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Analysis of the feasibility of using fertilizers based on fulvic acids in bioremediation of contaminated soil

Vladyslav Nedoroda[1]*, Ganna Trokhymenko[1], Oleh Kibarov[1]

[1] Admiral Makarov National University of Shipbuilding, 54007, Heroiv Ukrainy ave., 9, Mykolaiv, Ukraine

* Corresponding author: PhD, Vladyslav Nedoroda, nedorodavlad@gmail.com, ORCID iD: https://orcid.org/0000-0002-1046-5114

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Abstract

The use of bioremediation techniques makes it possible to restore the ecological functions of oil-contaminated soils, reduce the concentration of biologically available petroleum compounds, and prevent the spread of pollutants through erosion or groundwater. Enhancing bioremediation helps to accelerate the processes of oil destruction, improve soil structure, and increase the number and metabolic activity of microorganisms. The aim of this article is to outline the results of the study of the phytotoxicity of soil with a high level of oil pollution under the combined impact of fulvic acids and microorganisms. During the experiments, artificially polluted samples of soil were exposed to an oil-oxidizing consortium based on strains of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. The main goal was to determine the effect of fertilizers on the efficiency of microbiological destruction in the root zone of plants. The additional aim was to evaluate the effectiveness of biosurfactant in conjunction with organic fertilizers, those able to enhance plant growth and their development in contaminated soil by inducing growth on its own and lowering soil phytotoxicity through pollutant degradation. Utilizing *Sinapis arvensis* as a bioindicator, bioassay was used to assess the overall feasibility. The analysis of the phytotoxicity of soil samples shows a resulting decrease of 21.46–33.76%, depending on both pollutant and fulvic acid concentrations.

1. Introduction

Large-scale extraction and use of oil and oil products have made them high-priority environmental pollutants. Getting into the environment, petroleum hydrocarbons have a pronounced effect on all components of the ecosystem (Ablieieva et al., 2021). Among them, plants and microorganisms (producers and reducers) deserve special attention since they are the basic leading systems of synthesis and decomposition of organic compounds. The processes of accumulation, transformation, and degradation of petroleum hydrocarbons by plants and microorganisms are actively studied both for risk assessment and for the search for natural mechanisms of detoxification and degradation of anthropogenic pollutants (Ricci et al., 2019; Sinha et al., 2020). The results of such studies were, on the one hand, the identification of strains of destructive microorganisms, with the description of the pathways of microbial catabolism of hydrocarbons, and, on the other hand, the characteristics of the metabolism of some hydrocarbons in plants (Yateem et al., 2008). As a result, microbial degradation of hydrocarbons forms the basis of modern technology for the bioremediation of oil-contaminated environmental objects (Volkogon et al., 2010; Stanojevic et al., 2023). The study of newly identified bacterial strains and how they interact with various environmental conditions has been a long-standing and continuous topic of research for many years to come. Miscroorganism consortium studied in this context include various associations of *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Mycobacterium* and other species with confirmed petroleum biodegradation potential (Das and Chandran, 2011; Mekonnen et al., 2024).

The detection of the effect of increased biodegradation of pollutants in the root zone served as the reason for combining the efforts of plant physiologists and microbiologists in the development of phytoremediation biotechnology – the use of plants and microorganisms associated therewith to improve the soil ecosystem (Tonelli et al, 2022). The advantages of phytoremediation as an economically beneficial, ecologically safe, and aesthetically attractive biotechnology for pollution recovery have been shown by many researchers over the course of time (Hinchman et al., 1996; Sumiahadi and Acar, 2018). Plant-microbial associations and symbioses with flexible metabolism and unique enzyme systems have great advantages when living in adverse environmental conditions, due not only to increased tolerance to xenobiotics but also to the ability to actively remove toxicants (Surriya et al., 2015). Ecological conditions of plant growth (oil pollution of the soil, bioremediation with the introduction of a biosurfactant) affect growth and development and form a non-specific physiological and biochemical response, which is expressed in a change in morphological parameters, a decrease in productivity, the accumulation of pigments and secondary metabolites, the mitotic index of meristematic cells of roots, and degradation of chromosomes. Oil pollution of the soil changes the structure of the microbial complex of the rhizosphere of plants, wherein hydrocarbon microorganisms become dominant. Phytoremediation normalizes the structure of the microbial complex in the rhizosphere of plants, restores the number of cellulolytics, and reduces the number of phytopathogenic forms of microscopic fungi (Bais et al., 2006; Shi et al., 2023). Root exudates as well can improve nutrient availability by altering soil microbial communities, which in turn can enhance plant growth (Zhao, 2020). Root exudates can help in the removal of pollutants, which may enhance ecosystem resilience (Steinauer, 2016). The interactions between plants and microbes can be complex and context-dependent, making it challenging to predict outcomes (Pan, 2022).

An important feature of phytoremediation is its zone specificity, meaning that the use of plants in some soil and climatic conditions does not guarantee their successful use in others. Therefore, the expansion of the spectrum of plant species-phytoremediants and the clarification of the laws of this process are the basis for the successful spread and application of this ecological biotechnology. Scientific studies of plant-microbial associations under conditions of hydrocarbon pollution are based on the belief in the prospects of phytoremediation and are aimed at elucidating its mechanisms. They can serve as a foundation for solving new problems in ecology (Egamberdieva et al., 2017). The impact of petroleum hydrocarbons on soil microflora has been well investigated so far, but research is still ongoing to determine the ecological and functional responses of rhizosphere microbial communities to hydrocarbon pollution (Cherniak et al., 2023). At the same time, the features of the rhizosphere as an ecological niche (nutrient saturation, microorganism numbers, and their physiological activity) suggest a different nature of microflora reactions to pollution compared to soil without plants. The study of the structure and functions of the microbial community of the root zone of plants in conditions of hydrocarbon pollution offers a possibility to find out the causes and consequences of the increase in the degradation of organic pollutants in the soil with plants, which is the basis of the effect of phytoremediation (Da Rosa et al., 2015).

Realization of the adaptive and destructive potential of plants, as well as the microbial complexes associated with them in conditions of hydrocarbon pollution, can lead to increased cleaning of the environment as well as changes in plant-microbial interactions (Liu et al., 2011). The relationship between plants and microorganisms in the rhizosphere consists of mutual influences. The influence of plants on the formation of microbiocenoses in the soil is realized through the provision of a niche for living microorganisms (the root system itself) and root secretions that regulate the development of rhizomicroflora. In turn, the metabolic activity of microorganisms and their ability to produce biologically active substances largely ensure the growth and development of plants (Das and Chandran, 2011; Carolin et al., 2021). The use of fertilizers, for example, based on organic acids, can improve the development of the root structure of plants, which leads to an increase in the efficiency of rhizodegradation. Like humic acids, fulvic acids are high-molecular nitrogen-containing organic acids (Alister et al., 2020). The molecular weight of this acid is small enough to penetrate cell membranes. This contributes to the splitting of dissolved minerals and organic substances into ions. This acid is an excellent conductor of vitamins, and thanks to this, the percentage of assimilation of supplements at the cellular level reaches 95–99% (Ćwieląg-Piasecka et al., 2018; Goranov et al., 2022). The presence of fulvic acid enhances the activity of petroleum-degrading microorganisms, leading to improved degradation rates of hydrocarbons in the rhizosphere. Park et al. reported that the addition of fulvic acid can significantly boost the degradation of total petroleum hydrocarbons (TPH) in contaminated soils, with an increase in degradation efficiency from 30% to 45% (Park et al., 2011). The synergistic effect of fulvic acid with arbuscular mycorrhizal fungi and petroleum-degrading bacteria further enhances phytoremediation outcomes, as seen in various plant species (Germida et al., 2002; Alarcón et al., 2008). Doses of fulvic acids are selected in such a manner that they do not stimulate the transition of metals from the soil to groundwater and do not contribute to the leaching of nutrients. At moderate concentrations, the main effects of fertilizers based on fulvic acids are the regulation of energy and basic biochemical processes of plants, increasing the work of enzymes, normalizing metabolism, and freeing cells from decay products and toxins. Fulvic acid reacts with minerals and breaks them down into particles similar in size to ions, creating fulvates, the smallest possible form of minerals. In this form, minerals are easily absorbed by plant roots and distributed throughout all cells (Zhang et al., 2021). Plants are the basis of any biogeocenosis, and therefore deviations in the biochemical and physiological reactions of plants can be an indicator of ecosystem condition. Similar changes in the environment are used in the bioassay technique. This is an effective and relatively inexpensive approach based on the use of bio-indicators to assess the degree of environmental pollution (Bravin et al., 2010; Ossai et al, 2019).

The aim of the present work is to investigate the phytotoxicity of soil that has been contaminated by petroleum under the combined effect of fulvic acids and an oil-oxidizing consortium based on strains of *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus amyloliquefaciens subsp. Plantarum.*

2. Materials and methods

In order to investigate the effectiveness of the use of plants and oil-destroying microorganisms to reduce the phytotoxicity of contaminated soil, laboratory vegetation studies on phytoremediation of soils contaminated with oily sludge in various concentrations were conducted. Materials for the experiment (soil samples, oil sludge and fulvic acid) were provided by LLC&ARE "KARAT-BIO" (Sumy). For the purpose of the experiment standard soil type no. 2.4N according to good laboratory condition were used (LUFA Speyer, 2024). The artificially prepared soil mixture (the samples were thoroughly mixed with oil sludge and loosened to form a homogeneous loose layer weighing about

70 g each) contained 60%, 70%, and 80% of the pollutant. Fulvic acid (purity ≥ 95%), as a "KARAT-BIO" commercial bioresource-derived product consist of total C (40–52%), total O (40–48%), total H (4–6%), total N (2–6%), with pH in range of 6–8, ach content (15–19%) and water content (5–7%). The research was conducted in a laboratory with controlled lighting, temperature (24±2°C), under constant humidity of the substrate. All experiments had the same duration of 86 days. In the course of the experiment, microorganisms were inserted in combination with fertilizers based on fulvic acids into artificially polluted soil to accelerate the degradation of petroleum hydrocarbons. To determine the effectiveness of bioremediation, oil destroyers based on the *Bacillus* consortium (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus amyloliquefaciens subsp. Plantarum* isolated from Semirenkivsky GKR of Myrhorod district of Poltava region in Ukraine) were used. This consortium of microorganisms has the ability to decompose hydrocarbons by synthesizing surfactants in the presence of oil products as a carbon source. In previous studies, we compared the effectiveness of the biosurfactant at different concentrations (Nedoroda et al, 2021; Nedoroda et al., 2022). Based on the obtained results, a concentration at the level of 4% was chosen for further work. Exactly in this amount, the consortium of *Bacillus* provided the soil with the best growth-stimulating properties and the most effective reduction of phytotoxicity relative to the concentration of the biosurfactant itself. In order to maintain and accumulate the mass of microorganisms, moisture and bioaugmentation took place twice a week.

The effectiveness of phytoremediation was evaluated by the change in the level of phytotoxicity of the soil mixture using the Growth Test Method as one of the methods of bioassay (Hrytsak, 2017). On the basis of the analysis of literature data (Dobrochaeva and Kotova, 1987), Field Mustard (*Sinapis arvensis*) was selected as the most suitable bioindicator for assessing the soil phytotoxicity as one of the most popular native plants presented in the Poltava region, in which tested *Bacillus* strain were isolated.. The seeds of *Sinapis arvensis* were sown in the quantity of 40 per sample, in 4 rows of 10 seeds per row. Shoots height, root length, and weight of dry organic mass were chosen as the basis for soil toxicity assessment. These features were determined on five randomly chosen seedlings in each experiment repetition. All variants of the described experiments and analyses had triple repeatability. Average values were calculated for the obtained results, and for comparison, indicators of standard deviation and the smallest significant difference were used. Statistical processing of the obtained results was carried out at $P < 0.05$ using analysis of variance (ANOVA) in the Statgraph PRO software. Standard deviation and average values was calculated by Microsoft® Office Excel.

3. Results and discussion

Control was carried out for each individual pollution level (60%, 70%, and 80%) at different concentrations of fulvic acids (1%, 0.2%, and 0%). As shown in Figure 1, the effect of reducing phytotoxicity was pronounced when cultivating a bio-indicator under the influence of a biosurfactant in combination with organic fertilizers. It was especially clearly manifested in a change in the linear dimensions of the root system and thereby in its morphology in the direction of the formation of thinner and more branched roots that occupy a larger volume of root exudates. The toxic effect of hydrocarbons on the development of plants in oil-contaminated soil was accompanied by yellowing and drying, which could be associated with the transpiration of toxic compounds through the plant. These findings were consistent with other research on the phytotoxicity of contaminated soil (Cherniak et al., 2022). It is stated that the presence of petroleum products in soils can be used to determine the allowable level of soil contamination as well as the reversibility of degradation processes (Sivkov and Nikiforov, 2021). But such an effect is noted only in the first weeks of the experiment and depends on the period of the primary toxic effect of oil pollution. pH values remained stable throughout the experiment, with slight change from 7.2 to 7.1, which don't have a significant effect on the observed outcome. This stability can imply that other factors were more influential in

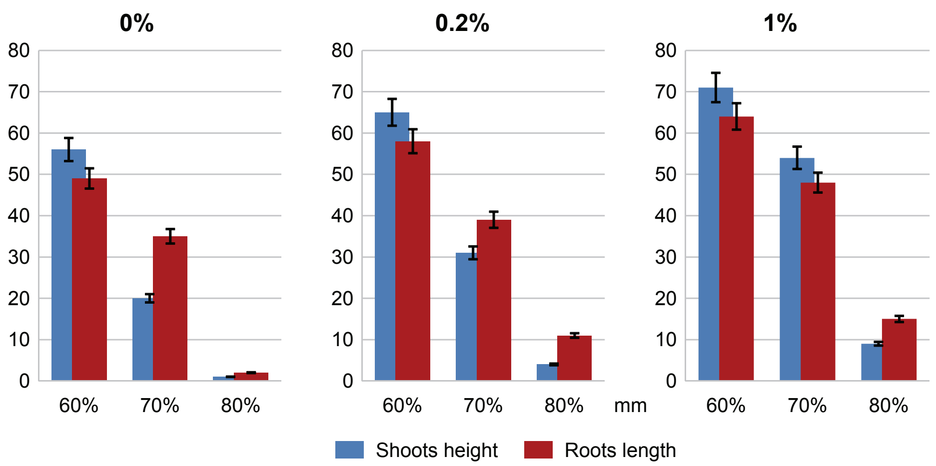

Fig. 1. Results of *Sinapis arvensis* sprouting in artificially polluted soil (60%, 70%, 80% of total petroleum concentration, 0%, 0,2%, 1% of fulvic acid concentration), mm

driving the results. During the experiment, the direct action of the biosurfactant (oil destruction) and the branching of plant roots (rhizodegradation) lead to a decrease in the phytotoxicity of the soil. This is important, first of all, to increase the density of rhizosphere microbial populations, which, in turn, leads to more favorable and stable rhizosphere conditions compared to soil without plants. Thus, the plant under conditions of pollution is a factor that supports and/or increases the number of soil microbial populations, providing them with a niche and additional nutrition for reproduction and performing protective functions against the influence of the pollutant, thereby intensifying the processes of soil recovery.

In addition to root length and shoot height, yielding ability is an important parameter that gives an assessment of environmental phytotoxicity (Table 1). The physiological structure of microbial communities in the rhizosphere is characterized by greater stability and is less prone to changes under the influence of pollutants than in soil without the influence of organic fertilizers due to the branched root system. Contamination of the soil with hydrocarbons leads to a violation of the balance of the main nutritional elements – carbon, nitrogen, phosphorus, etc., which entails significant changes in the physiological structure of the soil microbocenosis. Previous studies have shown that fulvic acid enhances microbial metabolic activities, including organic compound degradation and nitrogen fixation, which are essential for nutrient cycling in the rhizosphere (Zuo et al., 2021; Ling et al., 2022). However, these changes are ambiguous and depend on the presence and activity of microorganisms, whose release can change the balance of nutrients in the soil in different ways, which in turn leads to a change in the organic matter yield.

The change in the morphometric parameters of plants under the influence of hydrocarbons is associated with a change in their biochemical characteristics, which reflect the toxic effect of the pollutant and the plant's adaptive response to stress under conditions of pollution. The analysis of phytotoxicity showed (Table 2) that after 3 months of use of the oil destroyers, the toxicity in combination with fulvic acids decreased by 21.46–33.76%, and in the variant without the impact of biosurfactant, it decreased by 8.93–17.47%. This result is consistent with other research, which concluded that combined use of bacteria and fertilizer is more efficient than alone application of either for improving plant resistance or degradation of petroleum hydrocarbons (Darmayati and Afianti, 2018; Rafique et al., 2023). Calculated results showed there was a significant positive non-linear correlation in each experiment repetition (0,8301, 0,8372, 0,8165) between concentration of fulvic acids and total phytotoxicity effect.

Based on the results of soil toxicity measurements, it is clearly visible that at 60% oily sludge concentration, the effect of 0.2% and 1% fulvic acids is approximately the same. This means that with a similar level of pollution, a low concentration of fertilizers is enough to start the symbiotic process of oil destruction in the root zone of plants. But even with 70% oily sludge in the samples, the specified amount of fulvic acids is not enough; that is, approaching the maximum allowable concentration of the pollutant requires a more significant effect of organic fertilizers to start the same processes. Also, an increase in the concentration of pollution and the further use of fertilizers, biosurfactant and combinations thereof do not have a sufficient effect on plant survival. Therefore, at oily sludge pollution levels of 80% and above, fertilizers only lead to the fact that the seeds germinate, sometimes even earlier than at lower levels of pollution, but the shoots quickly wither and dry since the high concentration of oil products has a critical cumulative effect on the subsequent vegetation plant period. Taking into account such a result, the use of fertilizers at an extremely high concentration of oily sludge is impractical since it does not lead to the activation of the rhizodegradation effect and, as a result, does not improve the efficiency of oil destruction.

Table 1
Weight of dry organic mass / yield per sample, g

Concentration of fulvic acids	Concentration of oily sludge		
	60%	70%	80%
1%	1.403 ± 0.171	0.892 ± 0.088	0.085 ± 0.006
0.2%	1.274 ± 0.190	0.508 ± 0.069	0.063 ± 0.005
0%	0.726 ± 0.064	0.371 ± 0.032	–

Table 2
Phytotoxicity effect of oil-contaminated soil with the use of oil destroyers, %

Concentration of fulvic acids	Concentration of oily sludge		
	60%	70%	80%
1%	66.24 ± 3.05	78.54 ± 4.31	97.95 ± 1.17
0.2%	69.35 ± 4.41	87.80 ± 1.58	98.48 ± 1.39
0%	82.53 ± 2.80	91.07 ± 3.86	100

5. Conclusions

The conducted studies revealed differences in the reactions of microflora to environmental pollution with hydrocarbons, quantitative and qualitative advantages of microbial communities in the branched root zone of plants, which create the effectiveness of phytoremediation and its advantages of stability and longevity. The analysis of the phytotoxicity of soil samples under the conditions of a vegetation experiment shows that in hydrocarbon-contaminated soil, the rhizospheric effect was more significant when using a combination of biosurfactant and organic fertilizers. Thus, the use of fulvic acids in combination with oil destructors in the case of hydrocarbon contamination of the soil with heavy oil fractions (oily sludge) had a pronounced positive effect on vegetation. The most sensitive indicators of soil phytotoxicity were the germination readiness and accumulation of plant biomass. The nature of the phytotoxic effect of the soil depended, first of all, on the concentration of pollution, the use of a biosurfactant, as well as the presence and amount of fertilizers. Thus, a microorganism consortium based on *Bacillus* strain in combination with a fertilizer based on fulvic acids can be used for complex soil bioremediation. This improves the rate of plant growth in hydrocarbon-contaminated soil, both by stimulating soil microbiocenosis and by reducing phytotoxicity through biodegradation of the pollutant. The described studies demonstrated that both plants and microorganisms, and especially their associations, have a significant potential for adaptation and destructive activity in relation to petroleum hydrocarbons. Targeted use of this potential for soil improvement can have a significant effect on the system of phytoremediation technologies. Maximizing the potential of plant-microbial complexes for soil improvement requires the selection and testing of various microorganisms (depending on the deposit), taking into account the zonal specificity of plants, and applying organic fertilizers at certain pollutant concentrations.

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