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# Oil Contaminated Soils And Their Biological Recultivation

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***ABSTRACT: The following studies have shown the results of low, moderate, strong and very strong soil contamination as well as the change of soil morphological characteristics, the strains separation of oil-breaking bacteria and the development of recultivation technology. According to the results, the changes in soil morphological characteristics differ in contamination degree, and the morphological characteristics of very strong soil contamination (more 25 g/kg) have dramatically changed. The oil-breaking bacterial strains such as MFD-100 Pseudomonas stutzeri, MFD-200 Pseudomonas caryophyllis, MFD-5000 Bacillus subtilis are separated from oil-contaminated soils. Based on these strains, the three-stage recultivation technology has been developed, and as a result of the conducted recultivation, the soil cover is purified up to 81.8%. Soil purification rate is divided into the following efficiency indicators: 1 - soil purification rate 0-20%, 2 - soil purification rate 20-40%, 3 - soil purification rate 40-60%, 4 - soil purification rate 60-80% and 5 - soil purification rate 80-100%. After the soil recultivation, the productivity indicators such as nutrient and humus content as well as pH environment are improved.***

***Keywords: Soil, bacteria, contamination, oil, recultivation.***

## 1. INTRODUCTION

Oil contaminated soil is one of the most serious environmental problems in the world (Prostov et al., 2019, Mwaura et al., 2018), which is mostly caused by human activity and changes in soil properties. It affects biological (especially microbiological), chemical composition (Hewelke et al., 2018), as well as the physical properties of the microstructure (Yu et al., 2018). Along with oil contamination soils are also contaminated with heavy metals such as Pb, Fe, Zn, Cu, Cr, Ni and Cd (Orji et al., 2018).

The anthropogenic factor plays a major role in the pollution of soils with oil, including the increase in pollution caused by oil production and oil industry activity. It leads to changes in plants' phytotoxic properties and state, soil enzyme activity, nitrogen turnover in the soil and changes in soil microorganisms (Achuba et al., 2018). It is scientifically proven that catalase enzyme can be an indicator of soil contamination by oil and heavy metals (Titova et al.,

2019). Experiments have been shown that Medicago sativa plant is an indicator to determine contaminated soils (Glibovytska et al., 2019).

Soils with diesel fuel contamination may occur in a number of ways and may have different effects (Cuervo, 2018). Soil contaminated with oil products (diesel fuel) can be purified with using 5% opiates, while bioremediation, aeration, the amount and activity of microorganism are high and the purification process is efficient (Alvim et al., 2018). Rice bran has been used in oil contaminated soils and bioremediation has been observed and achieved high efficiency taking into account soil pH environment, organic carbon, organic matter, total nitrogen and mobile phosphorus content (Stanley et al., 2017). Fitoremediation methods have also been successful in purifying diesel fuel contaminated soils (Mita et al., 2018), based on phytemediation technology, Lathyrussativus species and Lenusculinarys species (Noori et al., 2012) and Chromolaena odorata plants give high efficiency (Devatha et al., 2019).

The use of electronic nose (E-nose) is successful in purifying soils contaminated with oil (Bieganowski et al., 2018). The process of biostimulation is highly effective in purifying soils contaminated with oil and heavy metals such as Cd, Pb, As using Brassica juncea and chicken compost (Makombe et al., 2018). The method of penetrating radar (GPR) is effective in studying hydrocarbon contamination of soils around oil fields (Agustine et al., 2019). In the treatment of polycyclic aromatic hydrocarbons, the bacterial strains Bacillus licheniformis ATHE9 and Bacillus mojavensis ATHE13 were highly effective, including Bacillus mojavensis ATHE13 strains during 120 hours breaking 16 types of hydrocarbons in different degree, including hydrocarbons such as Naphtalene, Acenaphtylene, Acenaphtene, Benzo(ghi)prylene, Dibenzo(ah)anthracene, Indeno pyrene decomposed by 100% (Eskandari et al., 2017).

The use of bioremediation, namely microorganism strains in the treatment of oil and oil contaminated soils, especially in the use of consortium tractors (Ghoreishi et al., 2017, Adams et al., 2017), has been shown to be highly effective with bacteria Pseudeomonas, Flavobacterium, Bacillus, Proteus and Klebsiella, strains of Penicillium, Aspergillus, Fusarium (Popoola et al., 2019), Cephalotheca, Lecanicillium and Septoriella (Wang et al., 2018), Rhodococcus sp strains were highly effective (Kis. et al. 2017). Ex-situ bioremediation has also been used to purify diesel fuel contaminated soils, with additional nutrient environments stimulating the population of microorganisms and degradation processes are purified up to 75% (Jabbar et al., 2018, Jabbarov et al. 2019).

Sinorhizobium, Promicromonospora, Novosphingobium, Georgenia, Ancylobacter, Roseomonas, Hansschlegelia are widely used for purifying oil contaminated soils (Sekkour et al., 2019). Improvement of physicochemical properties of soil, creation of conditions for microorganisms, provision of plant nutrients, restoration of soil fertility (Ogbeide et al., 2019), application of inorganic fertilizers, accounting of physical properties of soil play important role in purifying (Elechi et al., 2018).

Bioaugmentation is highly effective in bioremediation technology, with nitrogen fixing bacteria (NFBs) as biosurfactants improving soil properties, increasing the overall microorganism population, and reducing hydrocarbons by up to 80% (Pérez Vargas et al., 2017). The use of plant-bacterial symbiosis (plant-bacterial synergism) has been highly effective in purifying oil-contaminated soils, with the population of plant-based bacteria maximizing organic matter (Fatima et al., 2017, Ite et al., 2019). In situ use of Pseudomonas aeruginosa (JX100389) and P.plecoglossicida (JX149549) bacteria has been successful (Kumar et al., 2017). Moreover, the biosorbents of aerobic and anaerobic microorganisms have been successful in purifying oil contaminated soils, and their application has purified up to 90% of the soil cover (Khokhlov et al., 2018).

## 2. MATERIALS AND METHODS

The soil samples have been taken from oil contaminated grassy alluvial soils around the Mingbulak oilfield in Namangan region with 0.8, 2, 5, 15, 22 km away from the oil field. Oil decomposing bacteria were isolated from this area. Separated cultures kept in 500 mL tube with synthetic nutrient medium (g/l $KH_2PO_4$-0,85, $(NH_4)SO_4$ -5,0, $K_2HPO_4$-0,15, $MgSO_4 \cdot 7H_2O$-0,5, NaCI-0.1, $CaCI_2 \cdot 4H_2O$-0.1, distilled water-1000 ml, pH-6,0-7,8), to be determined by observing the decomposition activity of oil concentrations in the range 0.5-30.0%. The experiments were conducted for 3-5-7 and 30 days at 28°C. Three oil-sprayed MFD-100-Pseudomonas stutzeri, MFD-200-Pseudomonas caryophyllis, MFD-5000-Bacillus subtilis bacterial strains were isolated at 5 $m^2$ in specific field conditions with $10^{10}$-$10^{12}$ cells / $m^2$ in field conditions (fluid was used in the Raymon forage), providing additional conditions for air exchange and moisture. To activate the bioremediation process, mineral fertilizers in the amount of $N_{120-140}$, $P_{80-100}$, $K_{30-40}$ kg, and 8 t / ha fertilizer were used. Soil sampling, storage, laboratory, lysimetric experiments State Standard (SS): 17.4.3.01–83, sampling and storage of soil samples for chemical and bacteriological analyzes SS:17.4.4.02–84, determination of the amount of microorganisms in soil with Zvyagintsev Method (Zvyagintsev, 1991), chemical and physical properties of soils Pansu Method (Pansu et al., 2014), determination of oil and oil products in soils, Guidance Document (GD).118.3897485.13–92 methods for determination of oil products in soil by fluorimetric method, soil fertility and recultivation state standards (SS:17.5.1.01.-83; SS:17.5.3.04-83), the statistical analysis of the results obtained from the program "Statgraphics Centurion XVII".

## 3. RESULTS AND DISCUSSION

When soil was contaminated with oil, its morphological features were initially changed, including its color, density and structure changed dramatically. The morphological features of the soil cut from the study area were analyzed in the case of 08 cut:

08 cut. The irrigated area is located 0.8 km east of the oil field. Microrelief is uneven lands, noticeable slope, no vegetation cover (previously used for cotton). The top of the soil is dry and hard. Due to oil mixing, soil and oil slices are formed in various sizes (0-23 cm) (Figure 1).

Fig. 1: Oil residue content in oil contaminated soil and the extraction of oil from it

The morphological features of the soil cut are characterized by different layers, and each layer is different.

0-30 cm. Layer, dark, pale gray, dry, low-cut, dense, unstructured, with a large amount of oil residue, plant roots and insect nests are not seen, and density and color pass to the next layer.

30-45 cm. Dark gray (with more oil content than upper layer), partially damp, medium loam, very dense, plant roots and insect nests are not seen at all, while cutting, separeted with big slices and density and color pass to the next layer.

45-60 cm. Gray, damp, poorly structured, concentrated, oil residues are clearly visible, plant roots and insect nests are not seen, and the color goes to the next layer.

60-90 cm. Light gray, moderately damp, medium loam, almost unstructured, oil spots are rare, plant roots and insect nests are not seen, rust spots are rare, the color goes to the next layer.

90-150 cm. Compared with the upper layer, it is more light gray, damp, medium loam, fractured crust, black spots of oil are rare, rust spots are seen, and the color passes to the next layer.

150-196 cm. The color is lighter than before, with high moisture content, fractured crust, almost no oil residues, less dense, sandy gravel and rust spots are seen and ground water comes from 196-200 cm.

Based on the abovementioned data, as a result of oil contamination, the morphological features, physical and mechanical properties of the soil underwent a complete change. In addition, water retention, transfer and air properties have also been completely changed. Previously planted cotton fields and plants growing in different seasons of the year were completely destroyed.

The level of contamination was estimated in soil samples and based on results the amount of oil in the areas adjacent to the oil field reached 174g/kg in. Due to technical failures around the oil field and spillage into the environment, resulting in a large amount of oil retained in the soil. Soil contamination is 16 years, and oil in soil has been retained for 16 years, while the natural self-purification process of the soil is very slow (Figure 2).

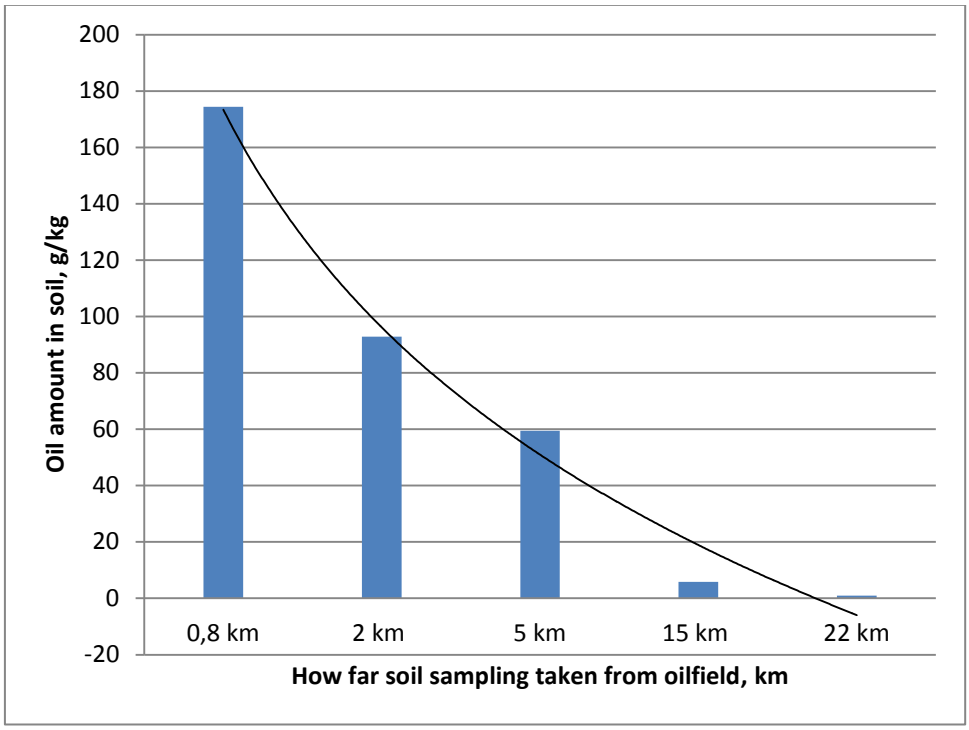



Fig. 2: The condition of soil contamination in Mingbulak oilfield, g/kg

Other soil samples from 2 and 5 km away from the study area were subject to similar changes, while the remaining 15 and 22 km distances were not significantly changing.

To purify these highly contaminated soils, isolated oil-producing bacteria cultures from the soils of the same region were identified and their oil decomposition properties at different concentrations of oil (Table 1).

TABLE 1: Feature of dissolved bacteria cultures to different oil concentrations

| Experiment variants | Oil concentration (%) | Soil cuts separated by bacterial cultures | Activity of Destructive Process (per day) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 28 | 34 |
| Control | 0,5 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Control | 6 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Control | 15 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Control | 25 | - | | - | - | - | - | - | - | - | - | - | - | - |
| A | 0,5 | 2 | ++ | ++ | ++ | ++ | | | | | | | | |
| A | 1 | 2 | - | + | + | ++ | | | | | | | | |
| A | 3 | 2 | ++ | +++ | +++ | ++++ | ++++ | | | | | | | |
| A | 6 | 2 | - | - | ++ | ++ | ++ | ++ | ++ | +++ | | | | |
| A | 10 | 2 | - | - | + | ++ | ++ | + | ++ | + | + | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | + | + | ++ | ++ | | | |
| A | 12 | 2 | - | - | ++ | ++ | ++ | ++ | +++ | +++ | ++++ | +++++ | | |
| A | 25 | 2 | - | - | - | + | + | ++ | ++ | +++ | ++++ | ++++ | +++ | +++ |
| B | 0,5 | 5 | + | ++ | +++ | | | | | | | | | |
| B | 1 | 5 | + | ++ | ++ | +++ | | | | | | | | |
| B | 3 | 5 | + | + | + | ++++ | +++ | | | | | | | |
| B | 6 | 5 | + | + | ++ | ++ | +++ | +++ | | | | | | |
| B | 10 | 5 | - | + | + | ++ | +++ | +++ | +++ | | | | | |
| B | 12 | 5 | - | ++ | ++ | ++ | ++ | +++ | +++ | ++++ | +++++ | | | |
| B | 25 | 5 | - | - | ++ | ++ | +++ | +++ | +++ | +++ | +++++ | ++++ | ++++ | |

**Note:** - decomposition does not occur; + passive; ++ average; +++ high; ++++ very high oil decomposition rate. A- Oil of Mingbulak oil field, V- Oil of Kokdumalak oil field.

In order to determine the oil breakdown properties of isolated bacterial cultures, 2 oil fields were used: the Oil of Mingbulak oil field, the Oil of Kokdumaluk oil field, which revealed bacteria's ability to decompose in oil contaminated soils of other places.

The different levels of oil decomposition of the oil-breaking bacteria isolated from soils were achieved for 34 days, including the use of non-concentrated oils in the nutrient environment as a control, with no destructive process observed. In the experiments, the concentration of oil ranged from 0.5% to 25%. The destructive process occurred at different times according to the amount of oil, namely 0.5% oil concentration was dissolved in Variant A for 8 days, Variant B for 6 days, and 1% oil in Variant A for 8 days and Variant B for 8 days. In the first 2 and 4 days, 10% of the oil was in the form of Variant A, the decay started on the 6th day and ended on the 18th day In Variant B, 10% oil decomposition was not observed for the first 2 days, but unlike Variant A there was a light decay on the 4th day and on the 14th day decay was ended. The dissolution of the highest oil content, that is 25% of the variant A, was not observed for 2, 4, 6 days, the destructive process started on the 8th day and ended on the 34th day. In Variant B, no destruction process was observed for the 2th, the

4th day, starting at the 6th day and ending at the 28th day. Based on these results, it can be concluded that oil contaminated soils contain bacteria with different destructive activity, and in laboratory experiments it is important to separate the most active ones.

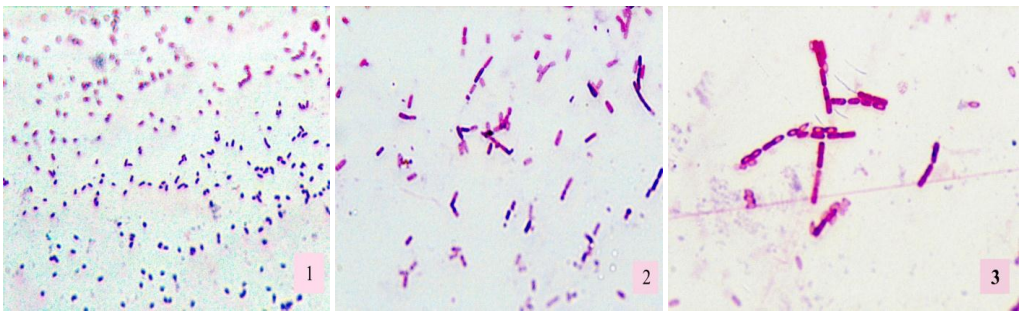
As a result of the research, 3 highly active oil-breakinging bacterial strains were isolated from grass-alluvial soils contaminated with oil. Their physiological characteristics are as follows:

**MFD-100 strain** has high oil decomposition activity, size 1.5-2x0.7-0.8 microns, single-celled, straight-shaped, edges rounded, mobile, aerobic, colored colony in nutritive "meat peptone agar", creates a white curtain in the nutrient environment "agar pepton soup". Gelatin has a clear hydrolysis process, high activity in milk and starchy nutrients, absorption of nitrates to nitrite. Sodium chloride grows well in up to 7% of the environment, with an optimal growth temperature of 20$^{o}$C-45$^{o}$C and survival at 50 $^{o}$C. It grows due to carbon reserves in oil.

**MFD-200 strain** has high oil decomposition activity, size 2-2.2x0.6-0.7 microns, single-celled, straight-edged, motile, aerobic, in "meat agar peptone" the colorful round edge colony, while in "meat peptone soup" forms a grayish-yellow curtain. Hydrolyzing process is high compared to gelatin, high activity in catalase, milk and starchy nutrients, absorption of nitrates to nitrite. In "Ashby" and "Fedorova" nutrient environments it grows well, have low acetone reactions, have higher activity than glucose, mannit, aratin, sucrose, ramnose but do not absorb dultsite. The absorption of phenylalanine is low, it growths fast in sodium chlorine up to 7%, and an optimal growth temperature of 20-45°C, it also growths at 50°C, minimum low temperature, where it growths is 10°C and it is active in the pH range of 5.0-8.5. It grows due to carbon reserves in oil.

**MFD-5000 strain** has high oil decomposition activity, 3-4x0.6-0.7 microns, straight shaped, spores, high growth in "fish peptone agar" and "meat peptone agar", aerobic, in the nutrient environment "fish peptone agar" forms colony, the absorption of Phenylalanine is high and it is neutral in sucrose. Its optimum growth temperature is 25-45°C, the growth limit is 55°C for high temperatures, the minimum growth limit is 10°C, enaerob, it lives in 6.0-8.5 pH, it increases in the concentration of salt to 0.5-7%.

The electron microfiography of bacterial strains isolated from the soil can be seen in Figure 3.




**Note:** 1) MFD-100 Pseudomonas stutzeri, 2) MFD-200 Pseudomonas caryophyllis, 3) MFD-5000 Bacillus subtilis strains

Fig. 3: Electron microfotography of oil-breaking bacteria strains

The technology of biological recultivation corresponding to the climatic conditions of the study area and soil properties was developed on the basis of the oil-breaking bacteria strains isolated from the soil. The recultivation technology is based on the use of strains of the oil-destroying bacteria, but the major stages of the development of the great oil refining technology are as follows:

The first stage is the preparation and pre-treatment phase, which takes into account the timing and extent of contamination, and the initial agro-technical treatment (softening, crushing, etc.), and the following scheme of experiments is carried out in the soil layers.

**Scheme of experiment:**

1. Oil contaminated soil, control ($Soil_{control}$);
2. Oil contaminated soil (agrotechnically treated and moisturized, $Soil_{a+m}$);
3. Oil contaminated soil + strains (Soil+Strains);
4. Oil contaminated soil + strains + nitrogen, phosphorus, potassium fertilizers (Soil + Strains + NPK);
5. Oil contaminated soil + strains + phosphorus, potassium fertilizers + fertilizer (Soil + Strains + NPK + F);

The second stage is the testing and sampling phase, which is to carry out the experiments based on the developed experimental schemes (nutrients, humidity, air conditioning etc.), to monitor the dynamics of the level of purification, to compare and create the necessary conditions. The application of oil-strain bacteria strains according to soil contamination and soil microorganisms. This phase lasts for 6-8 months;

The third stage continued the second phase with the most effective treatment variant. This phase took 24 months.

The table 2 shows the recultivation process by months and the dynamics of soil purification rate. The recultivation activities were carried out with the different variants of contamination degrees. Oil contamination of 5 g/kg is low; moderate contamination -12 g/kg; heavy contamination - 25 g/kg; at extremely high degree of contamination - more than 25g /kg. According to the results of the recultivation activities, the highest degree of soil purification was up to 82%, which was in weakly contaminated soils. It was achieved 17.04 times faster than the control, and the moderate contaminated variant was 80% and 15.06 times faster compared to the control. The rate of purification was 77.3% in the highly contaminated variant, it was 19.3 times faster than the control, and the degree of soil purification in the extremely high degree of contamination variant 36.1% and 13.8 times faster than the control.

TABLE 2

The purification rate of the different degree of oil contaminated soils by months and its dynamics, %

| Variants | Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 12 | 16 | 20 | 24 |
| | Low contaminated soil | | | | | | |
| $Soil_{control}$ | - | 1,3±0,03 | 2,3±0,02 | 3,1±0,09 | 3,6±0,10 | 4,0±0,12 | 4,8±0,13 |
| Soil+Strains + NPK+F | - | 34,5±1,02 | 43,7±1,21 | 58,7±1,70 | 65,4±1,96 | 74,6±2,23 | 81,8±2,45 |
| | Moderately contaminated grassland-alluvial soil | | | | | | |
| $Soil_{control}$ | - | 1,0±0,03 | 1,8±0,05 | 2,6±0,07 | 3,4±0,10 | 3,9±0,11 | 5,1±0,12 |
| Soil+Strains + NPK+F | - | 31,4±0,91 | 42,4±1,25 | 57,1±1,70 | 62,0±1,86 | 72,1±2,16 | 80,0±2,40 |
| | High contaminated grassland-alluvial soil | | | | | | |
| $Soil_{control}$ | - | 0,6±0,01 | 1,1±0,03 | 1,7±0,05 | 2,0±0,06 | 2,5±0,07 | 4,0±0,08 |
| Soil+Strains + NPK+F | - | 20,0±0,6 | 29,7±0,88 | 44,7±1,33 | 59,1±1,76 | 70,2±2,08 | 77,3±2,30 |

| | Very high contaminated grassland-alluvial soil | | | | | | |
|---|---|---|---|---|---|---|---|
| $Soil_{control}$ | - | 0,3±0,08 | 0,8±0,22 | 1,2±0,03 | 1,6±0,04 | 2,1±0,05 | 2,6±0,075 |
| Soil+Strains + NPK+F | - | 9,3±0,26 | 15,2±0,45 | 19,7±0,57 | 26,4±0,75 | 32,1±0,95 | 36,1±1,07 |

It is important to analyze the situation in the recultivation process. There is a dramatic increase in the level of soil purification during the early and middle periods of recultivation (4-12 months), and in the end of periods (16-24 months), it is observed that soil purification is declining. A distinctive feature of the developed recultivation technology is that the process continues during winter, spring, summer, and autumn seasons. Purification rates are much higher in spring, summer and autumn. Depending on the degree of purification, it is possible to increase the level of purification associated with time consumption, the higher the level of contamination, and the higher the time consuming contamination.

The degree of soil purification varied from 36.1% to 81.82% depending on the oil amount. Three-stage recultivation analysis revealed the effectiveness of soil purification (Table 3).

TABLE 3

Soil purification degree and efficiency of oil-contaminated soils (within 24 months)

| Variants | Oil amount before recultivation (g/kg). | Oil amount after recultivation (g/kg). | Soil purification degree (%). | Efficiency (score). |
|---|---|---|---|---|
| $Soil_{control}$ | 174,0±5,22 | 170,3±5,10 | 2,12±0,06 | 1 |
| Soil+S+NPK+F | 174,0±5,22 | 112,2±4,30 | 36,10±0,52 | 1 |
| Soil+S+NPK+F | 21,5±0,64 | 4,87±0,14 | 77,38±2,31 | 4 |
| Soil+S+NPK+F | 11,75±0,25 | 2,35±0,05 | 80,2±2,4 | 5 |
| Soil+S+NPK+F | 1,21±0,03 | 0,23±0,006 | 81,82±2,45 | 5 |

The following indicators were developed in order to represent the degree of soil purification after recultivation.

**Efficiency indicators:**

1 - soil purification rate - 0-20%;

2 - soil purification rate - 20-40%;

3 - soil purification rate - 40-60%;

4 - soil purification rate - 60-80%;

5 - soil purification rate - 80-100%;

As a result of decreasing oil concentrations in the soil, gradual recovery of its productivity was observed, including the improvement of humus content in the soil, nutrients, general microorganisms, physical and chemical properties. Studies have shown that the humus content in the soil was recovered, the increase in humus was dependent on the concentration of oil in the soil. Before recultivation, humus in 8 soil cut was 0.54%, after recultivation it increased by 0.95%, that is 1.75 times, in 15 soil cut, from 1.40% to 1.50% (increased by 1.07 times). It can be seen that humus recovery depends on the amount of oil in the soil, and the faster the soil is removed from oil, the higher the humus content (Table 4).

TABLE 4

In oil-contaminated soils change in humus content,% (before and after recultivation)

| Height of Soil Cut, cm | Soil cuts | | | | |
|---|---|---|---|---|---|
| | 0,8 | 2 | 5 | 15 | 22 |
| | Before recultivation | | | | |
| 0-30 | 0,54±0,02 | 1,76±0,03 | 1,30±0,04 | 1,40±0,039 | 1,42±0,05 |
| 31-60 | 0,59±0,02 | 0,78±0,02 | 0,84±0,03 | 0,91±0,250 | 0,98±0,03 |
| 61-90 | 0,40±0,01 | 0,51±0,01 | 0,68±0,02 | 0,73±0,200 | 0,76±0,03 |
| After recultivation | | | | | |
| 0-30 | 0,95±0,01 | 1,93±0,06 | 1,38±0,05 | 1,50±0,414 | 1,46±0,05 |
| 31-60 | 0,63±0,02 | 0,80±0,03 | 0,95±0,03 | 1,00±0,025 | 1,19±0,04 |
| 61-90 | 0,52±0,01 | 0,53±0,02 | 0,71±0,03 | 0,85±0,023 | 0,78±0,03 |

The gradual increase of humus content in the soil is due to improved soil environment, reduced oil concentration, increased microorganisms, and improved moisture, air regimes and physical properties.

There was also a recovery of nutrients and pH content in the soil, along with the purification of the soil, which resulted in an increase in total nitrogen, mobility phosphorus and potassium (Table 5).

TABLE 5
Improvement of soil nutrients and pH environment after recultivation

| Soil cuts | pH $H_2O$ | | Total nitrogen % | | Mobil $P_2O$ mg/kg | | Mobil $K_2O$ mg/kg | |
|---|---|---|---|---|---|---|---|---|
| | before | after | before | after | before | after | before | after |
| 0,8 | 6,2 | 6,8 | 0,057 | 0,073 | 19,69 | 21,72 | 198,3 | 218,7 |
| 2 | 6,5 | 7,0 | 0,065 | 0,088 | 20,54 | 24,72 | 192,8 | 221,2 |
| 5 | 6,8 | 7,0 | 0,067 | 0,084 | 24,32 | 27,83 | 195,7 | 224,7 |
| 15 | 6,4 | 6,7 | 0,049 | 0,062 | 32,87 | 37,08 | 188,0 | 236,1 |
| 22 | 5,9 | 6,8 | 0,070 | 0,078 | 19,45 | 21,04 | 196,6 | 216,4 |

According to the results, when the soil is contaminated with oil, its pH environment becomes acidic, and after recultivation it changes to neutral, for example, the pH of 0.8 soil cut is 6.2, after recultivation this figure is 6.8. In 2 soil cut, it changes from 6.5 to 7.0, in 5 soil cut it changes from 6.8 to 7.0, in 15 soil cut from 6.4 to 6.7, and in 22 soil cut 5.9 to 6.8. Total nitrogen also increased after recultivation, for example in 0.8 soil cut 0.057% before recultivation and 0.073% after recultivation, in 2 soil cut, it changes from 0.065% to 0.088%, in 5 soil cut it changes from 0.067% to 0.084%, in 15 soil cut from 0.049 to 0.062% and in 22 soil cut increased from 0.070% to 0.078%. The amount of mobile phosphorus was also significantly increased, as a result of recultivation it increased from 19.69 mg / kg to 21.72 in 0.8 soil cut, from 20.54 mg/kg to 24.72 mg/kg in 2 soil cut; from 24.32 mg/kg to 27.83 mg / kg in 5 soil cut, from 32.87 mg / kg to 37.08 mg / kg in 15 cuts, from 19.45 mg/kg to 21.04 mg/kg in 22 soil cut. The amount of mobile potassium also increased after recultivation, according to the results from 198.3 mg/kg to 218.7 mg/kg in 0.8 soil cut, from 192.8 mg/kg to 221.2 mg/kg in 2 soil cut, from 195.7 mg/kg to 224.7 mg/kg in 5 cuts, in 15 soil cut from 188.0 mg/kg to 236.1 mg/kg and in 22 soil cut from 196.6 mg/kg to 216.4 mg/kg. The above results show that the recovery of soil fertility performance is inextricably linked to the reduction of oil concentration in the soil, and it is also time-consuming, which means that over time the recovery of soil properties and productivity will intensify.

## 4. CONCLUSION

Very high oil contaminated soil (12 g/kg and more) contributes in strong change of the soil morphological features, getting dark black, intensifying density, and formation of large soil fragments. The separation and application of strains of oil-breaking microorganisms from oil contaminated soils is highly effective for recultivation of oil contaminated soils. Strains MFD-100 Pseudomonas stutzeri, MFD-200 Pseudomonas caryophyllis, and bacterial strains MFD-5000 Bacillus subtilis are separated in these studies. It can be concluded that bacterial strains separated from the soil of one region do not have high effect on the soil of the other region, so it is advisable to separate oil breaking bacteria strains from each contaminated soil. In the recultivation of very high oil-contaminated soils (25g/kg), the first mechanical treatment, namely the removal of large particles in the form of oil + soil, and agro-technical processing were highly effective. As a result of recultivation of oil contaminated soils, the soil was purified up to 81.82%, and after recultivation the soil fertility recovery was achieved.

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