

INFLUENCE OF FERTILIZATION WITH DAIRY SEWAGE SLUDGE SANITISED WITH COAL FLY ASH ON MICROBIOLOGICAL ACTIVITY AND CONCENTRATION OF HEAVY METALS IN GREY-BROWN PODZOLIC SOIL

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Abstract

The aim of the study was to determine how dairy sewage sludge (DSS) sanitised with coal fly ash affected microbiological properties of soil and level of heavy metals in soil. The experiment was performed under laboratory conditions (pot experiment). The experiment was carried out on grey-brown podzolic soil, formed from heavy loamy sand, of acidic reaction. Pots were filled with 4 kg of soil. The investigations were performed in three replications. Two rates of dairy sewage sludge fertilization: 1 and 2.5% DSS·kg⁻¹ of soil were applied. The soil in pots was watered to 60% of the total water capacity and incubated for 4 months. Analyses included assays of the total number of bacteria and fungi, number of cellulolytic bacteria, respiration and dehydrogenase activity and concentration of heavy metals.

It was confirmed that dairy sewage sludge had a significant effect on properties of soil. It positively affected microbiological activity of soil. The test doses (1; 2.5%·kg⁻¹) of dairy sewage sludge sanitised with lignite ash caused stimulation of the growth of bacteria and fungi in soil. Dairy sewage sludge had an inhibiting effect on the dehydrogenase activity and stimulated the respiratory activity in the soil under study. The incorporation of dairy sewage sludge caused a non-significant increase of heavy metal content in soil, which was much lower than the norms.

Key words: dairy sewage sludge, microbiological activity, microorganisms, soil.

WPLYW NAWOŻENIA OSADEM ŚCIEKÓW MLECZARSKICH HIGIENIZOWANYM POPIOŁEM WĘGLA BRUNATNEGO NA AKTYWNOŚĆ MIKROBIOLOGICZNĄ I ZAWARTOŚĆ METALI CIĘŻKICH W GLEBIE PŁOWEJ

Abstrakt

Celem badań było określenie wpływu osadu ścieków mleczarskich higienizowanego popiołem węgla brunatnego na właściwości mikrobiologiczne i zawartość metali ciężkich w glebie. Badania przeprowadzono w warunkach laboratoryjnych, w doświadczeniu wazonowym. Doświadczenie założono na glebie płowej, wytworzonej z piasku gliniastego mocnego, o odczynie kwaśnym. W wazonach umieszczono po 4 kg gleby. Badania prowadzono w trzech powtórzeniach. W badaniach zastosowano dwie dawki osadu ścieków mleczarskich: 1 i 2,5% osadu $\cdot \text{kg}^{-1}$ gleby. Glebę w wazonach nawodniono do poziomu 60% całkowitej pojemności wodnej i inkubowano przez 4 miesiące. Analizy obejmowały oznaczenie: ogólnej liczebności bakterii i grzybów, liczebności bakterii celulolitycznych oraz aktywności respiracyjnej i dehydrogenaz, a także poziomu metali ciężkich.

Stwierdzono, że osad ścieków mleczarskich istotnie wpływał na właściwości gleby. Korzystnie oddziaływał na mikrobiologiczne właściwości gleby. Zastosowane dawki (1; 2,5% $\cdot \text{kg}^{-1}$) osadu higienizowanego popiołem węglowym stymulowały rozwój bakterii i grzybów w glebie. Osad ścieków mleczarskich spowodował zahamowanie aktywności dehydrogenaz i stymulację aktywności respiracyjnej gleby. Wprowadzenie osadu spowodowało nieistotny wzrost zawartości metali ciężkich w badanej glebie, który był znacznie niższy niż przewidują dopuszczalne normy.

Słowa kluczowe: osad ścieków mleczarskich, aktywność mikrobiologiczna, mikroorganizmy, gleba.

INTRODUCTION

For productivity of agroecosystems and protection of the environment it is necessary to develop and implement management strategies that maintain the quality of soil, and these include conserving the amount of organic matter (SAVIOZZI et al. 1999). Sewage sludge is a useful source of major plant nutrients (nitrogen, phosphorus, sulphur and magnesium) and organic matter. However, sewage sludge can contain larger concentrations of heavy metals than most soils. There is a concern that once metals have been added to agricultural land and accumulate in the topsoil, they could have negative effects on soil fertility and microbial activity (GIBBS et al. 2006). For this reason it is very important to monitor the ecological state of soil after the application of such wastes (ROS et al. 2003, KIZILKAYA, BAYRAKLI 2005).

In many EU countries a notable increase can be currently observed in agricultural utilisation of sewage sludge from the food industry (BUTAREWICZ 2003, DAVIS, HALL 1997, PRZEWROCKI et al. 2004). Sewage sludge, rich in organic matter and biogens, has an effect on soil environment, soil microor-

ganisms included. Both counts of microorganisms and their biochemical activity undergo changes in soils subjected to the effect of such waste (SULLIVAN et al. 2005, JEZIEŃSKA-TYS, FRĄC 2005). Tests based on determination of microbial populations and biochemical activity are used for estimation of the fertility and productivity of soils, and provide us with comprehensive knowledge on changes taking place in soil environment (GOSTKOWSKA et al. 1998, KOBUS 1995, MYŚKÓW 1981). The concentration heavy metals in soil after incorporation of dairy sewage sludge helps to evaluate the risk of soil contamination by DSS (MORENO et al. 1999).

The objective of the present study was estimation of the effect of dairy sewage sludge sanitised with lignite ash on the total number of bacteria, fungi, cellulolytic bacteria, respiration activity and dehydrogenase activity and level of heavy metals in grey-brown podzolic soil.

MATERIALS AND METHODS

The study was conducted in a pot experiment in three replications. The object of the study was soil characterised by the grain size composition of sand fraction 65%, silt fraction 19%, fine silt and clay fraction 16%. The soil contained 0.45% of organic carbon and 0.036% of total nitrogen. The soil was amended with dairy sewage sludge sanitised with lignite ash. The sludge doses applied were 1 and 2.5% kg⁻¹. The soil in pots was brought to a moisture level of 60% total water capacity and incubated for 4 months in aerobic conditions. On the dates of analyses soil samples were taken from the pots and microbiological analyses were performed. Analyses were completed after 7, 14, 30, 60, 90 and 120 days of soil incubation. The scope of the microbiological analyses, performed with methods commonly used, covered total counts of bacteria, fungi and cellulolytic bacteria (RODINA 1968), the respiration activity (RÜHLING and TYLER 1973) and dehydrogenase activity (THALMANN 1968). In addition, the content of heavy metals in the soil samples was analysed using the AAS method after mineralization in concentrated nitric acid (V).

The results were processed statistically with ANOVA. The LSDs were calculated with Tukey's test at significance level of $\alpha = 0.05$. All statistical calculations were made with Statistica 7.1 Software.

RESULTS AND DISCUSSION

Figure 1 presents the results of the study on the effect of dairy sewage sludge doses applied in the experiment on counts of bacteria on particular dates of analysis, and their mean values for all experimental treatments.

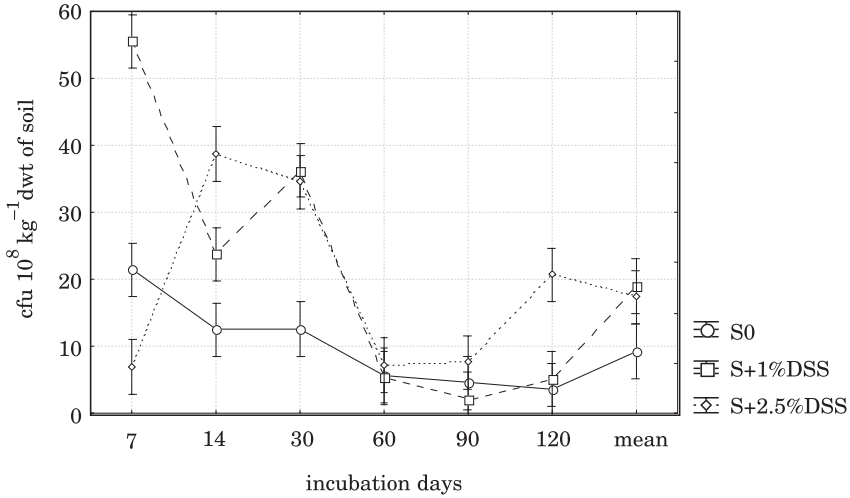


Fig. 1. Effect of dairy sewage sludge on total number of bacteria in soil

The data indicate that in the initial period of the experiment the sludge dose of 2.5% caused a decrease in the bacterial populations to levels considerably lower than the value obtained in the control treatment. The lower sludge dose (1%), on the other hand, caused a significant increase in the growth of the bacterial groups under study. In the subsequent stages of the study, i.e. on analysis dates II and III, a significant increase was observed in the bacterial populations under the effect of both sludge doses applied. On analysis dates IV and V, the populations of the microbial groups in treatments with sludge were similar or insignificantly higher than the values obtained in the control treatment. At the final stage of the experiment, in the treatment with the higher dose of sludge, a significant increase in the number of bacteria was recorded. The mean values of bacterial populations in the particular experimental treatments showed notable stimulation of the microbial groups by both sludge doses applied.

The data concerning the size of fungal populations (Figure 2) indicate that both sludge doses stimulated, throughout the whole study, the growth of this microbial group. A significant seasonal increase in the population of fungi affected the mean value for particular experimental treatments, as illustrated in Figure 2.

The effect of the doses of dairy sewage sludge on the number of microorganisms participating in mineralisation of cellulose is illustrated in Figure 3. The data show that on all the dates of analyses, with the exception of date V, stimulation was observed in the growth of cellulolytic bacteria, especially in the treatment with the higher dose of sludge. This is confirmed by the mean values for the particular treatments concerning the numbers of cellulolytic bacteria, as illustrated in Figure 3.

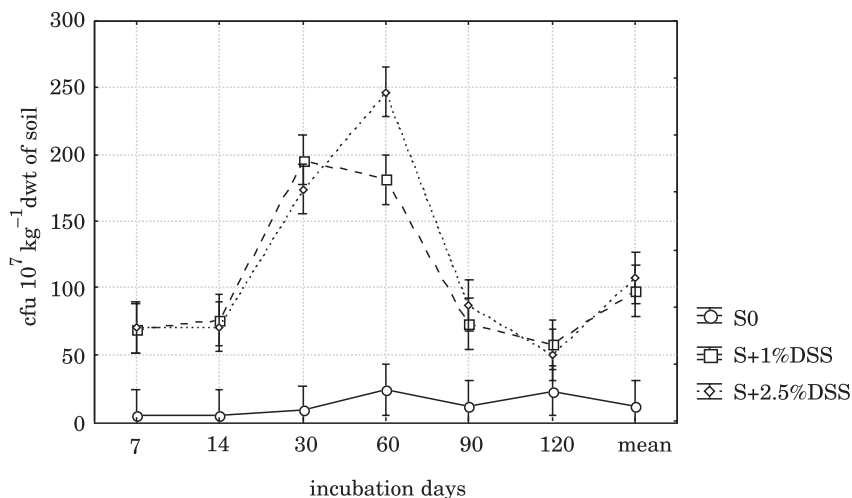


Fig. 2. Effect of dairy sewage sludge on total number of fungi in soil

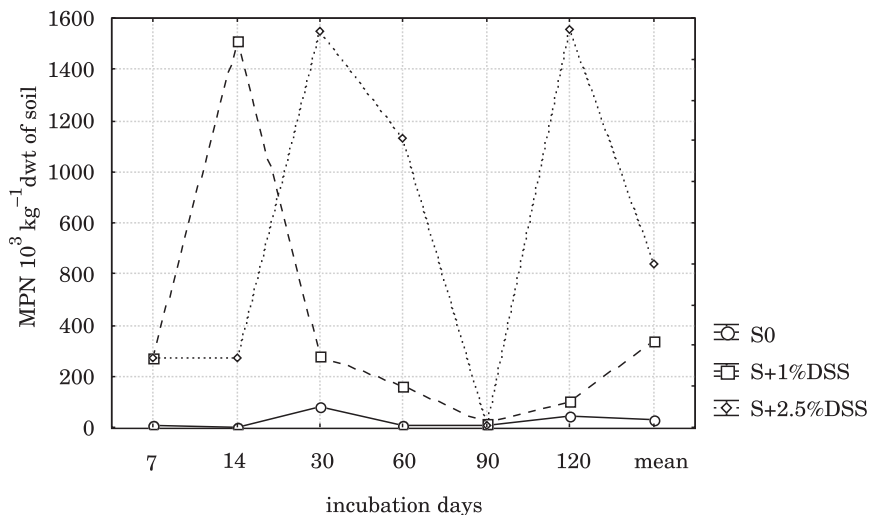


Fig. 3. Effect of dairy sewage sludge on the number of cellulolytic bacteria in soil

The respiration activity in the experimental treatments for the particular dates of analysis is illustrated in Figure 4. The highest respiration activity was characteristic of the treatment with the sludge dose of $2.5\% \cdot \text{kg}^{-1}$, but only until date III of analysis. A lower level of respiration activity was observed in the treatment with the sludge dose of 1% , but nevertheless it was higher than in the control treatment. On analysis dates IV and V the respiration activity in the treatments with sludge was significantly higher

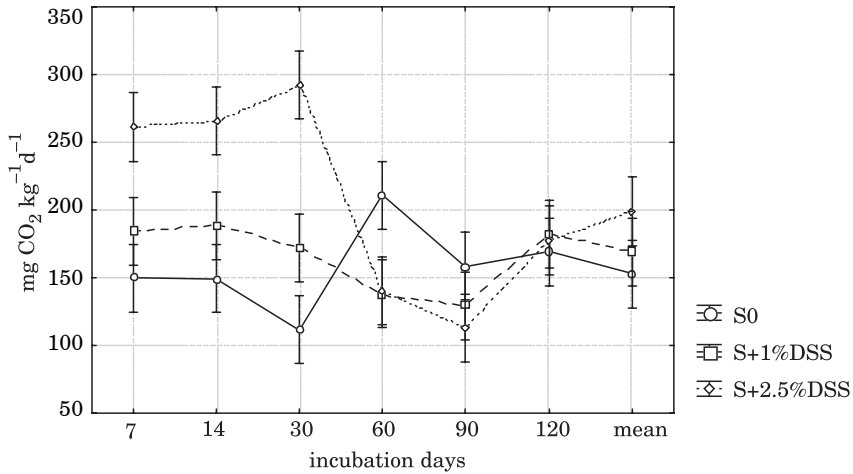


Fig. 4. Effect of dairy sewage sludge on respiration activity of soil

compared to the control treatment. In the final stage of the study, the respiratory activity of grey-brown podzolic soil with dairy sewage sludge was on a level slightly above the value obtained in the control. Mean values of respiration activity for the particular experimental treatments are given in Figure 4a. Notable stimulation of the respiration activity was caused by the sludge dose of 2.5%.

Figure 5 illustrates the data concerning the effect of sludge on dehydrogenase activity in the soil under study. Both the seasonal results and mean values for the experimental treatments indicate a decrease in this activity under the effect of both sludge doses (1 and 2.5%·kg⁻¹) compared to the values obtained in the control treatment.

The dairy sewage sludge had influence on the concentration of heavy metals in soil (Table 1). The highest values of heavy metals were observed in the soil amended with the higher dose of DSS, but in all the treatments the concentration of heavy metal was lower than permissible (Rozp. Min. Środ. 2002).

The total number of bacteria and fungi is an index of the biological activity of soil and is used for the determination of the biological status of soil environment (KUCHARSKI et al. 1992, LOC, GREINERT 2000, LOC, OBERTYŃSKA 2003). The present study shows that the doses of dairy sewage sludge sanitised with lignite ash had a stimulating effect on the total number of bacteria and fungi. The stimulation in the growth of the analysed microbial groups should be attributed to enrichment of grey-brown podzolic soil with organic matter and mineral components brought in with sludge. Increase in soil microbial populations caused by dairy sewage sludge was also observed by other authors (FURCZAK, JONIEC 2002, JEZIERSKA-TYS, FRĄC 2005, LIMA et al.

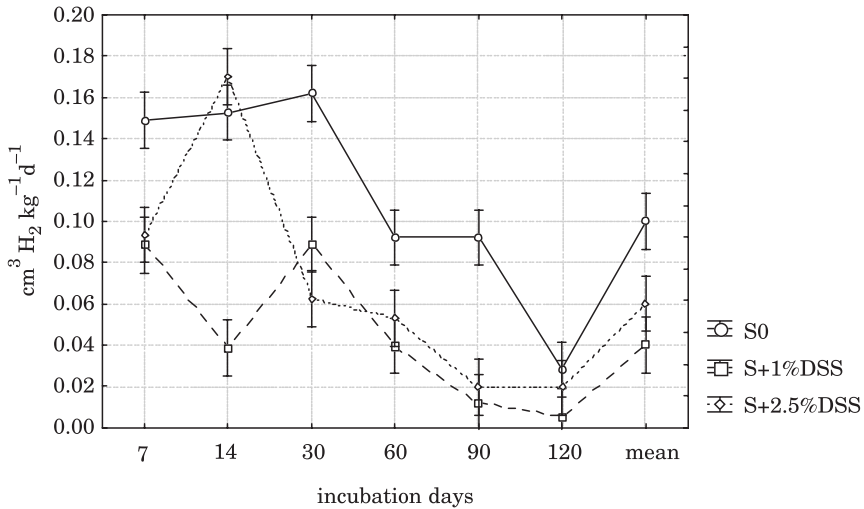


Fig. 5. Effect of dairy sewage sludge on dehydrogenase activity in soil

Table 1

Effect of dairy sewage sludge on concentration of heavy metals in soil ($\text{mg} \cdot \text{kg}^{-1}$ d.m. of soil)

Metal	Treatments		
	SO control soil	S+1%DSS	S+2.5%DSS
Maximum concentration of metal in grey-brown podzolic soil (Rozp. Min. Środ. 2002)			
Cd (1)	0.05	0.06	0.10
Cr (50)	2.45	2.44	12.8
Cu (25)	1.21	1.31	5.26
Ni (20)	1.00	1.10	3.62
Pb (40)	2.00	2.98	6.32
Zn (80)	2.23	5.26	17,1
Hg (0.8)	0.01	0.02	0.02

S – soil,

DSS – dairy sewage sludge

1996). The results obtained in our study indicate an effect of dairy sewage sludge on bacteria participating in mineralisation of cellulose, as evidenced by a significant increase in populations of cellulolytic bacteria following the application of sludge at both doses. Also FURCZAK and JONIEC (2005) observed increased populations of cellulolytic bacteria as a result of application of municipal sewage sludge.

Many authors (MYŚKÓW 1981, NANNIPIERI 1994, NANNIPIERI et al. 1999) claim that dehydrogenase activity may be used as an index of changes in biological activity in soil environment resulting from the application of mineral fertilisation, including amendment with sewage sludge. The results obtained in this study indicate a decrease in the dehydrogenase activity of the test soil under the effect of both doses of dairy sewage sludge sanitised with lignite ash. Also LAI et al. (1999) found that coal ash added to sewage sludge reduced dehydrogenase activity in soil. Examination of respiratory activity of soil is one of the methods for determination of activity of soil microorganisms and changes taking place under the effect of both natural and anthropogenic factors. The results of our study showed that dairy sewage sludge doses of 30 and 75 t ha⁻¹ caused stimulation of respiratory activity of grey-brown podzolic soil. This was probably caused by increased levels of organic matter in the test soil owing to the introduction of organic matter with sludge. Our earlier study (JEZIEJSKA-TYS and FRĄC 2005) also revealed a stimulating effect of dairy sewage sludge on the respiration activity of soil.

CONCLUSIONS

1. The test doses (1 and 2.5%) of dairy sewage sludge sanitised with lignite ash caused stimulation of the growth of bacteria and fungi in grey-brown podzolic soil.

2. Dairy sewage sludge had an inhibiting effect on the dehydrogenase activity and stimulated the respiratory activity in the soil under study.

3. The incorporation of dairy sewage sludge caused a non-significant increase in the content of heavy metals, which remained on a permissible level.

REFERENCES

- BUTAREWICZ A. 2003. *Higieniczne aspekty procesu kompostowania osadów ściekowych*. W: Nowe spojrzenie na osady ściekowe. *Odnawialne źródła energii*. Cz. I. *Konf. Nauk.-Tech.*, Częstochowa, 3-5 lutego, ss. 243-252.
- DAVIS R.D., HALL J.E. 1997. *Production, treatment and disposal of wastewater sludge in Europe from a UK perspective*. *Eur. Wat. Poll. Contr.*, 7(2): 9-17.
- FURCZAK J., JONIEC J. 2002. *Studies of the effects of the level of sewage sludge crumbling on microbial and biochemical activities of soil*. *Pol. J. Soil Sci.*, 35(1): 59-67.
- FURCZAK J., JONIEC J. 2005. *Wpływ osadu ścieków komunalno-przemysłowych na rozwój mikroorganizmów celuloリティcznych i mineralizację celulozy w glebie biellicowej*. *Konf. Nauk. Kształtowanie i ochrona środowiska – uwarunkowania przyrodnicze, techniczne i społeczno-ekonomiczne*. Olsztyn, 15-17 czerwca. *Inż. Ekol.*, 11: 136-137.

- GIBBS P.A., CHAMBERS B.J., CHAUDRI A.M., McGRATH S.P., CARLTON-SMITH C.H. 2006. *Initial results from long-term field studies at three sites on the effects of heavy metal-amended liquid sludges on soil microbial activity*. Soil Use Manage., 22: 1-8.
- GOSTKOWSKA K., FURCZAK J., DOMŻAŁ H., BIELIŃSKA E.J. 1998. *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., 31(2): 69-78.
- JEZERSKA-TYS S., FRAC M. 2005. *The effect of fertilization with sewage sludge from a dairy plant and with straw on the population numbers of selected microorganisms and respiration activity of brown soil*. Pol. J. Soil Sci., 38(2): 145-151.
- KIZILKAYA R., BAYRAKLI B. 2005. *Effects of N – enriched sewage sludge on soil enzyme activities*. Appl. Soil Ecol., 30: 192-202.
- KOBUS J. 1995. *Biologiczne procesy a kształtowanie żyzności gleby*. Zesz. Probl. Post. Nauk Rol., 421: 209-219.
- KUCHARSKI J., NIKLEWSKA-LARSKA T., NIEWOLAK T. 1992. *Wpływ substancji organicznej i niektórych grup drobnoustrojów na liczebność i aktywność mikroorganizmów glebowych. II. Liczebność grup fizjologicznych*. Zesz. Nauk. Akad. Rol.-Tech. Olsztyn, Agricult., 54: 23-41.
- LAI K.M., YE D.Y., WONG J.W.C. 1999. *Enzyme activities in a sandy soil amended with sewage sludge and coal fly ash*. Wat. Air Soil Poll., 113: 261-272.
- LIMA J.A., NAHAS E., GOMES A.C. 1996. *Microbial populations and activities in sewage sludge and phosphate fertilizer-amended soil*. Appl. Soil Ecol., 4: 75-82.
- LOC N.T.B., GREINERT H. 2000. *Wpływ osadu ściekowego na mikroflorę gleby oraz wzrost i skład chemiczny grochu siewnego (pisum sativum L.)*. Fol. Univ. Agric. Stetin. Agricult., 83: 119-124.
- LOC N.T.B., OBERTYŃSKA E. 2003. *Wpływ osadu ściekowego na niektóre mikroorganizmy glebowe pod uprawą kukurydzy (Zea mays L.)*. Zesz. Probl. Post. Nauk Rol., 494: 305-314.
- MORENO J.L., HERNANDEZ T., GARCIA C. 1999. *Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil*. Biol. Fertil. Soils, 28: 230-237.
- MYŚKÓW W. 1981. *Próby wykorzystania wskaźników aktywności mikrobiologicznej do oceny żyzności gleby*. Post. Mikrob., 20(3/4): 173-192.
- NANNIPIERI P. 1994. *The potential use of soil enzymes as indicators of productivity, sustainability and pollution*. In: *Soil biota management in sustainable farming systems*. (Eds C.E. PANKHU, B.M. DOUBE, V.V.S.R. GUP and P.R. GRACE). CSIRO, Melbourne, Australia.
- NANNIPIERI P., GREGO S., AND CECCANTI B. 1999. *Ecological significance of the biological activity in soil*. In: *Soil biochemistry*. (Eds J.M. BOLLAG and G. STOTZKY). New York Marcel Dekker, USA.
- PRZEWROCKI P., KULCZYCKA J., WZOREK Z., KOWALSKI Z., GORAZDA K., JODKO M. 2004. *Risk analysis of sewage sludge – Poland and EU comparative approach*. Pol. J. Envir. Stud., 13(2): 237-244.
- RODINA A. 1968. *Mikrobiologiczne metody badania wód*. PWRiL, Warszawa.
- ROS M., HERNANDEZ M. T., GARCIA C. 2003. *Bioremediation of soil degraded by sewage sludge: effects on soil properties and erosion losses*. Environ. Manage., 31(6): 741-747.
- Rozporządzenie Ministra Środowiska z dnia 01.08.2002 roku w sprawie komunalnych osadów ściekowych. Dz.U. Nr 134, poz. 1140.
- RÜHLING A., AND TYLER G. 1973. *Heavy metal pollution and decomposition of spruce needle litter*. Oikos, 24: 402-415.
- SAVIOZZI A., BIASCI A., RIFFALDI R., LEVI-MINZI R. 1999. *Long-term effects of farmyard manure and sewage sludge on some soil biochemical characteristics*. Biol. Fertil. Soils, 30: 100-106.

- SULLIVAN T. S., STROMBERGER M. E., PASCHKE M. W., IPPOLITO J. A. 2005. *Long-term impacts of infrequent biosolids applications on chemical and microbial properties of semi-arid rangeland soil*. Biol. Fert. Soils, 42(3): 258-266.
- THALMANN A. 1968. *Zur methodik der Bestimmung der Dehydrogenaseaktivität im Boden Mittels Triphenyltetrazoliumchlorid (TTC)*. Landwirtsch. Forsch., 21: 249-258.