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Selection of Plants as Possible Rhizoremediators for Restoration of Oil Contaminated Soil

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Abstract**—**In studying the possibility of using plants as rhizoremediators, barley and grass mixture which showed resistance to various concentrations of oil were selected. The minimum inhibitory effect of oil on these plants by morphological parameters such as survival of plants, length and biomass of shoot and root compared with the control was showed. In determining physiological parameters, a slight decrease in the number of chlorophyll a and b in the leaves of plants was noted. The differences in the ratio of the total surface of the roots to the work surface with the growth of plants in soil with oil in the study of adsorption of the root surface were showed.

Keywords**—**Length of shoot and root, biomass, chlorophyll a and b, adsorption surface, barley, grass mixture.

I. Introduction

IN the last 30 years of oil-oxidizing microorganism active research s, this coincided with a change in the fuel balance, when oil began to take a leading role as a source of energy. This has led to pollution of soil and water with oil and oil products. Sources of oil pollution are accidents at wells, pipelines and other industrial facilities, such as oil extraction, transportation, processing and storage of oil. Studies show that the loss of oil in producing countries accounts for about 30% of annual production. World oil losses are estimated at 25 million tons a year.

Despite the fact that the processes for oil production are constantly being improved and the level of service is on a higher level, the risk of accidental spills and the resulting consequences very relevant, especially in Kazakhstan, one of the largest suppliers of oil to the world market.

Currently, ways to intensify biodegradation of petroleum hydrocarbons in soil and water is being actively seeking. The most promising biotechnological methods based on the use of oil-oxidizing microorganisms, organic waste and plants. Plants used in the last stages of purification to return soil for agriculture areas.

The main key to determining the effectiveness of bioremediation using plants as rhizoremediators is the correct selection of species. Microbe-plant relationships are the main mechanism of bioremediation, contribute to the degradation of petroleum hydrocarbons [1]. Main processes take place in the root system of plants. In this case, root exudates stimulate survival and action of microorganisms, which leads to a more effective cleaning of oil-contaminated soils.

Compared with trees and shrubs, grass plants are often offered as an effective plant for cleaning contaminated soils, due to their extensive root system, which has a large surface area [2]. They are characterized by rapid growth, large biomass, and high resistance [3].

In Kazakhstan, for the solution of problems related to soil contamination by hydrocarbons of oil, bioremediation processes using active destructor microorganisms' oil are widely used. At the same time, rhizoremediation for advanced treatment and rehabilitation of contaminated soil were not carried out. In this regard, the purpose of work was the selection of plants with the possibility of their use as rhizoremediators for soil restoration.

II. Materials and Methods

For the experiment soil from Aktau region, Zhetybai deposit was used. Oil content of the contaminated soil samples was determined by liquid analyzer Fluorat - 02-3M, and ranged between 22.8-31.4g of oil / kg soil. Plants were grown in a 5L containers filled with 5 oil-contaminated and clean (control) soil. Each container was placed with 10 seeds per plant and grown in a growth room under the conditions - 14 of the light period and 10 hours of the dark period. Duration of cultivation was 20-60 days.

The survival rate of plants was calculated for each plant by counting viable plants after 20 days of cultivation. The survival rate was calculated by the formula:

$$\text{Survival Rate (\%)} = \frac{\text{Quantity of viable plants}}{\text{Number of seeds}} x100$$

For the measurement of shoot and root of plant, plant was extracted from soils, shoots and roots were separated from each other and their lengths were measured. To determine the biomass of plant, separated shoots and roots were dried at 70°C for 7 - 9 hours until dry and their weight were measured.

Chlorophyll a and b was determined by the conventional method, quantification was performed on a spectrophotometer at 647 and 665nm (Spectrophotometer PD-303, APEL).

General and the working surface of the absorbent roots were determined by Kolosov [4]. As the adsorbent, methylene blue was used, the absorption of which can be precisely

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defined by a colorimetric change in the concentration of the test solution. Roots extracted from the vessel with water, were dried carefully with filter paper and immersed sequentially in three glasses with methylene blue for 1.5min in each glass.

III. Results

A. Effects of Oil on the Morphological Characteristics of the Plant

Study of the effect of oil on the change in the length of roots and shoots, as well as the determination of their dry biomass is an important criterion for the use of plants in rhizoremediation [5], [6]. Therefore, an assessment on the impact of oil plants and analysis of morphological characteristics of the plants was done.

According to the study, the oil in different concentrations has different effects on the growth and development of selected plants. Oil at 2% (22.8g of oil / kg soil) caused a slight decrease in the concentration of plant survival compared with control.

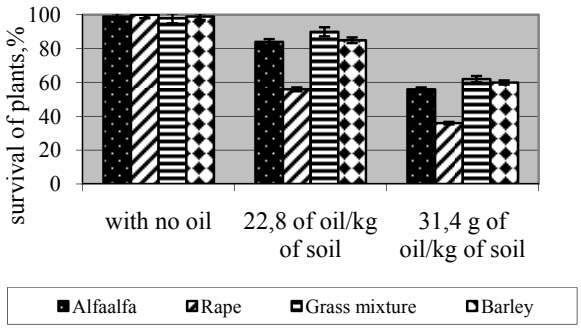

Fig. 1 Survival rate of plants during the growth on oil-contaminated soil

The highest survival rate (Fig. 1) was observed in barley and mixtures, the survival rate was up to 85.1 ± 1.3% and 90.2 ± 2.3%, respectively, rape and alfalfa this figure was in the range 56.2 ± 2.1% and 84.2 ± 1.5%. In studying the effects of oil at a concentration of 22.8g of oil / kg of soil on long shoots minimum inhibitory effect was observed for the mixtures, the high - the rape, the length of the aerial part decreased by 22.9% compared with the control (Fig. 2). Tables I and II show the effect of oil on the length of the above-ground and below-ground plant parts. The results obtained show that the length of the plant is reduced depending on the concentration of oil in the soil, however, the characteristic more to escape than the root.

At a concentration of 31.4g of oil / kg soil, negative impact of hydrocarbons on plant growth was showed. Compared with controls, the length of the underground part of rape declined by 51.7 ± 1.3%. Barley and grass mixture were more resistant to oil, the length of the aerial part was 16.85 ± 0.98cm and 51.82 ± 0.05cm, respectively, compared to the control decreased by more than 20%.

TABLE I
Length of Shoots and Roots of Plants during the Growth on Oil-Contaminated Soil

Plant	Non-contaminated Soil	Oil, g/ kg soil	
		22.8	31.4
Length of Shoot, (sm)			
Alfaalfa	56.1±2.65	51.3±1.17	37.4±1.65
Rape	95.4±2.08	72.5±0.56	43.7±0.22
Grass mixture	21.54±1.12	18.59±1.04	16.85±0.98
Barley	72.65±1.65	68.47±0.21	51.82±0.05
Length of Root , (sm)			
AlfaAlfa	4.82±0.15	4.25±0.96	2.39±0.09
Rape	12.35±0.05	9.68±0.58	7.84±0.68
Grass mixture	2.85±0.08	2.37±0.06	2.02±0.05
Barley	25.78±0.42	16.35±0.23	13.42±0.54

During measuring the root, the difference between the length of the root in unpolluted soil and root length with oil concentration of 20g / kg soil was minimal in alfalfa (Fig. 2); it shows a weak inhibitory effect of oil on the plant. However, at 30g / kg of soil, oil, the decline in 2 times to 2.39 ± 0.096 versus control 4.25 ± 0.96.

Control Soil+Oil (22.8g of oil/kg of soil)

(a) Alfaalfa

Control Soil+Oil(22.8g of oil/kg of soil)

(b) Barley

Fig. 2 Length of shoot and roots of (a) alfalfa (b) barley

With the oil concentration increasing the growth of plant roots inhibited. Root length of grass mixture and barley decreased by only 2.02 ± 0.05cm and 13.42 ± 0.54cm at the

oil content at 30g / kg of soil, so that an inhibitory effect of oil was less than in alfalfa.

Shoot and root biomass also decreased with increasing oil content in the soil (Table II). Depending on the amount of oil in the soil shoot biomass of all plants decreased. Thus, the observed reduction in biomass of rape decreased twice, alfalfa, barley and grass mixture showed resistance to oil, their biomass decrease at a concentration of 20g of oil / kg soil decreased slightly. However, the oil concentration of 30g of oil / kg soil caused a significant reduction in biomass of shoots in all plants.

In assessing the impact of oil on the biomass of roots, the following was observed. Under the action of oil, at a concentration of 20g of oil / kg of soil, there was a reduction of the root biomass of plants in the range 10-30%. Weight control for alfalfa equaled 0.156 ± 0.03g and 0.134 ± 0.026 for oil content 22.8g oil / kg soil. That is, 10% of the root biomass was lower. When oil concentration of 30g / kg of soil that parameter had fallen to 0.072 ± 0.058g.

TABLE II
INFLUENCE OF OIL ON BIOMASS OF SHOOT AND ROOT DURING THE GROWTH ON OIL-CONTAMINATED SOIL

Plants	Non-contaminated Soil	Oil, g/ kg soil	
		22.8	31.4
Biomass of shoot, g			
Alfaalfa	0.328±0.031	0.288±0.019	0.113±0.02
Rape	1.015±0.15	0.534±0.045	0.212±0.05
Grass mixture	0.215±0.01	0.184±0.017	0.12±0.01
Barley	0.612±0.10	0.374±0.05	0.158±0.02
Biomass of root, g			
Alfaalfa	0.156±0.003	0.134±0.026	0.072±0.058
Rape	0.192±0.012	0.125±0.01	0.074±0.011
Grass mixture	0.174±0.014	0.126±0.01	0.089±0.019
Barley	0.12±0.01	0.081±0.01	0.033±0.001

Thus, the growth of plants on contaminated soil, depending on its concentration, the oil has a negative effect on plant growth, but all studied plants grown in the presence of oil. It is very likely that these plants can be used as plant - rhizoremediators to restoration of oil - contaminated soil in order to involve them in agrocenosis.

B. Effect of Oil on the Physiological Characteristics of the Plants

Stressful conditions caused by the presence in the environment of petroleum hydrocarbons, influence on physiological parameters of plant growth (the photosynthetic apparatus, the enzyme activity, the quantitative change of the surface roots). State of the photosynthetic apparatus and change the root structure an indicator of the functional activity of plants. In addition, the study of the physiological parameters is necessary to clarify the relationship between the growth and development of plants and the effects of petroleum hydrocarbons [7], [8].

Soil+Oil(22.8 g of oil/kg of soil) Control

(a) Barley

Soil+Oil (22.8 g of oil/kg of soil) Control

(b) Alfaalfa

Fig. 3 Growth of plants on oil-contaminated soil (a) barley (b) alfalfa

During growing plants in oil-contaminated soils reduction of photosynthetic pigments, which manifested it in yellowing and wilting of the leaves, as well as reduction of the leaf area were visually observed (Fig. 3).

Determination of the main photosynthetic pigments (a, b and total chlorophyll content) showed a reduction in the number of chlorophyll in the leaves when they are grown on oil-contaminated soil (Table III). Reducing the amount of pigment varied within 20.1 - 25.2% as compared to control of two plants (barley, grass mixture) in alfalfa 65.2% chlorophyll in leaves was lower relative to the control (Table III). Chlorophyll b was lower in comparison with the content of chlorophyll a. For all plant samples indicated an even greater reduction in chlorophyll b.

So, for example, barley shows the decrease of chlorophyll b on average by 50.2% compared with the plants, the growth of which was produced in the soil without oil. For alfalfa, this difference was minimal (27.15 ± 0.40mg /g - in the control and 23.4 ± 0.25 - with an increase in oil-contaminated soil). The total chlorophyll content in the control was compared with samples grown plants in contaminated soils.

TABLE III
CONTENT OF PHOTOSYNTHESIS PIGMENTS (CHLOROPHYLL A, B AND TOTAL CHLOROPHYLL CONTENT) IN LEAVES OF PLANTS GROWN ON OIL-CONTAMINATED SOIL, MG/G

Variance of study	Chlorophyll a	Chlorophyll *b*	Total chlorophyll content
Barley			
Control	28.53±0.41	25.30±0.26	54.5±0.49
Contaminated soil	22.27±0.25	13.24±0.14	36.18±0.35
Grass mixture			
Control	24.15±0.35	20.42±0.22	46.5±0.45
Contaminated soil	18.0±0.18	13.56±0.14	31.2±0.39
Alfaalfa			
Control	32.4±0.45	27.15±0.40	60.2±0.25
Contaminated soil	20.7±0.19	23.4±0.25	44.1±0.42
Rape			
Control	32.15±0.45	29.48±0.15	62.1±0.48
Contaminated soil	24.8±0.35	15.4±0.17	39.0±0.43

Determination of adsorption of root surface (total and work) showed differences in the quantitative ratio of the total surface of the roots to the work surface with the growth of plants in the soil with different concentrations of oil (Table IV).

TABLE IV
CHANGES IN PHYSIOLOGICAL ACTIVITY OF ROOTS

Plants	Adsorption surface, m^{22}			
	Control (Non-contaminated soil)		22 g of oil/kg f soil	
	Total	Work	Total	Work
Rape	8.2±0.02	3.8±0.03	8.7±0.04	5.4±0.03
Barley	7.5±0.04	3.9±0.05	9.14±0.02	6.18±0.04
Alfaalfa	5.1±0.01	2.2±0.01	5.2±0.08	3.15±0.03
Grass mixture	5.3±0.02	3.15±0.02	6.43±0.07	4.38±0.08

For example, if there is no oil in the soil, the ratio of the total surface area of working in plants of oats was 8.2 ± 0.02 m^2 / 3.8 ± 0.03 m^2 (in percentage - 100/46), at a concentration of 20g of oil / kg of oil was 8.7 ± 0.04 m^2 / 5.4 ± 0.03 m^2 (100/62). Most common absorbent surface was observed in barley and was 9.14 ± 0.02 m^2. Results of studies have shown that the growth of plants in soil contaminated with oil observed wilting and yellowing of the leaves of the plants, reducing the content of chlorophyll, Differences in the quantitative ratio of the total surface of the roots to the work surface with the growth of plants in the soil with oil.

IV. CONCLUSION

In the selection of the potential of plants used for cleaning contaminated soils as indicators of survival in a polluted environment, researchers used various indicators such as germination and seed germination, length of root and shoot, as well as their dry biomass. So, Kirk et al. [9] in his works suggest using these parameters to assess the impact of petroleum hydrocarbons on plants. For the selection of plants as remediator in their studies Adam and Duncan [10] relied on the vigor and germination of seeds of plants, and have been shown a slight decrease in these parameters when exposed to oil. These works Issuofi et al. [11] talk about reducing the seed vigor with increasing concentrations of petroleum hydrocarbons in some host plants, maize, wheat, and soybean. Researchers as remediators suggested using those plants for cleaning oil-contaminated soils. So, screening of plants for application them as rhizoremediators is conducted by analysis of such parameters like lengths and biomass of shoots and roots, survival of plants.

We found that of among tested plant resistance to various concentrations of oil showed two plants: barley and grass mixture. Thus, the study estimates the impact of oil on the morphological parameters of the plant is established that any of the investigated plants decreased depending on the concentration of oil in the soil, increasing charge more for escape than to the root. Minimum inhibitory effect is shown for mixtures of oil, and barley, as compared to control reported negligible morphological changes.

In studying the effect of oil on the physiological parameters of these plants, they show the decrease of chlorophyll a and b in the leaves of plants a slight decrease in the amount of chlorophyll a and b as compared to control three plants (barley, grass mixture, rape) was showed. In determining the adsorption of root surface (total and operating), difference in the ratio of the total surface of the roots to the work surface with the growth of plants in soil with oil was found.

Thus, the selected plants expressed a resistance to oil concentrations exceed the maximum permissible concentration of soil. It is possible that they can be used as rhizoremediators for recovery of contaminated soils.

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