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MICROBIAL REMEDIATION OF PETROLEUM-POLLUTED SOIL

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ABSTRACT. The issues of land pollution, restoration, and return of land to agricultural cultivation are today. Especially, this is gaining new relevance in modern conditions of military action on the territory of Ukraine and other countries, which causes the reduction of cultivation areas. Therefore, there is a need for maximum cleaning and restoration of polluted soils to ensure environmental and food security. Petroleum hydrocarbons are classified as major environmental pollutants due to their stability and durability in the environment. The influence of petroleum hydrocarbons is caused by direct toxic activity and by the soil environment transformation. The research aimed: to study the effectiveness of probiotics in the technology of soil cleaning and remediation; evaluate the phytotoxic effect of oil-contaminated soil before and after the application of probiotics; to find the optimal concentration of probiotics for the effective cleaning and remediation of soil. The seedling method was used to evaluate the phytotoxic effect of contaminated soil before and after the application of probiotics. Research results showed an ambiguous impact on *Pisum sativum* and *Avéna satíva* at different times after pollution. In the initial phase, polluted soil has no significant influence on *Pisum sativum*. For *Avéna satíva* soil, become toxic right away after pollution. Phytotoxic effect of *Pisum sativum* and *Avéna sativa* decrease by the indexes of seed emergence, roots length, roots weight, underground part length and ground part weight due to probiotics treatment. The high efficiency of biological remediation by probiotics in comparison with soil cleaning in natural conditions is determined in the experiment. Probiotic concentration 1:10 is the most effective of all studied initial concentrations of pollutants. Reducing probiotic concentration leads to a decrease in the efficiency of soil cleaning from petroleum products.

Introduction

The level of environmental pollution increases with industrial growth. Petroleum hydrocarbons are classified as major environmental pollutants due to their stability and durability in the environment. These include alkanes, cycloalkanes, polycyclic aromatic hydrocarbons, and many other organic pollutants (Ambaye *et al.*, 2022). Petroleum hydrocarbons are considered one of the most ecologically dangerous soil pollutants. The source of this pollution is the enterprises of oil refining, petrochemical and chemical industries, transport, waste deposits *etc*. (Fanaei *et al.*, 2020). Soil can absorb many chemical compounds and hold them in the superficial fertile soil layer. Petroleum products change chemical, mechanical, biochemical, and physicochemical soil properties (Hung *et al.*, 2020). These modifications can lead to plant or microorganism death, which impacts soil self-cleaning (Wang *et al.*, 2017). The influence of petroleum products is caused by the direct toxic effect and by the transformation of the soil environment.

The issues of land pollution, restoration, and return of land to agricultural cultivation are today. Especially, this is gaining new relevance in modern conditions of military action on the territory of Ukraine and other countries, which causes the reduction of cultivation areas. Food shortages could cause a global food crisis. Therefore, there is a need for maximum cleaning and restoration of polluted soils to ensure environmental and food security. Soil petroleum pollution has direct and indirect influences on plants. The direct influence consists of the immediate effect of the pollutant on plants. The effect of oil carbohydrates on the cellular and physiological level is manifested by the alteration of photosynthesis and chloroplast structure. These compounds damage chloroplast, mitochondria and root cell membranes. The plants, which grow in the conditions of petroleum product pollution, can contain much more stress-protecting compounds (Mukome *et al.*, 2020). On the other hand, the indirect impact manifests through the change in morphological, physicochemical and biological properties of the soil (Hung *et al.*, 2020). In addition, petroleum pollution can have negative as well as stimulating action on plants. Hence, the determination of the phytotoxic effect is a relevant method to study petroleum-polluted soil.

The level of oil pollution affects not only plants but also microorganisms. As an important part of soil ecosystems, soil microorganisms are mainly involved in the soil material and energy cycles. There are many kinds of soil microorganisms, which mainly include prokaryotes such as bacteria and actinomycetes and eukaryotes such as fungi, algae and protozoa. Bacteria, as the largest proportion of soil microorganisms, mainly use heterotrophic nutrition. They are distributed in the surface soil and are the most active component of soil microorganisms. Actinomycetes are widely distributed in soils with high contents of alkaline and organic matter. Fungi are aerobic microorganisms that exist in the tillage layer. As opposed to bacteria and actinomycetes, fungi can grow and reproduce in acidic soil. Algae and protozoa are not widely distributed in soils. The growth of algae is greatly affected by light and water, and protozoa mainly feed on organic matter and can consume bacteria and algae (Cabral *et al.*, 2022). Therefore, we should choose optimal and environmentally-friendly remediation methods of petroleum polluted soil.

A wide range of methods and technologies for of remediation polluted soil is mentioned in the scientific literature (Asghar *et al.*, 2016; Wang *et al.*, 2018; Ossai *et al.*, 2020; Haider *et al.*, 2021), the usage of probiotics for soil cleaning is not studied thoroughly. The abandoned usage of probiotics is limited by their insufficient studies, the absence of scientific and research bases, the comparative analysis of probiotics, the determination of required doses as well as the profit to obtain a wanted result *etc*. Hence, there is a necessity to further petroleum product influence research on the vegetation and new methods establishment for polluted soil treatment by microorganisms.

The study aimed: 1) to study the effectiveness of probiotics in the technology of soil cleaning and remediation; 2) to evaluate the phytotoxic effect of oil-contaminated soil before and after the application of probiotics; 3) to find the optimal concentration of probiotics for the effective cleaning and remediation of soil.

Materials and Methods

An assessment of the phytotoxic effect of petroleum-polluted soil was carried out in the Laboratory of Agroecological monitoring of Poltava State Agrarian University, Ukraine. The seedling method was used to evaluate the phytotoxic effect of contaminated soil before and after the application of probiotics (ISO 11269-1:2012; ISO 11269-2:2012). This method is based on the test culture reaction to different contaminants in the soil. It permits to detection of the toxic activity or stimulating action of various compounds. As test cultures, we used plants that are capable or not fixing nitrogen and are typical species for contaminants*:* *Pisum sativum* and *Avéna satíva* (Hrytsaienko, 2003). The experiment was performed in four repetitions.

The experiment to a determination of phytotoxic effect on *Pisum sativum* and *Avéna satíva* included the following factors:

Factor A – the duration of petroleum pollution. Variant 1 – the sowing of test cultures on the 2nd day after petroleum pollution. Variant 2 – the sowing of test cultures on the 30th day after petroleum pollution. Variant 3 – the sowing of test cultures on the 180th day after petroleum pollution.

Factor B – petroleum products concentrations (1 000, 2 000, 5 000, 10 000 and 20 000 mg kg^{-1}). The concentration of petroleum products in control (natural condition for podzolic chernozem) was 40 mg kg^{-1}. The samples were taken on the 14th day after sowing.

Factor C – the application of probiotic Sviteco-Agrobiotic-01 (Chrisal NV, Lommel, Belgium; based on *Bacillus subtillis*) with the aim of soil bioremediation. The probiotic was applicated on the second day after petroleum pollution.

The determination of phytotoxicity was evaluated by the following indexes: the test culture seed emergence, roots length, roots weight, ground part length, and ground part weight.

The phytotoxic effect (*PE*) was determined by the Formula 1 (Pisarenko *et al.*, 2019):

$$PE = \left[\frac{Mo - Mk}{Mo}\right] \times 100\%, \tag{1}$$

where *Mo* – the mass or growth index of control soil; *Mk* – the mass or growth index of soil, treated by tested water (where the probiotic is present).

The determination of petroleum hydrocarbonates in soil was determined by infrared spectroscopy IRAFFINITY-1S (Shimadzu, Japan) after the 30th day of contamination (MVV 31-497058-009-2002).

The phytotoxic effect of more than 20% is considered significant. PE <20% – non-toxic; PE = 20–40% – low toxicity; PE = 40–60% – medium toxicity; PE = 60–80% – dangerously toxicity.

Results

Phytotoxic effect of petroleum polluted soil on *Pisum sativum*

The research results (Table 1) show that in Variant 1 petroleum pollution had no significant toxic effect on the *Pisum sativum* growth because the values of phytotoxic effect were less than 20% and variated in the range from 11.9 to 16.9%. This indicated the stimulation of the plant growth under the influence of the studied factors at all experimental concentrations.

Table 1. Phytotoxic effect of polluted soil on *Pisum sativum* before and after probiotic treatment

Time of petroleum pollution	The concentration of petroleum products, mg kg^{-1}	Phytotoxic effect, %									
		By seed emergence		By roots length		By roots weight		By ground part length		By ground part weight	
		Before[1]	After[2]	Before[1]	After[2]	Before[1]	After[2]	Before[1]	After[2]	Before[1]	After[2]
Variant 1	1 000	2.6	–1.2**	5.0	–2.5**	0.8	–1.1**	6.7	–1.1**	0.0	–1.2**
	2 000	2.6	–1.6**	9.3	–1.0	–0.8	–0.5**	–5.7**	–5.1**	–3.4**	–5.2**
	5 000	–2.6**	–3.2**	14.3	2.4	–0.5	–0.6**	3.7	–2.1**	–5.2**	–4.5**
	10 000	10.4	2.5	11.4	3.6	–9.3	–5.5**	9.8	3.1	–9.3**	–10.2**
	20 000	16.9	3.1	15.0	5.1	–11.9	–5.6**	13.6	4.2	–11,9**	–5.2**
Variant 2	1 000	2.7	–0.6**	–3.9**	–0.5**	2.6	–0.1**	1.7	–1.2**	0.7	–2.1**
	2 000	13.3	–0.4**	1.9	1.2	7.6	1.2	11.3	–3.1**	4.3	1.2
	5 000	9.3	1.2	3.9	1.5	5.7	1.5	**24.8***	1.1	10.1	5.1
	10 000	9.3	3.5	5.8	1.5	9.8	0.2	**22.6***	12.1	13.0	7.8
	20 000	13.3	5.7	8.3	2.2	–3.8	–1.1**	–0.2**	–1.1**	–6.5**	–2.5**
Variant 3	1 000	18.0	2.5	14.0	2.8	**25.0***$^{}$	5.1	1.8	–2.1**	2.0	–1.1**
	2 000	**28.8***	7.8	**53.7***	10.1	**39.1***	6.1	**21.2***	5.1	12.6	5.3
	5 000	**42.4***	10.2	**57.4***	12.2	**55.1***	6.8	**35.9***	10.1	**28.3***	9.1
	10 000	**67.0***	15.1	**72.8***	15.5	**75.1***	15.1	**65.2***	12.1	**60.3***	14.3
	20 000	**78.3***	18.2	**49.3***	18.1	**78.2***	**21.5**	**51.8***	15.3	**79.8***	**22.0***

*The phytotoxic effect of more than 20% was considered significant.
**Negative values of the phytotoxic effect indicate the stimulation of plant growth and development under the influence of the studied factors.
[1]Phytotoxic effect before probiotic application.
[2]Phytotoxic effect after probiotic application.

The following results were obtained in Variant 2 after the 30th day after petroleum pollution: the length and the weight of roots at all concentrations of pollutant were nearly the same and the phytotoxic effect was in the range of 8.3–9.8%. So, on the 30th day after pollution, the soil was not toxic to the root system of *Pisum sativum*. The length of the ground part of *Pisum sativum* decreased with the petroleum concentration increasing up to 5 000 mg kg^{-1}. At the petroleum concentrations of 5 000 and 10 000 mg kg^{-1}, the phytotoxic effect reached 24.8% and 22.6% respectively, which indicated the soil toxicity concerning the development of the ground part of plants. The values of phytotoxic effect in Variant 1 after the use of probiotics were in the range of –10.2 to 5.1%. The average value of phytotoxic effect after the application of the probiotic was 2.78%, which was two times lower compared to the values before the application of the probiotic. The soil pollution was not toxic at petroleum concentrations of 1 000 and 2 000 mg kg^{-1} after the probiotic application, conversely, observe the stimulation of *Pisum sativum* growth. The insignificant phytotoxic effect (2.4%) was at a petroleum concentration of 5 000 mg kg^{-1}. The phytotoxic effect values increased with increasing the pollution concentration up to 5.6% (at 20 000 mg kg^{-1}), but this was not considered significant.

The increase of petroleum concentration (to 20 000 mg kg^{-1}) in Variant 2 caused an increase in the underground parts of plants. The root's length becomes very close to the values of plants in the control conditions. The weight of the underground part of plants also changed, following a similar tendency. There were no significant differences from control plants at the petroleum concentration from 1 000 to 20 000 mg kg^{-1}. Hence, the research results of Variant 2 showed the considerable toxic effect of petroleum products on the underground part of the *Pisum sativum* at concentrations of 5 000 and 10 000 mg kg^{-1}. An average value of phytotoxic effect in Variant 2 after the application of probiotic was not significant (3.13%). The values were in a range from –3.1 to 12.1%. The research results showed that with the increase of petroleum concentration phytotoxic effect increased in Variant 2.

The growing of *Pisum sativum* in Variant 3 on the 180th day after soil pollution obtained the following results: the root length reduction was observed at a concentration of 2 000 mg kg^{-1}. The minimal value was spotted at 10 000 mg kg^{-1}, but there were no significant differences between pollutant concentrations (2 000, 5 000, 20 000 mg kg^{-1}). By increasing the starting pollutant concentration, the soil toxicity also increased. The maximal phytotoxic effect value (72.8%) was observed at 10 000 mg kg^{-1}. At the studied concentration of petroleum products of 2 000; 5 000 or 20 000 mg kg^{-1} phytotoxic effect corresponded to 53.7, 57.4 and 49.3%, respectively. The root weight decreased along with the petroleum concentration increase. The significant decrease in plant weight and phytotoxic effect (25%) occurred at a concentration of 1 000 mg kg^{-1}. The ground part length of a plant was changing at the same rate as a root length. A considerable decrease in plant height was already observed at the initial pollutant concentration of 2 000 mg kg^{-1}. The concentration of 1 000 mg kg^{-1} did not influence the plant sizes. The biggest phytotoxic effect (65.2%) corresponded to the petroleum concentration of 10 000 mg kg^{-1} and the minimal value (1.8%) – of 1 000 mg kg^{-1}. The underground part weight of a plant started to decrease at the petroleum concentration of 5 000 mg kg^{-1} and continued to decrease with the pollutant concentration increment. The maximum value of the phytotoxic effect (79.8%) was achieved at the petroleum concentration of 20 000 mg kg^{-1}. Consequently, the soil became toxic to *Pisum sativum* at all investigated concentrations on day 180 after contamination. The research results of Variant 3 after the use of probiotics showed the phytotoxic effect decrease of *Pisum sativum*. The soil toxicity was insignificant (–2.2...18.20%) at all studied petroleum concentrations. Except for the indexes of roots weight and underground part weight where the phytotoxic effect of *Pisum sativum* was significant at pollution concentrations of 20 000 mg kg^{-1} – 21.5 and 22.0% respectively.

Therefore, with the increasing duration of pollution and the petroleum concentration of soil, the phytotoxic effect of *Pisum sativum* increased by the indexes of seed emergence, roots length, roots weight, ground part length and weight. *Pisum sativum* soil becomes toxic only on the 30th day after the pollutant application (5 000 and 10 000 mg kg^{-1}) and the toxicity increased over time. The research results showed the decrease of the phytotoxic effect of *Pisum sativum* by the indexes of seed emergence, roots length, roots weight, ground part length, and ground part weight due to the application of probiotics.

Phytotoxic effect of polluted soil on *Avéna satíva*

The research results of *Avéna satíva* growing in Variant 1 showed the phytotoxic effect in the range from 1.4 to 46.0% (Table 2). At all petroleum concentrations, less than 5 000 mg kg^{-1} observed an insignificant phytotoxic effect (less than 20%) by studied indexes (except seed emergence and roots length). Starting at pollutant concentration 5 000 mg kg^{-1} the height of the ground part of a plant decreased relative to control plants. *Avéna satíva* had a significant phytotoxic effect at petroleum concentrations starting from 10 000 mg kg^{-1}. The petroleum concentration up to 20 000 mg kg^{-1} had no visible impact on the root weight. The weight of the ground part changed at the same rate as the root weight at different concentrations. The diminution of weight was only noticeable at 10 000 and 20 000 mg kg^{-1} and the phytotoxic effect was 20.7 and 35.6% respectively. The pollutant concentration of 20 000 mg kg^{-1} shows the maximal soil toxicity in Variant 1 by all studied indexes (34.8–40.9%). The application of probiotics in Variant 1 shows a decrease in soil toxicity. The values of the phytotoxic effect were in the range from –1.2 to 15.1%, which was not significant (less than 20%).

The research results of *Avéna satíva* growing on the 30th day after soil pollution (Variant 2) showed some decrease in phytotoxic effect compared to Variant 1. The values of the phytotoxic effect were in the range of 0.0 to 35.6%. The soil toxicity was not significant (less than 20%) at all studied pollutant concentrations by all studied indexes, except seed emergence and roots length. The *Avéna satíva* showed a significant phytotoxic response for these indicators at a petroleum concentration of 20 000 mg kg^{-1}. Thus, the soil on day 30, after contamination did not induce a toxic response of *Avéna satíva* at any of the concentrations tested. The results of probiotic application in Variant 2 showed the decreasing of phytotoxic effect about three times by all studied indexes and petroleum concentrations. Thus, the phytotoxic effect in Variant 2 after the use of probiotics was insignificant (–0.5...10.3%).

Table 2. Phytotoxic effect of polluted soil on *Avéna satíva* before and after probiotic treatment

Time of petroleum pollution	Concentration of petroleum products, mg kg^{-1}	Phytotoxic effect, %									
		By seed emergence		By roots length		By roots weight		By ground part length		By ground part weight	
		Before[1]	After[2]	Before[1]	After[2]	Before[1]	After[2]	Before[1]	After[2]	Before[1]	After[2]
	1 000	15.5	1.5	2.3	0.2	3.4	–1.2[**]	8.7	1.1	1.4	0.5
	2 000	16.9	3.5	12.4	5.1	5.1	0.6	10.7	5.2	9.4	1.1
Variant 1	5 000	**20.8**[*]	3.5	**22.2**[*]	6.5	5.1	2.2	18.8	3.6	13.8	1.5
	10 000	**23.9**[*]	7.8	**36.0**[*]	10.2	5.9	2.3	**25.5**[*]	8.9	**19.6**[*]	12.2
	20 000	**40.9**[*]	10.5	**40.1**[*]	12.3	**35.6**[*]	4.9	**46.0**[*]	8.9	**34.8**[*]	15.1
	1 000	7.5	1.2	3.3	0.2	2.2	–1.2[**]	–0.4[**]	–4.2[**]	2.7	0.2
	2 000	13.0	5.2	7.9	0.8	0.0	–0.5[**]	2.2	–3.2[**]	8.0	0.5
Variant 2	5 000	18.1	7.8	9.5	3.6	9.5	0.9	3.2	0.5	3.6	1.1
	10 000	17.8	8.1	11.5	5.2	16.8	1.5	4.1	0.9	5.3	1.6
	20 000	**35.6**[*]	10.3	19.2	7.1	**20.7**[*]	4.9	9.9	1.1	11.6	3.3
	1 000	1.5	–1.2[**]	–2.1[**]	–5.1[**]	1,6	0.5	3.4	–0.9[**]	1.9	–1.2[**]
	2 000	–6.2[**]	–5.2[**]	9.6	–1.2	4.7	3.5	1.7	1.2	4.6	–0.5[**]
Variant 3	5 000	–8.5[**]	–10.5[**]	2.1	0.6	7.9	3.9	9.1	3.3	1.0	–0.5[**]
	10 000	1.5	0.5	6.4	1.2	6.3	2.8	6.8	3.2	3.7	1.2
	20 000	**43.9**[*]	1.2	10.7	3.1	13.4	4.9	4.0	3.5	15.7	7.8

[*]The phytotoxic effect of more than 20% was considered significant.
[**]Negative values of the phytotoxic effect indicate the stimulation of plant growth and development under the influence of the studied factors.
[1]Phytotoxic effect before probiotic application.
[2]Phytotoxic effect after probiotic application.

The research results of *Avéna satíva* growing in Variant 3 showed insignificant soil toxicity by all studied indexes. The values were in the range from –8.5 to 43.9%. Only at the petroleum concentration of 20 000 mg kg^{-1} phytotoxic effect by seed emergence was significant – 43.9%. The phytotoxic effect increased by 11–13% at an initial pollutant concentration of 20 000 mg kg^{-1}. The petroleum products at studied concentrations did not influence the ground part and root system of *Avéna satíva*, growing on the 180th day after pollution. In this case, the phytotoxic effect was not higher than 16%. Thus, in Variant 3, polluted soil affects only the seed emergence of *Avéna satíva* starting at 20 000 mg kg^{-1} and had no impact on the biometric indexes of *Avéna satíva* at any studied concentration. The probiotic use in Variant 3 reduced the phytotoxic effect (up to 7.8%) and even had the stimulation effect of *Avéna satíva* growth by seed emergence (–1.2...–10.5%) and ground part weight (–0.5...–1.2%) at petroleum concentrations less than 5 000 mg kg^{-1}.

Therefore, with the increasing time of soil pollution, the phytotoxic effect of *Avéna sativa* decreased by the indexes of roots length, roots weight, ground part length, and ground part weight, except for seed emergence. For *Avéna sativa*, the soil was toxic only on the 2nd day after pollutant application at concentrations 10 000 and 20 000 mg kg^{-1} by all studied indexes. The research results showed a decrease in phytotoxic effect and even in some variants stimulation effect of *Avéna sativa* by the indexes of seed emergence, roots length, roots weight, underground part length, and ground part weight due to the use of probiotics.

Content of petroleum products in the soil and determination of the optimal probiotic concentration

The research on petroleum content in the soil at different probiotic concentrations and natural soil cleaning (Control) is shown in Table 3. The research results showed that the efficiency of soil cleaning from petroleum products in natural conditions was in the range of 18 to 41% depending on the initial pollutant concentration. With increasing initial pollutant concentration, we observed a decrease in the soil cleaning efficiency in natural conditions. Thus, in natural conditions, at an initial pollution concentration of 1 000 mg kg^{-1}, the petroleum content decreased up to 590 mg kg^{-1} (by 41%); at the initial pollution concentration of 2 000 mg kg^{-1} – decreased up to 1 240 mg kg^{-1} (by 38%); at the initial pollution concentration of 5 000 mg kg^{-1} – decreased up to 3 250 mg kg^{-1} (by 35%); at the initial pollution concentration of 10 000 mg kg^{-1} – decreased to 7 300 mg kg^{-1} (by 27%); at the initial pollution concentration of 20 000 mg kg^{-1} – decreased up to 16 400 mg kg^{-1} (by 18%). That was, then lower initial petroleum concentration in the soil, the natural processes of soil cleaning were better, the lower amount of petroleum products keep in the soil.

Table 3. Content of petroleum products (± standard error, mg kg^{-1}) in the soil before and after probiotic treatment

Initial petroleum concentration in the soil, mg kg^{-1}	Content of petroleum products, mg kg^{-1}			
	Control (remediation in natural conditions)	Probiotic use, dilution 1:10	Probiotic use, dilution 1:100	Probiotic use, dilution 1:1 000
1 000	590 ± 0.32	140 ± 0.20	220 ± 0.17	450 ± 0.22
2 000	1 240 ± 0.55	360 ± 0.38	520 ± 0.26	1 040 ± 0.60
5 000	3 250 ± 0.60	1 000 ± 0.27	1 500 ± 0.42	2 950 ± 0.45
10 000	7 300 ± 0.81	2 500 ± 0.35	3 200 ± 0.55	7 000 ± 0.68
20 000	16 400 ± 0.70	6 200 ± 0.54	10 400 ± 0.80	15 600 ± 0.54

The probiotic efficiency for the reduction of phytotoxic effect for the plants was established by the previous stages of our research. The different probiotic concentrations (1:10; 1:100; 1:1 000) were studied for the practical use of probiotics to remediate petroleum-polluted soil. The research results show that the greatest efficiency of soil cleaning from petroleum products observe at the probiotic concentration of 1:10 for all studied initial concentrations of pollutants. At all studied initial concentrations, the content of petroleum products decreased by 69–86%. The efficiency of probiotic use at concentration 1:10 was higher by 45–51% comparative with soil remediation in natural conditions. The results of probiotic use at concentration 1:100 showed a decrease in petroleum product content by 7–21%. The lowest efficiency of probiotic application was at a concentration of 1:1 000, content of petroleum products in the soil at all studied initial concentrations of pollutant decreased by 22–55%.

For all studied probiotic concentrations, results showed a decrease in the efficiency of soil cleaning from petroleum products with the increasing initial concentration of pollutants. Thus, at the initial concentration of pollutant 1 000 mg kg^{-1} content of petroleum products in the soil decreased by 55–86%; at the initial concentration of pollutant 2 000 mg kg^{-1} – by 48–82%; at the initial concentration of pollutant 5 000 mg kg^{-1} – by 41–80%; at the initial concentration of pollutant 10 000 mg kg^{-1} – by 30–75%; at the initial concentration of pollutant 20 000 mg kg^{-1} – by 22–69%.

Experiment showed high-efficiency probiotic use in the soil remediation from petroleum products in comparison with soiled cleaning in natural conditions. The probiotic concentration 1:10 was the most effective at all studied initial concentrations of pollutants. The reduction of probiotic concentration leads to a decrease in the efficiency of the soil cleaning from petroleum products.

Discussion

Our findings correspond to other studies conducted by Steliga and Kluk (2020) and Haider *et al.* (2021). Thus, Haider *et al.* (2021) have shown that petroleum hydrocarbons significantly induce severe phytotoxicity and cause inhibition in seed germination, seedling development, and photosynthesis activity in plants. Research results by Steliga and Kluk (2020) showed reducing in root length of *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum* by 65.1, 42.3 and 47.3%, respectively, with increasing petroleum hydrocarbon concentration in the soil by 7791 mg kg^{-1}.

The microbial remediation can completely mineralize organic pollutants into carbon dioxide, water, inorganic compounds and cell proteins, or convert complex organic pollutants into other simpler organics (Das, Chandran, 2011). Microorganisms can utilize petroleum hydrocarbons as their only source of carbon, allowing them to degrade organic pollutants in the soil. Microorganisms can destroy 62–75% of petroleum hydrocarbons in the soil in 150 to 270 days According to previous studies (Cai *et al.*, 2016; Iqbal *et al.*, 2019), most microorganisms and plants are suitable for soil remediation. Studies by Varjani and Upasani (2016), Pi *et al.* (2018) and Yuan *et al.* (2018) have demonstrated that bacteria, including *Rhodococcus sp.*, *Pseudomonas sp.*, and *Scedosporium boydii*, can degrade petroleum contaminants. Hydrocarbons are mostly degraded by bacteria via aerobic pathways. The pollution concentration dropped by 14 and 8%, respectively, after 45 days of degradation by *Pseudomonas aeruginosa* (Zhang *et al.*, 2020). *S. changbaiensis* and *P. stutzeri* may decompose 39.2 ± 1.9% and 47.2 ± 1.2% of TPH in soil, respectively within 30 days (the initial petroleum concentration is 1 026 ± 50 mg kg^{-1}) (Li *et al.*, 2020). *T. versicolor* can degrade 50% of TPH within 280 days (the initial petroleum content of the soil is 1 727 mg kg^{-1}) (Lladó *et al.*, 2012). Research results of Christopher *et al.* (2021) found that 61.80% of total petroleum hydrocarbon removal efficiency in treated soil by surface-modified lipopeptide biosurfactant produced using *Bacillus Malacitensis*. The germination of seeds increased from 60 to 100% and the phytotoxicity of root and shoot was reduced from 89.50 and 88.45% to12.55 and 11.87% respectively.

Our previous studies (Pisarenko *et al.,* 2019; Pysarenko *et al.,* 2021) have shown the possibility of probiotic application for soil cleaning of disposal sites and the use of antibacterial properties of probiotics (based on *Bacillus subtilis*) in agrocenosis. The first report about the utilization of petroleum hydrocarbons by *Bacillus subtilis* strains was made by Lily *et al.* (2009). Maximum degradation of petroleum compounds was approximately 84.66% after 24 hours and continued up to 28 days. These findings inferred that *Bacillus subtilis* strains are a very efficient degrader of petroleum hydrocarbons and it can degrade a wide range of petroleum hydrocarbons including naphthalene, anthracene, and dibenzothiophene. Therefore, *Bacillus subtilis* strains could serve as a better candidate for bioremediation of PAHs contaminated soil. Research results of Tao *et al.* (2016) revealed the promising potential of *Bacillus subtilis* strains for application to the degradation of crude oil. The degradation ratio (85.01%) was superior to the indigenous bacterial consortium (71.32%) after 7 days of incubation when the ratio of inoculation size of indigenous bacterial consortium and *Bacillus subtilis* was 2:1.

Our research confirms results by Cai *et al.* (2016) and Iqbal *et al.* (2019) and shows that the remediation potential of microorganisms is rapidly negatively affected when the concentration of petroleum pollutants in the soil increases. Thus, the efficiency of microbial remediation from petroleum products with an increasing initial concentration of the pollutant (from 1 000 to 20 000 mg kg^{-1}) decreases from 86 to 69%.

Conclusion

1. Petroleum pollution has an ambiguous impact on *Pisum sativum* and *Avéna satíva* at different times after pollution. In the initial phase, the polluted soil has no significant influence on *Pisum sativum*. Only on the 30^{th} day after pollutant introduction does soil become toxic at a concentration of 5 000 and 10 000 mg kg^{-1} for *Pisum sativum*. Soil toxicity grows over time of petroleum pollution in this case. For *Avéna satíva* soil become toxic at once after pollution only and does not increase with time of petroleum pollution.
2. The phytotoxic effect of *Pisum sativum* and *Avéna sativa* decrease by the indexes of seed emergence, roots length, roots weight, the ground part length and ground part weight due to the use of probiotics.
3. Determinate the high efficiency of biological remediation by probiotics in comparison with soil cleaning in natural conditions. Probiotic concentration 1:10 is the most effective of all studied initial concentrations of pollutants. Reducing probiotic concentration leads to a decrease in the efficiency of soil cleaning from petroleum products.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Author contributions

PP – the author of the idea, critical revision, and approval of the final manuscript.
MS –performed the data analysis, studied the conception and interpretation of data.
AT – the corresponding author, discussion of the results and drafted the manuscript.
YT –collected data and acquisition of data.
ST – made literature search.
All authors read and approved the final manuscript

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