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ANALYSIS OF THE PHYTOTOXIC EFFECTS OF DIESEL FUEL CONTAMINATED DERNO-PODZOLUIVISOLIC SOILS ON PLANTS AND THE IMPACT OF BIOPREPARATION *PSEUDOMIN* IN DETOXIFICATION UNDER DIFFERENT SOIL MOISTURE CONDITIONS

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ABSTRACT
This article examines the phytotoxic effect of diesel fuel contamination on plants growing on contaminated soils under different conditions of soil moisture, and the impact of petroleum hydrocarbon oxidizing microorganisms in mitigating the toxic effects of diesel fuel contamination. After 12 weeks of experiment, results of the research showed a significant increase in the percentage of germinated oat seedlings of up to 100%; and the length of oat seedlings to 11 cm under favorable conditions of soil moisture content of 60%, which is optimal for soil microbial activities. Under deficient moisture conditions (30%) the percentage of germinated seedlings increased only up to 60%, and the length of oat seedlings to 7.7 cm.

KEYWORDS: Diesel fuel, derno-podzoluivisolic soil, bioremediation, biological preparation «Pseudomin», Pseudomonas putida, phytotoxicity, phytoremediation, oat seeds.

INTRODUCTION

The development and expansion of the petroleum industry has brought invaluable benefits to mankind and civilization. However, the inevitable accidental spillages that occur during the storage and transportation of crude oil and its derivatives have led to varying degrees of environmental devastation and contamination, as well as the reduction of the area land meant for agricultural purposes [1,4,5,12]. Plant growth in crude oil contaminated soil is adversely affected due to changes in the nutrient status of the soil and disruption of microbial activities. Crude oil spillage on land results in oil entering into the leaves of plants through their pores and hampering the processes of photosynthesis and evapo-transpiration [6, 10]. The interference of oil to soil air and water is another means of inhibiting seed germination. It has been noted that the toxic effect of crude oil coupled with poor aeration and the altered wet ability of the soil due to oil spillage results in poor seed germination[7]. Consequently, it is necessary to study the toxic effects of petroleum hydrocarbon contamination on plants, in order to optimize the best possible technique of bioremediation. Several studies on analyzing the effects of crude oil contamination on plants have been carried out, using various bio-test plants as specimens; analyzing the effects of various types of petroleum products on seed germination and plant Growth, as well as extensive research on phytoremediation [6,11]. However, there are no available data on the phytotoxic effects of diesel contaminated derno-podzoluivisolic soil on seed germination and plant growth under different soil moisture conditions, and no specific recommendations as well, on the use of biopreparation *Pseudomin* for bioremediation and detoxification under conditions of different soil moisture content.

Hence the aim of the research is to study the phytotoxic effects of diesel fuel contamination on plants under three different levels of soil moisture conditions (maximum water holding capacity (MWHC), 60% and 30% of MWHC) and the impact of *Pseudomin* in the bioremediation and detoxification of the understudy soil from the central region of Russia.

MATERIALS & METHOD

Materials for this research included:

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 (Tab. 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) was used as the petroleum hydrocarbon degradation agent, which is contained in the bio-preparation *Pseudomin*. The strain was first isolated from oil contaminated derno-podzoluivisolic soils, by the department of Microbiology and Immunology of the Russian State Agrarian University – MTAA named after K.A. Timiryazev.

Bio-test Specimen: Oat seeds (*Avena sativa*).

METHODOLOGY

To conduct this study, soil phytotoxicity test was carried out at regular intervals by method of bio-testing on seedlings of oats (*Avena sativa*)[2, 8]. The research was carried out 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»

Experimental Design: The soil phytotoxicity test was performed by directly sowing the seeds of oats in to the soil samples [2, 8]. Five pieces of oat seeds each were sown into Petri dishes containing 20 g of the understudy derno-podzoluivisolic soil samples according to the experimental scheme (tab. 2). The plates were incubated for 7 days at room temperature, after which seedling germination was analyzed. Sample replication of the experiment was done 3 times.

The analysis was performed three times - in the first, seventh and twelfth week of the experiment. The impact of the understudy factors was evaluated based on the degree of suppression / inhibition of seedling germination (in %); and by measuring the length of seedling growth of the test plant (in cm) growing in the understudy soil.

TABLE 2. Experimental Scheme

Soil moisture content, %		Experimental Variants	Initial Petroleum Hydrocarbon Content, mg/g	Amount of Petroleum Hydrocarbon Added, mg/g
100	1	Uncontaminated soil	0.01	15.0
	2	Contaminated soil		
	3	Uncontaminated soil + *Pseudomonas*		
	4	Contaminated soil + *Pseudomonas*		
60	5	Uncontaminated soil	0.01	15.0
	6	Contaminated soil		
	7	Uncontaminated soil + *Pseudomonas*		
	8	Contaminated soil + *Pseudomonas*		
30	9	Uncontaminated soil	0.01	15.0
	10	Contaminated soil		
	11	Uncontaminated soil + *Pseudomonas*		
	12	Contaminated soil + *Pseudomonas*		

TABLE 3. The phytotoxic effects of diesel fuel contaminated under study derno-podzoluivisolic soil on the growth of oat seedlings under different soil moisture conditions

Soil moisture content, %		Experimental Variants	Week 1		Week 7		Week 12	
			Number of seedlings, %	Maximum length of seedling, cm	Number of seedlings, %	Maximum length of seedling, cm	Number of seedling, %	Maximum length of seedling, cm
100	1	Uncontaminated soil	100	13,0	100	10,0	100	10,2
	2	Contaminated soil	0,0	-	20	2,5	80	5,3
	3	Uncontaminated soil + *Pseudomonas*	100	13,5	100	11,0	100	10,5
	4	Contaminated soil + *Pseudomonas*	20	1,2	60	6,0	100	7,3
60	5	Uncontaminated soil	100	12,0	100	9,1	100	11,0
	6	Contaminated soil	0,0	-	80	7,2	100	6,2
	7	Uncontaminated soil + *Pseudomonas*	100	6,0	100	11,0	100	10,6
	8	Contaminated soil + *Pseudomonas*	20	1,4	100	8,5	100	7,8
30	9	Uncontaminated soil	60	1,7	60	6,0	60	6,4
	10	Contaminated soil	0,0	-	40	4,8	60	4,0
	11	Uncontaminated soil + *Pseudomonas*	20	0,1	60	5,1	60	7,7
	12	Contaminated soil + *Pseudomonas*	0,0	-	60	7,0	60	7,3

P_{05}- 0.27

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

DISCUSSIONS

After week 1 of the experiment, results of the analysis on the phytotoxicity of the soil samples on the growth of oat seedlings showed a low percentage of germinated seedlings and a reduction in length of oat seedlings in the experimental variants contaminated by diesel fuel (variants 2, 6, 10, 12) (Tab. 3).

A complete inhibition of seedling germination was observed, but over time, the toxic effect was weakened due to the decomposition of diesel fuel by the actions of the *Pseudomonas putida* contained in the bio-preparation, and the normal soil micro-biota. Subsequently, towards the end of the experiment (week 12), a gradual increase in the percentage of germinated oat seedlings of up to 60-100%; and in the length of oat seedlings of up to 11 cm, was observed. It should be noted that, the best results were recorded under soil moisture conditions of 60%, were the percentage of seedling germination was 100%, and the length of seedlings of 11 cm was the highest recorded.

In the variants with a deficiency of soil moisture (30%), the percentage of germinated oat seedlings increased only up to 60%, and the length of oat seedlings up to 7.7 cm. Under conditions of total moisture saturation, the excess availability of water obviously played a vital role in the outcome of the seedling germination and length, but nevertheless results were not as impressive as the optimal conditions.

Thus, analysis of the phytotoxicic effect of diesel fuel contaminated derno-podzoluivisolic soil on the germination and growth of oat seedlins, have shown a positive impact of the hydrocarbon oxidizing microorganism (*Pseudomonas putida*) contained in the biopreparation Pseudomin, in the reduction of the toxic effect of diesel fuel contaminated understudy derno-podzoluivisolic soil on the growth of the test plant, especially at the level of soil moisture in the range of 60%, which is optimal for soil microbial activities.

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