Environment Protection Engineering

Vol. 35 2009 No. 4

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BIOLOGICAL INDICATORS OF SOIL QUALITY AFTER APPLICATION OF DAIRY SEWAGE SLUDGE

Microbiological indicators can be used for the assessment of the ecological condition of soils affected by various environmental and anthropogenic factors. The objective of the study was to estimate the causative effect of sludge from dairy sewage treatment plant on enzymatic activity of dehydrogenases, protease and urease and on nitrogen transformations (intensity of ammonification and nitrification) in a brown soil. The microbiological and biochemical tests applied proved to be sensitive indicators of the biological properties of soil fertilised with sludge from a dairy sewage treatment plant.

1. INTRODUCTION

Enzymatic activity of soils can be accepted as an indicator of overall microbiological activity [1]–[3]. It is one of the indices used for the estimation of both fertility and productivity of soils and permits the acquisition of comprehensive knowledge on changes taking place in soil environment following the introduction of, e.g., sewage sludge [3]–[6]. The analyses of the soil environment make use also of such processes as ammonification and nitrification that provide information on the transformations of soil nitrogen [7]–[9]. In view of the role of the above parameters, their rate or intensity is considered to be an important indicator of the biological activity of soil and is frequently employed for determining the effect of various factors on the biological status of the soil environment [10], [11].

Numerous authors have studied the effect of sewage sludge on the enzymatic activity of soil [4], [12]–[16] and on the intensity of the processes of ammonification and nitrification in soil amended with sewage sludge [10], [17], [18]. Sewage sludge introduced into soil may cause notable changes in its enzymatic activity and in the intensity of proc-

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esses taking place in the soil environment, whose rate and direction depend on the type of the sludge applied, its dose, and on the biochemical parameter studied [13].

In the available literature, there are few studies concerning the effect of dairy sewage sludge on the microbiological and biochemical activity of soil, and those studies that had been undertaken were conducted mostly under laboratory conditions [19]–[23]. Therefore, the objective of the study reported herein was to determine the causative effect of soil fertilisation with sludge from a dairy sewage treatment plant on the enzymatic activity of dehydrogenases, urease, protease, intensity of ammonification and nitrification, and on the content of organic carbon and total nitrogen in a field experiment set up on a brown soil.

2. MATERIAL AND METHODS

The study on the fertilising effect of dairy sewage sludge on the microbiological and biochemical activity of soil was performed on the basis of a field experiment covering the area of 1 ha (10 plots of 200 m^2 in surface area for each experimental treatment selected in a fully random way). Each year the soil on experimental plots was amended with sludge at the dose of 22 t ha^{-1} (4 t d.m. ha^{-1}), which gave the following experimental treatments: 1 – control soil, without sludge (G0); 2 – soil in the first year after sludge application (G1); 3 – soil in the second year after sludge application (G2); 4 – soil in the third year after sludge application (G3); 5 – soil in the fourth year after sludge application (G4). Soil samples for analyses were taken in the fourth year of the experiment, at various stages of plant vegetation: stalk shooting (F1), heading (F2), milk ripeness (F3) and full ripeness (F4). The soil was sown with winter wheat. From each experimental plot 20 soil samples were taken, thus a representative averaged sample was obtained. Such a model of the experiment permitted the study of the sequential effect of the sludge on the microbiological and biochemical activity of the soil.

The study was conducted on a brown soil developed from a silt-clay formation with the following grain-size composition: 8% of sand fraction (1.0–0.1 mm), 47% of silt fraction (0.1–0.02 mm) and 45% of washable particles (<0.02 mm). The sludge was drawn from the Regional Dairy Cooperative in Krasnystaw, with an active sediment system and a mechanical excess sediment dehydration station equipped with a centrifuge. Sewage treatment with the method of active sediment consists in introducing an adequate amount of oxygen into the sewage and in maintaining the sediment in suspension by keeping the liquid in constant motion due to the application of vertical and horizontal aerators. Thanks to the absorption capabilities of the floccules of the active sediment, organic contaminants contained in the sewage get absorbed by micro-organisms included in the sediment. In spite of the great load of contaminants that reach the sewage treatment plant (2000–4000 kg BZT_5 day^{-1}), the loading of the active sediment does not exceed the level of 0.3 kg BZT_5 kg^{-1} d.m. of sludge due to the high concentration of the active sediment, i.e. 4000–6000 mg d.m. dm^3 in the biological troughs, and due to their considerable capacity that ensures sewage retention times of ca. 3.5 days. The very high reduction of contaminants, reaching 98% for BOD_5 and 96% for COD, results in the formation of notable amounts of excess sediment that has to be removed from the system for the process of sewage treatment to proceed under optimum conditions. The dairy sewage sludge did not contain pathogenic micro-organisms, helminth ova or toxic substances (PAHs, PCBs), and the content of heavy metals was considerably below the permissible standards. Basic characteristics of the soil and the sludge used in the study presented are given in table 1.

Table 1

Characterictics of soil and 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	–

The study included determination of dehydrogenase activity with the method of THALMANN [24], in modification introduced by ALEF [25]. Protease activity was determined according to the method of LADD and BUTLER [26], in modification proposed by ALEF and NANNIPIERI [27], and urease activity with the method of ZANTUA and BREMNER [28]. The intensity of ammonification and nitrification was determined in accordance with the Polish Standard PN-ISO [29], determining the content of NH_4^+ ions with the use of Nessler's reagent, and those of NO_3^- with the brucine method [30]; pH was determined potentiometrically. In the final stage of the experiment, chemical analyses of the soil were performed: the content of organic carbon with the Tiurin method and the content of total nitrogen with the method of spectrophotometry.

To study the multi-year fertilising effect of the sludge and the effect of the dates of the analyses on the values of the microbiological, biochemical and chemical features of the soil, two-factor analyses of variance were performed (ANOVA). Mean values of the traits under study for the experimental treatments and the dates of analyses were compared by means of the Tukey 95% intervals of confidence, at the significance level $\alpha = 0.05$. Statistical analyses were made using the software STATISTICA 7.1PL (StatSoft Inc. 2006).

3. RESULTS AND DISCUSSION

Organic matter introduced into soil usually increases its enzymatic activity and stimulates microbiological activity, among others that of dehydrogenases [31].

In the soil under study, dehydrogenase activity remained on a stable very low level in all treatments and throughout the experiment time (test times F1, F2 and F3) (figure 1). As follows from the data collected, significant differences in the activity of the enzyme studied occurred only on the last date of analyses, i.e. in the full ripeness phase, which also had an effect on mean levels of dehydrogenase activity. Statistical evaluation of the results obtained showed that average dehydrogenase activity was the highest in the fourth year after sludge application – treatment G4 (figure 3). Probably, the reduction in dehydrogenase activity in the first year of sludge fertilization was due to the presence of hard-to-decompose organic compounds, which inhibited both the microbial growth and of the activity of the enzyme in question. As the process of degradation of those compounds progressed and simpler substances became available for micro-organisms, the dehydrogenase activity increased, reaching the highest level in the fourth year of sludge application. Similar results were obtained by JEZIERSKA-TYS and FRĄC [19].

The activity of enzymes that control nitrogen transformations in soil may be an indicator of biological activity of the soil environment, may be used for estimating the effect of anthropogenic factors on changes taking place in soil, and may also provide evidence of the intensity of nitrogen compounds transformations in the environment and may be an index of nitrogen availability for plants [27]. In the study, among the enzymes studied the highest sensitivity to fertilisation with dairy sewage sludge was displayed by protease (figures 1 and 3). The dairy sewage sludge introduced into the soil had a significant effect on proteolytic activity, both in the particular years of its effect and in the various vegetation phases of the plants. The analysis of the mean values of protease activity for the particular experimental treatments shows that the dairy sewage sludge applied had a significant stimulating effect on changes in its activity. Comparing the average protease activity in the experimental treatments one can observe that the highest activity of that enzyme was characteristic of the soil in the first and third years of the dairy sewage sludge application (treatments G1 and G3), and the lowest in the fourth year – treatment G4 (figure 3). A stimulating effect of dairy sewage sludge on protease activity was also demonstrated in the studies by JEZIERSKA-TYS and FRĄC [21]. Similar trends in the effect of dairy sewage sludge on protease activity were observed by ZAMAN et al. [32].

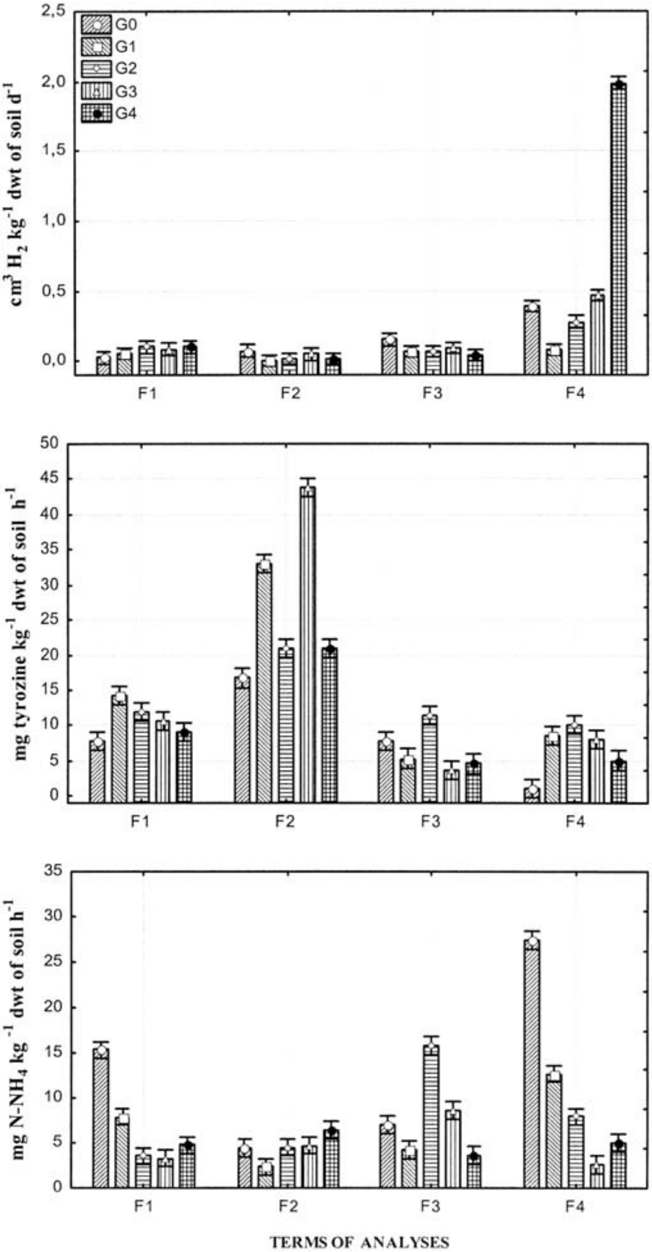

Fig. 1. Dehydrogenases, protease and urease activity of soil fertilised with dairy sewage sludge
Explanations: G0 – control soil, without sludge, G1 – soil in the first year after sludge application, G2 – soil in the second year after sludge application, G3 – soil in the third year after sludge application, G4 – soil in the fourth year after sludge application. Terms of analyses: F1 – stalk shooting phase, F2 – heading phase, F3 – milk ripeness phase and F4 – full ripeness phase

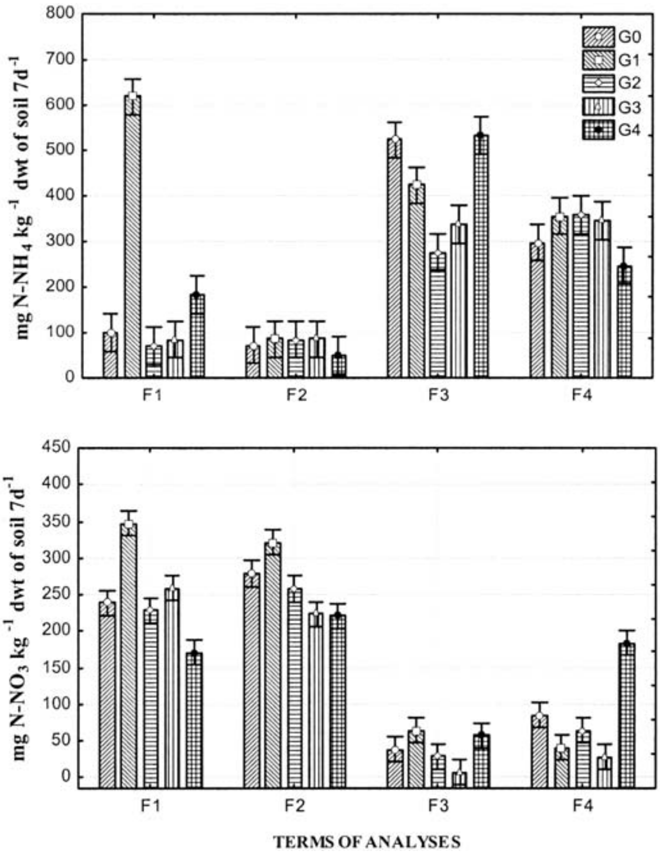

Fig. 2. Ammonification and nitrification intensity of soil fertilised with dairy sewage sludge. Explanations: see fig. 1

Certain authors [28], [33] are of the opinion that urease activity depends on the soil reaction and its content of organic matter, and that the enzyme plays a significant role in soil nitrogen transformations. The available literature contains numerous studies [13], [26], [34] on the effect of municipal sewage sludge on urolytic activity. However, there is little data [19], [21] concerning the effect of dairy sewage sludge on the activity of the enzyme in question. Urease activity in the soil fertilised with dairy sewage sludge remained on a notably lower or similar level compared with that in the control soil, throughout the period of the experiment (figure 1). The analysis of mean values of urease activity for the particular experimental treatments supports its significant lowering in treatments with the sludge in relation to the values obtained for the control (figure 3). It should emphasized that the high mean level of urease activity in the control soil, compared with the treatments with sludge amendment, was a consequence of the high urease activity recorded in that treatment in analysis times I and

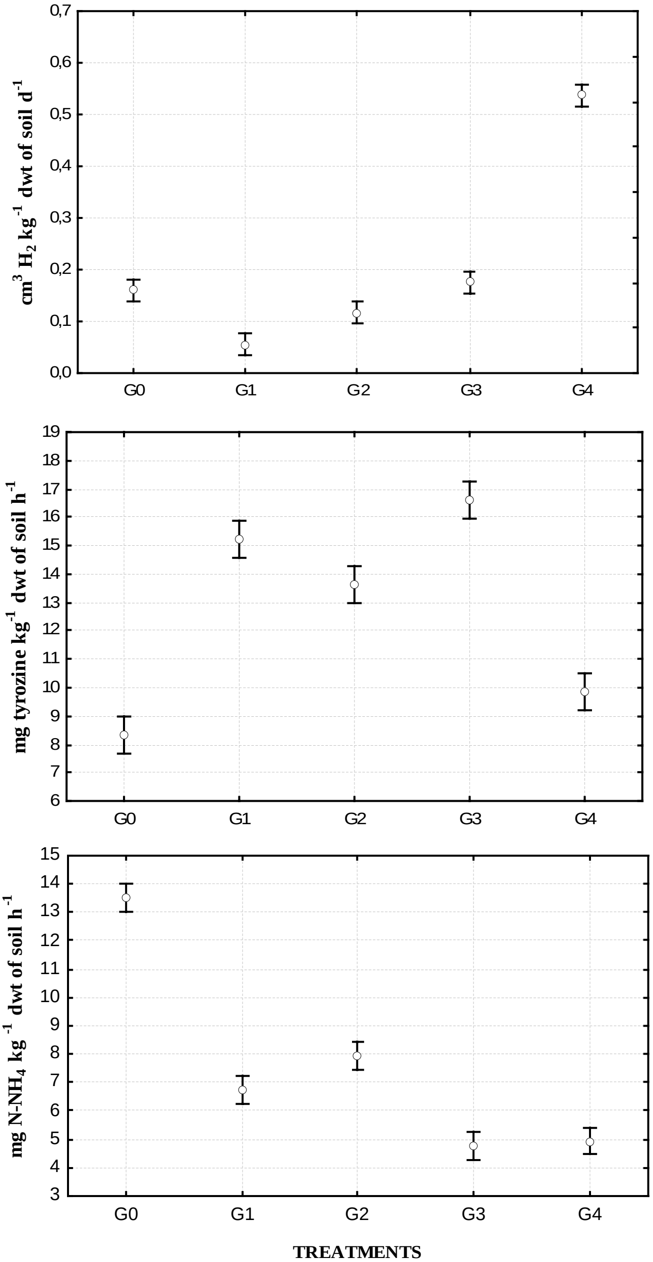

Fig. 3. Mean dehydrogenases, protease and urease activity in particular treatments
Explanations: G0 – control soil, without sludge, G1 – soil in the first year after sludge application, G2 – soil in the second year after sludge application, G3 – soil in the third year after sludge application, G4 – soil in the fourth year after sludge application

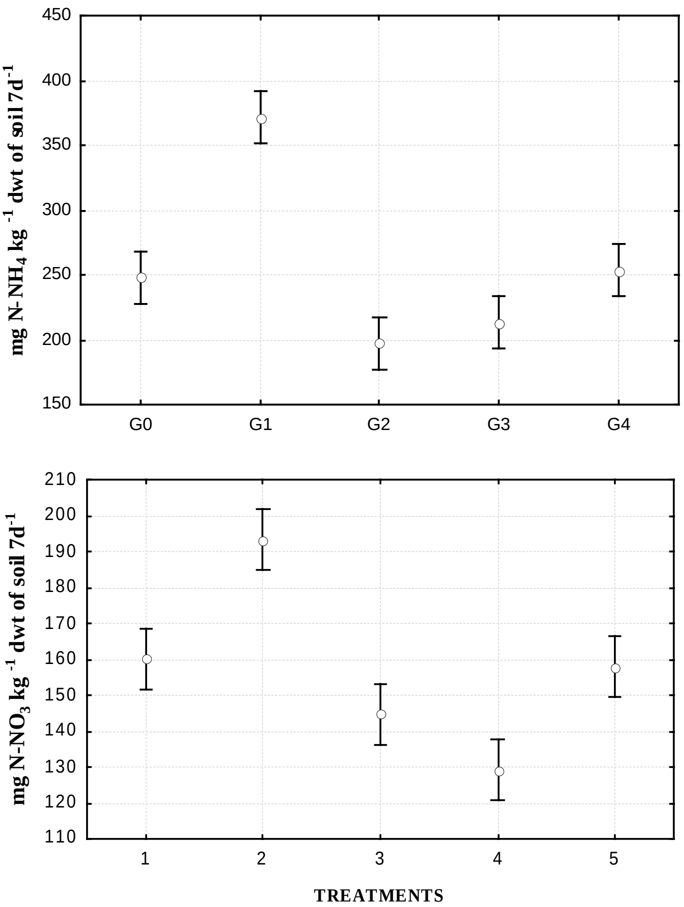

Fig. 4. Mean ammonification and nitrification intensity in particular treatments
Explanations: see fig. 3

IV (F1 and F4). The urease activity was probably caused by lowering in pH of the soil in the treatments with the dairy sewage sludge.

The intensity of ammonification was subject to periodic oscillations in the particular experimental treatments and was characterised by a certain dynamics of changes during experiment (figure 2). Mean values of ammonification rate for the particular experimental treatments indicate that the sewage sludge introduced into the soil significantly stimulated the rate of organic nitrogen mineralisation only in treatment G1, i.e. in the first year of the sludge fertilization (figure 4). It should be noted that the high mean value of ammonification rate in the experimental treatment in question was caused by the high rate of that process in analysis time I (F1). In the other experimental treatments (G2 and G3), the rate of the process examined decreased or slightly increased (treatment G4) compared with the values obtained in the control soil. The recorded values of amonification rate testify to a tendency towards mineral nitrogen immobilisation in the soil, which is usually unfavourable for crop plants, though – on the other hand – it reduces oxidation of $N\text{-}NH_4$ to $N\text{-}NO_3$ in the process of nitrification, thus limiting potential losses of nitrogen [36].

Stimulation of the rate of nitrification was recorded only in treatment G1, i.e. in the first year of dairy sludge effect on the soil. An increase in the content of nitrates(V) was probably due to the nitrifying activity of microorganisms occurring in the sludge introduced into the soil [20]. In the remaining treatments, the rate of the process in question was similar to or even lower than that obtained for the control soil. In the successive times of analyses, a rapid decrease was recorded in the rate of that process in all experimental treatments, with a significantly higher nitrifying activity compared with the control treatment, being observed only in analysis time IV (F4), i.e. in the phase of full ripeness of winter wheat, in the fourth year after the sludge application (treatment G4). The analysis of the mean values of nitrification rate in particular experimental treatments indicates that the dairy swage sludge is responsible for the inhibition of nitrification, with the exception of treatment G1 in which the rate of this process was on a significantly higher level compared with the rates obtained in the remaining experimental treatments and in the control soil (figure 4). A favourable effect of fertilising agents on nitrification activity of soils was observed by GOSTKOWSKA et al. [33]. Those authors attributed that effect to soil enrichment with organic substances and to increase in soil pH. Also JEZIERSKA-TYS and FRĄC [19] mentioned a significant stimulating effect of dairy sewage sludge on the rate of ammonification and nitrification.

The soil reaction varied, depending on the duration of the experiments with dairy sewage sludge and on the time of analysis (table 2). Soil fertilisation with the sewage sludge decreased significantly pH of the soil, especially in the analysis times I (F1) and IV (F4). In the analysis time I (F1), a distinct acidifying effect of the dairy sewage sludge was observed in the treatments G2, G3 and G4. Only in the treatment G1, i.e. in the first year after sludge application, a soil pH recorded was higher compared with that in the control soil. In the second analysis time (F2), a lowering in pH in relation to the control soil was recorded only in treatment G3, i.e. in the third year of the experiment. In the remaining treatments (G1, G2 and G4), soil reaction was neutral or slightly alkaline (pH 7.00–7.30). Comparing the changes in soil reaction in the milk ripeness phase (F3), a considerably lower pH value can be observed in the treatments G1 and G4, and a higher value in the treatments G2 and G3 in relation to the control. In the final stage of the experiment (F4), acidifying effect of the waste applied became evident in all the treatments, with the exception of G1, i.e. the first year of the sludge effect. In that treatment (G1) a considerably higher pH value was recorded, compared to the remaining experimental treatments. The highest pH value (7.53) was recorded at the beginning of the experimental period, i.e. in the analysis time I (F1) (stalk shooting phase), in the treatment G1. Whereas, the lowest pH value (4.63) was recorded in the same treatment in the milk ripeness phase of wheat (F3). In the field experiment, soil reaction was subject to notable variation, which was probably also due to climatic factors, as was found by JEZIERSKA-TYS and FRĄC [37].

Table 2

Changes of pH_{KCl} in particular treatments during the experiment (pH)

Treatments	Terms of analyses			
	F1	F2	F3	F4
G0	7.00	6.98	6.22	6.62
G1	7.53	7.00	4.63	7.24
G2	5.74	7.18	7.17	6.15
G3	5.84	5.99	7.01	4.96
G4	5.15	7.30	4.61	6.02

Explanations: G0 – control soil, without sludge, G1 – soil in the first year after sludge application, G2 – soil in the second year after sludge application, G3 – soil in the third year after sludge application, G4 – soil in the fourth year after sludge application. Terms of analyses: F1 – stalk shooting phase, F2 – heading phase, F3 – milk ripeness phase and F4 – full ripeness phase.

Table 3

Content of organic carbon and total nitrogen in particular treatments at the end of the experiment (g kg^{-1} dwt of soil)

Properties of soil	Treatments				
	G0	G1	G2	G3	G4
C-organic	10.55	8.74	7.83	7.83	14.47
N-total	1.62	1.33	1.12	1.20	1.99
C:N	6.51	6.57	6.99	6.52	7.27

Explanations: see table 2.

Organic carbon content was higher in the control soil only in the treatment G4, i.e. in the fourth year of the sludge application (table 3). This content in the treatment in question increased by 37.1% in relation to the control soil. In the remaining treatments with the sewage sludge, the content of organic carbon was below the values obtained in the control. As in the case of organic matter, the level of total nitrogen in the soil with the sludge was higher than that in the control only in treatment G4, i.e. in the fourth year of sludge amendment. Total nitrogen content in that treatment was by 22.8% higher in comparison with that in the control. In the other treatments, the content of total nitrogen was lower in relation to the control soil. The content of organic carbon and total nitrogen had an effect on the C:N ratio in the soil under study. In the treatments G1, G2 and G3, the value of this ratio proved to be slightly higher than that in the control, and only in the treatment G4 the C:N ratio increased considerably as a result of the dairy sewage sludge introduced into the soil.

4. CONCLUSIONS

1. The activity of the soil enzymes under study and the rate of biochemical processes associated with nitrogen transformations were generally stimulated by the dairy sewage sludge introduced into the soil. Dehydrogenase and protease revealed the highest activity in the fourth and in the third years, respectively, after sludge application. The dairy sewage sludge decreased urease activity.

2. Following the application of dairy sewage sludge, nitrogen can remain in soils in small amounts for many years, and a gradual mineralisation and transformation of mineral nitrogen forms can occur.

3. The activity of the soil enzymes under study was subject to seasonal variations in the vegetation period of wheat.

4. The microbiological and biochemical tests applied proved to be sensitive indicators of the biological properties of soil fertilised with sludge from a dairy sewage treatment plant.

REFERENCES

[1] ALKORTA I., AIZPURUA A., RIGA P., ALBIZU I., AMEZAGA I., GARBISU C., *Soil enzyme activities as biological indicators of soil health*, Rev. Environ. Health., 2003, 18, 1, 65–73.

[2] KOPER J., PIOTROWSKA A., *Influence of long-term organic fertilization on the enzymatic activity*, Acta Agrophys., 2001, 52, 133–140.

[3] NANNIPIERI P., ASCHER J., CECCHERINI M.T., LANDI L., PIETRAMELLARA G., RENELLA G., *Microbial diversity and soil functions*, Eur. J. Soil Sci., 2003, 54, 655–670.

[4] GARCIA-GIL J.C., PLAZA C., POLO A., *Sewage sludge effects on biological and biochemical parameters in a degraded soil*, Waste Manag. Environ., 2002, 56, 341–350.

[5] JANVIER C., VILLENEUVE F., ALABOUVETTE C., EDEL-HERMANN V., MATEILLE T., STEINBERG C., *Soil health through soil disease suppression: Which strategy from descriptors to indicators?* Soil Biol. Biochem., 2007, 39, 1–23.

[6] ZAHIR Z.A., ATTEEQ UR REHMAN MALIK M., ARSHAD M., *Soil enzymes research: a review*, J. Biol. Sci., 2001, 1, 5, 299–307.

[7] EMMERLING C., SCHLOTER M., HARTMANN A., KANDELER E., 2002, *Functional diversity of soil organisms – a review of recent research activities in Germany*, J. Plant Nutr. Soil Sci., 2002, 165, 408–420.

[8] HABTESELASSIE M.Y., STARK J.M., MILLER B.E., THACKER S.G., NORTON J.M., *Gross nitrogen transformations in an agricultural soil after repeated dairy-waste application*, Soil Soc. Am. J., 2006, 70, 1338–1348.

[9] SIMON J., *Enzymology and bioenergetics of respiratory nitrite ammonification*, FEMS Microbiol., 2002, 26, 285–309.

[10] HERNANDEZ T., MORAL R., PEREZ-ESPINOSA A., MORENO-CASELLES J., PEREZ-MURCIA M.D., GARCIA C., *Nitrogen mineralisation potential in calcareous soils amended with sewage sludge*, Biores. Tech., 2002, 83, 3, 213–219.

[11] SHI W., MILLER B.E., STARK J.M., NORTON J.M., *Microbial nitrogen transformations in response to treated dairy waste in agricultural soils*, Soil Soc. Am. J., 2004, 68, 1867–1874.

[12] ALBIACH R., CANET R., POMARES F., INGELMO F., *Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil*, Biores. Tech., 2000, 75, 1, 43–48.

[13] ALBIACH R., CANET R., POMARES F., INGELMO F., *Organic matter components, aggregate stability and biological activity in a horticultural soil fertilized with different rates of two sewage sludges during ten years*, Biores. Tech., 2001, 77, 2, 109–114.

[14] KUCHARSKI J., WYSZKOWSKA J., NOWAK G., HARMS H., *Activity of enzymes in soil treated with sewage sludge*, Pol. J. Soil Sci., 2000, 33, 1, 29–36.

[15] MORENO J.L., GARCIA C., HERNANDEZ T., *Toxic effect of cadmium and nickel on soil enzymes and the influence of adding sewage sludge*, Eur. J. Soil Sci., 2003, 54, 377–386.

[16] PASCUAL I., ANTOLIN M.C., GARCIA C., POLO A., SANCHEZ-DIAZ M., *Effect of water deficit on microbial characteristics in soil amended with sewage sludge or inorganic fertilizer under laboratory conditions*, Biores. Technol., 2007, 98, 1, 140–144.

[17] ALVA A., PARAMASIVAM S., SAJWAN K., *Nitrogen transformation from three soil organic amendments in a sandy soil*, Arch. Agron. Soil Sci., 2006, 52, 3, 321–331.

[18] NIEKERK C., CLAASSENS A., *N transformation in incubated sewage sludge and commercial fertilizer enriched soil*, Commun. Soil Sci. Plan., 2005, 36, 743–757.

[19] JEZIERSKA-TYS S., FRĄC M., *Changes in the enzymatic activity and the number of proteolytic microorganisms in brown soil under the influence of organic fertilization and cultivation of spring wheat*, Pol. J. Soil Sci., 38, 1, 61–68.

[20] JEZIERSKA-TYS S., FRĄC M., *Studies into the effect of sewage sludge from dairy plant on nitrogen transformation in brown soil*, Pol. J. Soil Sci., 2005, 38, 1, 69–75.

[21] JEZIERSKA-TYS S., FRĄC M., *Enzymatic activity of grey-brown podsolic soil enriched with sewage sludge from a dairy plant*, Pol. J. Soil Sci., 2006, 39, 1, 33–42.

[22] JEZIERSKA-TYS S., FRĄC M., *The influence of dairy sewage sludge and FYM on the numbers and biochemical activity of microorganisms that participate in the transformations of soil nitrogen*, Pol. J. Environ. Stud., 2007, 16, 2A, 3, 686–693.

[23] JEZIERSKA-TYS S., FRĄC M., *Microbiological indices of soil quality fertilised with dairy sewage sludge*, Int. Agrophys., 2008, 22, 3, 215–219.

[24] THALMANN A., *Zur Methodik der Bestimmung der Dehydrogenaseactivität im Boden Mittels Triphenyltetrazoliumchlorid (TTC)*, Landwirtsh. Forsch., 1968, 21, 249–258.

[25] ALEF K., *Dehydrogenase activity*, [in:] Alef K., Nannipieri P. (eds.), *Methods in applied soil microbiology and biochemistry*, London Academic Press, 1995, 228–231.

[26] LADD J.N., BUTLER J.H.A., *Short-terms assays of soil proteolytic enzyme activities using proteins and dipetide derivaties as substrates*, Soil Biol. Biochem., 1972, 4, 19–30.

[27] ALEF K., NANNIPIERI P., *Protease activity*, [in:] Alef K., Nannipieri P. (eds.), *Methods in applied soil microbiology and biochemistry*, London Academic Press, 1995, 313–315.

[28] ZANTUA M.J., BREMNER J.M., *Comparison of methods of assaying urease activity in soils*, Soil Biol. Biochem., 1975, 7, 291–295.

[29] Polska Norma, PN-ISO 14238, 2000, *Soil quality. Biological methods. Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes* (in Polish).

[30] NOWOSIELSKI O., *Methods of determining fertilization requirements* (in Polish), PWRiL, Warszawa, 1981.

[31] NANNIPIERI P., GREGO S., CECCANTI B., *Ecological significance of the biological activity in soil*, [in:] Bollag J.M., Stotzky G. (eds.), *Soil biochemistry*, Marcel Dekker, New York, 1990, 293–354.

[32] ZAMAN M., MATSUSHIMA M., CHANG S.X., INUBUSHI K., NGUYEN L., GOTO S., KANEKO F., YONEYAMA T., *Nitrogen mineralization, N_2O production and soil microbiological properties as affected by long-term applications of sewage sludge composts*, Biol. Fertil. Soils., 2004, 40, 101–109.

[33] GOSTKOWSKA K., FURCZAK J., DOMŻAŁ H., BIELIŃSKA E.J., *Suitability of some biochemical tests and microbiological tests for the evaluation of the degradation degree of podzolic soil on the background of its differentiated usage*, Pol. J. Soil Sci., 1998, 31, 2, 69–78.
[34] GARCIA-GIL J.C., PLAZA C., SENESI N., BRUNETTI G., *Effects of sewage sludge amendment on humic acids and microbiological properties of a semiarid Mediterranean soil*, Biol. Fertil. Soils., 2004, 39, 320–328.
[35] MARSCHNER P., KANDELER E., MARSCHNER B., *Structure and function of the soil microbial community in a long-term fertilizer experiment*, Soil Biol. Biochem., 2003, 35, 3, 453–461.
[36] MAZUR T., *Nitrogen in arable soils* (in Polish), PWN, Warszawa, 1991.
[37] JEZIERSKA-TYS S., FRĄC M., FIDECKI M., *Influence of fertilization with dairy sewage sludge on enzymatic activity of brown soil* (in Polish), Ann. UMCS Sect., 2004, E. 59, 3, 1175–1181.