

ECOTOXICOLOGICAL EFFECTS OF BIODIESEL IN THE SOIL

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ABSTRACT

The paper analysed the toxic effect of the presence of biodiesel in the soil. The study involved tests with microorganisms that evaluated changes in their number and activity, and phytotoxicity tests with garden cress (*Lepidium sativum*) and spring barley (*Hordeum vulgare*). Biodiesel produced in laboratory conditions and biofuel purchased at a petrol station were introduced to the soil. Two levels of contamination were used – 1% and 5% (per dry mass of the soil). Based on the results, it was discovered that biofuels both stimulated and reduced the number and activity of microorganisms. The changes observed depended on the type of biofuel and, most often, on its dose. Laboratory biodiesel exhibited more toxic effects, especially for actinobacteria and fungi. The tested plants showed diverse sensitivity to the presence of biodiesel. Given the determined value of the germination index, laboratory biodiesel was more toxic to spring barley and commercial biofuel to garden cress. In both cases, toxicity increased with an increase in the amount of biofuel.

Keywords: biodiesel, soil, microorganisms, microbial biomass, bioassays, ecotoxicity.

INTRODUCTION

Petroleum fuels are a major source of environmental pollution [Head and Swannell 1999]. Petroleum hydrocarbons modify the physico-chemical, biochemical, and microbiological properties of soils [Wyszkowska and Kucharski 2000, Merkl et al. 2005, Agbogidi et al. 2007, Tejada et al. 2008], adversely affect plants and also cause contamination of groundwater [Siddiqui and Adams 2002, Serrano et al. 2009]. Biofuels are an alternative to traditional fuels. The intensification of efforts to increase their share as an energy source is mainly caused by economic reasons (increasing prices of crude oil and its derivatives, difficulties with supplies or depletion of resources) as well as environmental ones (traditional fuels' negative impact on the environment).

The most important biofuels are, among others, bioethanol, methanol, methane and biodiesel produced by transesterification of vegetable oils or animal fat. Biodiesel can be used as a fuel itself (B100) or a bio-component in a mixture with diesel oil. The use of biodiesel reduces emissions

of hydrocarbons, carbon monoxide and volatile organic compounds [Ramadhas et al. 2005, Usta 2005, Fernando et al. 2006, Murugesan et al. 2009]. However, the results of analysing the biological effects related to the presence of biodiesel in the environment are ambiguous. Experiments conducted by Peterson and Reece [1994] indicate that it is much less harmful to the species of *Daphnia magna* than conventional fuel. According to Khan et al. [2007], the increase of biodiesel content in diesel fuel reduces mortality of these crustaceans. On the other hand, Bünger et al. [2000] noted a four-times higher toxicity of biodiesel exhaust gases, compared to conventional fuel. Tamada et al. [2012] have observed high toxicity of biodiesel and its metabolites to earthworms of the species *Eisenia andrei*. Kooter et al. [2011] revealed a significant higher relative cytotoxicity of the biodiesel compared to diesel fuel. Liu et al. [2009] compared extracts of gaseous emissions from diesel fuel and the biodiesel blend – in the Microtox test B10 had a higher acute toxicity and cytotoxicity than diesel. The impact of biodiesel on the microorgan-

isms whose activity is extremely important for its proper functioning, is ambiguous [Winding et al. 2005]. Biofuel can increase respiration proportionally to the increase of its amount, stimulate, or restrict dehydrogenase activity and microbial biomass content [Lapinskiene et al. 2006, Hawrot-Paw and Martynus 2011]. The differences in test results regarding toxicity induced by biodiesel may arise, among other reasons, from the different chemical composition of biofuels, which depends on the substrate from which they were produced. Even the particular batch of fuel can matter [Bluhm et al. 2012].

The use of biological tests for the toxicity of biofuels can not only allow the detection of contamination, but also provides information about the possibility of restoring soils their function as a living environment for organisms. The aim of this study was to assess the changes caused by the presence of biodiesel in the soil. We analysed the reaction of soil microflora based on the number and activity of microorganisms and plants (phytotoxicity tests).

MATERIALS AND METHODS

Materials

The study was conducted on loamy sand. The material was collected from a depth of 0–15 cm from the level of agricultural-humic soil in the Experimental Station in Lipnik, belonging to the West Pomeranian University of Technology in Szczecin. The study used biodiesel prepared in the laboratory in the process of methanol transesterification of rapeseed oil (BDI) and commercial biodiesel bought at petrol station (BDII). In the phytotoxicity test, as bioindicators of contamination, spring barley *Hordeum vulgare* and garden cress *Lepidium sativum* were used.

Methods

Based on actual moisture, soil samples were brought to 50% of their capillary water capacity. This moisture was maintained during the entire experiment and any losses were refilled with distilled water. The fuel was introduced into the soil in the amount of 1% and 5% (per dry mass of the soil), leaving one object uncontaminated as a control sample (C). Soil samples were incubated at temp. 20 ± 1 °C for four weeks. The ecotoxicological tests, including microbiological analysis and

phytotoxicity tests, were performed on the 28th day of the studies.

Ecotoxicological tests

The microbiological analyses involved evaluating changes in the number of bacteria, actinobacteria, and fungi, as well as the biomass content of living organisms. The number was determined by plating dilutions of soil using media suitable for different groups of microorganisms – bacteria after three days of incubation on Bunt and Rovira's medium [1955], for actinobacteria after seven days on Cyganow and Žukov's medium [1964] and for fungi after five days on Martin's medium [1950] in 25 °C. The results were expressed as CFU (colony forming units) per 1 g dry mass of the soil. The content of the biomass was estimated by the SIR method according to Anderson and Domsch [1978]. All microbiological analyses were performed in three repetitions.

In the phytotoxicity test, as bioindicators of contamination, spring barley *Hordeum vulgare* and garden cress *Lepidium sativum* were used. In the experiment, the modified test of germination / root elongation [Włodkovic and Tomaszewska 2003] was employed, calculating the germination index according to the formula proposed by Barbero et al. [2001]:

$$\%GI = \frac{100 \cdot (G_S \cdot L_S)}{(G_C \cdot L_C)}$$

where: G and G is the number of seeds that germinated in the research sample and in the controls sample,

L and L is the length of roots in the research sample and in the controls sample.

Phytotoxicity tests were performed in three repetitions, using 10 seeds in each repetition.

Statistical analysis

The analysis of variance (ANOVA) and the Newman-Keuls test at the $P < 0.05$ level were used to analyse the experimental results. Statistical calculation were carried out using Statistica 10.0 program (StatSoft, Poland).

RESULTS AND DISCUSSION

The soil has the ability to renew its resources, which are essential to the growth and development of plants and other organisms, among other

reasons, due to the presence of microorganisms [Russel 2005]. All factors that adversely affect microorganisms can also have a negative impact on the quality of the soil.

Changes in the number and activity of microorganisms under the influence of biodiesel are shown in Figure 1(A–D). Statistical analysis showed a significant effect of the type of biofuel and its amount on the numbers of actinobacteria, fungi, and biomass content. In the case of bacteria, regardless of the amount, biofuels stimulated their growth compared to the control sample. Values shown in BDI and BDII were approx. 20–40% higher than in control samples, but these differences were not statistically significant. Biofuels used in the study negatively affected actinobacteria, which, due to significant enzymatic activity of these microorganisms, can cause a reduction in the rate of mineralization of the soil’s organic matter [Paul and Clark 2000]. A significant

reduction in their numbers, as the amount of biodiesel increased, was observed mainly in the BDI sample. Significant changes were also found after the introduction of commercial biodiesel into the soil. However, the degree of inhibition in this case was lower (up to 65–69% with over 90% in BDI₅). Biodiesel prepared in the laboratory inhibited fungal growth, and soil where commercial biofuel was introduced revealed a significant stimulation, in particular in BDII₅. The growth in the number of fungi in the presence of biodiesel has also been reported in previous studies [Hawrot-Paw 2011].

According to Gil-Sotres et al. [2005], biomass is an important indicator of the quality of soils, including those contaminated with fuels [Brohon et al. 2001]. The content of biomass of living organisms in the soil contaminated by hydrocarbons changed depending on the kind of biofuel and its dosage. At a concentration of 1%, biodiesel ad-

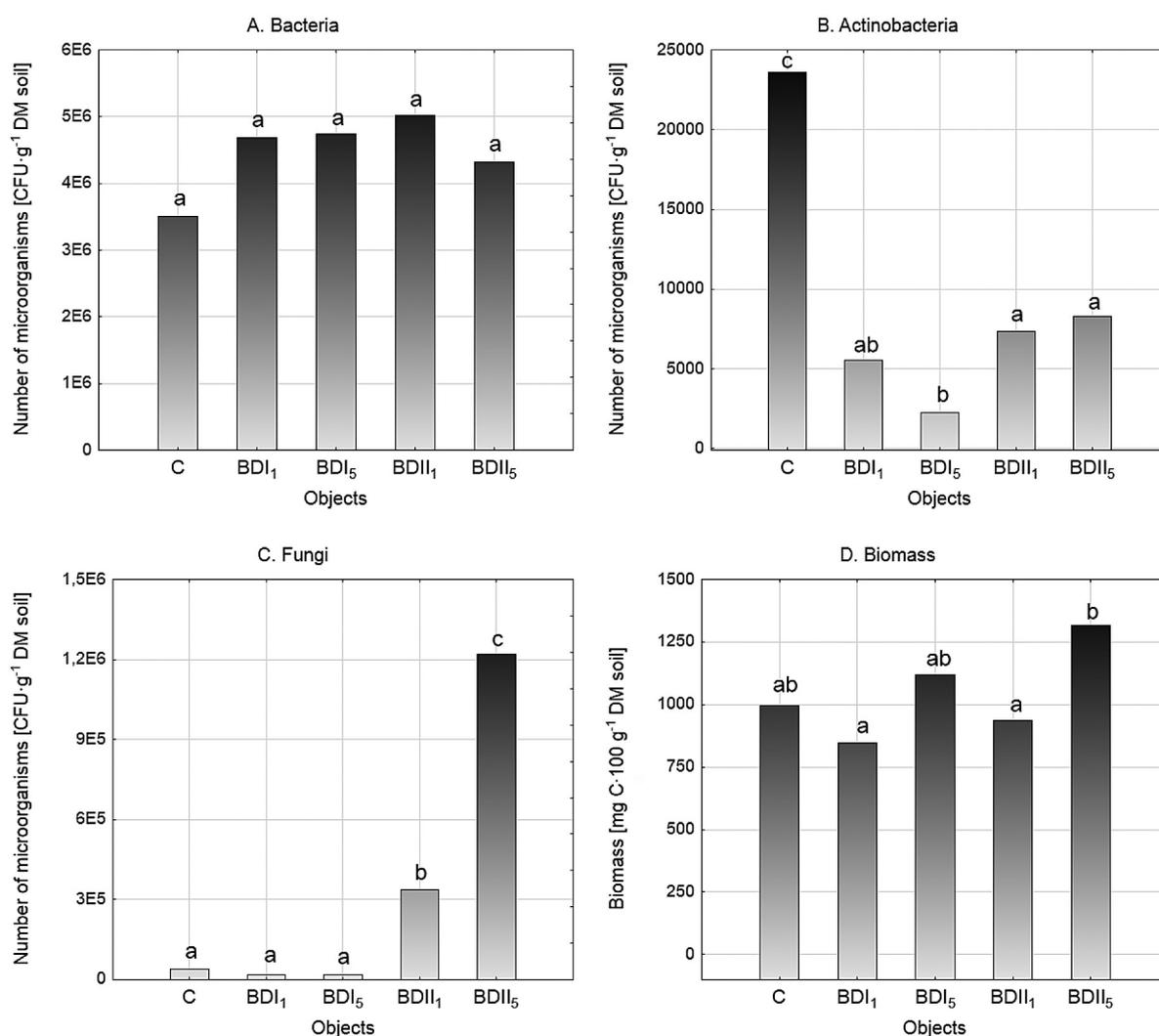


Figure 1. The number of microorganisms and the biomass content in biodiesel contaminated soil (mean over each column not marked with the same letter is significantly different at $P < 0.05$)

Table 1. The germination index value in particular experience objects

Plants	Germination index [GI%]				
	C	BDI ₁	BDI ₅	BDII ₁	BDII ₅
Spring barley (<i>Hordeum vulgare</i> L.)	100	69	43	123	67
Garden cress (<i>Lepidium sativum</i> L.)	100	121	68	82	69

versely affected both BDI and BDII, and in the presence of 5% contamination, the biomass content in soil contaminated with commercial fuel increased compared to the control sample. In the experiments carried out by Lapinskiene et al. [2006] biodiesel even in a concentration of 12%, did not adversely affect the activity of the microorganisms. In previous studies of Hawrot-Paw et al. [2010] a 10% biodiesel dose caused a reduction of the biomass content below the control values (inhibition persisted for 112 days of the experiment), while in the soil contaminated with 5% biodiesel, the content of the biomass during incubation varied. In these experiments, we used a biofuel obtained directly from the manufacturer.

In evaluating the phytotoxic effect of biodiesel, we used seeds of garden cress (*Lepidium sativum*) and spring barley (*Hordeum vulgare*), which have successfully been used in the monitoring of the level of contamination of soils with conventional fuels [Maila and Cleote 2002, Kolwzan 2005, Hawrot-Paw 2011]. The specified germination index value, regardless of plant species or kind of biofuel, decreased with the increasing degree of contamination. Tamada et al. [2012] noted that commercial biodiesel showed far more phytotoxicity for the seeds of arugula (*Eruca sativa*) and lettuce (*Lactuca sativa*) than soybean oil used in their tests. In this study, garden cress was more sensitive to the presence of BDII biodiesel.

The toxicity of biodiesel may be generated by introducing chemical substances into it, whose aim is to prevent its oxidation [Tamada et al. 2012]. Synthetic antioxidants that may adversely affect various levels of biological life [Wiley 1994, Tseng and Tseng 1995, Aluyor et al. 2009], include, among others: butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and esters of gallic acid [Kruszewski et al. 2013]. Even pure vegetable oils may cause adverse changes, which result from waste products forming as a result of their biodegradation [Tamada et al. 2012]. Soil contaminated with biodiesel may include toxic methanol, which is formed by inverting the transesterification re-

action and is present in its water-soluble fraction [Leite et al. 2011, da Cruz et al. 2012].

CONCLUSIONS

Fuel elements can be a valuable source of carbon and energy for microorganisms, necessary for their growth and development, but they can also be toxic to the same microorganisms. Antioxidants are added to vegetable oils, and in the production of biodiesel stabilizers, depressants, or antimicrobial agents, which may adversely affect some organisms, are also added. Biodiesel produced in laboratory conditions in most cases exerted more negative influence than commercial biofuel towards, for example, actinobacteria or fungi. These results may indicate that the response of test organisms used in the study may result directly from the different chemical composition of biofuels or result indirectly from physico-chemical changes caused by biodiesel in the soil. These factors may have a significant impact on phytotoxicity of biodiesel, as indicated by the diverse reaction of garden cress (*Lepidium sativum*) and spring barley (*Hordeum vulgare*). In the context of the presented results of the ecotoxicological effects of the biofuels, the related research should be continued.

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