Bioassay as a method of integral assessment for reclamation of oil-contaminated ecosystems

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The development of new technologies and improvement of existing ones for the restoration of oil-contaminated lands, the neutralization and disposal of oil drilling waste are important measures to solve environmental problems. Bioassay is successfully used to determine the danger of pollution of environmental objects by oil and oil industry wastes. With the help of biotests, it is possible to assess the state of contaminated objects, which is not always possible to do by chemical-analytical methods, considering the complex chemical composition of petroleum hydrocarbons. An analytical review confirms the need to develop a biotesting system to assess the state of ecosystem components in the event of oil pollution and to determine the effectiveness of measures to restore them. Taking into account the nature of the pollution, presented in the article by a number of provisions on the chemical characteristics of petroleum hydrocarbons and the duration of the recovery processes, bioassay should be carried out at each stage of the reclamation process, in dynamics. Determination of the degree of neutralization of contaminated objects consists in a step-by-step transfer of the hazard level from a higher class to a lower one. Bioassay, in this case, remains a mandatory method for determining the total toxicity. In the course of sample preparation for biotesting, it is advisable to consider the possibility of increasing the bioavailability of hydrocarbon components. A prerequisite for the use of biotesting is the use of eluate and contact approaches. The main methods that can be included in the abbreviated scheme for determining the efficiency of remediation of oil-contaminated objects can be considered the method of biotesting using hydrobionts in the eluate (water extract) and phytotesting performed using the contact and eluate approaches. The battery of biological tests included in the extended scheme should be developed taking into account the specific case, taking into account the specifics of the ecosystem components, soil and climatic conditions, the methods of reclamation used, etc. The strategy for the development of biotesting is closely related to the solution of issues on the assessment of the real danger of oil pollution and the neutralization of oil drilling waste, which are among the priority ones.

Keywords: bioassay, phytoassay, drilling slurries, reclamation, hazard class.

Биотестирование как способ интегральной оценки приёмов рекультивации загрязнённых нефтью экосистем

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Разработка новых и совершенствование существующих технологий по восстановлению нефтезагрязнённых земель, обезвреживание и утилизация нефтебуровых отходов – важные меры по решению экологических проблем. Биотестирование успешно применяется для определения опасности загрязнения объектов окружающей среды нефтью и отходами нефтяной промышленности. С помощью биотестов можно оценить состояние загрязнённых объектов, что не всегда возможно сделать химико-аналитическими методами, учитывая сложный химический состав нефтяных углеводородов. Аналитический обзор подтверждает необходимость разработки системы биотестирования для оценки состояния компонентов экосистемы в случае нефтезагрязнения и определения эффективности мер по их восстановлению. Принимая во внимание характер загрязнения, представленный в статье рядом положений по химической характеристике нефтяных углеводородов и длительность процессов восстановления, биотестирование следует проводить на каждом этапе процесса рекультивации, в динамике. Определение степени обезвреживания загрязнённых объектов заключается в поэтапном переводе уровня опасности из более высокого класса в более низкий. Биотестирование, в данном случае, остаётся обязательным методом определения суммарной токсичности. В ходе пробоподготовки образца для биотестирования целесообразно рассмотреть возможность увеличения биодоступности углеводородных компонентов. Обязательным условием применения биотестирования является использование элюатного и контактного подходов. Основными методами, которые могут входить в сокращённую схему определения эффективности рекультивации нефтезагрязнённых объектов, можно считать метод биотестирования с использованием гидробионтов в элюате (водная вытяжка) и фитотестирование, выполненное с применением контактного и элюатного подходов. Батарея биологических тестов, входящих в расширенную схему, должна быть разработана с учётом конкретного случая, учитывая специфику компонентов экосистемы, почвенно-климатических условий, используемых способов рекультивации и др. Стратегия развития биотестирования теснейшим образом связана с решением вопросов по оценке реальной опасности нефтяных загрязнений и обезвреживанию нефтебуровых отходов, относящихся к числу приоритетных.

Ключевые слова: биотестирование, фитотестирование, отходы нефтедобывающей промышленности, рекультивация, класс опасности, экотоксикология.

According to the official statistics of the Ministry of Energy of Russia, the level of oil production in 2020 amounted to 512.8 million tons. In 2019, more than 17 thousand accidents with oil spills occurred at the enterprises of the fuel and energy complex. Of these, 10.5 thousand cases were at oil pipelines [1]. The main sources of pollution by oil and oil products include: oil leaks during transportation, tie-in; oil drilling sludge, untimely liquidation of sludge pits, etc. In the presented review, bioassay is considered mainly in relation to terrestrial soil ecosystems. Natural systems have the ability to regenerate even with high levels of pollution. The process of self-cleaning of natural objects takes quite a long time, especially in extreme climatic conditions [2, 3]. In this case, a special protective function is assigned to soils, in connection with which the use of biotesting methods for assessing the integral toxicity of soil samples is an urgent issue.

Monitoring and control of the remediation process is important at all stages, and assessment of the state of oil-contaminated soils is possible using biological methods. When carrying out biotesting, it is necessary to take into account specific methodological nuances, which are not always clearly spelled out in the methodological recommendations.

For example, the death of Daphnia in a water extract from the waste, established within 48 hours, may not exceed 10%, but the morphological state of the test object indicates a significant negative impact: individuals of Daphnia are smaller than in the control test, their trophic activity is minimal, and only with a longer exposure (96 h) leads to the death of the test organism, indicating an insufficiently objective characterization of the hazard of waste only in terms of lethality [4].

The system of biotesting methods for assessing the effectiveness of methods for reclamation of soil contaminated with oil and waste from the oil industry, first of all, includes a set of biotests recommended by the regulatory document for determining the hazard class of waste (SanPiN 2.1.7.1386-03), but even for them there are nuances that associated with the specificity of the pollutant, which should be paid attention to.

The toxicity of soils, determined by the phytotesting method, directly in the soil is higher than the toxicity of aqueous extracts from the same soils [5]. The difference in the results for soil contamination with toxicants that are poorly soluble in water (oil and products of its transformation) is large. There are disagreements when choosing the optimal indicators for establishing the toxic effect. At the same time, enough experimental material has been accumulated for their scientific substantiation.

The purpose of the review is to summarize the available experimental data on the effectiveness of using biotesting methods to establish toxicity in environmental objects during the remediation of oil-contaminated ecosystem components.

Objects and research methods

This review uses publications of both domestic and foreign authors, as well as information published on Internet resources (74 sources). The materials cover the time period from 2000 to 2021. Relevance in information search was obtained by accessing the following scientific search engines: Russian scientific electronic library eLIBRARY.RU, Google scholar, Scopus/ScienceDirect. The works that meet the search queries: “biotesting”, “phytotesting”, “oil drilling waste”, “recultivation of oil-polluted lands”, “hazard class of oil waste” (in Russian) were analyzed. Foreign scientific electronic resources: Elsevier, Springer, ResearchGate, PubMed, Mendeley were searched by keywords and phrases: “bioassay”, “drilling slurries”, “reclamation”, “pollution of oil products”, “bioassay of oil-contaminated soils ".

Chemical components of oil affecting the toxicity of oil-contaminated samples

Petroleum hydrocarbons, represented by persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs), have a serious impact on the environment, polluting soil and water, and pose a threat to both humans and ecosystem components as a whole [6–9]. Of the entire list of PAHs, as a rule, only benzo[a]pyrene is standardized, which is a carcinogen and belongs to the 1st hazard class. However, this PAH has poor solubility, and chemically more active and readily soluble PAHs are not taken into account in Russian regulatory documents [10]. Thus, the toxicity of petroleum products is determined by the hydrocarbon composition, and the toxicity of a mixture of hydrocarbons is higher than that of its individual components [11]. On the one hand, these provisions force the development of biotesting methods that allow determining possible toxic effects [12] and, on the other hand, resort to the use of sample preparation techniques for biotesting with possible preliminary dissolution of the organic compounds present, thereby increasing the toxicity index of the sample [13]. For example, methanol allows less soluble or bound pollutants to be desorbed and thus can help establish toxicity due to poorly soluble organic pollutants. In some cases, they resort to the use of catalysts capable of increasing the destruction of poorly soluble pollutants in an aqueous extract and not having a negative effect on the results of biotesting [14].

Alkanes, naphthenes and aromatic components can be processed by microorganisms, and the lighter fractions of these compounds can be completely degraded. Cyclic hydrocarbons are heavier fractions that are resistant to bacterial attack [15]. In connection with these provisions on the characterization of hydrocarbon pollution, the battery of biotests is expanding, bacterial test systems are widely used [16]. In addition to hydrocarbon pollution, during accidental oil spills, readily soluble mineral salts are often supplied to the soil, which are present in crude oil in the composition of formation waters and can also have a negative impact on biogeocenoses [17].

The study of the composition of organic compounds contained in oil sludge, which is necessary to determine the toxicity of these samples [18, 19], can be performed at the final stage, based on the results of biotesting, which will significantly reduce the time and financial costs for the chemical analysis of hydrocarbons.

Bioassay as a stage of control of efficiency of recultivation of oil-contaminated areas

In soil reclamation, the following methods are used: mechanical, physicochemical, agrotechnical, microbiological, phytomeliorative [20]. The goal of reclamation is to reduce the oil content in soil and water to safe concentrations. Land reclamation should be carried out in accordance with Russian State Standard GOST R 57447-2017 in two main stages: technical and biological [21]. The decision on reclamation is made on the basis of regional and municipal limit standards for the permissible residual content of oil and its transformation products in soils. This indicator acts as the main and fundamental criterion after reclamation and other restoration work [22]. Determination of the mass fraction of petroleum products in soils according to the regulatory document PND F 16.1:2.2.22-98 is relevant, but the expediency of referring to this indicator is justified provided that the main volumes of the liquid fraction of petroleum products are removed, in cases of monitoring the effectiveness of reclamation, determining further stages of disposal of contaminated samples or the way of operation contaminated object. When quantitatively assessing the level of oil pollution, the fluorimetric method is widely used using the Fluorat-02 liquid analyzer (PND F 16.1: 2.21-98), as well as the methods of gas and gas-liquid chromatography [23].

Another widespread and promising reclamation technology is bioremediation. It is a process in which bacteria, fungi and plants decompose, transform and help remove pollutants while maintaining the integrity of the ecosystem. An increase in microbial density through the introduction of organic substances (compost, sewage sludge, etc.), promotes the acceleration of the decomposition of pollutants. Bioremediation can be carried out using biostimulation of aboriginal microflora or by introducing specialized microbial preparations designed to cleanse polluted ecosystems [24, 25]. Preparations, ameliorants and materials used in this technology are preliminarily evaluated in laboratory conditions using biotest cultures [26].

The regulation of pollutants and the introduction of an ecosystem approach, implying the use of a biotesting system for the remediation of oil-polluted ecosystems, make it possible to realistically assess the detrimental effects on ecosystem components due to the presence of a complex of polluting chemicals that are not determined by chemical analyzes. Not all chemically hazardous compounds can be known, and their metabolites can be formed during biogeochemical processes [27]. At present, biotesting is widely used to control natural environments when justifying the permissible exposure to pollutants [28–30]. Determination of the degree of neutralization of contaminated objects consists in a step-by-step transfer of the hazard level from a higher class to a lower one

[31]. Obviously, to determine the dynamics of the hazard level of oil pollution of ecosystem components, one can use biological tests included in the scheme for the experimental determination of the hazard class of waste (methods of biotesting on hydrobionts and in phytotest mentioned in the regulatory document SP 2.1.7.1386-03). An important condition in determining the hazard class is the exact adherence to the protocol of the procedure in order to accurately determine the toxicity of the sample. According to the Federal Classification Catalog of Wastes [32], most of the names of drill cuttings generated during oil production belong to the fourth class of hazard to the environment, and waste from the production of petroleum products belongs to the third class. This paradoxical information on individual components is in no way consistent with serious environmental disturbances, which once again emphasizes the relevance of choosing additional biotesting methods and revising the criteria for an adequate assessment of the state of the polluted environment (water, soil) with their help. It seems possible to use a reduced and extended biotesting scheme for an integrated assessment of the remediation of ecosystem components. The abbreviated scheme has a strictly limited number of tests (2–3 biotests). Taking into account the specifics of oil pollution (complex hydrocarbon composition, possible presence of saline solutions and other pollutants), it is important that this limited set of tests be performed taking into account the necessary sample preparation and / or methodological approach: in an aqueous medium using a water extract – eluate approach; in a solid sample (for example, oil-contaminated soil) – the contact approach.

The expediency of a combination of eluate and contact biotesting

It is important to take into account that biotesting in an aqueous extract can be accompanied by significantly underestimated results in determining the degree of toxicity, while substrate biotesting allows a more complete assessment of the level of contamination. The main advantage of direct contact is that there is an interaction between the soil and the experimental test organisms, which increases the bioavailability of the contaminant. It is the use of the contact approach in biotesting increases the probability of accounting for presence of heavy metals in oil sludge increasing its toxicity. In this connection, the comparative analysis of phytotoxicity established using different approaches (eluate and applicate) differs significantly [33].

In [34], a comparative analysis of the sensitivity of two approaches (contact and eluate) in determining the toxicity of excess activated sludge of biological treatment of oily wastewater is presented. Biotesting using radish (*Raphanes sativus* L.) and watercress (*Lepidium sativum* L.) seeds showed that aqueous sludge extracts at concentrations of 100, 50, and 25% were non-toxic. During testing by the contact method, directly on the sludge, a suppression of seed germination (100% phytotoxicity) was observed.

Contact tests, in contrast to eluate tests, can increase the bioavailability of pollutants and, thus, increase the toxicity index. The use of substrate bioassay, which provides direct contact of the tested organism with the test sample, allows one to establish the level of cumulative exposure from pollutants present in solid substrates [35, 36].

Thus, a bioassay system for determining soil toxicity should include both eluate and contact methods. Their combination will make it possible to give an objective assessment of soil pollution by firmly fixed or poorly soluble toxicants that create chronic toxicity.

The use of aquatic organisms to assess the toxicity of oil-contaminated objects

The essence of the biotesting method is to determine the effect of toxicants on specially selected test organisms with a sensitivity established under standard conditions with the registration of various behavioral, physiological, or biochemical parameters (test reactions) [37]. Although biotests fail to identify specific toxic compounds, the combination of various test systems used is indicative (but not always sufficient) to determine the ecotoxicity of soils, both contaminated with oil hydrocarbons and those that have been reclaimed [13].

There is evidence that the biotesting method makes it possible to record the toxic effect of aqueous filtrates of oil-contaminated peat, even in cases where the oil content is not detected by instrumental methods (IR spectrophotometry) [38].

In cases where there is a danger of oily products entering water bodies, the use of aquatic organisms in biotesting is justified. Moreover, it should be borne in mind that along with the risk of migration of pollutants into groundwater, lateral washout into water bodies can also occur.

One of the most common test cultures used in ecotoxicological research is *Daphnia magna* Straus. It is a sensitive test organism that reacts sensitively to the presence of oil products of various fractions and mass concentrations in water [39–41]. In experiments [42], the test response was observed in the range of concentrations of oil products from 0.012 to 200 mg/dm^3. *D. magna* was most affected by the heaviest oil fractions of petroleum products. 100% reaction to the effects of a toxicant (ascent to the surface, rotation on the surface on the side, convulsions, impaired respiratory functions) during the first hour from the moment of the experiment was observed at a kerosene concentration of 20 mg/dm^3, oily waste – 0.45 mg/dm^3. Biotesting of drill cuttings and drilling mud using daphnia showed that both toxicants in the concentration range of 1.0–2.0 g/L are toxic, and only the content of 0.5 g/L in water did not have a negative effect on the vital activity of crustaceans – they remained active, reacted positively to light, and made vertical migrations. An increase in exposure to 25 days (chronic experiment) increased the negative effect of drill cuttings on daphnia, and at the maximum concentration for 10 days, all individuals died. By the end of the experiment, the death of daphnia at a concentration of 1.0 and 0.5 g/L was 50 and 10%, respectively [43].

The death of 50% daphnia was observed at a concentration of 0.25 mL/L of heating oil, 1.3 mL/L of diesel fuel and 5.5 mL/L of aviation gasoline. Oil was characterized by a sufficiently high toxicity for cladocerans; the death of 50% of test objects was recorded at a concentration of 0.86 mL/L. The minimum inoperative were 6.1 • 10^{-3} mL/L of fuel oil, 3.7 • 10^{-2} mL/L of diesel fuel, 0.53 mL/L of aviation gasoline and 1.8 • 10^{-3} mL/L of oil [44].

In an experiment with the removal of oil-contaminated soil from lakes, an improvement in the ecological state of water bodies was observed, which affected the life of *D. magna*. After cleaning the lake, neither acute nor chronic lethal effect on *D. magna* was observed, the survival rate of crustaceans in an acute experiment (4 days) was 100%, in a chronic one (30 days) – slightly (by 10%) below the control or at the control level [45].

Based on the results of biotesting on the *D. magna* test culture, it was found that the actual level of harmless concentrations of oil products dissolved and dispersed in natural water at sampling points within the oil pollution spot on the Serebryanaya Volozhka channel (Astrakhan, Russia) is 1.4– 1.5 times lower than the MPC for oil products for fishery water bodies [46].

In all research materials with this test culture, great importance is attached to the duration of the experiment, that is, in an acute or chronic experiment, significant deviations from the control in terms of culture survival were found.

In addition to the lethality of aquatic organisms, indicators such as fertility, growth rate, change in linear dimensions, etc. can be assessed.

Paramecium caudatum Ehrenberg is another crop used in the experimental determination of the hazard class of waste and for biotesting samples contaminated with oil and oil products.

In a series of experiments on the impact of crude oil from the Luginetskoye field, the number of freshwater ciliates of the *P. caudatum* species was determined in laboratory conditions for 18 days until 50% mortality was established [47]. These changes in the number of ciliates show that all the concentrations of crude oil used in the experiment (50, 100 and 200 mg/L) cause an increase in the number of Paramecia on certain days of observation and reduce their number. For ciliates at a concentration of 50 mg/L, the mortality rate of 50% of individuals was established on 18 days of observation, at a concentration of 75 mg/L – on 12 days, with the introduction of 100 mg/L and 200 mL/L of oil – on 6 and 5 days, respectively. Due to the high sensitivity, this test culture and the possibility of automatic fixation of the death of *P. caudatum* [48], the reproducibility of the results significantly increases, which makes this method very promising.

Another indicator of toxicity when using the biotesting method with a culture of Paramecia can be the chemotaxis reaction based on the ability of ciliates to move in the direction or from the source of chemical exposure. Intact paramecium was placed in a clean drop of water connected to the second drop of a suspension of oil-contaminated soils. On day 1, the control individuals were almost evenly distributed in both drops of water; later they were concentrated in one of the drops of pure water [49].

The problem of secondary pollution often arises after the remediation of oil spill lands. The obtained results of secondary pollution showed that the adsorptive preservation of oil in the soil increases its toxicity, and this has a detrimental effect on living organisms and on the state of the soil as a whole. Changes in the survival rate of *P. caudatum* ciliates were studied at different periods of storage of samples of extracts from soils with different periods of oil pollution. It was found that with prolonged conservation of oil-contaminated soils (up to 6 months), the survival rate of ciliates decreased, and the toxicity of aqueous extracts increased [50].

Water extracts of oil-contaminated soils, containing 300–10000 mg / L of oil products, slowed down the reproduction processes and caused the death of some of the least resistant individuals of the protozoan population; compared with a diluted nutrient medium). Thus, we can talk about the manifestation of adaptations of resistant individuals [49].

It is known that one of the regularities of the toxic effect of oil pollution on protozoa is the alternation of suppression and stimulation of their biological functions (abundance, chemotaxis, phagocytosis). The stimulating effect of a potentially toxic substance is provided due to the mobilization of the body's reserve resources, and if the negative effect does not exceed this adaptive resource, then the stimulation can persist for a long time [51].

The test using a representative of the simplest – ciliary ciliates tetrahymen (*Tetrahymena pyriformis* Ehrenberg) is now widespread and generally accepted, convenient in cultivation and testing. Due to the fact that the ciliate is both a cell and an organism, it is possible to assess the effect of toxicants both at the cellular level and at the highest level of organization [53].

Thus, among the considered tests using aquatic organisms, especially in cases associated with oil pollution and potentially possible migration pollution of waters, cultures of *D. magna*, *P. caudatum*, *T. pyriformis* can be recommended for assessing toxicity at all stages of work on recultivation of oil-contaminated areas.

Use of higher plants for toxicity assessment of oil-contaminated facilities

One of the available methods of biotesting contaminated soils is phytotesting – diagnostics using plant organisms, which is carried out under controlled conditions on test plants according to known and measurable characteristics [54].

Studies of the phytotoxicity of oil-contaminated soils using different plant species on different soils are of scientific interest [55]. Phytotesting makes it possible to assess the total pollution, ie, not only oil, but also from other by-substances that pollute the soil during oil production [56].

In [57], an express phytotest was proposed to measure the germination of seeds of white clover (*Trifolium repens* L.). The phytotest was developed on the example of a gray forest soil contaminated with diesel fuel or copper(II), and tested in the course of many years of experiments on the adsorption bioremediation of oil-contaminated soils. The sensitivity of the proposed method is quite high, since it can record a 10% increase in phytotoxicity of oil-contaminated soil at a concentration of the total amount of petroleum hydrocarbons of 1–5 g/kg, which is close to their MPC (1 g/kg) for reclaimed technogenic soils.

Considering that the germination of plant seeds in oil-contaminated soil is mainly determined by the availability of water and oxygen for them, and not by the toxicity of oil [58], plants that are sensitive to this pollution are used as test cultures. For oil products and PAHs, these can be seeds of lettuce (*Lactuca sativa* L.), sorghum (*Sorghum saccharatum* L.), and mustard (*Sinapis alba* L.) [37]. It was found that the rate of decrease in the content of petroleum hydrocarbons at subsequent stages of the recovery process correlates with the level of phytotoxicity, and therefore, these crops can be used to assess toxicity at all stages of bioremediation of soil contaminated with diesel fuel [59]. There is information about a good response of spring wheat seeds to the toxic effect of oil pollutants in the soil [29, 60].

The effect of oil on plants during soil contamination can be divided into direct toxic (stimulating) effect of hydrocarbons and other substances contained in oil, and indirect, in which changes in soil properties and transformation of the soil microbial community are possible [61]. Therefore, to assess the toxicity of samples with oil pollution, it is advisable to include in the phytotesting scheme, in addition to laboratory express methods, long-term chronic (vegetation) experiments.

In long-term experiments, when determining the degree of pollution impact on plants, a combination of factors influencing the bioavailability of pollutants is reflected. For example, as the concentration of oil increases, as well as in soil without plants, the number of saprotrophic micromycetes increases in the rhizosphere and on the surface of plant roots (rhizoplane); however, in the zone of the rhizosphere and rhizoplana this increase is more significant [62, 63].

The discrepancies in the results of assessing the toxicity of oil by the phytotesting method can be dictated by the lack of a unified control, i. e., the changes are associated with the activity of the substrate into which this pollutant enters. The control can be both standard soils provided in a number of regulatory documents for biotesting, consisting of coalinite, sand and high-moor peat, and a substrate with reference additives of nutrients. It has been proven that it is more acceptable to use soil with poor fertilizing nutrient characteristics (GOST R ISO 22030-2009).

Evaluation criteria should be at least two indicators: seed germination and plant growth at the juvenile stage. Germination depends on the internal energy of the plant's seed. Phytotesting methods based on the response of plants to the negative impact of pollutants are capable of providing reliable information about the quality of soils, have high sensitivity, versatility, integrity, and simplicity [54, 64].

The work [65] presents data on phytotesting of oil-contaminated soil by the method of “water extract” and “soil plates”. Phytotesting carried out using the test of the culture of cress (*Lepidium sativum* L.) made it possible to establish patterns in the change in the level of pollution and the state of oil-contaminated substrate during reclamation work using sorbents. Oil was added to the soil at concentrations of 1, 5, and 10% of its mass. During the entire study period, in variants with the use of sorbents, a tendency to an increase in the biometric parameters of watercress seedlings was observed. A vivid example of the effective use of phytotesting in the recovery of highly oil-contaminated soils is the results of studies of samples taken from lagoons-settling tanks using six species of higher plants: rye (*Secale cereal* L.), lettuce (*Lactuca sativa* L.), corn (*Zea mays* L.), watercress (*Lepidium sativum* L.), wheat (*Triticum vulgare* L.), and cabbage (*Brassica oleracea* L.) [13]. In the ecotoxicological analysis, two test parameters were investigated: seed germination and root length. The phytotesting results were compared with the results for acute toxicity according to other biotests: with luminescent bacteria *Vibrio fischeri*, ciliated protozoa *Spirostomum ambiguum* Ehrenberg, with newborn freshwater crustaceans ostracods *Heterocypris incongruens* Ramdohr. The authors, along with the comparability of the results, emphasize that during biogeochemical processes in the soil, more toxic metabolites or compounds can be formed.

Analysis of literature data confirms that phytotesting can be successfully used to detect oil pollution, as well as to assess and control the processes of neutralization of toxicants and remediation of oil-contaminated soils. It is important to use test cultures that are most sensitive to this type of contamination.

Use of additional analyzes to assess the toxicity of oil-contaminated objects

There is growing interest in the inclusion of several toxicity tests during remediation at the same time (with a battery of different analyzes), for a more complete ecotoxicological assessment of contaminated soils.

Such a search was carried out in an experimental work on the screening of the toxic hazard of oil-contaminated bottom sediments, in which the authors used the eluate test for *P. caudatum* in conjunction with the contact test for crustaceans *Ceriodaphnia affinis* Lilljeborg and *Hyalella azteca* Saussure [66]. A group of authors has developed a test system consisting of the microbial strain *Pseudomonas putida* [67]. In another work [68], it is proposed to use the gram-positive bacterium *Bacillus pumilus* KM-21 as a test culture to determine the toxicity of HM and the possibility of this method for assessing the toxicity of oil waste. The authors of [69] propose to use three test objects for ecotoxicological assessment: hydrobionts (*D. magna*), soil microorganisms (soil respiration), and higher plants (*Avena sativa*). In world practice, an integral estimate is widely used, calculated taking into account the results of a set of methods used – the Triad approach [70]. This approach, along with biotesting, which reflects the ecotoxicological characteristics, includes methods of chemical analysis and bioindication parameters taking into account the biological activity and response of microbial communities.

A number of researchers have resorted to the characterization of additional test systems, including a complex of soil enzymes [8]. The presence of these methods is quite justified, since the biological activity of the soil as a "receiver" of the toxicant reflects the soil's ability to transform the pollutant.

Studies by a number of authors [71, 72] indicate the promising application of biotesting soils and water bodies contaminated with oil and oil products not only with the help of higher plants, but also with the help of a bioluminescent test for photobacteria. The use of these methods, which determine the reducing activity of microorganisms in conjunction with growth tests on bacteria and microalgae, reflects the pollution of water and soil with oil products. The sensitivity of contact and eluate tests using microbial test organisms *V. fischeri* (bioluminescence inhibition test) and *Azomonas agilis* Beijerinck (dehydrogenase activity test) has also been reported [73].

Among all potential indicators that could be used to assess the efficiency of bioremediation methods, one can note the activity of soil enzymes, the content of malonic aldehyde, the activity of superoxide dismutase, and the luminescence of *Photobacterium phosphoreum* [27].

The toxicity found in tests based on the use of direct contact with the sample (applicate testing methods) correlates more closely with the results of physicochemical analyzes. Currently, it is the “tests of direct contact” with the soil, for example, with the earthworms *Eisenia foetida* Michaelsen, the standard test GOST 33036–2014, in which bioindicators are in close contact with toxic substances adsorbed on soil particles, that are quite promising. Soil pollution with oil has a long-term negative effect on soil animals, causing their almost complete elimination in the obligate zone of pollution and a sharp decline in numbers even with weak pollution. The toxic effect of oil on earthworms is determined not only by the intensity of pollution, but also by the morpho-ecological characteristics of certain species and their belonging to different natural and climatic zones [74].

Bioassaying aquatic organisms and higher plants to assess oil pollution are well-studied, but not the only methods. The use of bacteria and enzymes as test organisms is currently being actively studied due to the fact that these test organisms are highly sensitive to the pollutants under study and grow well in laboratory conditions. These data can be used to create mandatory methods for biotesting oil-contaminated objects and are included in the biotest system, in which the presence of representatives of all the main kingdoms of the living is important: bacteria, fungi, plants and animals.

Conclusions

Despite the fact that there is no universal test system for the determination of all existing toxicants, biotesting as a method of integral assessment is successfully used as a tool for environmental monitoring. Biotesting has a good potential for evaluating the effectiveness of technologies for cleaning and restoring oil-contaminated components of the ecosystem, which is advisable to carry out in dynamics.

Taking into account the low bioavailability of pollutants (hydrophobic organic toxicants), it is advisable to use eluate and contact biotesting approaches as complementary to each other. To improve the accuracy of biotesting results in the course of determining the toxicity of heterogeneous oily components in combination with other toxicants, the possibility of using sample preparation reagents for dissolving/leaching poorly soluble organic compounds should be considered.

The biotesting system can be represented by reduced (basic version) and extended schemes. To select the battery of biotests used in the abbreviated scheme, it is possible to propose methods using aquatic organisms and plants, which are recommended in environmental and hygienic legislation. Hydrobionts *Daphnia magna* Straus or *Paramecium caudatum* Ehrenberg (*Tetrahymena pyriformis* is considered as an alternative test culture) are sensitive test organisms and are able to diagnose both acute and chronic toxicity of aqueous extracts of oil-contaminated objects. Higher plants *Lactuca sativa* L., *Sorghum saccharatum* L., *Avena sativa* L., and *Sinapis alba* L. are widely tested and are used as a test culture for phytotesting oil-contaminated soils. These biotesting methods comply with Russian state standards and methodological recommendations, have a clear execution protocol and are used to assess the effectiveness of reclamation techniques.

The extended research scheme is carried out taking into account the specifics of the contaminated objects, reclamation measures and according to the results obtained in the abbreviated scheme. The extended scheme covers a wide range of tested organisms and their reaction levels: higher plants, soil biota, protozoa, hydrobionts, microorganisms, luminescent bacterial tests, biochemical enzymatic methods, etc. Selection of a complex of sensitive and physiologically different test cultures, determination of informative test indicators, the choice of highly reproducible methods, a summary approach to effective assessment, processing of results, automation of methods are the necessary conditions for the biotesting system to assess the effectiveness of reclamation measures.

It is advisable to perform a chemical quantitative assessment of the hazardous pollutants present on the basis of preliminary results established by the biotesting system, however, this requires the development of an individual approach.

To develop a unified strategy for classifying oil pollutants and determining their real hazard, scientific institutions need to establish communication with regulatory organs that are involved in the prevention of environmental hazards.

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