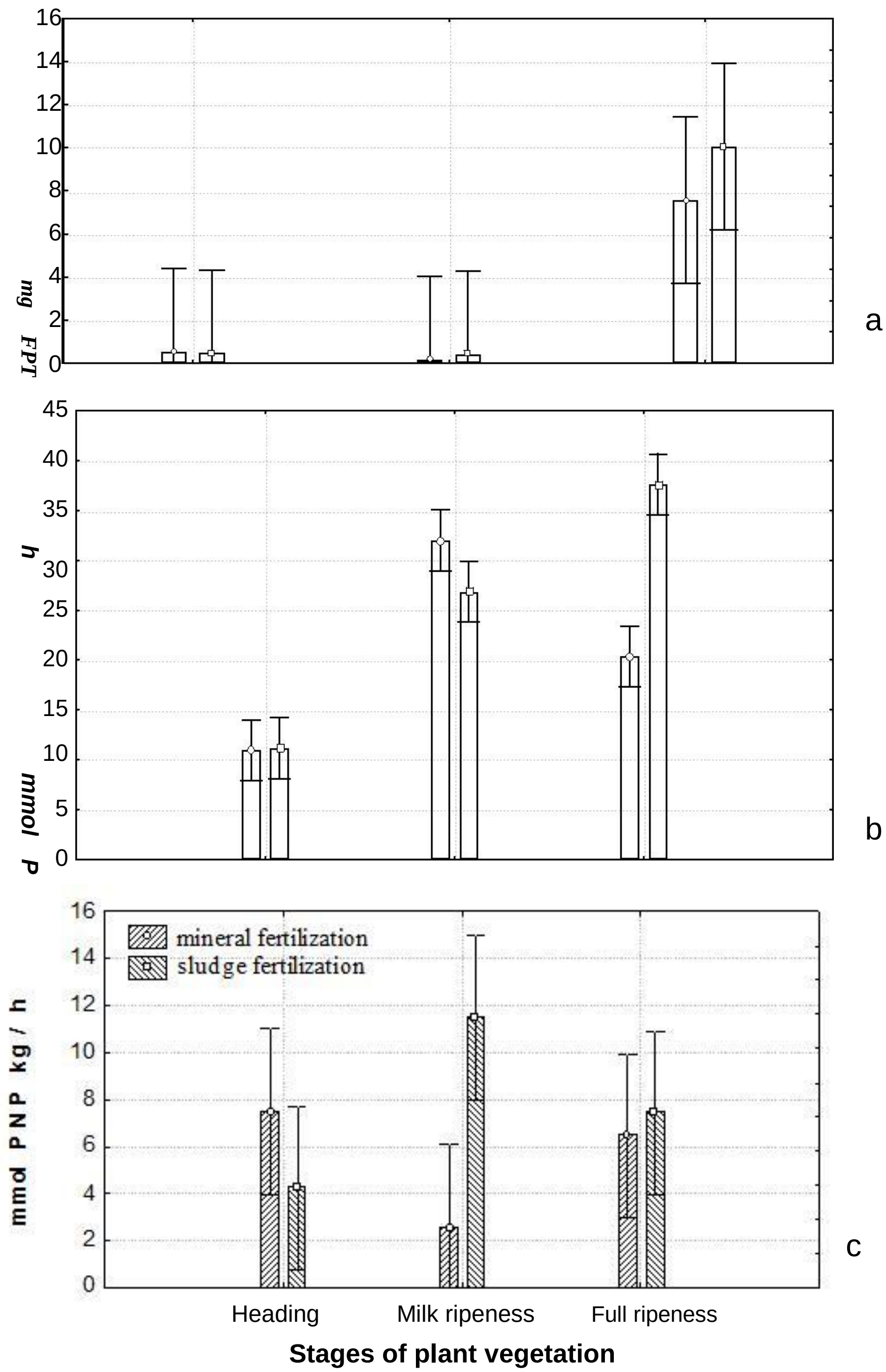

Figure 2. Activity of (a) dehydrogenases, (b) acid phosphatase and (c) alkaline phosphatase.

are enzymes that occur in soil and are responsible for the mineralisation of organic phosphorus compounds (Huang and Shindo, 2000). Among the hydrolases, the activity of acid and alkaline phosphormonoesterases is frequently used for the determination of changes in soil quality, due to their high sensitivity to contaminants (Gil-Sotres et al., 2005). The activity of phosphatases may be disturbed by the presence of contaminants in the environment, such as Pb (Gil-Sotres et al., 2005) and other heavy metals like Cd, Zn, Cu (Ranella et al., 2003). Considering the activity of those enzymes one can observe that they are a good indicator of the level and quality of organic matter in soil. It can be predicted that an increase in the content of organic matter in soil is accompanied by an increase in the activity of the enzymes in question (Alvarenga et al., 2008).

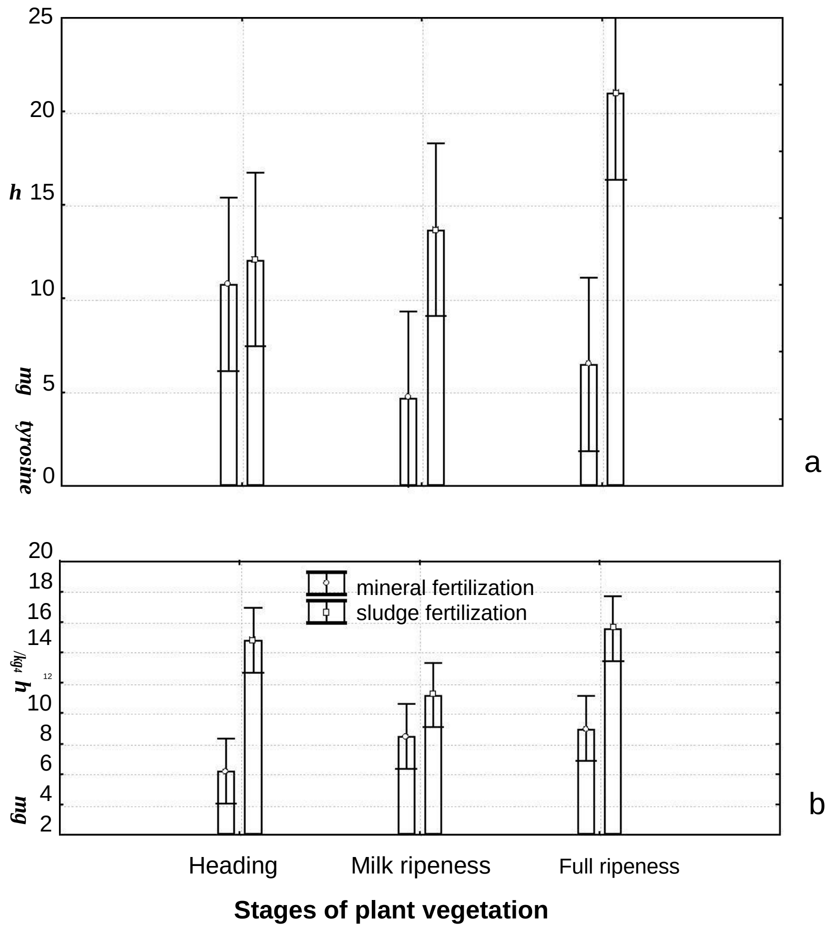

Figure 3. Activity of (a) protease and (b) urease.

In our study, the high activity of phosphatases in the soil with the sludge may have been caused by higher content of organic matter introduced with the sludge compared to that in the soil with mineral fertilisation. A high level of phosphatase activity caused by increased organic matter content in soil was also observed by Halasz et al. (2008) and Alvarenga et al. (2008, 2009).

Protease is an enzyme catalysing the hydrolysis of proteins to peptides and amino acids, while urease catalyses the hydrolysis of urea to CO2 and NH3 (Alef and Nannipieri, 1995). Comparing the relative increase in all the enzymatic activities studied, one can observe that the activity of the two latter enzymes (protease and urease) was stimulated most strongly by the dairy sewage sludge introduced in the soil. That effect could have been caused by the high content of organic nitrogen in the sludge (Table 1), affecting the activity of enzymes involved in nitrogen circulation. Ammonification and nitrification are processes that take place in soil with participation of microorganisms involved in nitrogen transformations (Nugroho et al., 2009). Analysing the results obtained concerning the processes of ammonification and nitrification, one can conclude that the sewage sludge introduced in the soil did not cause any interference with the run of those processes. With a low level of N-NH4 ions on the 1st and 3rd dates of analyses, a high level of N-NO3 ions was observed in the soil studied. On the second date of analyses, with a fairly high level of N-NH4 ions, in both experimental treatments a low level of N-NO3 was noted. According to Nugroho et al. (2009), changes in the intensity of the processes under discussion are related also with the presence and species composition of microbial populations in the soil environment, as the activity of microbial groups participating in nitrogen transformations depends on the presence of nitrates, ammonia and other nitrogen compounds in the environment (Ginovart et al., 2005).

The study demonstrated that the enzymatic activity in the soil studied was significantly affected by the experimental factors applied, that is, fertilisation and the development phases of the experimental crop plant. The results of the study indicate a stimulating effect of dairy sewage sludge on the soil microorganisms, which was apparent also as an increase in the activity of the enzymes studied. That effect should be attributed to soil enrichment in organic matter, total nitrogen and minerals.

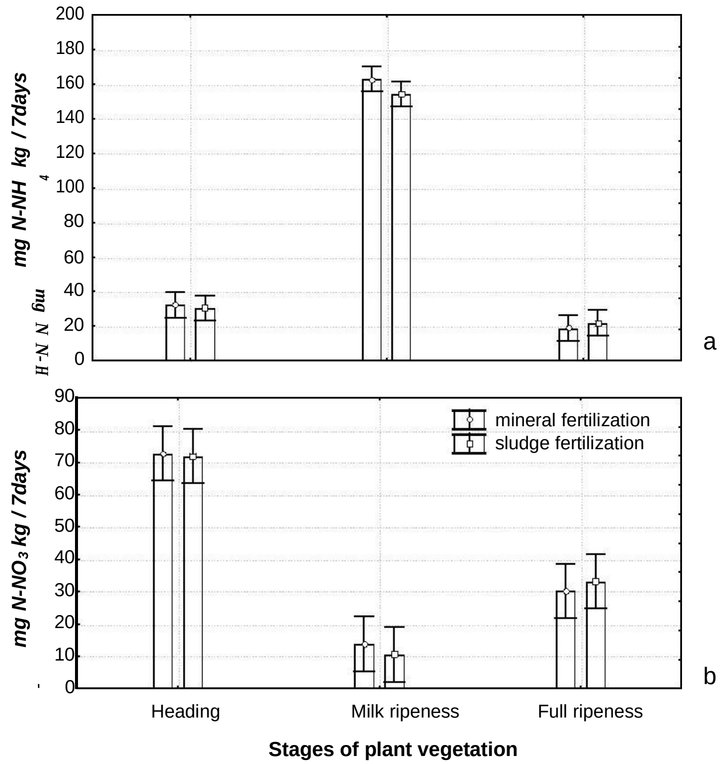

Figure 4. Intensity of (a) ammonification and (b) nitrification stages of plant vegetation: heading, milk ripeness and full ripeness phases of wheat.

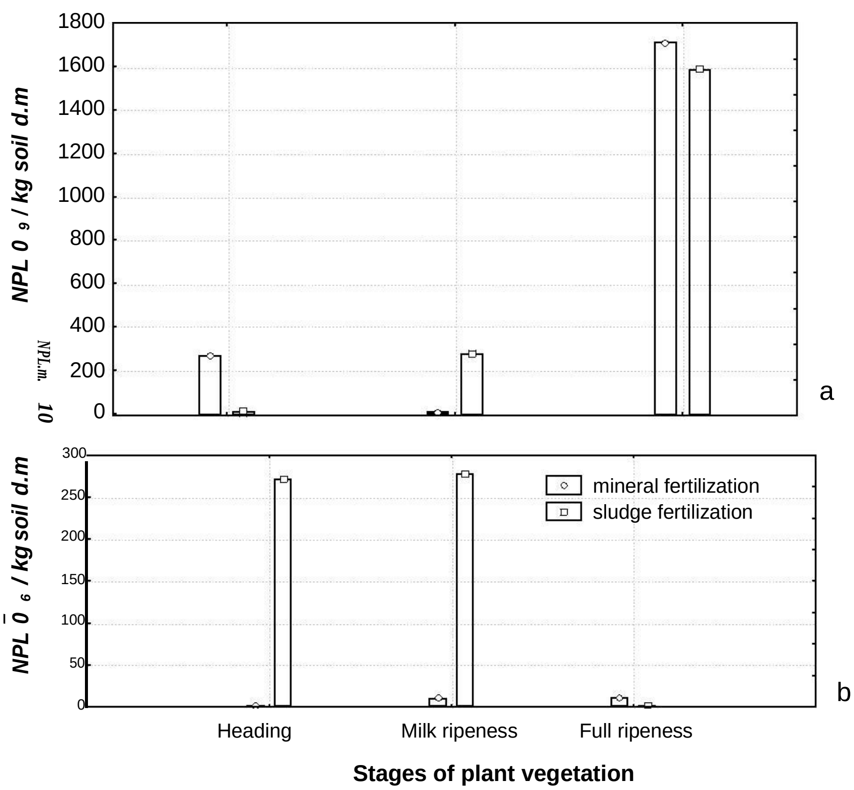

Figure 5. Numbers of (a) ammonifying and (b) nitrifying bacteria.

The study showed that dairy sewage sludge has a favourable effect on the microbiological and biochemical properties of soil and can be accepted as a product characterised by notable fertiliser value. The microbiological and biochemical tests applied proved to be sensitive indicators of the biological properties of soil amended with dairy sewage sludge and can be used in the assessment of soil fertility.

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