Letter to Editor

Effects of Different Soil Treatments on Diesel Fuel Biodegradation

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Abstract

The effect of soil modification on diesel fuel removal from soil in field conditions was studied.

The experiment was established in the form of microplots. Diesel fuel was introduced into the soil at the concentration of 5% (ww. – in count on layer 15 cm depth of plot 1x1 m size). Particular experimental variants were modified by means of fertilization, stirring and inoculation with microorganisms active in the process of biodegradation of petroleum-derived pollutions. Diesel fuel content of soil was determined with the method of ether extraction, i.e. by assaying the concentration of total hydrocarbons. The results obtained were elaborated statistically using analysis of variance.

The research carried out demonstrated that the efficiency of diesel fuel biodegradation increased with respect to the modifications applied. The greatest changes of extractable hydrocarbons were observed after the introduction of select microorganisms into fertilized and stirred soil – to 89% biodegradation of diesel fuel.

Keywords: remediation, biodegradation, diesel fuel, microorganisms, soil, petroleum hydrocarbons.

Introduction

Following the rule of environmental realism [1], investigations into the pollution of ecosystems should be carried out directly in the environment. This suggestion supports the application of bioremediation *in situ*, i.e. at the site where pollution appears [2-4]. It should be taken into account, however, that under field conditions the efficiency of contamination removal is determined not only by hydrocarbon susceptibility to degradation but also by the conditions of the course of the biodegradation process, including the type of contaminated soil and climatic parameters [5, 6].

Biological degradation of hydrocarbons in the environment is linked to a number of physical and chemical factors, including the concentration and chemical structure of contaminant, physicochemical properties of soil, the content of biogenic salts, moisture content, oxygen and other terminal electron acceptor availability, organic compounds level, temperature and pH of soil [7-11]. The rate and efficiency of the purification process of soil depends on the occurrence of adequately numerous and active microflora in the contaminated soil [12], among other things. In the biodegradation of petroleum-derived contamination, use is made of either natural autochtonous microflora of the soil treated or specialized strains of microorganisms isolated from environments contaminated with petroleum hydrocarbons for a longer period of time.

The effect of soil treatment on the biodegradation process ranges from small changes to high stimulation [8, 13-15]. Therefore, the aim of our study was to evaluate the efficiency of selected treatments on changes in the concentration of ether-extractable diesel fuel hydrocarbons in soil under field conditions.

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Experimental Procedures

The experiment was carried out at the Experimental Station in Lipnik at the Agricultural University of Szczecin. It was established in the form of microplots (1x1 m) with method of completely randomized blocks in 4 replications. With respect to the mechanical composition, the soil to be examined demonstrated the composition of light loamy silty sand with pH_{KCl} 6.53.

Diesel fuel was introduced to the soil at a concentration of 5% (ww.) – when converted into the depth of 15 cm – and the following experimental plots were established: 0 – contaminated, non-modified soil (control); I – contaminated, fertilized, stirred soil; and II – contaminated, fertilized and stirred soil inoculated with microorganisms. Fertilization was carried out at the beginning of the research and after 3 months. The following treatments were applied: nitrogen fertilization – NH_4NO_3 (100 kg $N \cdot ha^{-1}$), phosphorus fertilization in the form of granulated superphosphate (80 kg $P \cdot ha^{-1}$), and potassium fertilization in the form of potassium salt (100 kg $K \cdot ha^{-1}$). Stiring (digging up) was carried out every 14 days. The usability of those strains, marked as BS 101 (*Pseudomonas sp.*), BS 126 and BS 135 (*Bacillus sp.*), to be applied for biodegradation of diesel fuel was evaluated and described in a paper by Hawrot and Nowak [16]. Bioaugmentation was carried out at the beginning of the experiment by introducing 2 L of liquid culture medium with bacteria number suitable for biodegradation (10^7 cells$\cdot mL^{-1}$) to the soil. Cultures were prepared on mineral medium with the following composition [$g \cdot l^{-1}$]: K_2HPO_4 – 4.35; KH_2PO_4 – 1.7; $MgSO_4$ – 0.2; NH_4Cl – 2.1; $MnSO_4$ – 0.05; $FeSO_4$ x H_2O – 0.01; $CaCl_2$ x $2H_2O$ – 0.03, and distilled water – 1000 mL.

Soil moisture (23%) was determined by Kopecky's method [17]. The soil was kept in fallow in order to eliminate the effect of plants on the efficiency of biodegradation. A collective soil sample (ca. 500 g) was collected at a depth of 0-15 cm from each experimental plot every 30 days. In order to evaluate the potential migration of contamination inside the soil, soil samples were additionally collected at a depth of 15-30 cm. Climatic conditions (average monthly air temperature and average monthly precipitation) prevailing over the plot experiment period were presented in Fig. 1.

Quantitative determinations of diesel fuel in the soil were carried out following the Polish Norm PN-75/C-04573/10 for assays of the contents of substances extractable with organic solvents. The etheric extraction method using Soxhlet's apparatus was applied for determination of total petroleum hydrocarbon (TPH).

The results obtained were subjected to a statistical analysis of variance. A combination of effects and errors was applied, which enabled us to consider block variability.

Results

At the beginning of the research, in soil samples contaminated with 5% diesel fuel and collected from a depth of 0-15 cm, ether extraction revealed 3.75% of petroleum products extractable with petroleum ether. Thus, the efficiency of extraction accounted for 75%. In soil samples collected at a depth of 15-30 cm, 0.02-0.06% of ether extracts were determined in all of measurement date, even at increased precipitation in July and September (Fig. 1). During the study, soil moisture and pH in KCl ranged between 21.6-42.8% (mean 29.4%) and 6.50-6.75 (in plot 0, I) and 6.25-6.35 (in fertilized, stirred, inoculated soil), respectively.

The content of diesel fuel in the soil was expressed in percentages as compared to values reported for plot 0 (control – contaminated, untreated soil).

In plot 0, the concentration of diesel fuel was observed to decrease gradually with time. The relatively greatest changes were reported between day 90 and day 150 of measurements when diesel fuel concentration ranged from 3.50% to 2.60% (31% decrease in the petroleum hydrocarbon concentration). Fertilization and stirring increased the efficiency of biodegradation, thereby decreasing the amount of petroleum hydrocarbons extracted with petroleum ether. This amount gradually decreased from the first measurement up to 1.68% of diesel fuel on the last date, which was 45% of initial content (Fig. 2). Treat-

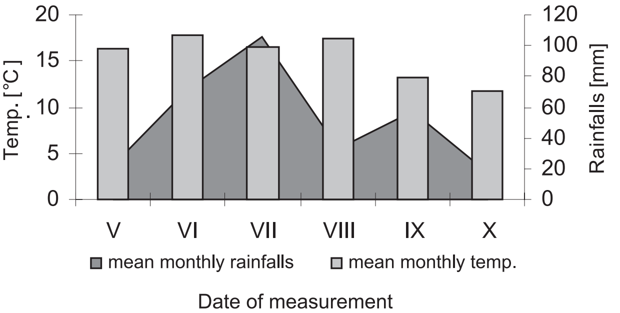

Fig. 1. Characteristics of climatic conditions during the experimental period.

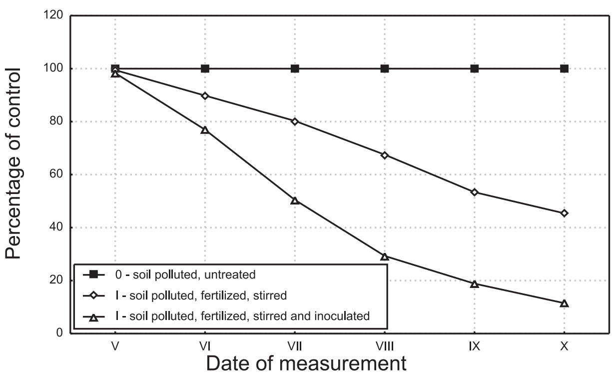

Fig. 2. Effect of various treatments on changes in the concentration of ether-extractable diesel fuel hydrocarbons (percentages in relation to the untreated soil).

Table 1. Results of a statistical analysis of the level of substances extractable with petroleum ether in soil, in a plot experiment.

Variation source	Sum of squares	Degrees of freedom	Mean of squares	F Value	P Value
Blocks	0.013884	3	0.004628		
1	62.99966	3	20.99989	5239.957	0.00**
2	8.387779	5	1.677556	418.589	0.00**
1*2	6.702780	15	0.446852	111.500	0.00**
Error	0.276528	69	0.004008		

Factors: 1 – bioremediation treatment, 2 – measurement period, ** – the highly significant effect of a factor (at $P<0.05$)

ment of contaminated soil with fertilization, stirring and selected microorganisms produced the highest reduction in extractable hydrocarbons. The level of diesel fuel significantly decreased starting from the first measurement up to 0.4% on the last date. It was only 11% of the values reported in the first measurement and indicated an 89% decline in petroleum hydrocarbons in the soil examined, compared to the initial level, within only 150 days of the experiment.

The bioaugmentation increased biodegradation of the diesel fuel by 34% in comparison to fertilization and stirring alone.

An analysis of variance confirmed a highly significant effect of the treatments applied in contaminated soil bioremediation on the concentration of ether extracts (Table 1).

Discussion of Results

The presence of bacteria enables the biotransformation and elimination of contamination from our environment. Compared to a number of other remediation treatments, the biological methods are relatively inexpensive, uncomplicated and enable effective degradation of pollution without great interference in the environment.

A crucial element to increase the efficiency of biodegradation is biostimulation, including fertilization, aeration or bioaugmentation – introduction of microorganisms active in the process of petroleum-derived hydrocarbons to the environment [18, 19]. In fertilization, it is vital to provide an appropriate ratio of carbon introduced with the contamination to the amount of nitrogen. As postulated by Xu et al. [20], in soils contaminated with petroleum-derived products the losses of nitrogen, mainly as a result of denitrification, are substantially higher than those reported in the non-contaminated soils. The efficiency of biodegradation may be limited by another factor as well, namely phosphorus content [21]. During biodegradation of petroleum hydrocarbons under field conditions, the level of degradation is limited, to a great extent, not only by a suitable content of nutrients but also by oxygen availability, which may pose multiple problems with respect to strictly controlled laboratory conditions [18].

Fertilization and stirring increased the diesel fuel loss in the soil 55% compared to the initial level and 24% compared to the unfertilized, unstirred control. The observation that the efficiency of hydrocarbon biodegradation increased with increasing treatment ranging from simple fertilization and mixing to bioaugmentation confirms the observations of others [8, 14, 15]. Carmichael and Pfaender [13] and Palmroth et al. [22] did not observe any significant effect of hydrocarbon biodegradation following nutrient addition alone. Maybe the nutrients stimulated the growth of heterotrophic microorganisms and they did not influence microorganisms active in the biodegradation process.

The availability of suitable terminal electron acceptor (for example oxygen, nitrate, iron, manganese, sulfate, etc.) rather than nutrients alone, will also be a factor that determines the extent of biodegradation. Stirring provided oxygen and the nitrogen fertilizer provided nitrate.

Fertilization, stirring and bioaugmentation increase the percentage of biodegradation up to 89% and accounted for a 34% increase in bioremediation compared to just fertilizing and stirring alone and 58% increase compared to untreated control. The effectiveness of the applied mixed bacterial inoculum under laboratory conditions and during soil bioremediation with the *ex situ* method has also been confirmed in previous experiments of Nowak and Hawrot [23] as well as Hawrot and Nowak [24].

Similar results have been obtained by many authors [6, 8, 25-28]; however, another [29] did not observe differences between fertilized objects and those additionally inoculated. In both cases they got about 60-65% of the decrease of diesel fuel (in relation of contaminated soil, without modification).

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