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MICROBIOME OF SOIL CONTAMINATED BY FUELS

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Streszczenie. Celem prowadzonych badań było określenie wpływu obecności paliw w glebie na liczebność wybranych grup mikroorganizmów glebowych, w tym bakterii, promieniowców, grzybów, kopiotrofów i oligotrofów. W doświadczeniu wykorzystano olej napędowy (ON), biodiesel (BD) oraz mieszaninę obu paliw, w której udział biodiesla wynosił 10% (BD10). Zastosowano dwa poziomy skażenia: 10 i 100 g · kg^{-1} s.m. gleby. Liczebność mikroorganizmów oceniano metodą posiewu rozcieńczeń glebowych z zastosowaniem podłoży hodowlanych odpowiednich dla poszczególnych grup drobnoustrojów. Na podstawie otrzymanych wyników badań stwierdzono zróżnicowaną wrażliwość badanych mikroorganizmów na obecność paliw w glebie. Rodzaj paliwa i jego dawka miały istotny wpływ na liczebność bakterii, promieniowców i grzybów. Nie stwierdzono zależności pomiędzy tymi czynnikami doświadczenia a liczebnością mikroorganizmów kopiotroficznych i oligotroficznych.

Key words: biodiesel, diesel fuel, microorganisms, soil.
Słowa kluczowe: biodiesel, diesel, gleba, mikroorganizmy.

INTRODUCTION

Soil is a natural habitat for microorganisms whose activity is essential for maintaining the continuity of transforming matter in the environment (Winding et al. 2005). Microorganisms are involved in shaping fertility of the soil, providing nutrients to plants, and participate in detoxification of harmful chemical substances (Galus-Barchan and Paśmionka 2014). Among the many pollutants that enter the soil environment, the most common are petroleum products. Their presence in the soil can be associated with the processes of mining, processing in refineries or failures that occur, for example, during fuel storage (Jansson et al. 2000, Pena et al. 2007, Nowak 2008). Fuel influences physical, chemical, biochemical and microbiological properties of soil, it may be toxic to plants, it is also a source of groundwater pollution, which in consequence may negatively affect the health and lives of people (Hawrot-Paw 2011). Soil microorganisms are very sensitive to changes occurring in the soil (Schloter et al. 2003, Andreoni et al. 2004) and may be used as indicators of its health (Hayat et al. 2002, Eibes et al. 2006). Petroleum substances may limit the number of microorganisms or

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eliminate them from the contaminated environment (Wyszkowska and Kucharski 2001, Kucharski and Jastrzębska 2005, Hawrot-Paw and Ryłów 2008), but may also stimulate their growth and development (Michalcewicz 1995, Nowak et al. 2008, Hawrot-Paw 2011).

In recent years, much attention has been given to issues related to enriching diesel oil with bio-components of plant origin (Mańczak et al. 2010). One of such additives is biodiesel, prepared in the process of trans-esterification, which can also be used as a stand-alone fuel. Although biodiesel is considered to be an easily biodegradable substance, it does not occur naturally in the environment (Hawrot-Paw 2011), and its presence in the soil can affect its biological balance and modify the activity of the resident microorganisms.

The aim of the study was to specify changes in the population of selected groups of soil microorganisms in soil contaminated with biodiesel, diesel and a mixture of these fuels.

MATERIAL AND METHODS

The study was conducted in loamy sand. The material was obtained in the Experimental Station in Lipnik belonging to the West Pomeranian University of Technology in Szczecin, from a depth of 0–15 cm up to the level of hummus/arable land. At the laboratory the soil was dried with air and then sieved through a sieve with apertures of 2 mm. Based on current humidity, as determined by the drying-weighing method, moisture content of the soil was brought to the level corresponding to 60% capillary water capacity, and such humidity was maintained throughout the study.

During the experiment biodiesel (BD100) and diesel (DO) from the distributor of a gas station, and mixture of the two fuels was also prepared in a laboratory by introducing a 10 per cent biodiesel additive (BD10) to conventional fuel. The soil material has been divided into samples of 500 grams, and then the appropriate amount of fuel was added: dose 1 – 10 g · kg^{-1} d.m. of soil, dose 2 – 100 g · kg^{-1} d.m. of soil. Soil uncontaminated with fuel was the control object (K). The experiment was conducted for 28 days. Scheduled microbiological analyses were performed on the day when the soil was contaminated with fuels, and then after 14 and 28 days of incubation.

Microbiological analyses included determination of the number of selected groups of soil microorganisms by the method of inoculating soil dilutions, which were repeated three times. Results were converted to 1 g dry mass of the soil and are given as colony forming units (CFU).

The scope of the study included determining the number of:

- bacteria on the medium of Bunt and Rovira (1955) at 20ºC after three days of incubation,
- actinobacteria on the medium of Cyganow and Žukov (1964) at 20ºC after 7 days of incubation,
- fungi on Martin's medium (1950) at 20ºC after 5 days of incubation,
- copiotrophs on NB (nutrient broth) medium after 7 days incubation at 28ºC (Ohta and Hattori 1980),
- oligotrophs on the DNB (dilution nutrient broth) medium after 14 days incubation at 28ºC (Ohta and Hattori 1980).

The results were statistically analysed using the analysis of variance and significance tests of Newman-Keuls. The analysis programme used was Statistica v. 10.0 by StatSoft Poland.

RESULTS AND DISCUSSION

Petroleum substances that leak into the soil environment can interfere with the natural cycle of matter and energy circulation and thus affect the biological composition of the soil (Kołwzan 2005, Hawrot-Paw 2011). The dynamics of change in the number of individual groups of microorganisms under the influence of fuels is shown in Figs. 1–5, and the average values of the entire incubation period in Fig. 6 A–E. Basing on these results we can say that applying contamination to the soil caused shifts in the quantitative composition of microflora. Depending on the group of microorganisms, the number increased or decreased. For selected groups of microorganisms, significant effects of the type of contamination and the dose, as well as the time of inspection were reported. The relationship between the dose of contamination and number of soil microorganisms is also supported by previous studies of Hawrot-Paw (2011). In general, the number of microorganisms in soil contaminated with petroleum products varies in time (Song and Bartha 1990). When the experiment was performed, fluctuations in the number of microorganisms were observed. Significant changes in individual testing sites regarding bacteria, actinobacteria and fungi were marked after 14 days of incubation. A transient increase in the number of the number of copiotrophs and oligotrophs was observed, however, the observed stimulation was not statistically significant and, as in the case of other groups of microorganisms, limited in time.

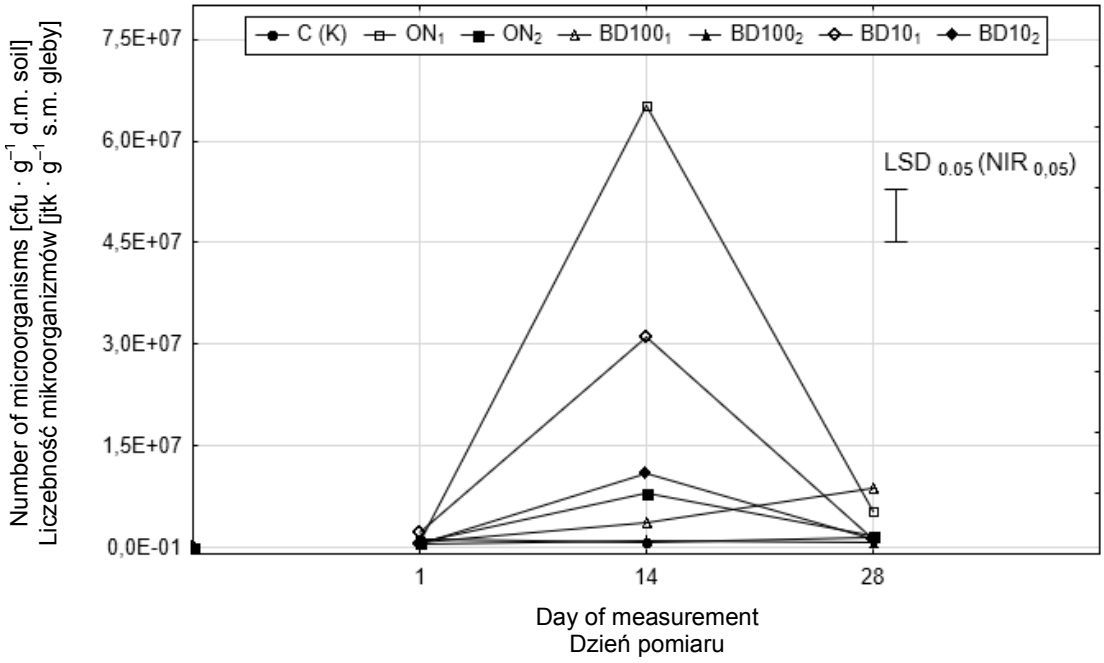

Fig. 1. The number of bacteria in soil of particular objects
Rys. 1. Liczebność bakterii w glebie poszczególnych obiektów

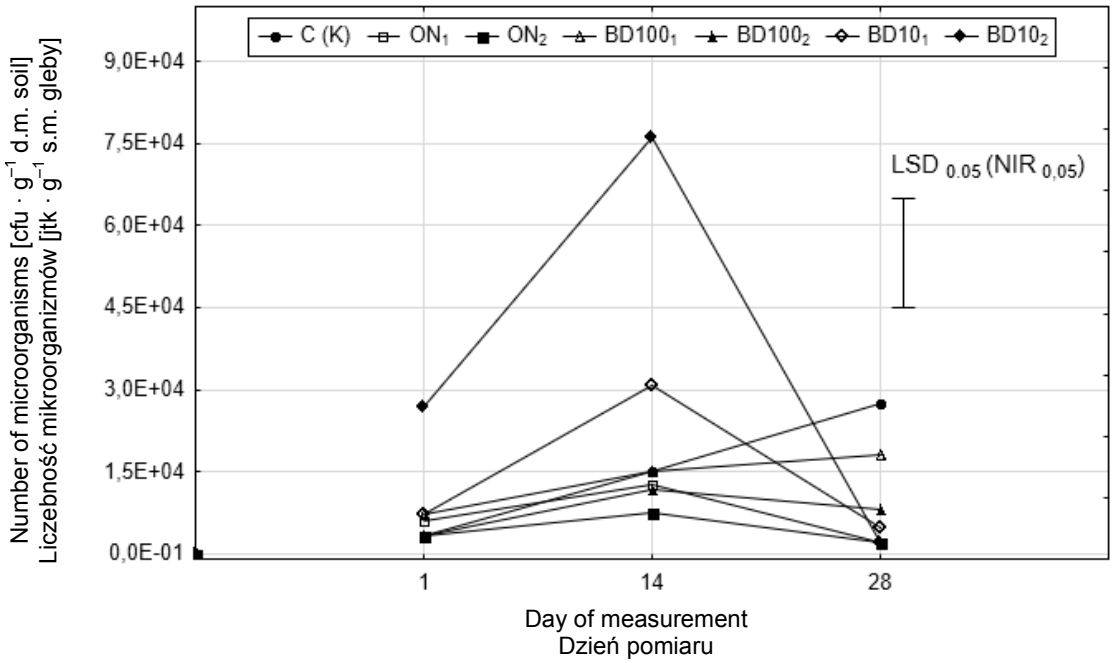

Fig. 2. The number of actinobacteria in soil of particular objects
Rys. 2. Liczebność promieniowców w glebie poszczególnych obiektów

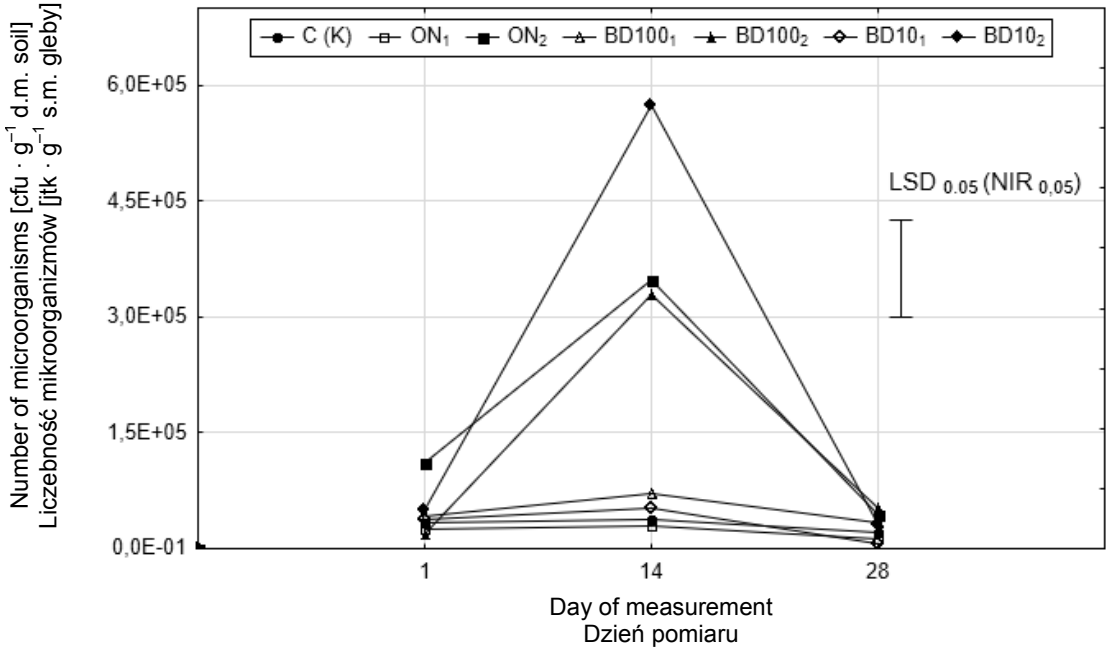

Fig. 3. The number of fungi in soil of particular objects
Rys. 3. Liczebność grzybów w glebie poszczególnych obiektów

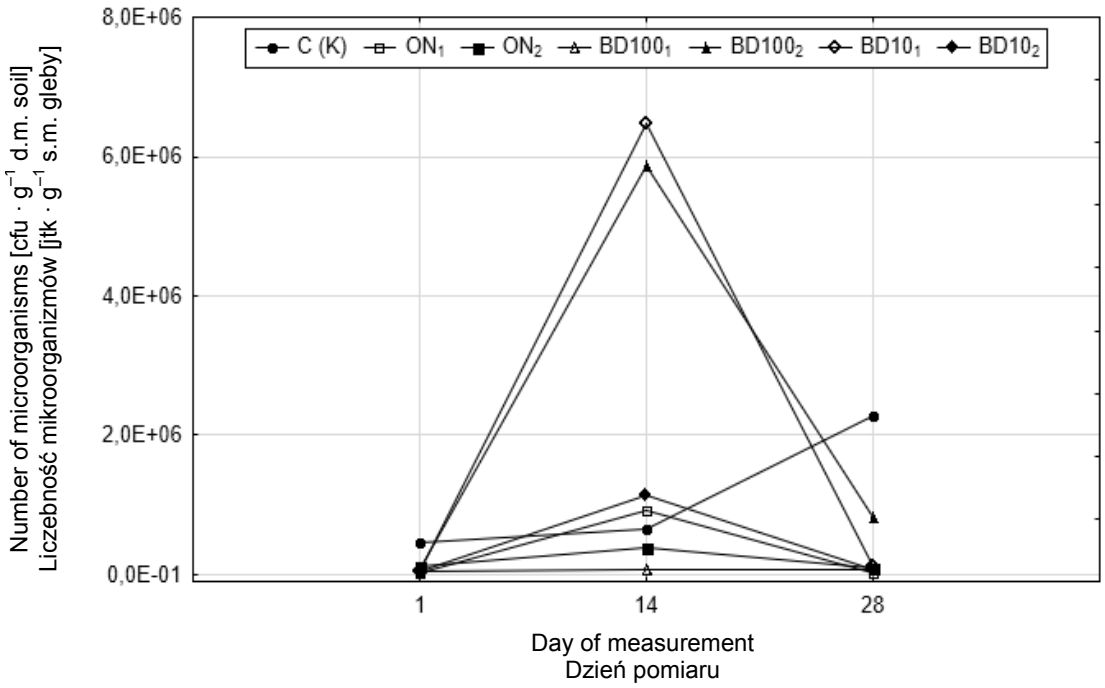

Fig. 4. The number of copiotrophics microorganisms in soil of particular objects
Rys. 4. Liczebność kopiotrofów w glebie poszczególnych obiektów

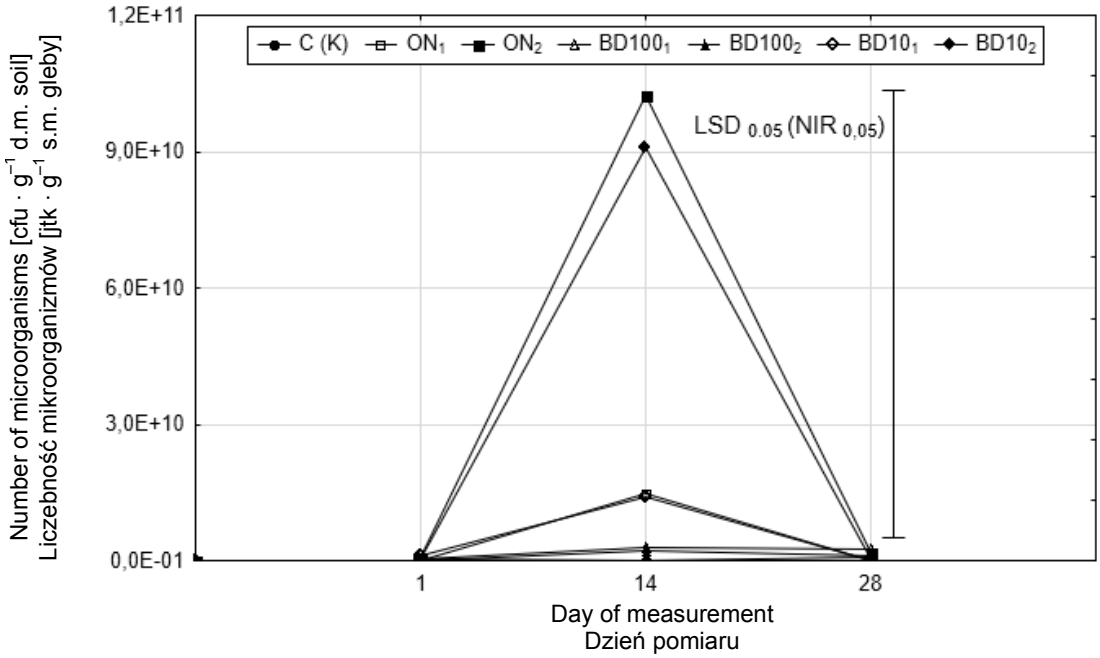

Fig. 5. The number of oligotrophics microorganisms in soil of particular objects
Rys. 5. Liczebność oligotrofów w glebie poszczególnych obiektów

Considering the mean values throughout the incubation period it has been found that diesel oil stimulated the growth of bacteria and oligotrophic microorganisms, and in a dose of 100 g · kg^{-1} d.m. of soil, also of fungi. This may mean that they found fuel to be an easily digestible nutrient. This stimulating effect of conventional fuel was also observed by other

authors (Molina-Barahona et al. 2005, Hawrot-Paw 2012). The increasing amount of fuel limited the number of actinobacteria. There has also been a negative impact on copiotrophs, and in research of Kucharski and Wyszkowska (2001) the number of these microorganisms under the influence of diesel increased. Kucharski and Jastrzębska (2005) observed a stimulating effect on both fuel oil copiotrophs and oligotrophs.

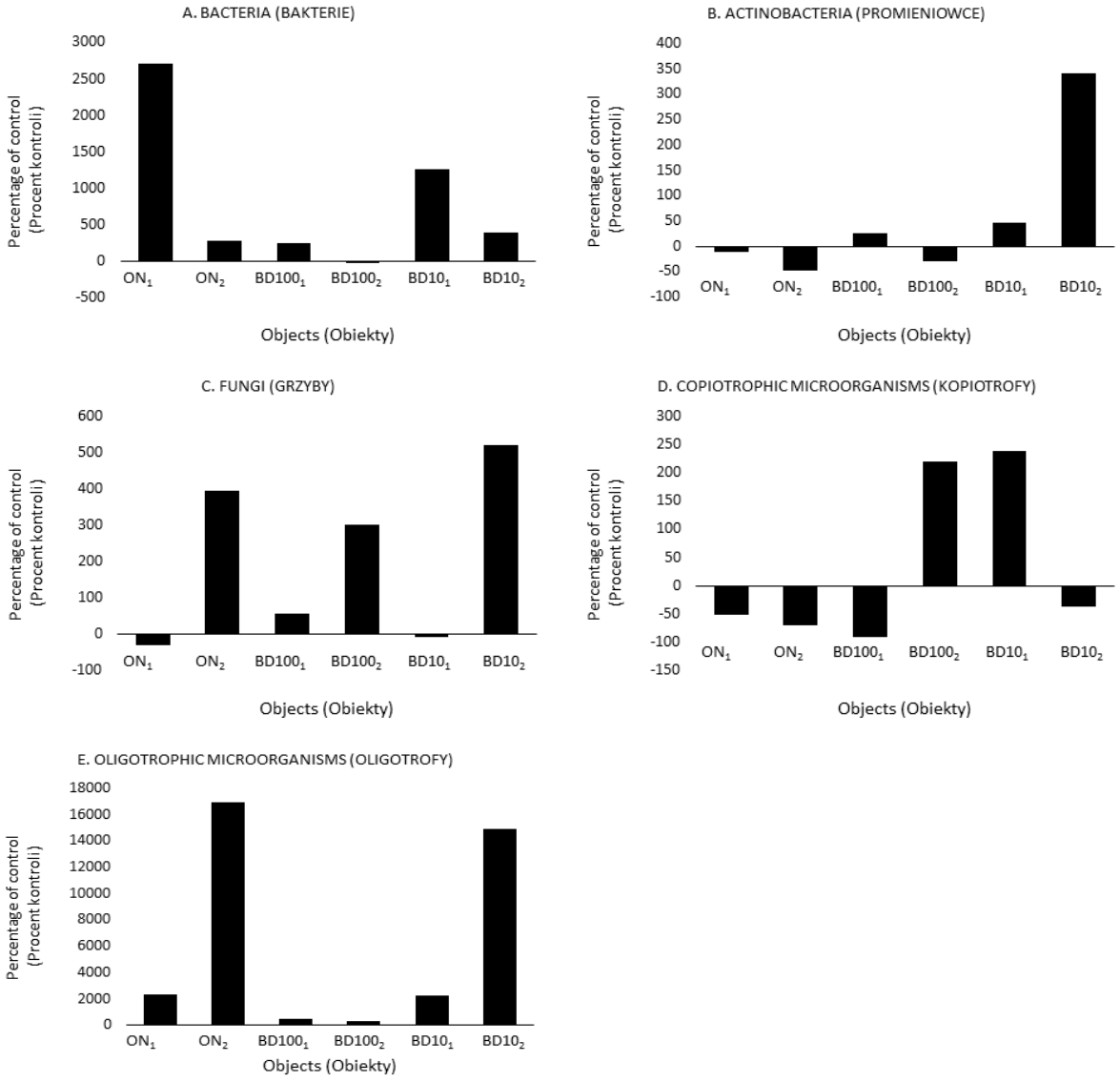

Fig. 6 A–E. The average number of microorganisms as percent of control
Rys. 6 A–E. Średnia liczebność mikroorganizmów jako procent kontroli

Favourable effects of the mixture of both tested fuels (objects BD10) were observed as regards bacteria, and as to actinobacteria and oligotrophs – their numbers further increased with increasing the level of contamination. The stimulatory effect of the dose of 100 g · kg^{-1} d.m. of soil was also recorded for fungi. In object $BD10_2$ their numbers increased by over 500% compared to the control object.

In the presence of pure biodiesel, regardless of the dose, an increase in the number of fungi and to a lesser extent, oligotrophic microorganisms was observed. Increased fuel concentration in the soil negatively affected bacteria and actinobacteria, reducing their numbers by approx. 30–35%, compared to the control object. The negative reaction of some microorganisms could be a response to biodiesel additives, for example, antioxidants. However, studies carried out by Lapinskiene et. al. (2006) showed that up to 12 per cent dose of biofuel did not impair the activity of microorganisms. Differences in results may result from, among others, variable composition of vegetable oils, out of which biodiesel is produced (Srivastava and Prasad 2000, Pinto et al. 2005, Hawrot-Paw 2011). In the case of conventional fuel, hydrocarbons present in them may be a source of carbon and energy for some microorganisms, and other may be toxic (Hawrot-Paw et al. 2015). The reaction of microorganisms may be the result of the effect of pollutants on their cells or an indirect result of changes caused by the presence of fuel in the soil environment.

CONCLUSIONS

Soil contamination caused by biodiesel, a mixture of diesel with biodiesel disrupts the biological balance of the soil. Microorganisms have different sensitivity to the presence of fuel, depending on their type. The dose of contamination is also important – in studies, the increasing the level of pollution caused an increase or reduction in the number of particular groups of microorganisms.

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Abstract. The aim of the study was to determine the influence of the presence of fuels in the soil on the number of selected groups of soil microorganisms, including bacteria, actinobacteria, fungi, copiotrophs and oligotrophs. In the experiment, diesel oil (DO), biodiesel (BD) and a mixture of both fuels, in which the share of biodiesel was 10% (BD10), were used. Two levels of contamination were applied: 10 and 100 g · kg^{-1} d.m. of soil. The number of microorganisms was assessed with the method of plating dilutions of the soil on culture media suitable for particular groups of microorganisms. Based on the obtained results, varying sensibility of microorganisms to the presence of fuels in the soil was discovered. The kind of fuel and its dose had a significant impact on the numbers of bacteria, actinobacteria and fungi. There was no correlation between these factors and copiotrophic and oligotrophic microorganisms.