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Short Communication

The Influence of Diesel Fuel and Biodiesel on Soil Microbial Biomass

Małgorzata Hawrot-Paw*, Małgorzata Martynus

Department of Microbiology and Environmental Biotechnology,
Słowackiego 17, 71-434 Szczecin, Poland

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Abstract

Our study aimed to determine the effects of conventional diesel fuel modification with biodiesel addition on the activity of soil microbiota. Diesel fuel, biodiesel and their mixture in a concentration of 5% (w/w) were introduced into soils – light loamy silty sand and light silty loam. Based on the obtained results, reduction in the content of live microbial biomass was found, irrespective of the soil type and contamination. Despite the introduction of biodiesel into soils, inhibition was observed in all examined treatments throughout the entire incubation.

Keywords: diesel fuel, biodiesel, microbial biomass, soil

Introduction

Petroleum hydrocarbons are one of the most frequently occurring environmental contaminants [1, 2]. Due to the depletion of fossil fuels in the world alternative fuels have attracted increasing attention [3]. Biofuels, which come from natural components, are recognised as renewable energy sources and environmentally friendly. Of particular interest is biodiesel obtained during the transesterification process from rapeseed oil [4]. Experiments evaluating its toxicity carried out by Peterson and Reece [5] showed that it was 15 times less harmful for *Daphnia magna* than diesel oil. In experiments prepared by von Wedel [6], LC_{50} for the early developmental stage of *Menidia beryllina* was 578 ppm for biodiesel when compared to 27 ppm for diesel oil. According to Lapinskiene et al. [7], biodiesel is non-toxic up to a concentration of 12%, whereas diesel oil exhibits toxic properties just above 3% of its soil content. Soil microorganisms are very sensitive to any ecosystem perturbation [8], and measurement of microbial parameters (soil microbial biomass or enzyme activities) may serve as indicators of soil pollution and soil health [9, 10]. In the case of using rapeseed oil methyl ester (RME), the emission of hydrocarbons, carbon monoxide, and particulate solids is respectively lower by 20%, 13%, and 16% in relation to diesel fuel [11].

The aim of this study was to determine the effects of diesel fuel modified with biodiesel on the content of live microbial biomass. Diesel fuel, biodiesel and their mixture were introduced into two soils with different granulometric composition and organic matter contents.

Experimental Procedures

In order to carry out our examination, sandy soil (light loamy silty sand) and loamy soil (light silty loam) were used. Material was sampled from a depth of 0-15 cm of humus horizon. The characteristic of soils is presented in Table 1.

Fuel diesel was sampled from a fuel station pump. The liquid was clear, of straw-yellow colour. Biodiesel was purchased from a petroleum station too. It is a mixture of various methyl esters of polyunsaturated fatty acids, e.g.

*e-mail: Malgorzata.Hawrot-Paw@zut.edu.pl

Table 1. Soil particle size grading, total carbon and nitrogen contents in studied soils.

Soil type	Fraction content [%]			$C_{tot.}$ [%]	N [%]
	1.0-0.1	0.1-0.02	<0.02		
A. Brown-rusty soil (light loamy silty sand)	61	26	13	1	0.09
B. Chernozem (light silty loam)	46	26	28	1.9	0.15

Table 2. Results of statistical analysis for content of viable microorganism biomass.

Factor No.	Number of independent variables	Mean square sum	Number of independent variables for error	Mean square sum for error	Value F	Value P≤0.05
SANDY SOIL						
1	5	76,078.8	96	4,497.274	16.916	0.00*
2	7	287,426.7	96	4,497.274	63.911	0.00*
1·2	35	11,397.8	96	4,497.274	2.534	0.00*
LOAMY SOIL						
1	5	121,608.3	96	2,130.431	57.081	0.00*
2	7	379,820.3	96	2,130.431	178.283	0.00*
1·2	35	20,988.0	96	2,130.431	9.851	0.00*

Factors: 1 – measurement period, 2 – treatments
* a significant effect of a factor

$C_{17}H_{33}COOCH_3$. This product does not contain components classified as dangerous and is not harmful for human health or the natural environment (PKN, Orlen).

Soils were adjusted to 50% water-holding capacity (WHC) and this moisture was preserved throughout the whole experiment; losses were replenished with distilled water. Each soil material was divided into 500 g samples. The soil contamination was carried out by adding fuel (5% w/w) with the following volume percent composition of the diesel/biodiesel blends: 100/0 (object D 100); 95/5 (D-BD 95:5), 80:20 (D-BD 80:20); 50/50 (D-BD 50:50); 20:80 (D-BD 20:80); 5/95 (D-BD 5:95); 0/100 (BD 100), leaving one uncontaminated sample as a control. Soil material was incubated at room temperature. Analyses were made on contamination day and successively after 7, 14, 28, 56, and 112 days. The content of live microbial biomass in soil was examined using the physiological method developed by Anderson and Domsch [12]. In brief, this method measures the initial change in the soil respiration rate after the addition of an easily available substrate (source of carbon). All measurements were made in three replications.

The findings were subjected to statistical evaluation using Statistica computer software package, applying multi-factor analysis of variance to test for significant differences in amounts of microbial biomass. To establish if differences between individual treatments were significant (P≤0.05), the Newman-Keuls test for least significance difference (LSD) was used.

Results

The tested fuels inhibited soil live microbial biomass (Figs. 1 and 2). This inhibitory effect was noticeable immediately after contamination and persisted 112 days after pollution (in both types of soil). Definitely most unfavourable changes were observed after the introduction of diesel fuel into soil (reduction of live microbial biomass content maximum to 92% in sandy soil and 90% in loamy). Based on the statistical analysis of findings, it was found that a dose

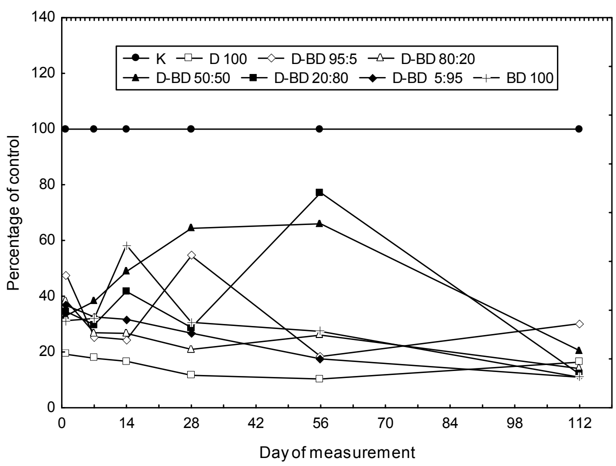

Fig. 1. The content of viable microorganism biomass in sandy soil as a percentage in relation to control (K – control, D – diesel, BD – biodiesel).

Table 3. The amount of microbial biomass of soil contaminated with biodiesel/diesel blends [mg C·100 g^{-1}].

Treatments	Incubation days						Mean
	0	7	14	28	56	112	
SANDY SOIL							
K	449	629	375	393	651	658	526
D 100	49	53	46	30	109	352	106
D-BD 95:5	72	58	46	57	359	371	160
D-BD 80:20	86	102	61	95	299	296	156
D-BD 50:50	94	151	76	68	285	174	141
D-BD 20:80	83	136	103	79	9	41	89
D-BD 5:95	56	135	97	68	306	53	119
BD 100	78	172	83	98	72	49	92
Mean	121	180	111	111	271	249	
$LSD_{0.05}$ Incubation days (ID) 39 Treatments (T) 48 Interaction (ID)·(T) 162							
LOAMY SOIL							
K	581	648	444	448	470	344	489
D 100	111	115	74	52	48	56	76
D-BD 95:5	277	165	109	245	93	104	165
D-BD 80:20	222	174	119	94	125	48	130
D-BD 50:50	193	247	217	288	310	71	221
D-BD 20:80	200	193	185	129	363	41	185
D-BD 5:95	213	211	141	120	83	38	134
BD 100	182	207	259	137	129	38	159
Mean	247	245	194	189	203	93	
$LSD_{0.05}$ Incubation days (ID) 57 Treatments (T) 70 Interaction (ID)·(T) 235							

of introduced substance, time, and interaction of examined factors significantly affected the content of live microbial biomass in both examined soils (Table 2).

Highest mean values were observed in the object with a fuel mixture at a ratio of 95:5 and 80:20 in sandy soil (160 and 156 mg C·100 g^{-1}), as well as in 50:50 and 20:80 objects in loamy soil (221 and 185 mg C·100 g^{-1}). In general, the content of live microbial biomass in all objects with biodiesel addition was higher in relation to the quantity observed in soil containing diesel fuel only (Table 3).

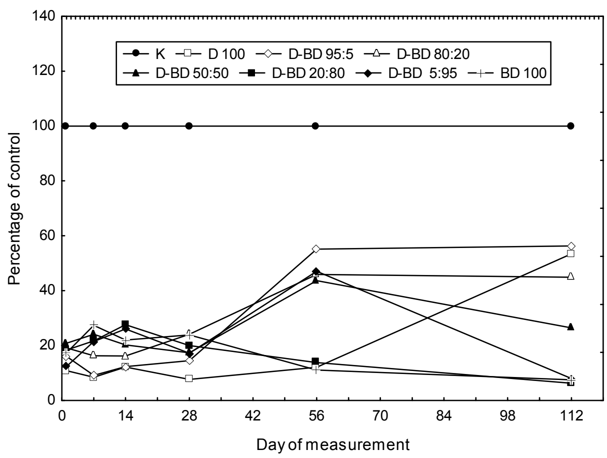

Fig. 2. The content of viable microorganism biomass in loamy soil as a percentage in relation to control (K – control, D – diesel, BD – biodiesel).

Discussion of Results

The effect of contamination with petroleum products on soil microbiota is not simple. A considerable quantity of hydrocarbons may favour development of microorganisms, for which they are a valuable source of carbon and energy [13-16], or induce inhibition of soil microbiota number and activity [17-19], depending on dose and contamination type, as well as soil properties.

In our experiment, the introduction of diesel fuel, biodiesel, and their mixture into soil induced a reduction in the content of live microbial biomass in relation to control values. The first weeks of incubation were characterized by some fluctuation, i.e. repeated increase and decrease, particularly in objects with the biodiesel. The fuels affect cell metabolism of various groups of microorganisms. Biodiesel, mostly composed of fatty acid esters that are synthesized in nature [20], could stimulate the activity of biofuel-degrading microorganisms. The presence of a readily assimilable carbon source could also increase the activity of hydrocarbon-degrading microorganisms capable of cometabolism. At the same time, strong development of one group of organisms could lead to a reduction of other, more sensitive groups. The observed changes were independent of soil type. A similar response to the presence of petroleum derivatives in soil was reported by Michalcewicz [17], and Hawrot and Nowak [19]. In the ecotoxicological study carried out by Lapinskiene et al. [7], referring to dehydrogenases and soil respiration, diesel fuel showed a negative effect in a concentration above 3% (w/w), whereas biodiesel was not toxic even in a concentration of 12% (w/w).

In the presented study, inhibition was observed to be persistent throughout the whole period of incubation in both sandy and loamy soils. In the study of Labud et al. [10], petroleum carbohydrates also reduced the content of live microbial biomass. These authors observed differences between the values determined in sandy (70% of reduction) and loamy (to 50% of reduction) soils. Organic matter has an ability to adsorb pollutants, decreasing their concentration in soil [9], which can protect microorganisms against the unfavourable effect of hydrocarbons.

Among biodiesel qualities, apart from technical values [4, 21, 22], emphasizes its biodegrability [5, 23-25]. Certain studies, however, point to the negative effect of this biofuel. Bünger et al. [22], after comparison of the toxicity of exhaust gases from an engine fueled with biodiesel and diesel fuel in mice, found four-times-higher toxicity of exhaust gases from biodiesel. As indicated by the findings presented in this paper, biofuel is a foreign substance for the natural environment, similar to conventional fuel, and its presence in soil may unfavourably affect the activity of soil microorganisms.

Conclusions

The contamination of soil with conventional and alternative fuels has a negative effect on soil biological activity. Microbial biomass is reduced in both test soils. The inhibitory effect persisted until the end of the incubation period, irrespective of the type of contamination. It can be concluded that, in general terms, diesel oil was more toxic than biodiesel. The measurements of live microbial biomass seems to be a useful indicator of diesel/biodiesel contamination.

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