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ECOLOGICAL EVALUATION OF THE EFFECTS OF DIFFERENT SOIL MOISTURE CONDITIONS ON THE EFFICACY OF *PSUEDOMIN* IN THE BIOREMEDIATION OF DIESEL FUEL CONTAMINATED DERNO-PODZOLUIVISOLIC SOIL

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ABSTRACT
This article examines the critical effect of soil moisture content on the rate of petroleum hydrocarbon degradation, and on the performance of the biopreparation Pseudomin in the clean-up of diesel fuel contaminated derno-podzoluvisolic soils. Results of the research showed that the optimal condition in enhancing the efficacy of Pseudomin was noted under conditions of around (60%) of maximum soil moisture capacity. The rate of petroleum hydrocarbon degradation in the soil samples under this soil moisture condition was accelerated up to 5 times, in comparison with soil moisture conditions of around (30%) and conditions of complete saturation, which significantly slowed down the process of bioremediation in the soil samples.

KEYWORDS: Diesel fuel, derno-podzoluivisolic soil, bioremediation, biological preparation, Pseudomin, *Pseudomonas putida*, phytotoxicity.

INTRODUCTION

The importance of bioremediation for the clean-up of oil and oil product contaminated soils is becoming more prominent with each year, as a result of the ever increasing volume of crude oil production, and consequently incidence of oil spillage into the environment [1, 2, 9, 10, 11]. To successfully implement the bioremediation of oil contaminated soils and achieve desired results, it is important to comply with all the measures and consider all the factors that influence this process. One of the determining factors in the successful implementation of bioremediation is the soil moisture content of the soil [4, 7, 10, 11]. Soil moisture plays a very important role in the biodegradation process of crude oil and crude oil products contaminated soils. The content of moisture directly affects the physical and chemical properties of the soil, as well as the metabolic activity of the hydrocarbon oxidizing microorganisms involved in the process of bioremediation. Therefore, the amount and availability of water determines the rate of occurrence of the bioremediation process of crude oil contaminated soils [1, 7, 10]. Consequently it is necessary to study the effects of soil moisture on the efficacy of the biopreparation Pseudomin, and determine the best optimal moisture condition for the biological product.

A considerable amount of research has been done on the efficacy of Pseudomin in the effective clean-up of crude oil spilled soils, studies ranging from the perspectives of using different strains of bacteria of the genera *Pseudomonas putida* as an hydrocarbon-oxidizing microorganisms in the bioremediation of crude oil contaminated soils; to the evaluation of the performance of pseudomin (and other similar biopreparations based on *Pseudomonas putida*) in comparison to other biological products (of different microbial components) in the biodegradation of soil and water contaminated by petroleum hydrocarbon[8,10]. However, there are no specific recommendations on the use of Pseudomin for bioremediation of contaminated soils in certain soil and climatic areas where the availability (in excess, or in short supply), or lack of soil moisture plays a very important role in the process of bioremediation. Hence the aim of this research, which is to study the effects of three different levels of soil moisture conditions (maximum water holding capacity (MWHC), 60% and 30% of MWHC) on the biodegradation rate, and performance of Pseudomin in the bioremediation of diesel fuel contaminated derno-podzoluivisolic soils of the central region of Russia.

MATERIALS & METHODS

Soil: Mixed samples of the top humic-accumulative horizon (A_1), and it's transit horizon (A_1A_2) of a loamy, completely podzolized derno-podzoluivisolic soil, collected from Area 4 of the ecological monitoring site, located in the experimental forest reserve of the Russian State Agrarian University – MTAA named after K.A. Timiryazev (Table- 1).

TABLE 1. Summary of the properties of the understudy derno-podzoluivisolic soil[3]

Topography and location (coordinates) of the area of sample collection	Horizon	Depth	Corg,%	pH_{H2O}	pH_{KCL}	H, mg-eqv/100g soil

The sample was collected at the middle of a slightly steep slope (N55° 48' 59.5" E037° 32' 59.4")	$A_1 + A_1A_2$	0-28	1,26	4,09	3,61	11,90

Pollutant

Diesel fuel as the pollutant (75% of which consists of light hydrocarbons, primarily alkanes which are easily assimilated by many microorganisms), density: 840 kg/m^3, boiling point: 40 °C, freezing point: 35 °C.

Microorganism

Pseudomonas putida (strain 91-96) as the petroleum hydrocarbon degradation microorganism (the strain was first isolated from oil contaminated derno-podzoluivisolic soil, by the department of Microbiology and Immunology of the Russian State Agrarian University – MTAA named after K.A. Timiryazev).

Methods of research

The analysis of the Total Petroleum Hydrocarbons (TPH) content using IR-spectrometry method on «*Fluorat-02*» equipment was carried out at regular intervals [12]. The research was carried out in the laboratory of the agro-ecological monitoring, modeling and ecosystems forecasting; and in the microbiology laboratory of the Russian State Agrarian University-MTAA named after K.A. Timiryazev. Statistical analysis of the research results were performed by «Statistic 8.0».

TABLE 2. Experimental Scheme

Soil Moisture Content	No. of Variant	Soil Sample
100%	1	Uncontaminated soil
	2	Contaminated soil
	3	Uncontaminated soil + *Pseudomonas*
	4	Contaminated soil + *Pseudomonas*
60%	5	Uncontaminated soil
	6	Contaminated soil
	7	Uncontaminated soil + *Pseudomonas*
	8	Contaminated soil + *Pseudomonas*
30%	9	Uncontaminated soil
	10	Contaminated soil
	11	Uncontaminated soil + *Pseudomonas*
	12	Contaminated soil + *Pseudomonas*

TABLE 3. The rate of diesel fuel degradation in the mixed samples of the top humic-accumulative horizon (A_1), and transit (A_1A_2) horizon of the understudy derno-podzoluivisolic soil

Soil moisture content %		Experimental Variants	Initial Petroleum Hydrocarbon Content, mg/g	Amount of Petroleum Hydrocarbon Added, mg/g	Total Petroleum Hydrocarbon, mg/g (Weeks)					
					1	2	3	4	8	12
100	1	Contaminated Soil	0.01	15.0	1.86	1.59	1.23	0.85	0.32	0.13
	2	Contaminated Soil + *Pseudomonas*			1.38	0.91	0.77	0.42	0.13	0.11
60	3	Contaminated Soil	0.01	15.0	9.08	7.44	6.09	5.10	0.68	0.15
	4	Contaminated Soil + *Pseudomonas*			8.68	7.08	5.49	4.80	0.59	0.12
30	5	Contaminated Soil	0.01	15.0	9.37	8.27	6.40	5.35	1.36	0.70
	6	Contaminated Soil + *Pseudomonas*			9.13	7.87	6.21	5.07	1.13	0.64
Approximate Safe Exposure Level			4mg/g							

P_{05}- 0.03

Experimental Design

The derno-podzoluivisolic soil sample having been dried and sieved (through hand held sieves with diameter of 1 mm) were placed into 3 different containers for mixing, one for each level of soil moisture according to the experimental plan (maximum water holding capacity (MWHC), 60% and 30% of MWHC) 3.6 kg of soil was placed in each bucket.

According to the experimental scheme, the variants to be contaminated were polluted by the addition of diesel at a concentration of 15 mg/g of soil, which corresponds to 5% by weight of air-dry soil. In the variants to be inoculated with hydrocarbon oxidizing microorganism, a 10 ml suspension of *Pseudomonas putida* was added, with titer value of 1.35×10^7 CFU/ml. Finally the soil was carefully mixed and placed in plastic containers in an amount of 300

g of soil per container. The soil moisture (according to the scheme) was set to MWHC, 26% and 13% respectively of MWHC, which had a value of 43.5%, and maintained daily by gravimetrical methods. The total number of experimental variants – 12, and replications – (Table 2).

RESULTS

Analysis of the total petroleum hydrocarbon content in the understudy derno-podzoluivisolic soil is displayed in the table below.

DISCUSSION

Results of the research on the effect of three different levels of soil moisture content on the rate of diesel fuel degradation by Pseudomin in the contaminated soil sample showed a remarkable reduction in the content of diesel fuel in all the variants of the soil sample after only 12 weeks of the experiment. By comparing the Total petroleum Hydrocarbon content at the start and end of the experiment, the variants with maximum soil moisture saturation (100%) showed an accelerated rate of diesel fuel degradation of up to 12-14 times; in the variants with optimal soil moisture conditions (60%) the rate of diesel fuel degradation showed the most significant values of accelerated degradation of up to 60-70 times. While in the variants with low moisture content (30%) an accelerated rate of diesel fuel degradation of up to 13-14 times was observed. This proves the impact of different levels of soil moisture content on the degradation rate of diesel fuel in the test sample derno-podzoluvisol. The best results of accelerated degradation were observed in the variants with soil moisture content of 60% (variants 3 & 4). This can be attributed to the optimal ratio of the liquid and gaseous phases of the soil, which is ideal for microbial activity, as well as the mineralization of organic matter in the soil. By analyzing the results of the variants with maximum soil moisture content a noticeable trend of significantly lower values of the Total Petroleum Hydrocarbon content from the 1st to the 12th week of the experiment is observed. This was as a result of the constant floating of the diesel fuel on the surface of the water saturated soil sample, and its subsequent evaporation, which resulted in the reduced presence / diluted effects of the diesel fuel in the soil samples taken for analysis. As noted by many researchers in their work [5,6,10], the initial stage of the bioremediation process of diesel fuel contaminated soils, is characterized by the evaporation of the volatile fractions (light fraction of petroleum hydrocarbons) as a result of physical and chemical weathering, *i.e.*, about 50% of diesel fuel decomposes without the action of hydrocarbon oxidizing microorganisms. This trend was also observed especially in variants 3-6 of the experiment, i.e., by comparing the total petroleum hydrocarbon content at the 1st week of the experiment to the total amount of diesel fuel added. Results of the research also showed a marked difference in the rate of diesel fuel degradation between the soil samples inoculated with *Pseudomonas* and the samples without inoculation. The rate of diesel fuel degradation was 1.25 times faster in the inoculated variants compared to the non-inoculated variants (Fig. 1).

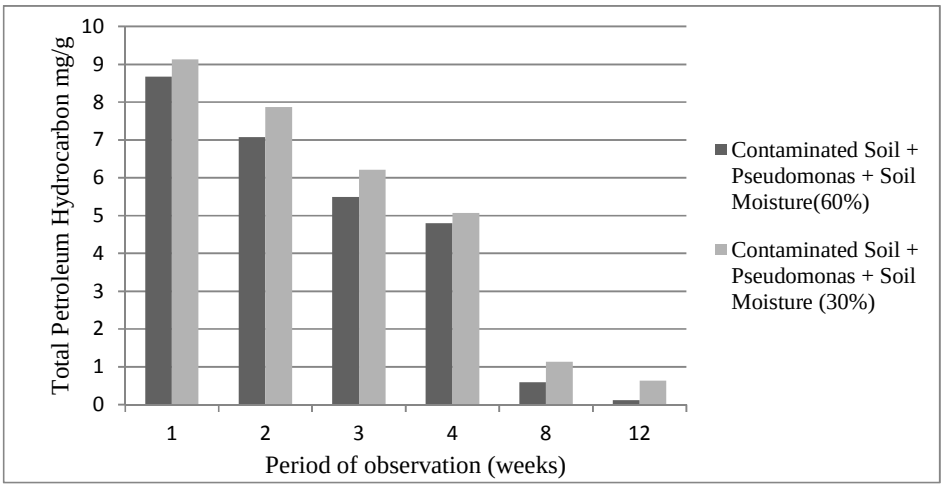

FIGURE 1. Graphical representation of the efficacy of Pseudomin in the bioremediation of the diesel contaminated derno-podzoluivisols under two different conditions of soil moisture

The results of the research have shown the significant impact of soil moisture content on the process of petroleum hydrocarbon degradation and the efficacy of Pseudomin. Under optimal conditions of soil moisture (60%) the degradation of the petroleum product was 5 times faster and more intense, when compared to conditions of total soil moisture saturation and insufficient soil moisture (30%), which were considerably slower. In general the application of the biopreparation pseudomin for the decomposition of petroleum hydrocarbons, showed a very high efficiency rate, especially under soil moisture conditions of (60%). This is because in only 12 weeks of the experiment, the biopreparation was able to reduce the total petroleum hydrocarbon content up to 99.2%. The residual amounts of petroleum hydrocarbon in the soil after the experiment were significantly lower than the minimum acceptable concentration levels for soils of the mid and southern taiga.

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