

EWA BEATA GÓRSKA^{1*}, WOJCIECH STĘPIEŃ²,
IZABELLA OLEJNICZAK³, STEFAN PIETKIEWICZ⁴,
HAZEM M. KALAJI⁴, PAWEŁ KOWALCZYK⁵

Microbial properties of soil fertilized by sewage sludge and cultivated with energy crops

Summary

The microbial activity of soil enriched with sewage sludge and cultivated with energy crops, is little known. The aim of this study was to assess the impact of sewage sludge fertilization on selected microorganisms in soil cultivated with the following crops: Miscanthus (*Miscanthus x giganteus* Greef et Deu), Virginia mallow (*Sida hermaphrodita* (L.) Rusby) and Common Osier willow (*Salix viminalis* L.). Sewage sludge was used in two rates 10 and 20 t/ha dry weight (DW). The numbers of total coliforms bacteria, sulphate-reducing spore-forming bacteria, *Proteus* sp., saprophytic, thermophilic and aerobic spore-forming bacteria were examined. Sewage sludge increased the number of coliforms and sulphate-reducing spore-forming bacteria, and stimulated the growth of saprophytic and thermophilic bacteria. Cultivation of Miscanthus limited the number of coliforms bacteria,

¹ Faculty of Agriculture and Biology, Warsaw University of Life Sciences – SGGW, Autonomus Department of Biology of Microorganisms, Nowoursynowska 166, 02-787 Warsaw, * e.b.gorska@wp.pl

² Faculty of Agriculture and Biology, Warsaw University of Life Sciences – SGGW, Department of Soil Sciences, Nowoursynowska 166, 02-787 Warsaw

³ Faculty of Christian Philosophy, Cardinal Stefan Wyszyński University in Warsaw, Institute of Ecology and Bioethics, Wóycickiego 1/3, 01-938 Warsaw

⁴ Faculty of Agriculture and Biology, Warsaw University of Life Sciences – SGGW, Department of Plant Physiology, Nowoursynowska 166, 02-787 Warsaw

⁵ Bionicum LTD, Chełmska 21, 00-724, Warsaw

while Virginia mallow and *Miscanthus* reduced the number of sulphate-reducing spore-forming bacteria. Common Osier willow stimulated the growth of saprophytic bacteria in the soil, while Virginia mallow the number of spore-forming bacteria. Our results revealed that microbial activity of soil expressed as the number of selected bacterial groups, depends not only on the applied rate of sewage sludge fertilizer, but also on the cultivated energy crop.

Key words: organic fertilizer, *Miscanthus*, microorganisms, Virginia mallow, Common osier willow

1. Introduction

The organic wastes, such as sewage sludge are very often used in fertilization of crops. The organic matter introduced with sewage sludge modifies physicochemical and biological properties (involving numbers of microorganisms) of the soil. Due to the presence of some pathogens, parasites and heavy metals and another toxics compounds, introducing sewage sludge into the soil may cause a potential risk to the environmental conditions. Many studies described the effects of fertilization with sewage sludge on plants, soil, and yield quality of energy crops (Augustynowicz *et al.* 2009, Iżewska 2009, Blanco-Canqui 2010, Černý *et al.* 2012, Seleiman *et al.* 2012; Stępień *et al.* 2014). There are still very few data in literature concerning microbial properties in the soil under energy crop plantations (Baum *et al.* 2009, Stępień *et al.* 2014). Thus, the aim of this study was to assess the impact of fertilization with sewage sludge on the population size of selected physiological groups of bacteria in soil, under three most prospective species as energy crops for Poland, representing each of basic types of C4 grasses (C4G), high perennial plants (HPP), and short rotation coppices (SRC).

2. Materials and methods

Soil samples for analyses were collected in 2009–2011 from an experiment with three energy crops (*Miscanthus x giganteus* Greef et Deu),

Virginia mallow (*Sida hermaphrodita* (L.) Rusby), and Common Osier willow (*Salix viminalis* L.) established in 2006 at a field of the Skierniewice Experimental Station, Warsaw University of Life Science (SGGW). Each year, sewage sludge from the municipal wastewater treatment plant was applied in two rates: 10 and 20 t DW per ha. The experiment was conducted on a podsollic soil formed from heavy loamy sand, containing very little humus, weakly acidic (pH 6.1), and medium-rich in available forms of macronutrients. Soil samples were analyzed for pH in 1 M KCl (ISO 10390: 1997), total amounts of organic carbon (ISO 10694: 2002), and total nitrogen (ISO 11261: 2002), available P by Egner-Riehm method (Indiati and Rossi 1999) and exchangeable forms of K, Mg and Ca by Ostrowska's method (Stępień *et al.* 2014) (Table 1).

Table 1. Physicochemical properties of sewage sludge used in the experiment.

pH	Humidity	g/kg					mg/kg						
		N	P	K	Mg	Ca	Cd	Zn	Hg	Ni	Cu	Cr	Pb
9.1	781	40.3	15.1	1.7	5.52	17.7	2.25	960	0.8	42	144	75	47.2
							20*	2500*	16*	300*	1000*	500*	750*

* Norms of the content of heavy metals according to official gazette on 2015 (article 257). Regulation of Ministry of the Environments on 6.02.2015 related to sewage sludge.

In 2009, representative samples were taken from the surface layer (5–20 cm) of the soil in each experimental combination (treatment) both for physicochemical and microbiological examinations. The chemical properties of sewage sludge were examined using the same methods as the soil samples. Additionally, the content of the heavy metals in sewage sludge was also assessed. The waste product was rich in nitrogen, phosphorus and organic matter. The levels of heavy metals in the sewage sludge used in the experiment never exceed the permitted limits (Table 1). Furthermore, analyses performed in the Katowice Epidemiological Sanitary Station proved that, there was no bacteria of the genus *Salmonella*, eggs of *Ascaris* sp., *Trichuris* sp., and *Toxocara* sp. in the sludge. The microbial tests were conducted

to determine the Most Probable Number (MPN) of following indicators: total coliforms bacteria in Eijkman lactose broth (Himedia), sulphate-reducing spore-forming bacteria on medium of Wilson-Blair (Himedia); *Proteus* sp. on nutrient agar medium (Himedia). The cultures were incubated for 24–72 hours at 37°C. Nutrient agar was also used to determine the number of saprophytic and spore-forming bacteria (24–72 hours at 28°C), and thermophilic bacteria (24 hours at 55°C). To determine the sulphate-reducing spore-forming bacteria inoculation of medium was preceded by pasteurizing the soil dilutions for 15 minutes at 85°C. For bacteriological analysis, ten-fold dilutions of the studied soil samples were prepared using sterilized physiological saline solution (0.85 % NaCl). The tests were conducted in triplicate. MPN values for the bacteria were read off using McCready's tables (de Man 1983). The number of bacteria was calculated per gram of dry weight of soil and expressed as colony-forming units (CFU).

Results were verified by means of uni- or multivariate analysis of variance using Statgraphics (ver. Plus 4.1); homogeneous groups were determined with Tukey's test for $\alpha = 0.05$.

3. Results

The sewage sludge incorporated into the soil planted with energy crops significantly increased pH and the content of nutrients in soils (Table 2), and changed the number of all investigated microorganisms groups (Tables 3, 4).

Table 2. Physicochemical properties of investigated soils (where: 0 – control soil; 1 – soil with sewage sludge 10 t DW/ha; 2 – soil with sewage sludge 20 t DW/ha; a, b, c – homogeneous groups in columns; DW – dry weight).

Experimental combination	pH	C (g/kg)	N (g/kg)	P (mg/kg)	K (mg/kg)
0	5.9a	7.62a	0.83a	65a	86a
1	6.0b	8.41b	0.86b	86b	88b
2	6.2b	9.08c	0.92b	121c	92b
LSD $\alpha=0.05$	0.569	0.745	0.055	7.079	5.767

With the exception of sulphate-reducing spore-forming bacteria (tendency to increase not significant), the largest number of all groups of bacteria was recorded in the soil fertilized with the larger rate of sludge (20 t DW/ha). A lower sludge rate only increased the numbers of the total coliforms bacteria and sulphate-reducing spore-forming bacteria in relation to the control, while the tendencies to increase have not been proven (Table 3).

Table 3. Effect of sewage sludge on the number of selected groups of bacteria in the soil under energy crops (where: 0 – control soil; 1 – soil with sewage sludge 10 t DW/ha; 2 – soil with sewage sludge 20 t DM/ha; a, b, c – homogeneous groups in columns; MPN – Most Probably Number, CFU – colony-forming units, DW – dry weight).

Sewage sludge level	MPN of bacteria per kg DW of soil					
	Total coliforms bacteria CFU × 10 ⁶	Sulphate- reducing spore-forming bacteria CFU × 10 ⁸	<i>Proteus</i> sp. CFU × 10 ⁶	Saprophytic bacteria CFU × 10 ⁹	Thermophilic bacteria CFU × 10 ⁶	Aerobic spore- forming bacteria CFU × 10 ⁷
0	10.7a	6.4a	1.0a	6.9a	3.8a	32.7a
1	36.1b	51.4b	9.2a	13.9a	8.0a	49.9a
2	89.3c	80.8b	90.3b	312.8b	75.4b	71.8b
LSD _{a=0.05}	18.11	29.65	43.22	255	32.8	18.7

Microbiological analyses showed that the size of the rate of sewage sludge applied to soil planted with the different species as energy crops did affect the population size of the investigated groups of bacteria. The higher rate of sewage sludge introduced into the soil under the three energy crops significantly increased the population size of all tested groups of microorganisms compared to the soil that never been fertilized (Table 4).

Table 4. Effect of sewage sludge fertilization on the MPN of bacteria per kg dry weight of soil cultivated with chosen energy crops in Skiermiewice (where: 0 – control soil; 1 – soil with sewage sludge 10 t DW/ha; 2 – soil with sewage sludge 20 t DM/ha; a, b, c – homogeneous groups in columns; MPN – Most Probably Number, CFU – colony-forming units, DW – dry weight).

Plants	Treatment	Total coliforms bacteria CFU ' 10 ⁶	Sulphate reducing spore-forming bacteria CFU ' 10 ⁸	<i>Proteus</i> sp. CFU ' 10 ⁶	Saprophytic CFU ' 10 ⁹	Thermophilic CFU ' 10 ⁶	Aerobic spore-forming CFU ' 10 ⁷
Miscanthus	0	2.5a	12.0a	1.0a	7.0a	0.9a	3.0a
(<i>Miscanthus x giganteus</i>)	1	5.0b	13.5b	4.3b	14.5b	1.2b	11.5b
	2	27.5c	27.5c	16.0c	22.8c	1.9c	18.8c
LSD _{a=0.05}		0.18	0.09	0.08	1.77	0.1187	2.5501
Virginia mallow (<i>Sida hermaphrodita</i>)	0	25a	2.5a	5.1a	5.1a	9.4a	90.4a
	1	50b	23.0b	19.0b	9.2b	14.9b	127.6b
	2	131c	51.0c	215.0c	16.4c	141.5c	177.5c
LSD _{a=0.05}		0.27	0.076	0.11	1.15	5.73	16.25
Common Osier willow (<i>Salix viminalis</i>)	0	1.4a	2.0a	0.5a	9.0a	1.0a	4.3a
	1	57.5b	120.0b	0.5a	20.4a	5.5a	12.0b
	2	126.0c	169.0c	40.0c	900.0c	82.0b	19.5c
LSD _{a=0.05}		0.04	0.0152	0.0187	19.661	7.1181	2.2573

The energy crops themselves also significantly differentiated population sizes of the selected physiological groups of bacteria in soil (Table 5). The lowest number of the coliforms bacteria was found in soil under *Miscanthus*. The largest numbers of sulphate-reducing spore-forming bacteria and saprophytic bacteria were found in the soil under the Common Osier willow plantation, while *Proteus* sp. and aerobic spore-forming bacteria were most numerous in the soil cultivated with Virginia mallow (Table 5).

Table 5. The number of selected groups of bacteria in soil per kg dry weight of soil under chosen energy crops in Skierniewice (where: a, b, c – homogeneous groups in columns; MPN – Most Probably Number, CFU – colony-forming units, DW – dry weight).

Plants	MPN of bacteria per kg DW of soil					
	Total coliforms CFU · 10 ⁶	Sulphate reducing spore-forming CFU · 10 ⁸	<i>Proteus</i> sp. CFU · 10 ⁶	Saprophytic CFU · 10 ⁹	Thermophilic CFU · 10 ⁶	Spore-forming CFU · 10 ⁷
<i>Miscanthus</i> (<i>Miscanthus x giganteus</i>)	11.2a	16.5a	6.9a	14.6a	1.4a	10.9a
Virginia mallow (<i>Sida hermaphrodita</i>)	64.9b	24.2a	80.2b	10.2a	55.3b	131.8b
Common Osier willow (<i>Salix viminalis</i>)	59.8b	97.9b	13.5a	308.9b	30.6ab	11.8a
LSD _{α=0.05}	37.93	42.37	58.51	255.1	33	18.7

4. Discussion

Our results confirm that the organic matter introduced with sewage sludge into the soil changes physicochemical and biological properties of soil (Joniec and Furczak 2008, Frąć and Jezierska-Tys 2009, 2011, Iżewska 2009, Jezierska-Tys and Frąć 2009, Stępień *et al.* 2014).

Fertilization with sewage sludge applied over three years in our experiment increased the number of microorganisms in the soil cultivated with energy crops (Table 4). The size of the rate of the applied fertilizer, and thus the amount of organic matter introduced into the soil affected the population size of heterotrophic microorganisms, which is consistent with the data of other research (Szanser *et al.* 2011, Jezierska-Tys and Frąć, 2009, Joniec and Furczak 2007). Joniec and Furczak (2007) showed a stimulating effect of fertilization of podsolic soil with different rates of sewage sludge on the number of heterotrophic bacteria in the soil under *Salix viminalis*. Wielgosz (1996) examined the number of microorganisms (cellulolytic, ammonifying and nitrifying bacteria) in a soil fertilized with sewage sludge and subjected to two years of transformation by energy crops and found an increase in their number under Virginia mallow. Our results showed that C4G *Miscanthus x giganteus* reduced the number of saprophytic bacteria and sulphate-reducing spore-forming bacteria, same as HPP Virginia mallow, compared with SRC willow. *Miscanthus* was the most effective in reducing the number of coliform bacteria, which means that, compared with other plants, it improved the sanitary condition of the soil (Table 5). The root system of plants of the genus *Miscanthus* secretes acid metabolites, including citric acid (Kayama 2001, Vranová *et al.* 2010). Our findings lead to the conclusion that acidic metabolites of the *Miscanthus* do affect the microbiological properties of soils, which manifests itself in a decrease in the number of coliform bacteria in comparison with the other energy crops.

The considerable biomass of living organisms constitutes an extremely active, in metabolic terms, mechanism that enriches soils in biogenic elements (through biogeochemical cycles), growth and antibiotic substances, and other biologically active substances. While

making these transformations, these organisms participate in creating the soil and determining its fertility, and make it more suitable for plants. Wielgosz (1996) investigated the number of microorganisms in soil cultivated with Virginia mallow (*S. hermaphrodita*), Common Osier willow (*S. viminalis*) and American willow (*Salix americana*). He found that all those plants stimulated the growth of fungi, and Virginia mallow was doing that to the greatest extent. However, the population sizes of cellulolytic, lipolytic and ammonifying bacteria of this soil were at a higher level compared with the other crops. This matches the results presented in this paper, which show that in relation to other plants the highest numbers of saprophytic bacteria and sulphate-reducing spore-forming bacteria were found in the soil of willow plantation but aerobic spore-forming bacteria and *Proteus* sp. in Virginia mallow plantation. The results of the study confirm that the number of soil microorganisms is affected by the energy crops used for cultivation. The use of appropriate energy crops on the soil fertilized with sewage sludge can decrease number of coliforms bacteria and sulphate-reducing spore-forming bacteria including *Clostridium perfringens* being indicators of soil sanitary state (Niewolak *et al.* 2002).

Summing up, our results showed that the number of microorganisms as a measure of soil microbial activity depends on the organic matter introduced into the soil with sewage sludge, the rate of sludge, and the planted energy crop. Addition of sewage sludge to the soil stimulated the growth of saprophytic and thermophilic bacteria. Sewage sludge increased the number of coliform bacteria and sulphate-reducing spore-forming bacteria (i.a. *C. perfringens*) known as indicators of sanitary conditions of the soil. The energy crops modified the sanitary conditions of soils, for example Miscanthus reduces the number of total coliforms bacteria, while on the other hand, willow stimulates growth of heterotrophic bacteria, and Virginia mallow the number of aerobic spore-forming bacteria in the soil.

Acknowledgements: This work was funded by Ministry of Science and Higher Education of Poland, grant nr NN 310 308734, Agricultural

productivity and energy-emission value of biomass of energy crops grown under differentiated soil moisture conditions.

References

- Augustynowicz J., Pietkiewicz S., Kalaji, M.H., Russel S., 2009, *The effect of sludge fertilization on chosen parameters of chlorophyll fluorescence and biomass yield of Jerusalem artichoke (Helianthus tuberosus L.)*, in: W. Sądej (ed.), *Sewages and waste materials in environment*, Department of Land Reclamation and Environmental Management, UWM in Olsztyn, Poland, 129–139.
- Baum C., Leinweber P., Weih M., Lamersdorf N., Dimitriou I., 2009, *Effects of short rotation coppice with willows and poplar on soil ecology*, *Agri. For. Res.* 3, 183–196.
- Blanco-Canqui H., 2010, *Energy crops and their implications on soil and environment*, *Agron. J.* 102, 403–419.
- Černý J., Balík J., Kulhánek M., Vašák F., Peklová L., Sedlář O., 2012, *The effect of mineral N fertilizer and sewage sludge on yield and nitrogen efficiency of silage maize*, *Plant Soil Environ.* 58, 76–83.
- De Man J.C., 1983, *The probability of most probable numbers*, *Eur. J. Appl. Microbiol.* 17, 301–305.
- Frąc M., Jezierska-Tys S., 2009, *Biological indicators of soil quality after application of dairy sewage sludge*. *Environ. Protect. Eng.* 1, 49–61.
- Frąc M., Jezierska-Tys S., 2011, *Agricultural utilization of dairy sewage sludge: Its effect on enzymatic activity and microorganisms of the soil environment*, *African J. Microb. Res.* 5, 1755–1762.
- Indiati R., Rossi N., 1999, *Extractability of residual phosphorus from highly manured soils*, *Ital. J. Agron.* 3, 63–73.
- ISO 11261: 2002, *Determination of total nitrogen – modified Kjeldahl*.
- ISO 10390: 1997, *Soil quality – Determination of pH*.
- ISO 10694: 2002, *Determination of organic and total carbon after dry combustion (elementary analysis)*.
- Iżewska A., 2009, *The impact of manure, municipal sewage sludge and compost prepared from municipal sewage sludge on crop yield*

- and content of Mn, Zn, Cu, Ni, Pb, Cd in spring rape and spring triticale, *J. Elementol.* 14, 449–456.
- Jeziarska-Tys S., Frąc M., 2008, *Examination of the effect of milk sewage treatment plants deposits on microbiological and biochemical activity of soil*, Treatises and Monographs 3, Acta Agrophysica, 160 (in Polish)
- Jeziarska-Tys S., Frąc M., 2009, *Seasonal changes in microbial activity of brown soil fertilized with dairy sewage sludge*, *Ecol. Chem. Eng. A*, 1, 1273–1282.
- Joniec J., Furczak J., 2007, *Liczebność wybranych grup drobnoustrojów w glebie bielkowej pod uprawą wierzby (Salix viminalis L.) użyźnionej osadem ściekowym, w drugim roku jego działania*, *Ann. UMCS Lublin*, 62, 93–104.
- Joniec J., Furczak J., 2008, *Counts and activity of microorganisms participating in nitrogen transformations in soil, four years after application of sewage sludge*, *J. Elementol.* 13, 545–557.
- Kayama M., 2001, *Comparison of the aluminium tolerance of Miscanthus sinensis Andress and Miscanthus sacchariflorus Benthams in hydroculture*, *Int. J. Plant Sci.* 162, 1025–1031.
- Niewolak S., Tucholski S., Radziejewska E., 2002, *Sanitary-Bacteriological evaluation of meadow soils irrigated with biologically treated sewage*, *Polish J. Environ. Stud.* 11, 141–149.
- Seleiman M.F., Santanen A., Stoddard F.L., Mäkelä P., 2012, *Feedstock quality and growth of bioenergy crops fertilized with sewage sludge*, *Chemosphere*, 89, 1211–1217.
- Stępień W., Górska E.B., Pietkiewicz S., Kalaji M.H., 2014, *Long-term mineral fertilization impact on chemical and microbiological properties of soil and Miscanthus x giganteus yield*, *Plant Soil Environ.* 60, 117–122.
- Szanser M., Ilieva-Makulec K., Kajak A., Górska E., Kusińska A., Kisiel M., Olejniczak I., Russel S., Sieminiak D., Wojewoda D., 2011, *Impact of litter species diversity on decomposition processes and communities of soil organisms*, *Soil Biol. Biochem.* 43, 9–19.

Wielgosz E., 1996, *Liczebność i niektóre parametry aktywności drobnoustrojów w osadzie ścieków komunalnych pod uprawą różnych roślin*, Zesz. Probl. Post. Nauk Roln. 437, 338–340.

Vranová V., Kaňová H., Rejšek K., Formánek P., 2010, *Dominant amino acids, organic acids and sugars in water-soluble root exudates of C4 plants: a mini-review*, Acta Univ. Agric. Silviculture Mendelianae Brunensis, 58, 441–446.

Właściwości mikrobiologiczne gleby nawożonej osadem ściekowym spod uprawy roślin energetycznych

Streszczenie

Właściwości mikrobiologiczne gleby z dodatkiem osadu ściekowego, na której uprawiane są rośliny energetyczne, są mało poznane. Celem naszych badań była ocena wpływu nawożenia osadami ściekowymi na wybrane grupy fizjologiczne mikroorganizmów w glebie na której uprawiano następujące rośliny: miskant (miskant olbrzymi Greef et Ger), ślazowiec pensylwański (ślazowiec pensylwański (L.) Rusby) oraz wierzba (*Alix viminalis* L.). Osady ściekowe stosowano w dwóch dawkach 10 i 20 t / ha suchej masy (DW). W glebie oznaczono ogólną liczebność bakterii grupy *coli*, przetrwalnikujących bakterii redukujących siarczany, *Proteus* sp., saprofitycznych, termofilnych i tlenowych bakterii tworzących formy przetrwalnikowe. W zależności od dawki osad ściekowy zwiększył liczebność bakterii grupy *coli* i endosporowych bakterii redukujących siarczany i stymulował wzrost bakterii saprofitycznych i termofilnych. Uprawa miskanta ograniczyła liczbę bakterii z grupy *coli* natomiast ślazowca pensylwańskiego i miskanta zmniejszyła liczbę bakterii przetrwalnikujących i redukujących siarczany. Wierzba stymulowała wzrost bakterii saprofitycznych w glebie a ślazowiec pensylwański liczbę bakterii przetrwalnikowych. Nasze wyniki wykazały, że aktywność mikrobiologiczna gleby wyrażona liczebnością wybranych grup bakterii, zależy nie tylko od stosowanej dawki osadu ściekowego, lecz również od uprawianej rośliny energetycznej.

Słowa kluczowe: nawozy organiczne, miskant, mikroorganizmy, ślazowiec pensylwański, wierzba