Int. Agrophysics, 2009, 23, 31-37

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Impact of dairy sewage sludge on enzymatic activity and inorganic nitrogen concentrations in the soils**

S. Jezierska-Tys[1] *and M. Frąc*[2]*

[1]Department of Agricultural Microbiology, University of Life Sciences, Leszczyńskiego 7, 20-069 Lublin, Poland
[2]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

Received June 30, 2008; accepted August 5, 2008

A b s t r a c t. Sewage sludge from the treatment wastewater contains nutrients but also contaminants, such as metals, pathogens and organic chemicals. On the contrary, dairy sewage sludges (DSS) usually do not contain these contaminants. We have studied the effect of adding the different doses of DSS (0, 30, 60, 80, 120, 200, 300 and 600 Mg ha^{-1}) on activities dehydrogenase, protease and urease and content of exchangeable NH_4^+, NO_3^-, NO_2^- in a brown soil and a grey-brown podzolic soil in a 240 days experiment. Generally, after the application of DSS enzyme activities initially increased and this increase depended on the dairy sewage sludge added to soil. Afterwards, these values decreased, but generally they remained higher than those of the unamended soils. The decrease in NH_4^+ concentration coincided with a subsequent increase in the NO_3^- level. The incorporation of DSS can have a positive effect on the microbial functioning of both grey-brown podzolic and brown soil but the effects should also be verified under field conditions.

K e y w o r d s: dairy sewage sludge, soil microbial activity, enzymatic activity, nitrogen transformations

INTRODUCTION

Sewage sludges are rich of organic matter and plant nutrients, such as nitrogen, phosphorus, sulphur, magnesium (Bozkurt *et al.,* 2006; Przewrocki *et al.,* 2004) and their application to soil can improve soil structure, increase soil-water capacity and stimulate microbiological activity (Albiach *et al.,* 2001; Fernandes *et al.,* 2005; Garcia-Gil *et al.,* 2004). However, generally sewage sludge can also contain heavy metals, organic chemicals and pathogens (Harrison *et al.,* 2006; Karaca *et al.,* 2002; Shrivastava and Banerjee, 2004), except these dairy sewage sludge produced by milk factories (Jezierska-Tys and Frąc, 2005).

Measurements of enzymes activities and biochemical nitrogen transformations can be sensitive indicators of soil microbial activity, which plays a major role in affecting soil quality (Beyer *et al.,*1992; Burns, 1982; Emmerling *et al.,* 2002; Nannipieri *et al.,* 2003). The response of different soils to sewage sludge addition has been extensively studied (Niekerk and Claassens, 2005; Pascual *et al.,* 2007; Saviozzi *et al.,* 1999, 2002; Vieria *et al.,* 2003; Wong *et al.,* 1998), but the effect of dairy sewage sludge on enzyme activities and N transformations is poorly known.

Oxidoreductases, transferases and hydrolases, have been the most studied enzymes activities of soil because of their role in the oxidation and release of inorganic nutrients from organic matter (Nannipieri *et al.,* 1990). Dehydrogenases are enzymes that function only intracellularly and their activity can represent the energy transfer of oxidative activities; for this reason this activity can be used as the index of overall microbial activity of soil (Nannipieri, 1994).

The aim of present study was to investigate the effect of different doses of the dairy sewage sludge application on enzyme activities and N transformations of soil, in order to develop management practices for using these organic wastes on a large scale. We have measured dehydrogenase activity because it is an index of microbial activity and protease, urease activity because they are involved in the N cycle in soil. Inorganic nitrogen concentrations ($N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$) were also measured. Since our aim was to study the lasting effect of the sludge on the measured parameters, measurements urea carried out after 14 days.

*Corresponding author's e-mail: m.frac@poczta.fm
**This scientific work was financed from Polish national budget for science in years 2007-2009 as the research project No. 2 P06S 042 30.

MATERIAL AND METHODS

Soil, sludge and experimental design

The study was carried out in a complete randomization pot experiment with two different soils: brown soil and grey brown podzolic soil. Dairy sewage sludge (DSS) was from the Regional Dairy Cooperative in Krasnystaw, Lublin Voivodeship added to soil. The main characteristics of soils and dairy sewage sludge are showed in Table 1. The soil was sampled from the 0-15 cm soil layer, sieved at 2 mm and mixed with the different doses of fresh matter of dairy sewage sludge. Each pot contained 4 kg of an individually prepared mixture. An unamendment soil was used as a control. The pot experiment design included on each soil eight treatments with three replicates on each treatment. The treatments were:

1a – control brown soil without amendment (B0), 2a – brown soil with DSS 30 (B30), 3a – 60 (B60), 4a – 80 (B80), 5a – 120 (B120), 6a – 200 (B200), 7a – 300 (B300), 8a – 600 Mg ha^{-1} (B600), respectively;

1b – control grey-brown podzolic soil without amendment (G0), 2b – grey-brown podzolic soil with DSS 30 (G30), 3b – 60 (G60), 4b – 80 (G80), 5b – 120 (G120), 6b – 200 (G200), 7b – 300 (G300), 8b – 600 Mg ha^{-1} (G600), respectively.

Sludge amended soils were incubated for 240 days in the dark at 22°C. The soil moisture content was adjusted to 50-60% of its water-holding capacity and this value was kept during the incubation by adding water when needed.

Enzyme activities and chemical analysis

Dehydrogenase activity was determined by the reduction of 2,3,5 triphenylotetrazolium chloride (TTC) to triphenyl formazan (TPF) using the method of Thalmann (1968), modified by Alef (1995). Briefly 5 g soil were incubated with 5 ml 1% TTC in buffer 1.1 M TRIS-HCl at pH 7.4 (for brown soil) and pH 7.8 (for grey-brown podzolic soil) for 96 h at 30ºC. The TPF was extracted with 10 ml methanol under shaking for 5 min, then the mixture was filtered through a filter paper. The TPF was measured spectrophotometrically at 485 nm.

Protease activity was determined by the method Ladd and Butler (1972), modified by Alef and Nannipieri (1995), with measurement of the concentration of tyrosine released by soil after 1 h incubation at 50ºC with a 1.1 M TRIS-HCl (pH 8.1) casein solution. The tyrosine concentration was measured at 578 nm.

Urease activity was determined according to Zantua and Bremner (1975) with 1% water urea solution as a substrate. This activity was determined by the NH_4^+ released after 18 h at 37ºC. The concentration of NH_4^+ was measured at 410 nm by colorimetric method.

After extraction of soil with 0.03 M acetic acid solution, NH_4^+ concentration was determined by the Nessler method and NO_3^- by the brucine's method (Nowosielski, 1981) with spectrophotometric measurements at 410 and 470 nm, respectively. The concentration of NO_2^- was determined as reported by Marczenko (1968) using sulfanilic acid and 1-naphthylamine. The concentration of NO_2^- was measured at 520 nm.

T a b l e 1. Characteristics of soils and dairy sewage sludge

Properties	Brown soil	Grey-brown podzolic soil	Dairy sewage sludge (DSS)
pH	6.4	4.8	8.5
C (g kg^{-1})	13.5	4.5	400.0
N (g kg^{-1})	1.6	0.4	33.2
C:N	8.3	12.5	12.0
	Macronutrients (total, g kg^{-1})		
P	18.3	5.3	11.5
K	26.8	8.7	2.5
	Heavy metals (mg kg^{-1})		
Zn	28.7	11.7	95.0
Cd	0.16	<0.16	2.6
Cu	7.16	2.21	19.8
Pb	10.3	7.08	9.0
Ni	10.1	2.98	12.6
Cr	18.4	8.10	36.8
Hg	0.09	0.03	0.22

The pH was measured on a soil suspension prepared by shaking 10 g of soil with 25 ml 1 M KCl.

An analysis of variance (ANOVA) was carried out to determine the treatment effects on the measured parameters. Least significant difference values (LSD) were calculated at $p < 0.05$. The correlations between parameters were also carried out. All statistical analysis were made with the Statistica 7.1.

RESULTS

Generally the organic amendment increased enzyme activities of soil, and all enzyme activities showed significant temporal variations (Fig. 1). Usually the dehydrogenase activity of the treated brown soil was significantly higher than that of the treated grey-brown podzolic soil; the dehydrogenase activity of the latter soil only increased at the

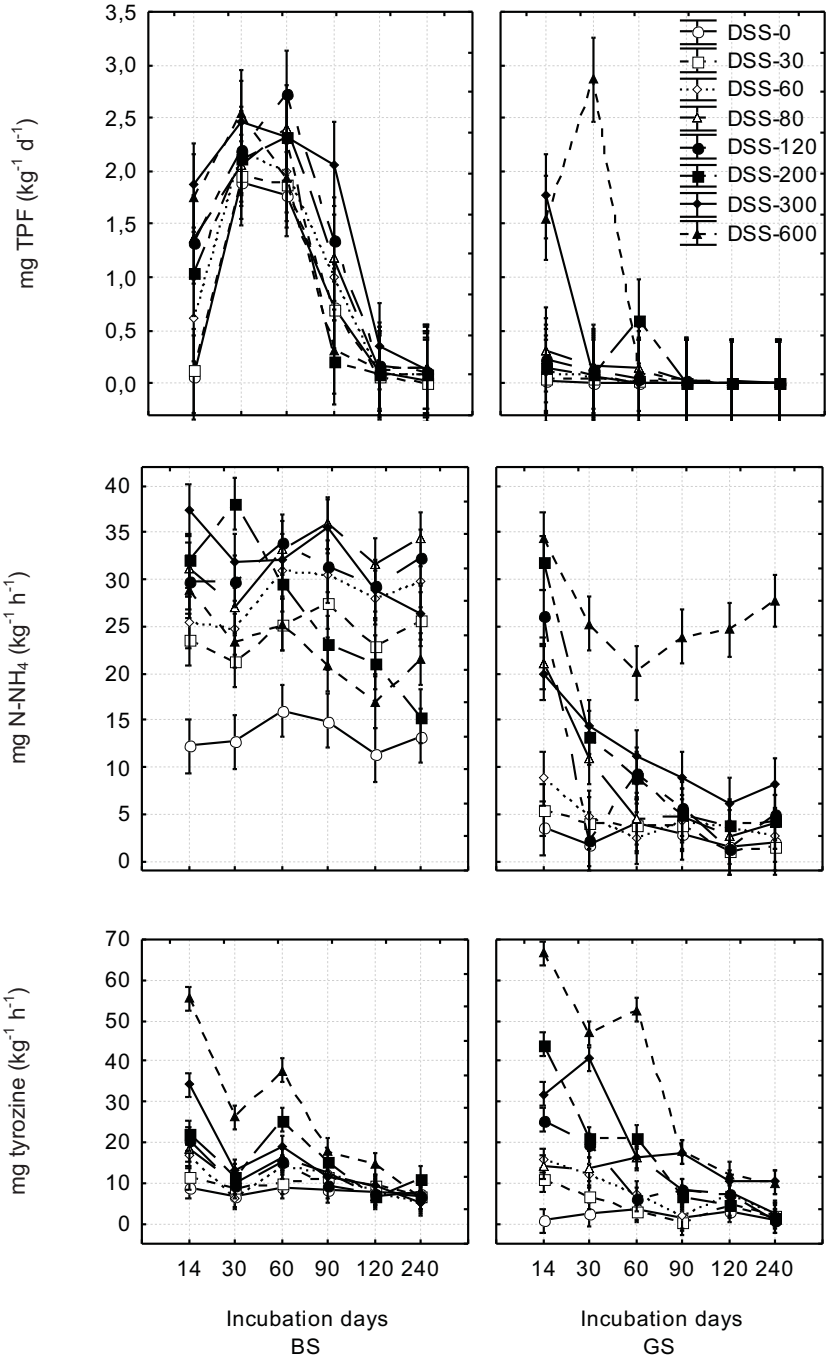

Fig. 1. Dehydrogenase, urease and protease activity in soils, BS – brown soil; GS – grey-brown podzolic soil; DSS – dairy sewage sludge.

highest sludge application rates (300 and 600 Mg ha^{-1}). The stimulation of dehydrogenase activity in both soils by sludge addition occurred in the first 60 days and then the enzyme activity decreased (Fig. 1).

Protease activity of both soils increased significantly ($p < 0.05$) compared to that of the control soils especially at the highest rates (Fig. 1). In both soils the highest protease activity was found with the highest application rate (600 Mg ha^{-1}).

Urease activity also was significantly ($p < 0.05$) stimulated by the addition of sludge to soil but values were generally higher in the brown than in the grey-brown podzolic soil. The highest urease activity was observed in the brown soil, treated at 200 Mg ha^{-1} and in the grey-brown podzolic soil, treated at 600 Mg ha^{-1}. The lowest values of urease activity were found in both control soils.

In the brown soil the highest concentration of exchangeable NH_4^+ was measured at 600 Mg ha^{-1} after 14 days whereas in the grey-brown podzolic soil, lower application rates were also effective in increasing the concentration of exchangeable NH_4^+ (Fig. 2). The increases in exchangeable NH_4^+ concentrations lasted longer in the grey-brown podzolic soil than in brown soil. The increase of NO_3^- concentrations lasted for all incubation period and concerned all treated soil (Fig. 2). In the brown soil the NO_2^- was measured at detectable concentrations after 14, 30 and 120 days with sewage sludge added at 300 and 600 Mg ha^{-1}. In the grey brown podzolic soil there was a high peak of NO_2^- after 30 days with sewage sludges added at 300-600 Mg ha^{-1}.

The pH values of the brown soil were not affected by treatments through out and whereas those of grey-brown podzolic were markedly increased (Fig. 2).

Dehydrogenase activity was significantly correlated with the dose of sludge, protease and urease activity, pH ($p < 0.001$), respectively, and NH_4^+ concentrations ($p < 0.01$). Urease activity was significantly correlated with the dose of sludge, dehydrogenase activity, protease activity, pH ($p < 0.001$), respectively; NH_4^+ concentrations ($p < 0.01$) and NO_3^- concentrations ($p < 0.05$). Protease activity was significantly correlated with dose of sludge, dehydrogenase and urease activity ($p < 0.001$), respectively; NH_4^+ and NO_2^- concentrations ($p < 0.01$ and 0.001), respectively and with pH ($p < 0.001$) (Table 2).

DISCUSSION

As already mentioned dehydrogenase activity is due to intracellular enzymes and it can be used to evaluate microbial activity of soil (Garcia *et al.,* 1994; Nannipieri, 1994). It has also found to be significantly correlated with soil microbial biomass (Garcia-Gil *et al.,* 2000). Dehydrogenase activity increased by increasing the dose of dairy sewage sludge applied to soil. At the end of the experiment in both soils the values of dehydrogenase activity of the amended soils were close to those of the control soil, suggesting that microbial activity decreased probably due to the decreased availability of easily degradable substrates after the initial mineralization of organic matter. Similar results have been obtained in sewage sludge amended soils (Pascual *et al.,* 1998; Reddy and Faza, 1989; Saviozzi *et al.,* 2002).

Protease activity of soil, measured as casein hydrolyzing activity, probably reflect the activity of extracellular enzymes (Ladd and Butler, 1972). The higher protease activity of DSS-amended soils than the control soils, particularly at the greater doses probably depended on the added of proteins, that stimulated both microbial growth and microbial syntheses of proteases. These results confirm what already found in sewage sludge and dairy sewage sludge treated soil (Garcia-Gil *et al.,* 2000; Jezierska-Tys and Frąc, 2006; Saviozzi *et al.,* 2002; Zaman *et al.,* 2002). The decrease in protease activity of both treated soils after 90 to 240 days was possibly due to the decrease of available proteins and also to decrease of available nutrient for microbial growth. Similar results have been observed by Ros *et al.* (2003) in organically amended soil.

The higher values of urease activity in DSS-amendment soils compared to the control was probably the results of microbial growth caused by the addition of nutrients by the dairy sewage sludge (Fig. 1). Similar results have been reported by Jezierska-Tys and Frąc (2006) and Zaman *et al.* (2002) in soil amended with dairy organic wastes. At the end of the incubation urease activity was decreased probably for the reason which caused the decrease of the other enzyme activities and by the presence of the high concentration of metabolites, such as NH_4^+ (Zantua and Bremner, 1975).

These results indicate that high dehydrogenase, protease and urease activity of soil are stimulated by the application of dairy sewage sludge could only be maintained through the constant supply of organic matter. The significant of the applied dose with each of the measured indicate that the relative stimulation probably depend on the same cause: the stimulation of microbial growth.

In the brown soil net N mineralization was significantly ($p < 0.05$) improved, only at the highest dose of DSS, at the beginning of the experiment as shown by the highest concentration of exchangeable NH_4^+ (Fig. 2). In the grey-brown podzolic soil net N mineralization rate was probably increased by the all doses of DSS, but only at the beginning of the experiment. Afterwards, the concentrations of exchangeable NH_4^+ of both soils decreased of rate probably as the result of NH_4^+ immobilization and nitrification. Zaman and Chang (2004) also observed the decrease of net N mineralisation rate during incubation study. Pascual *et al.* (1997) explained that the low N-mineralisation was due to the presence of toxic compounds inhibiting microbial reaction. However, the decrease in the concentration of exchangeable NH_4^+, generally coincided with an increase in NO_3^- levels, thus confirming what reported by Nieckerk and Claassens (2005) in soil treated with sewage sludge. Therefore it is reasonable to hypothesize that nitrification occurred. The

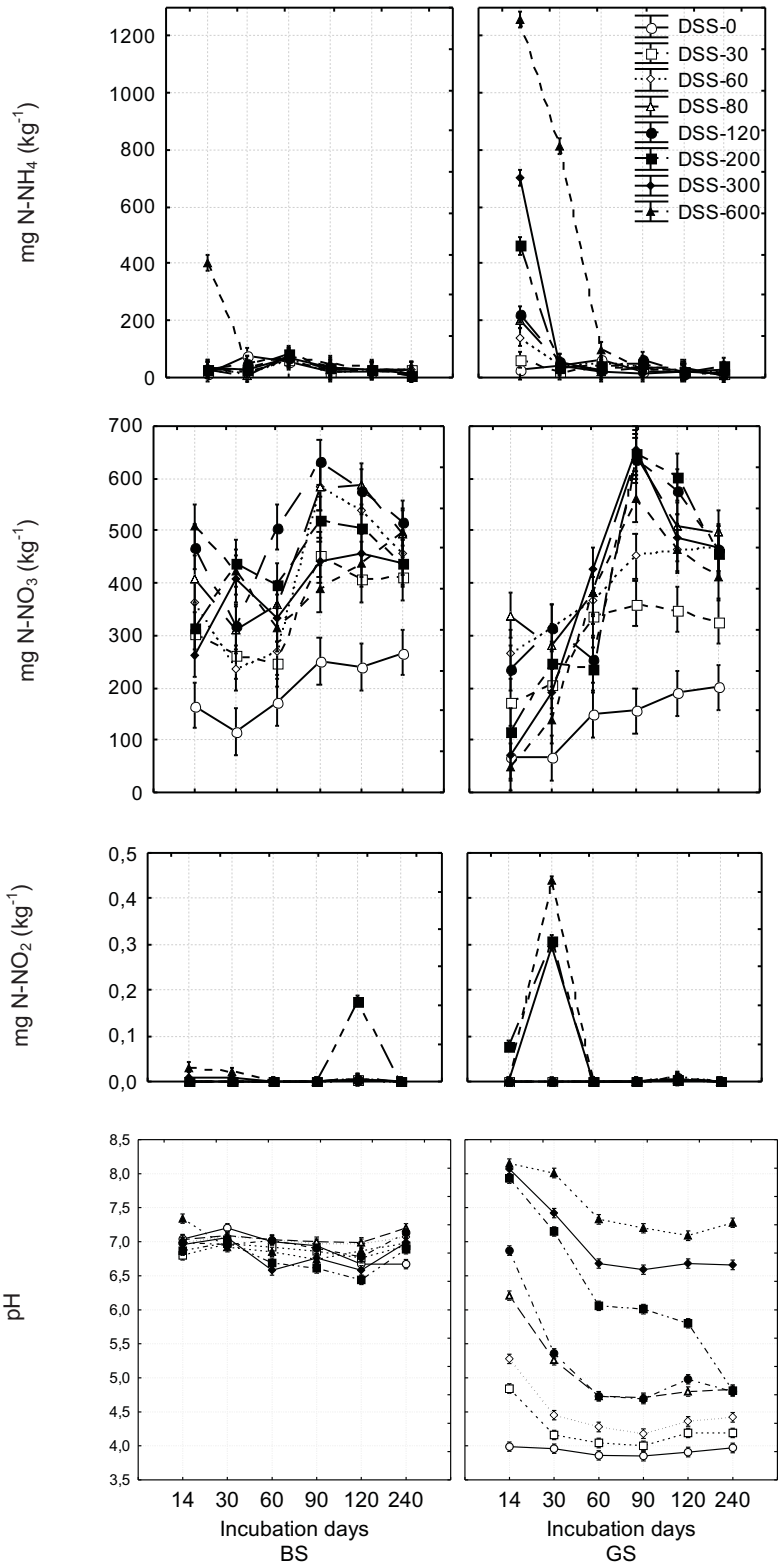

Fig. 2. NH_4^+, NO_3^-, NO_2^- concentrations in soils. Explanations as in Fig. 1.

T a b l e 2. Correlation coefficients

Factors	DSS	ADh	AP	AU	NH_4^+	NO_3^-	NO_2^-	pH
DSS	-	0.20 ***	0.56 ***	0.37 ***	0.28 ***	0.33 ***	0.24 ***	0.49 ***
ADh		-	0.36 ***	0.56 ***	0.28 **	-0.16 **	n.s.	0.47 ***
AP			-	0.40 ***	0.69 **	-0.22 ***	0.35 ***	0.54 ***
AU				-	0.21 **	0.13 *	n.s.	0.80 ***
NH_4^+					-	-0.37 ***	0.31 ***	0.32 ***
NO_3^-						-	-0.19 **	n.s.
NO_2^-							-	0.22 ***
pH								-

*, **, *** – indicate significance at the 5, 1, and 0.1% level, respectively; n.s. – no significant. DSS – dose of dairy sewage sludge, ADh – dehydrogenase activity, AP – protease activity, AU – urease activity.

high NO_3^- concentration in soil treated with the higher DSS doses, especially in the grey-brown podzolic soil, indicates that potential of leaching losses. Nitrite level peaked (0.4 mg N kg^{-1}) after 30 days in grey-brown podzolic soil at the highest DSS doses (300 and 600 Mg ha^{-1}) and probably this depended on the inhibition of the oxidation from NO_2^- to NO_3^- by the highest concentrations of exchangeable NH_4^+ and by the high pH values (Burns *et al.,* 1996). However these hypoteses do not explain the peak in NO_2^- concentrations in the brown soil. It is important to underline that NO_2^- is the precursor of the toxic nitrosamines (Rostkowska *et al.,* 1998).

The increase in the pH values of the grey-brown podzolic soil but not in the brown soil after DSS additions confirm what were reported by Jezierska-Tys and Frąc (2005; 2006) and indicate a lower buffering capacity of the former than the latter soil.

CONCLUSIONS

1. The application of dairy sewage sludge generally increased soil enzymatic activity and nitrogen content in soil.

2. The incorporation of DSS seems to be more effective in grey-brown podzolic soil than in brown soil. In this soil also pH values were also significantly higher after the application of DSS. Therefore the application of organic wastes, such as dairy sewage sludge, should be practiced, especially into light acid soil.

3. The dairy sewage sludge should be used as a safe organic fertilizer due to a beneficial impact on the soil environment and a low content of heavy metals. It is one of the methods of reducing the quantity of the industry sewage sludge and its disposal.

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