Influence of oil pollution on the surface of the microbial community and catalase activity of sod-podzolic soil

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Abstract. The article provides data on the influence of different levels of oil pollution on the structure of the microbial community and the activity of catalase in sod-podzolic soil during the cultivation of barley and wheat. It also presents data on the effect of biopreparations based on oil-oxidizing bacteria on the total number of microorganisms, microscopic fungi, actinomycetes and catalase activity. Based on the analysis of the microflora of oil-contaminated soddy-podzolic soil, it was found that oil pollution affects the total number of microorganisms, the qualitative and quantitative composition of micromycetes, and actinomycetes. It was also found that small concentrations of oil stimulate the number of microbiota and catalase activity. The introduction of biological products based on oil-oxidizing bacteria has a beneficial effect on the indicators of the general biological activity of the soil. It is noted that the selection of a phytomeliorant is of great importance in the reclamation of non-contaminated soil.

1. Introduction

Some of the most dangerous environmental pollutants are oil and oil products. They are especially dangerous for soil ecosystems, changing their physicochemical properties and biological activity.

Oil has a varied composition, due to which there may be some difficulties in cleaning oil-contaminated soils, and against which there is a wide range of microorganisms that use various oil components for nutrition [1-2].

At present, biological methods of soil purification are the most promising. It is noted that the plants grown on soils contaminated with oil have a lower content of pigments involved in photosynthesis and a higher amount of carotenoids [3]. It is this fact that subsequently affects the increase in the yield, slowing it down.

The main part of the soil microbiocenosis is made up of heterotrophic microorganisms. Most of them are represented by actinobacteria and micromycetes, and the share of hydrocarbon-oxidizing microorganisms is 0.01% [4-5]. Hydrocarbon oxidizing microorganisms are capable of producing biosuractants. These compounds are chemically diverse, multifunctional, and are characterized by reduced toxicity compared to synthetic surfactants. Currently, biosuractants of microbial origin are widely used in industry and bioremediation [6]. According to [7-9], bioremediation is a cost-effective way to reduce oil pollution in the soil.

When studying the oil-decomposing ability of mangrove deposits, it was noted that fungi more efficiently and quickly decompose almost all fractions of oil in comparison with other microorganisms.
Data on the communities of micromycetes in reclaimed soils are often limited to data on the content of fungal propagules in the soil. It is known that in oil-contaminated soils, the total number of fungi, in particular, toxin-forming fungi, increases. Their soil enzymes are the most informative indicator for the remediation of oil-contaminated soil is catalase, which characterizes the potential ability of the system to maintain homeostasis. The use of biological methods in soil reclamation is practically impossible without knowledge of the specifics of microbial communities at various levels of oil-contaminated soils. The study of the quantitative and qualitative composition of micromycetes, actinomycetes and soil enzymes remains an especially insufficiently studied issue.

The purpose of the research is to assess the effect of various concentrations of oil on the structure of the microbial community and the activity of catalase in sod-podzolic soil using microbiological destructors.

2. Materials and methods

Studies to study the effect of oil pollution on the structure of the microbial community and catalase activity were carried out in 2016-2019 at the experimental field of St. Pure oil was used as a pollutant. The experience included the use of biologics, Lenoil SHP and Destroil. Kirsanov's vessels (5 kg capacity) were used to set up the experiments. In a separate container, the soil was contaminated with oil, mixed, and a suspension of biological products was added according to the manufacturers' recommendations. Oil was added in the following concentrations: 10 ml (2000 mg / kg), 30 ml (6000 mg / kg), 50 ml (10000 mg / kg). Mineral fertilizers (according to Knop) were added to the vessels per vessel: potassium chloride (KCl) - 1 g and double superphosphate (Ca (H2PO4) 2 x H2O) - 1.2 g, ammonium nitrate (NH4NO3) - 1.2 g / vessel. Soil samples for microbiological studies were taken 15 and 90 days after the experiments were set up. The total number of microorganisms was determined in the following concentrations: 10 ml (2000 mg / kg), 30 ml (6000 mg / kg), 50 ml (10000 mg / kg). Mineral fertilizers (according to Knop) were added to the vessels per vessel: potassium chloride (KCl) - 1 g and double superphosphate (Ca (H2PO4) 2 x H2O) - 1.2 g, ammonium nitrate (NH4NO3) - 1.2 g / vessel. Soil samples for microbiological studies were taken 15 and 90 days after the experiments were set up. The total number of microorganisms was determined by the method of quantitative dilution (according to Koch). To determine the quantitative and qualitative composition: micromycetes used Czapek's medium, oat agar - for actinomycetes. Catalase activity was determined by the method of A.Sh. Galstyan using barley cv Belogorskiy and wheat cv Darya as test cultures.

Characteristics of the used bioperates.

The bacterial preparation Lenoil SCHP - consists of freeze-dried bacteria *Acinobacter calcoaceticus* and *Ochrobactrum intermedium*. Biological product Destroil - consists of microorganisms with carbon-oxidizing activity.

3. Results

The general assessment of the dynamics of the number of bacteria in the course of the experiment showed the dependence of the qualitative and quantitative composition of the soil microbiocenosis on the concentration of oil products and the exposure time. It was found that the introduction of a hydrocarbon pollutant of 2000 mg / kg stimulates the growth of the total number of bacteria, which is associated with the possibility of oil utilization by the soil microbial complex and an unexpressed toxic effect.

During the experiments, we noticed that at oil concentrations in the range from 6000-10000 mg / kg, it already acts as an active modifier of the microbiological properties of the soil. At this level of concentration of oil and oil products, there is a significant decrease and redistribution in the composition of the microbiocenosis in comparison with the control and the NPK-background variant when growing both test-object cultures (table 1).

It was found that the introduction of biological products stimulated the growth of the number of microorganisms. Moreover, the biological product Lenoil SHP proved to be the most effective. Perhaps this is due to the high destructive activity of microorganisms that are part of this drug, during the decomposition of oil hydrocarbons, which in turn contributes to the development of the total number of microorganisms.
The data obtained in the study demonstrate that 15 days after the experiment was set up, the maximum decrease in the number of microbiota in the oil-contaminated soil is observed, and after 90 days, its increase, and to a large extent in the variants with the use of biodestructors (Table 1).

Table 1. Influence of oil pollution on the total number of microorganisms.

| Experience options                  | The number of microorganisms thousand CFU per 1 g of soil |
|-------------------------------------|----------------------------------------------------------|
|                                     | barley 15 day | 90 day | wheat 15 day | 90 day |
| Control                             | 288           | 367    | 267           | 356    |
| NPK background                      | 520           | 677    | 450           | 600    |
| Background + 2000 mg / kg oil       | 347           | 466    | 304           | 359    |
| Background + 6000 mg / kg oil       | 111           | 190    | 102           | 116    |
| Background + 10000 mg / kg oil      | 27            | 10     | 12            | 5      |
| Background + 2000 mg / kg oil + Destroil | 297       | 338    | 385           | 320    |
| Background + 6000 mg / kg oil + Destroil | 180       | 350    | 132           | 140    |
| Background + 10000 mg / kg oil + Destroil | 29         | 17     | 28            | 54     |
| Background + 2000 mg / kg oil + Lenoil CKhP | 536        | 689    | 512           | 610    |
| Background + 6000 mg / kg oil + Lenoil CKhP | 277       | 440    | 280           | 307    |
| Background + 10000 mg / kg oil + Lenoil CKhP | 67         | 110    | 52            | 82     |
| HCP 0.5                             | 24            | 7      | 10            | 16     |

Our data show that in the NPK + 10,000 mg / kg variant of the entire experiment, the minimum amount of the total number of microorganisms was observed.

It was revealed that when growing barley, the total number of micropopulations in all variants of the experiment, except for variants with the introduction of the biological product Destroil, is higher than when growing wheat, which indicates a greater resistance of the crop to oil pollution.

The total number of soil micromycetes corresponds to the ecological state of the soil.

One of the goals of our work was to identify the number of soil micromycetes under the conditions of the use of biological products for the restoration of oil-contaminated soil when growing plants, i.e. assessment of the influence of three factors (pollution, the introduction of biological products and the use of a test culture) on micromycetes of sod-podzolic soil.

During the experiment, it was found that 15 days after the experiment, in contrast to the total number of microorganisms, the number of micromycetes also increased with the addition of 30 ml of oil compared with the control option (clean soil) and with the NPK option (Table 2). The maximum number of micromycetes was noted with the introduction of the biological product Lenoil CXP. The results of our experiments showed that in relation to microscopic fungi, oil was not a strong toxicant, and the used pollutant practically does not affect their amount. On the contrary, the introduction of 10 and 30 ml of concentration created favorable conditions for the development of micromycetes. In the opinion of many authors, individual oil components in this concentration range act as biological stimulants. Apparently, the increase in the number of microscopic fungi is associated with increased sporulation under stress conditions (the presence of high concentrations of oil). The process of sporulation contributes to the protection of the spore-forming microflora from the negative influence of xenobiotics; in this regard, micromycetes develop intensively in oil-contaminated soil, exceeding the control level in numbers.

It should be noted that when growing barley, the number of micromycetes is higher than when growing wheat. The peak of their number was noted when using the biological product Lenoil SKhP in the variant of 6000 mg / kg of oil on the 90th day of the experiment when growing barley.
Table 2. Influence of oil pollution on the number of microscopic fungi of sod-podzolic soil when polluted with oil.

| Experience options                                      | The number of microscopic fungi, 1 g of wet soil, thousand CFU |
|--------------------------------------------------------|-----------------------------------------------------------------|
|                                                        | barley after 15 days | after 90 days | wheat after 15 days | after 90 days |
| Control                                                | 22                  | 30            | 18                  | 31           |
| NPK background                                         | 27                  | 47            | 36                  | 28           |
| Background + Background + 2000 mg / kg oil             | 34                  | 40            | 26                  | 21           |
| Background + 6000 mg / kg oil                          | 40                  | 44            | 32                  | 6            |
| Background + 10000 mg / kg oil                         | 28                  | 16            | 14                  | 7            |
| Background + 2000 mg / kg oil + Destroil                | 25                  | 48            | 19                  | 15           |
| Background + 6000 mg / kg oil + Destroil                | 35                  | 45            | 17                  | 26           |
| Background + 10000 mg / kg oil + Destroil               | 27                  | 17            | 7                   | 10           |
| Background + 2000 mg / kg oil + Lenoil CKhP             | 38                  | 58            | 34                  | 47           |
| Background + 6000 mg / kg oil + Lenoil CKhP             | 48                  | 56            | 43                  | 51           |
| Background + 10000 mg / kg oil + Lenoil CKhP            | 36                  | 44            | 16                  | 14           |
| НСР 0.5                                                | 4                   | 3             | 5                   | 3            |

Identification of colonies grown on Czapek’s medium revealed that *Penicillium* and *Aspergillus* proved to be dominant in the micromycete complex in the stress (oil pollution) zone.

The study of the number of actinomycetes showed that with an increase in the concentration of xenobiotics, a decrease in the number of this group of organisms was observed.

This phenomenon is possibly associated with the formation of an oil film and, as a consequence, a violation of redox processes in the soil, as well as a change in the C: N ratio towards creating immobilization of nitrogen and a deterioration in nitrogen nutrition.

The introduction of biological products favorably affected the number of this group of bacteria. Moreover, the effect of the introduction of the destructor was largely manifested on the 90th day of the experiment, especially in the variant with the use of Lenoil CXP against the background of the introduction of 2000 mg / kg of oil. Perhaps this is due to the fact that for actinomycetes, as well as for fungi, oil is an available source of both nitrogen and carbon. In addition, an increase in the number of actinobiota at the end of the growing season may be due to the appearance in the soil of exudates of plant roots and fresh dead plant residues rich in readily soluble organic carbon compounds, which serve as a source of nutrition for this group of organisms.

The above studies allow us to assert that the complex of actinomycetes was not distinguished by a variety of species. In variants with the introduction of 2000 and 6000 mg / kg, streptomycete colonies were observed. It is known from the literature that bacteria belonging to this genus are the most resistant to oil pollution.

The next stage of our research was the assessment of the catalase activity of soils. It is known that in certain types of soils the so-called “pool” of enzymes accumulates, each of which is characterized by a special qualitative and quantitative composition.

In the course of research, it was found that the activity of catalase increased when the soil was contaminated with oil at concentrations of 2000 mg / kg and 6000 mg / kg. Against the background of the introduction of biodestructors, an increase in the catalase activity was observed in the same variants and retained the same tendency towards the end of the experiment (on the 90th day). Moreover, in the variant with the use of the biological product Lenoil SCHP, the highest catalase activity was noted on the scale of comparative assessment of biological activity in both test cultures. It was also revealed that the activity of catalase in the cultivation of barley was almost two times higher than in the cultivation of wheat (table 4).
Table 3. Influence of oil pollution on the number of actinomycetes of sod-podzolic soil with oil pollution.

| Experience options | The number of actinomycetes in 1 g of wet soil, thousand CFU | barley | after 15 days | after 90 days | wheat | after 15 days | after 90 days |
|---------------------|-------------------------------------------------------------|--------|---------------|--------------|--------|---------------|--------------|
| Control             | 208                                                         | 305    | 198           | 22          |        |               |              |
| NPK background      | 290                                                         | 409    | 255           | 38          |        |               |              |
| Background + Background + 2000 mg / kg oil | 166                                                         | 302    | 266           | 30          |        |               |              |
| Background + 6000 mg / kg oil | 108                                                         | 85     | 120           | 9           |        |               |              |
| Background + 10000 mg / kg oil | 16                                                          | 7      | 9             | 2           |        |               |              |
| Background + 2000 mg / kg oil + Destroil | 277                                                         | 327    | 334           | 385         |        |               |              |
| Background + 6000 mg / kg oil + Destroil | 130                                                         | 18     | 117           | 128         |        |               |              |
| Background + 10000 mg / kg oil + Destroil | 31                                                          | 12     | 14            | 21          |        |               |              |
| Background + 2000 mg / kg oil + Lenoil CKhP | 245                                                         | 380    | 305           | 398         |        |               |              |
| Background + 6000 mg / kg oil + Lenoil CKhP | 167                                                         | 190    | 156           | 202         |        |               |              |
| Background + 10000 mg / kg oil + Lenoil CKhP | 77                                                          | 24     | 32            | 67          |        |               |              |
| НСР 0.5            | 14                                                          | 11     | 13            | 6           |        |               |              |

Table 4. Dynamics of catalase activity in oil-contaminated soil using oil biodegradants.

| Experience options | Catalase activity in ml of O₂ released in 2 min per 1 g of soil | barley | after 15 day | after 90 day | wheat | after 15 day | after 90 day |
|---------------------|---------------------------------------------------------------|--------|--------------|--------------|--------|--------------|--------------|
| Control             | 2.2                                                         | 2.4    | 2.3          | 2.2          |        |               |              |
| NPK background      | 6.0                                                         | 7.4    | 5.7          | 6.6          |        |               |              |
| Background + Background + 2000 mg / kg oil | 7.0                                                         | 7.6    | 3.4          | 7.0          |        |               |              |
| Background + 6000 mg / kg oil | 2.7                                                         | 8.6    | 3.0          | 9.4          |        |               |              |
| Background + 10000 mg / kg oil | 1.1                                                          | 0.9    | 0.9          | 1.6          |        |               |              |
| Background + 2000 mg / kg oil + Destroil | 9.0                                                         | 10     | 8.6          | 12.0         |        |               |              |
| Background + 6000 mg / kg oil + Destroil | 7.4                                                         | 16.5   | 7.0          | 14.1         |        |               |              |
| Background + 10000 mg / kg oil + Destroil | 1.1                                                          | 1.3    | 0.6          | 1.8          |        |               |              |
| Background + 2000 mg / kg oil + Lenoil CKhP | 6.9                                                         | 14.2   | 6.9          | 15.4         |        |               |              |
| Background + 6000 mg / kg oil + Lenoil CKhP | 9.7                                                         | 20.6   | 10.6         | 19.3         |        |               |              |
| Background + 10000 mg / kg oil + Lenoil CKhP | 2.2                                                         | 2.7    | 1.4          | 1.9          |        |               |              |
| НСР 0.5            | 0.7                                                          | 0.9    | 0.5          | 0.6          |        |               |              |

Thus, the use of biodegradants significantly increases the catalase activity, in particular, the biological product Lenoil SCHP.

4. Discussion

Our studies to assess the effect of different concentrations of oil pollution on the structure of the microbial community and catalase activity in soddy-podzolic soil showed that the studied microorganisms react differently to the pollutant. For example, the number of fungi increases and actinobiota decreases.

It was also found that oil pollution at a level of 2000 mg / kg is not toxic to most soil microorganisms. The manifestation of the Arndt-Schultz effect, which consists in the fact that toxic oil compounds, accumulating on the cell surface in small concentrations, change the membrane
permeability, thereby disrupting its barrier and protective functions, determines the free flow of nutrients into the cell and, accordingly, an increase in metabolism.

Redox enzymes also play an important role in the decomposition of petroleum products. The highest activity of this enzyme, as well as of microbial communities, was noted in the variant with the use of the biological product Lenoil SCHP, since favorable conditions are created for the growth and development of aboriginal microflora, and this, in turn, affects the intensity of biodegradation processes.

Based on all the results obtained, it can be summarized that the composition of microorganisms included in biological products is important in accelerating the process of oil biodegradation. The correct selection of the test culture is also important. In our experiment, barley proved to be the most tolerant to oil pollution. Perhaps this is due to the genetic characteristics of the stability of this culture.

5. Conclusion
Based on the analysis of various aspects of the structure of the microflora of oil-contaminated soddy-podzolic soil, the following conclusion can be drawn: oil pollution affects the number of microorganisms, the qualitative and quantitative composition of micromycetes (in the form of an increase in their number), actinomycetes (in the form of a decrease in their number). It was also revealed that small concentrations (2000 mg / kg) of oil stimulate the number of microbiota and catalase activity. The introduction of biological products favorably affected the general biological activity of oil-contaminated soil, in particular, a biological product based on the oil-oxidizing bacteria Acinobacter calcoaceticus and Ochrobactrum intermedium - Lenoil SKHP - proved to be the most effective. It was noted that barley is more resistant to oil pollution than wheat. These results can be used to diagnose and predict the microbiological state of sod-podzolic soil contaminated with oil hydrocarbons.

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