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Studying oil products' degradation in soil by consortia of autochthonous strains from the black soils of Northern Kazakhstan

Sabit Kabdyshevich Kokanov, Gulnara Batyrbekovna Yunussova, Indira Saltanovna Beishova, Tatiana Vladimirovna Poddudinskaya, Rustem Salatanovich Beyshov,
The Kostanay State University A. Baitursynov, 11000, Kazakhstan, Kostanay, Baitursynov st., 47

Svetlana Aitkeldinovna Aitkeldiyeva, Elmira Ramazanovna Faizulina, Larisa Gennadievna Tatarkina,
RSE "Institute of Microbiology and Virology" MAS RK, 050056, Kazakhstan, Almaty, Bogenbai Batyr st., 103

Abstract
The possibility of using the selected active associations of hydrocarbon-oxidizing microorganisms for cleaning soil from oil products - diesel fuel, waste motor oil, axle oil and gear lube - has been studied. A model experiment in cleaning black soils of Northern Kazakhstan contaminated with oil products showed the efficiency of the created associations of hydrocarbon-oxidizing microorganisms. 2 months after introducing the associations, degradation of diesel fuel was 81.3 to 83.4%. Subsidence of axle oil, waste motor oil and gear lube during the same period was 42.2-45.3%, 38.6-39.2%, and 38.6-42.1%, respectively. Bioremediation of soils with the selected active associations resulted in decreasing their phytotoxicity. Thus, in the experimental variants, radish seed germination was 80 to 90%, and the average plant stem length varied between 73.5 mm and 104.8 mm. In the reference variants with germination of 50-70%, seedlings height did not exceed 72 mm.
Keywords: petroleum products' decomposition, soil phytotoxicity, black soil, Northern Kazakhstan.

INTRODUCTION

The necessity of finding and using efficient ways of cleaning contaminated lands is caused by their uncontrolled growth on many sources. Only in the areas of oil production in Kazakhstan, 200 thousand hectares polluted with oil products have been detected [1, 2]. In terms of the area of hydrocarbon pollution, larger sources may be human activities such as storage, processing, transportation and use of petroleum products [3, 4, 5]. The problem of hydrocarbon pollution is aggravated in Northern Kazakhstan, since agricultural and arable lands, as well as meadows and pastures are polluted there, which are particularly valuable for grain production and livestock breeding in the Republic and in the context of the climate changes.

Autochthonous strains of hydrocarbon-oxidizing microorganisms (HOM) had been formerly isolated from the black soils of Northern Kazakhstan, their species association was established using molecular-genetic methods, and consortia suitable for cleaning soil from oil products were selected [6]. In this paper, we studied the possibility of using the selected active HOM associations for decomposing petroleum products: diesel fuel, waste automotive oil, axle oil and gear lubricant.

METHODS AND MATERIALS

In the experiment, we used two associations that had shown the best hydrocarbon-oxidizing activity when grown in liquid mineral medium with oil products: Association 2 (strains *Candidafermentati* + *Rhodococcuserythropolis*) and Association 5 (strains *Candidafermentati* + *Arthrobactersiderocapsulatus* + *Rhodococcuserythropolis* + *Arthrobactersoli* + *Arthrobacterviscosus*) [6]. To assess the efficiency of the studied associations, model experiments were laid for purification of soil polluted by various petroleum products. Plastic containers with 300 g of soil in each were used in the model experiments. The soil was southern black soil artificially polluted with oil products. Diesel fuel was added at the rate of 50 g/kg, axle oil and waste oil – at the rate of 30 g/kg, and gear lubricant – at the rate of 10 g/kg. 7 days after the contamination, the studied associations were added into each container in the form of suspension with the 10^9 cells/ml titer at the rate of 30 ml per container. For preparing the associations, the cultures included into their composition had been individually grown in a nutrient broth for 2 days, then their equal shares were mixed.

Reference was soil contaminated with petroleum products without microorganisms.

The experiment was held for 2 months at room temperature of 25±2. The soil was periodically aerated by loosening, and moistened to 60% of the total moisture capacity.

The number of major groups of microorganisms in the soil was determined by standard microbiological method by inoculating onto appropriate nutrient medium [7, 8, 9].

Residual oil content in soil was measured by the gravimetric method using a gas chromatograph after extraction with hexane and chloroform.

After soil purification with HOM associations, its phytotoxicity was checked using the commonly used method of bioindication [10]. For this purpose, seeds of radish were sown into the soil at the rate of 10 seeds per container. Toxicity was assessed by the number of germinated seeds and the height of sprouts. Changes in seed germination and the average length of sprouts, compared to the reference, expressed in %, were the indicator of phytotoxicity of studied soils.

RESULTS AND DISCUSSION

Testing the HOM consortium in model experiments. The experiment included the following variants:

1) Soil + 5% of diesel fuel (reference 1);
2) Soil + 5% of diesel fuel + Association 2 (Ass. 2);
3) Soil + 5% of diesel fuel + Association 5 (Ass. 5);
4) Soil + 3% axle oil (reference 2);
5) Soil + 3% axle oil + Association 2;
6) Soil + 3% axle oil + Association 5;
7) Soil + 3% waste oil (reference 3);
8) Soil + 3% waste oil + Association 2;
9) Soil + 3% waste oil + Association 5;
10) Soil + 1% gear lube (reference 4);
11) Soil + 1% gear lube + Association 2;
12) Soil + 1% gear lube + Association 5;

Before introducing the studied associations, the number of major groups of microorganisms was determined in the soil samples contaminated by various oil products (Table 1).

Table 1 shows that soil contamination with oil products did not result in any significant changes in the abundance of major groups of soil microorganisms. Only increased HOM number was observed.

After 1 month of the experiment, the number of bacteria increased in all experiment variants, and the content of HOM increased by 2 to 4 powers (Table 2). The content of actinomycetes and filamentous fungi did not significantly change.

Two months after introducing the associations, the quantitative indicators of soil microflora remained almost at the same level as after 1 month of the study (Table 3). And only in the soil contaminated with waste motor oil and gear lube the number of the HOM increased by one power.

The residual content of oil products in soil was measured 1 and 2 months after introducing the studied associations of microorganisms (Table 4). Fuel oil was decomposed most intensively. Thus, after 1 month in the soil treated with Association 2, it was 30.8%, and in the soil treated with Association 5 – 34.4%. By the end of the experiment, oil product utilization was 81.8 to 83.4%. Association 2 showed slightly higher activity.

Other studied oil products were more resistant to biodegradation. Thus, the content of axle oil in soil after two months decreased only by 42.2-45.3%, and of waste oil – by 38.6-39.2%. It should be noted that while Association 5 was more active to axle oil during the first month, after two months, its activity slightly decreased, compared to Association 2, which decomposed it by 3% more.

Table 1 – The number of main groups of microorganisms in the soil samples contaminated with oil products

Oil products	Heterotrophic bacteria, CFU/g	Actinomycetes, CFU/g	Filamentous fungi, CFU/g	HOM, MPN, cells/g
Diesel fuel	$(2.1\pm0.1)\times10^{7}$	$(4.2\pm0.8)\times10^{5}$	$(3.5\pm0.5)\times10^{4}$	1.3×10^{3}
Axle oil	$(3.2\pm0.2)\times10^{7}$	$(4.6\pm0.4)\times10^{5}$	$(4.0\pm0.1)\times10^{4}$	2.5×10^{3}
Waste automotive oil	$(3.0\pm0.2)\times10^{7}$	$(3.4\pm0.4)\times10^{5}$	$(3.8\pm0.4)\times10^{4}$	1.3×10^{3}
Gear lubricant	$(3.0\pm0.2)\times10^{7}$	$(2.7\pm0.3)\times10^{5}$	$(2.0\pm0.3)\times10^{4}$	6.0×10^{2}
Uncontaminated soil	$(2.8\pm0.5)\times10^{7}$	$(3.0\pm0.3)\times10^{5}$	$(2.7\pm0.4)\times10^{4}$	2.5×10^{2}

Table 2 – The number of main groups of microorganisms in soil samples after 1 month

Oil products	Heterotrophic bacteria, CFU/g	Actinomycetes, CFU/g	Filamentous fungi, CFU/g	HOM, MPN, cells/g
DT + Ass. 2	$(5.27\pm0.2)\times10^{8}$	$(1.8\pm0.2)\times10^{6}$	$(1.5\pm0.1)\times10^{5}$	2.5×10^{7}
DT + Ass. 5	$(1.77\pm0.1)\times10^{8}$	$(4.3\pm0.2)\times10^{5}$	$(2.7\pm0.2)\times10^{5}$	2.5×10^{7}
Diesel fuel	$(1.97\pm0.1)\times10^{7}$	$(4.5\pm0.4)\times10^{5}$	$(3.3\pm0.2)\times10^{4}$	5.0×10^{3}
Axle oil + Ass. 2	$(3.65\pm0.2)\times10^{8}$	$(2.2\pm0.3)\times10^{5}$	$(2.5\pm0.2)\times10^{4}$	2.5×10^{7}
Axle oil + Ass. 5	$(6.98\pm0.1)\times10^{7}$	$(2.7\pm0.1)\times10^{5}$	$(4.0\pm0.4)\times10^{4}$	6.0×10^{6}
Axle oil	$(1.60\pm0.1)\times10^{7}$	$(2.4\pm0.2)\times10^{5}$	$(3.4\pm0.1)\times10^{4}$	6.0×10^{3}
Waste automotive oil + Ass. 2	$(3.65\pm0.2)\times10^{8}$	$(3.3\pm0.4)\times10^{5}$	$(1.7\pm0.1)\times10^{4}$	1.3×10^{5}
Waste automotive oil + Ass. 5	$(6.98\pm0.1)\times10^{7}$	$(1.9\pm0.1)\times10^{5}$	$(3.9\pm0.3)\times10^{4}$	2.5×10^{5}
Waste automotive oil	$(1.36\pm0.1)\times10^{7}$	$(4.8\pm0.5)\times10^{5}$	$(2.1\pm0.2)\times10^{4}$	6.0×10^{2}
Gear lubricant + Ass. 2	$(3.42\pm0.4)\times10^{8}$	$(8.5\pm0.3)\times10^{5}$	$(1.4\pm0.1)\times10^{4}$	6.0×10^{5}
Gear lubricant + Ass. 5	$(3.17\pm0.1)\times10^{8}$	$(3.0\pm0.2)\times10^{5}$	$(3.6\pm0.2)\times10^{4}$	6.0×10^{5}
Gear lubricant	$(1.49\pm0.1)\times10^{7}$	$(4.7\pm0.3)\times10^{5}$	$(1.0\pm0.1)\times10^{4}$	5.0×10^{2}

Table 3 – The number of main groups of microorganisms in soil samples after 2 month

+Variant	Heterotrophic bacteria, CFU/g	Actinomycetes, CFU/g	Filamentous fungi, CFU/g	HOM, MPN, cells/g
DT + Ass. 2	$(2.5\pm0.3)\times10^{8}$	$(6.6\pm0.5)\times10^{5}$	$(6.2\pm0.2)\times10^{5}$	2.5×10^{7}
DT + Ass. 5	$(1.1\pm0.2)\times10^{8}$	$(3.5\pm0.2)\times10^{5}$	$(6.4\pm0.5)\times10^{5}$	2.5×10^{7}
Diesel fuel	$(7.4\pm0.5)\times10^{6}$	$(3.3\pm0.3)\times10^{5}$	$(1.5\pm0.1)\times10^{4}$	5.0×10^{3}
Axle oil + Ass. 2	$(2.5\pm0.2)\times10^{8}$	$(4.7\pm0.2)\times10^{5}$	$(4.2\pm0.3)\times10^{4}$	2.5×10^{7}
Axle oil + Ass. 5	$(5.0\pm0.3)\times10^{8}$	$(3.6\pm0.1)\times10^{5}$	$(2.8\pm0.2)\times10^{4}$	6.0×10^{6}
Axle oil	$(8.2\pm0.2)\times10^{6}$	$(5.5\pm0.4)\times10^{5}$	$(2.0\pm0.1)\times10^{4}$	6.0×10^{4}
Waste automotive oil + Ass. 2	$(2.5\pm0.1)\times10^{8}$	$(6.6\pm0.4)\times10^{5}$	$(6.9\pm0.1)\times10^{4}$	6.0×10^{5}
Waste automotive oil + Ass. 5	$(1.8\pm0.1)\times10^{8}$	$(2.7\pm0.2)\times10^{5}$	$(4.7\pm0.2)\times10^{4}$	1.3×10^{6}
Waste automotive oil	$(1.0\pm0.1)\times10^{7}$	$(3.5\pm0.2)\times10^{5}$	$(1.6\pm0.1)\times10^{4}$	1.3×10^{3}
Gear lubricant + Ass. 2	$(1.6\pm0.2)\times10^{8}$	$(1.6\pm0.1)\times10^{6}$	$(3.3\pm0.3)\times10^{4}$	2.5×10^{6}
Gear lubricant + Ass. 5	$(1.3\pm0.1)\times10^{8}$	$(3.9\pm0.3)\times10^{5}$	$(4.8\pm0.2)\times10^{4}$	1.3×10^{6}
Gear lubricant	$(7.8\pm0.4)\times10^{6}$	$(4.0\pm0.2)\times10^{5}$	$(1.4\pm0.1)\times10^{4}$	1.3×10^{3}

Table 4 – Oil products' content in the soil, %

Oil products	Oil products' content in the soil, %					
	after 1 month			after 2 months		
	Association 2	Association 5	reference	Association 2	Association 5	reference
Diesel fuel	69.2	65.6	29.6	83.4	81.8	38.8
Waste oil	37	37.7	8.8	39.2	38.6	13.4
Axle oil	36.9	39.4	11.2	45.3	42.2	15.6
Gear lube	36	33.9	7.9	38.6	42.1	11.6

Gear lube proved to be less affected by HOM associations. With its initial content of 1%, the decline after one month was 33.9 to 36.0%, after two months - 38.6 to 42.1%. A month after the introduction, gear lube was slightly more efficiently decomposed by Association 2, while after 2 months, Association 5 showed higher activity.

It should be noted that decreasing the number of all oil products in the soil occurred more intensively within 1 month. After that, the destruction process slowed down. The subsidence of diesel fuel during the 2nd month was 14.2 to 16.2%, and of axle oil – 2.8 to 8.4%, of waste oil – 0.9 to 2.2%, and of gear lube – 2.6 to 8.2%. Evidently, the lighter hydrocarbons contained in oil products were decomposed during the first month, while decomposition of the remaining heavily degradable hydrocarbons required more time.

Chromatographic analysis showed that under the influence of the studied associations, changes occur in the component composition of oil products, primarily by reducing the number of n-alkanes (Figures 1-12).

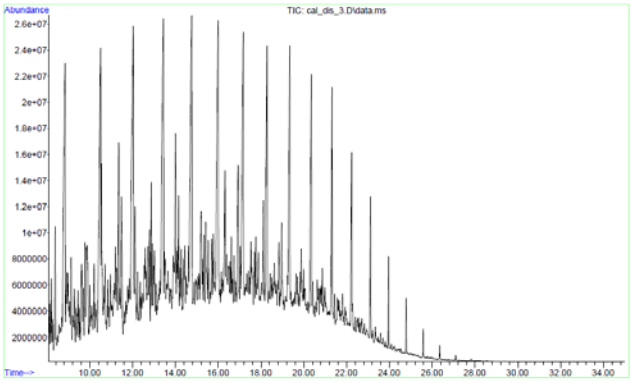

Figure 1 – Chromatogram of the total hydrocarbon content in diesel fuel

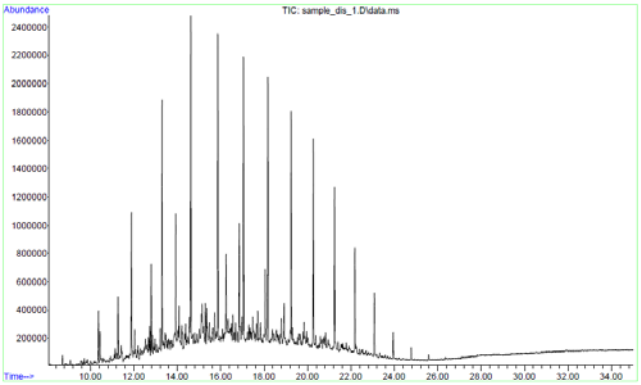

Figure 2 – Chromatogram of the total hydrocarbon content in the soil sample with diesel fuel after introducing Association 2

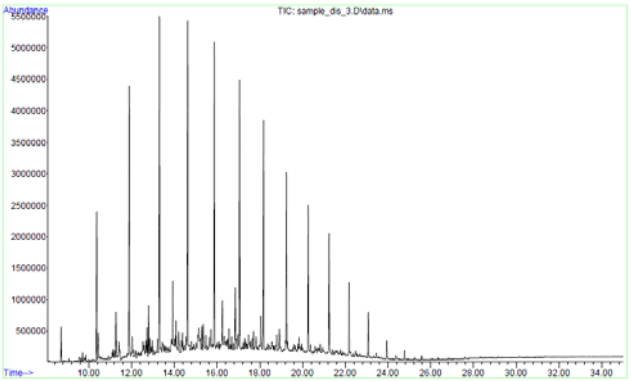

Figure 3 – Chromatogram of the total hydrocarbon content in the reference soil sample with diesel fuel

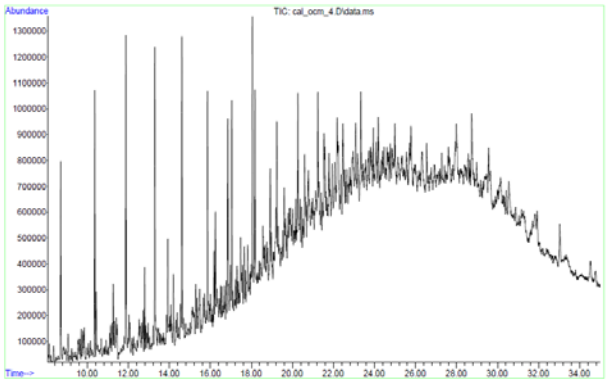

Figure 4 – Chromatogram of the total hydrocarbon content in axle oil

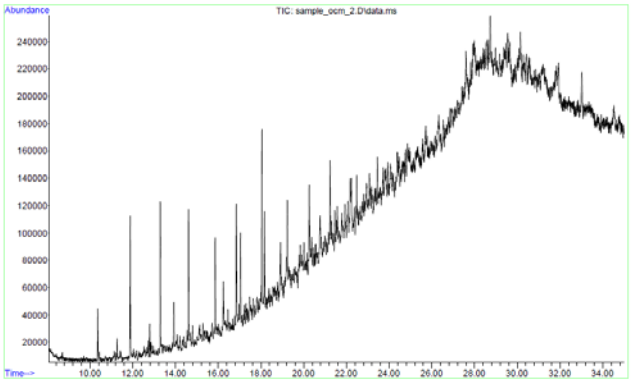

Figure 5 – Chromatogram of the total hydrocarbon content in a soil sample with axle oil after introducing Association 2

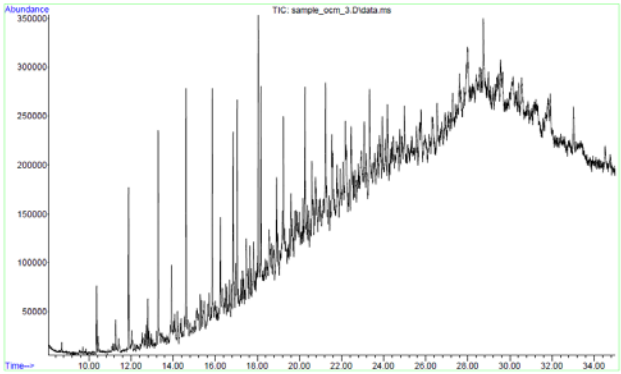

Figure 6 – Chromatogram of the total hydrocarbon content in the reference soil sample with axle oil

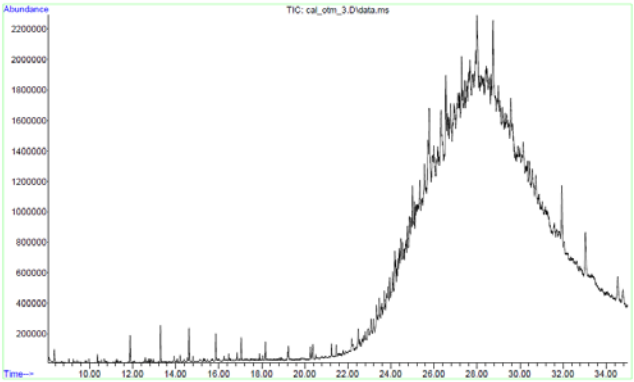

Figure 7 – Chromatogram of the total hydrocarbon content in the waste axle oil

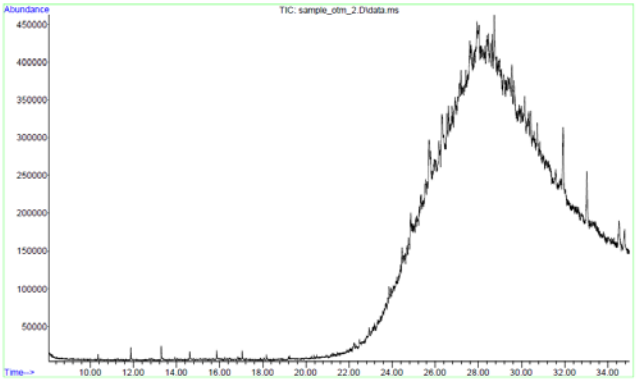

Figure 8 – Chromatogram of the total hydrocarbon content in the soil sample with axle oil after introducing Association 2

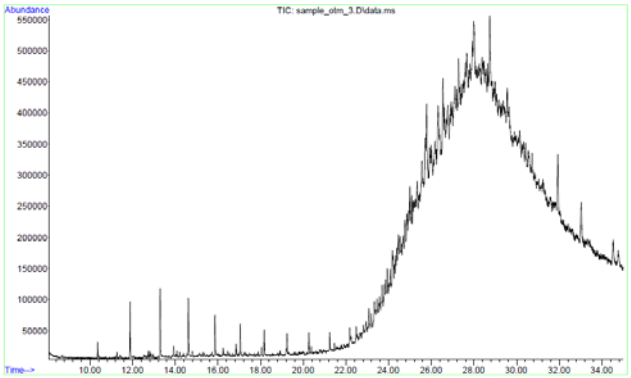

Figure 9 – Chromatogram of the total hydrocarbon content in the reference soil sample with waste axle oil

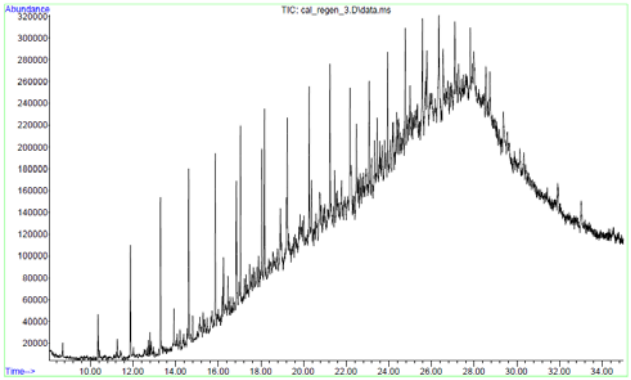

Figure 10 – Chromatogram of the total hydrocarbon content in gear lube

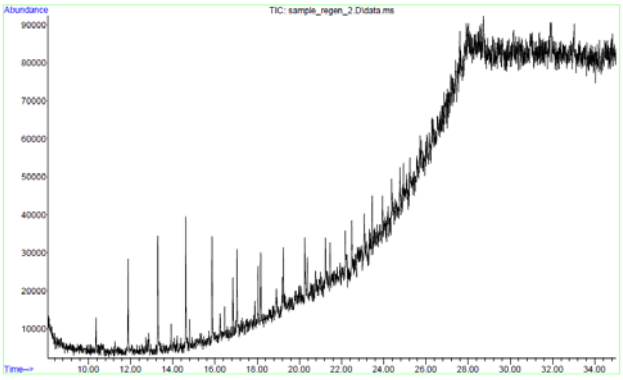

Figure 11 – Chromatogram of the total hydrocarbon content in a soil sample with gear lubricant after introducing Association 5

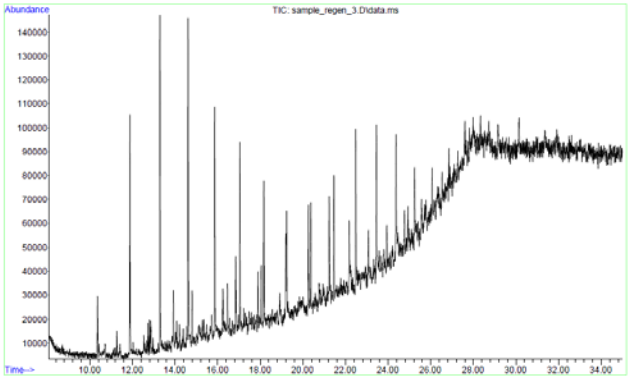

Figure 12 – Chromatogram of the total hydrocarbon content in a reference soil sample with gear lubricant

After cleaning soil from oil products with the help of Associations 2 and 5, its phytotoxicity was determined by germination of radish seeds (Table 5). The results of the study showed that in the contaminated soil treated with Association 2, seed germination was 90%, and only in soil with gear grease their number was less by 5%. In the soil with the introduced Association 5, germination was 80 to 90%. Most seeds germinated in the soil with diesel fuel and gear lube.

In the reference variants, 5 to 7 seeds germinated on the average. The highest germination rate was observed in the soil contaminated with diesel fuel, the lowest one - in the soil with waste oil.

The average stem length in the soil treated with Association 2 was 73.5 to 104.8 mm, in the soil treated with Association 5 – 77.3 to 102.5 mm. In the reference variants, seedlings height did not exceed 72 mm.

Table 5 – Radish seeds' germination rate in the model experiment

Variant		Seed germination rate, %	Average stem length, mm
Diesel fuel	association 2	90	96.2±12.6
	association 5	90	102.5±15.1
	reference	70	68.6±13.5
Axle oil	association 2	90	104.8±17.2
	association 5	85	78.6±14.6
	reference	60	71.4±8.5
Waste oil	association 2	90	81.4±13.6
	association 5	80	77.8±11.3
	reference	50	59.3±6.6
Gear lube	association 2	85	73.5±9.2
	association 5	90	77.3±6.4
	reference	60	63.2±8.3

The phytotoxicity reduction after treating the contaminated soil by consortia of HOM (Association 2, Association 5) is shown in Figure 13. Seeds germination rate increased from 29% to 80%, the average stem length, mm – from 10% to 49%.

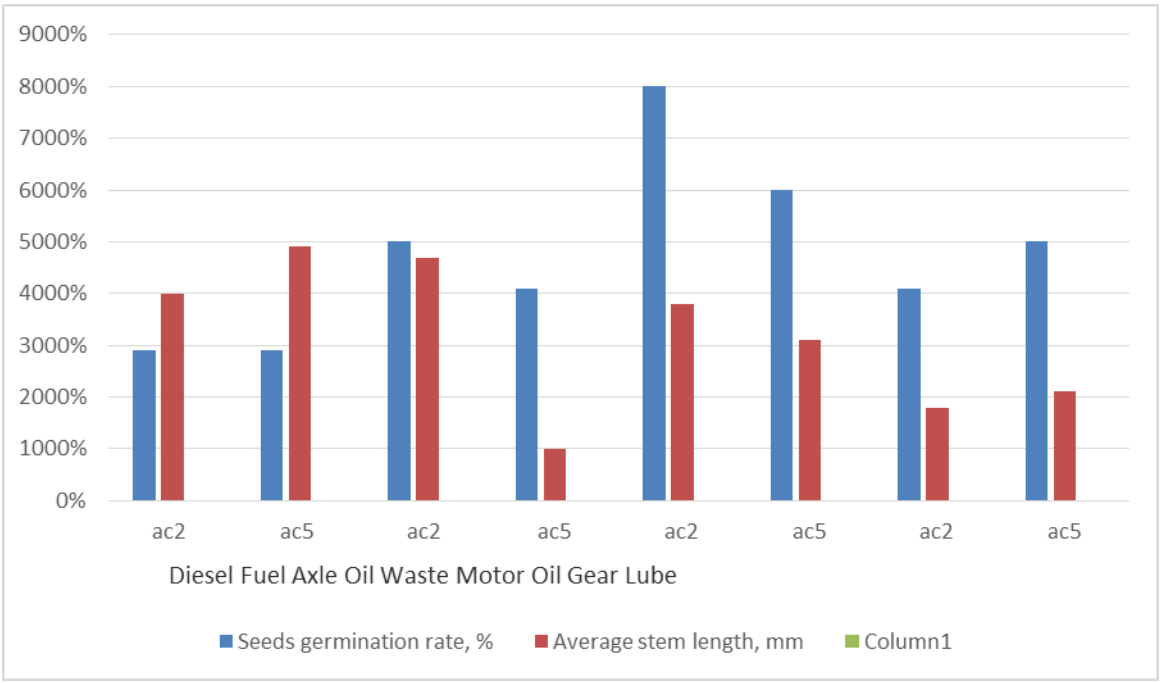

Figure 13 – Oil-contaminated soils' phytotoxicity reduction in the model experiment, %

Thus, the results of the study showed efficiency of the created HOM associations for purification of soils contaminated with oil products. Diesel fuel was most easily decomposed. With the initial content of 5% after 2 months, its subsidence was 81.3-83.4%. With the content of 3%, over the same time period axle oil and waste oil were decomposed by 42.2 to 45.3% and 38.6 to 39.2%, respectively. Gear lube was more resistant to microbial decomposition. With the contamination of 1%, its amount in soil decreased by 38.6-42.1% after 2 months. Therefore, complete cleaning of contaminated soil from heavier oil products requires more time. Also, it should be noted that many parameters have to be taken into account as it was reported before for aquatic biosystems [11, 12].

Conclusion

Based on the performed research, the following has been established**:**

1. The isolated consortia of active strains of hydrocarbon microorganisms, which are autochthonous to black soils of Northern Kazakhstan, are well able to decompose the oil products widely spread in the region, i.e. diesel fuel, waste motor oil, axle oil and gear lube.
2. The efficiency of the created HOM associations differed a little. Association 2 was more efficient in decomposing diesel fuel, waste motor oil and axle oil, while Association 5 – in decomposing gear lube.
3. It has been generally confirmed that consortia of microorganisms removed light oil products (diesel fuel) from soil faster than heavy oil products (waste motor oil, axle oil, gear lube). Good results of oil products' degradation in black soil have been achieved. Thus, 2 months after introducing the associations, degradation of diesel fuel in the model experiment was 81.3 to 83.4%. Subsidence of axle oil, waste motor oil and gear lube during the same period was 42.2-45.3%, 38.6-39.2%, and 38.6-42.1%, respectively.
4. Bioremediation of soils with the selected active associations resulted in decreasing their phytotoxicity. Thus, in the experimental variants, radish seed germination was 80 to 90%, and the average plant stem length varied between 73.5 mm and 104.8 mm. In the reference variants with germination of 50-70%, seedlings height did not exceed 72 mm.

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