Joint Effect of Natural Sorbent and Hydrocarbon-Oxidizing Bacteria on Phytotoxicity of Petroleum-Contaminated Soils

Olga V. Nechaeva, Dinara M. Uspanova, Elena I. Tikhomirova, Elena V. Glinskaya, Olga V. Abrosimova, Natalya V. Bespalova

For citation:
Nechaeva O.V., UspanovaD.M., Tikhomirova E.I., Glinskaya E.V., Abrosimova O.V., Bespalova N.V. Joint Effect of Natural Sorbent and Hydrocarbon-Oxidizing Bacteria on Phytotoxicity of Petroleum-Contaminated Soils. *Scientific Research and Innovation. 2021;2(4):120–126*
DOI:10.34986/MAKAO.2021.4.4.003

Authors' credentials:
Olga V. Nechaeva, DSc, Associate Professor; Yuri Gagarin State Technical University of Saratov, 77 Politekhnicheskaya St., Saratov 410054, Russia (olgav.nechaeva@mail.ru)

Dinara M. Uspanova Graduate Student; Yuri Gagarin State Technical University of Saratov, 77 Politekhnicheskaya St., Saratov 410054, Russia

Elena I. Tikhomirova, DSc, Professor, Yuri Gagarin State Technical University of Saratov, 77 Politekhnicheskaya St., Saratov 410054, Russia

Elena V. Glinskaya, PhD, Associate Professor; Saratov National Research University, 83 Astrakhanskaya St., Saratov 410012, Russia (elenavg-2007@ya.ru)

Olga V. Abrosimova, PhD, Associate Professor, Yuri Gagarin State Technical University of Saratov, 77 Politekhnicheskaya St., Saratov 410054, Russia

Natalya V. Bespalova, PhD, Associate Professor, Yuri Gagarin State Technical University of Saratov, 77 Politekhnicheskaya St., Saratov, 410054, Russia

Conflict of Interest:
The authors declare no conflicts of interest.

Received: 31 August 2021
Revised: 13 December 2021
Published: 29 December 2021

Abstract: Biological methods of processing petroleum products are the most effective and environmentally friendly. Use of biological products containing hydrocarbon-oxidizing microorganisms for restoration of anthropogenically altered soils may reduce their general toxicity and restore their fertility. The article presents data on the effect of the saprophytic strain of *Bacillus pumilus*, a natural sorbent (bentonite) and their combination on the phytotoxicity indicators of petroleum-contaminated soils. The choice of *B. pumilus* as a model microorganism was associated with its high enzymatic activity, particularly with respect to petroleum hydrocarbons, its biological safety and high adhesive ability, which makes it possible to obtain immobilized forms of a biological product on sorbent granules. Our laboratory tests established that the most pronounced decrease in phytotoxicity indicators of petroleum-contaminated soils was observed in samples with high concentrations of bentonite sorbent and *B. pumilus* bacteria introduced into the sample. Besides, the level of phytotoxicity in soil samples depended on the duration of the presence of both sorbent and bacteria. The obtained results yielded the prospects designing innovative biological products based on the saprophytic bacterium, *B. pumilus,* and the natural sorbent, bentonite, for the effective restoration of soils exposed to the petroleum pollution.

Keywords: petroleum pollution, biodegradation, phytotoxicity, *Bacillus pumilus*, bentonite.

Introduction

Environmental pollution with petroleum and its derivatives is a global environmental problem [1, 2]. Petroleum products have a toxic effect on plant communities and microbiota, which contributes to a decrease in soil productivity, a change in its enzymatic activity; and in some cases, it leads to the soil exclusion from agricultural use [3, 4]. Various methods are used to rehabilitate petroleum-contaminated soils. However, according to the Federal Standard No. R 57447-2017, biotechnological methods are the most effective and environmentally safe [5, 6, 7]. Although natural self-purification in soils occurs under existing environmental conditions, this is a long-term process, which, on one hand, is limited by the presence of light petroleum fractions that are most toxic for living organisms; and on the other hand, by the presence of complex aromatic structures that are not prone to the biodegradation [8, 9, 10, 11]. To intensify the process, biological products are employed, which include hydrocarbon-oxidizing microorganisms [12, 13, 14]. Incorporating certain components of petroleum in their metabolic processes, they help reducing soil contamination and restoring soil quality. However, the search for new biodegradable bacterial strains remains topical. Previous studies have made it possible to establish high hydrocarbon oxidizing ability in saprophytic bacteria, *Bacillus pumilus* [15, 16, 17]. Hence, the goal of our study was to assess the effect of *B. pumilus* strain and natural sorbent (bentonite) on phytotoxicity of soils with long-term petroleum pollution.

Materials and Methods

Determination of phytotoxicity in petroleum-contaminated soil samples was carried out on the basis of the Scientific and Educational Center, *Industrial Ecology*, at Yuri Gagarin State Technical University (SSTU). The object of our study was petroleum-contaminated soil obtained from the landfill (Village of Kolontaevo, Moscow Oblast). The previously investigated soil sample was autoclaved at 1 atm to destroy the asporogenic and sporogenic indigenous microflora. A bacterial culture of *B. pumilus* from the collection of the Department of Microbiology and Plant Physiology of Saratov National Research University, a natural sorbent (bentonite), as well as their combinations, the optimal concentrations of which were selected experimentally, were introduced into petroleum-contaminated soil samples. The choice of the bacterial strain of *B. pumilus* was due to the fact that previous studies have established its biological safety, high enzymatic and adhesive capabilities, which made it possible to consider it as a component of a biological product, as well as to design its immobilized form on bentonite granules, the choice of which was associated with its high porosity and sorption capacity [18, 19]. The bacteria were grown on Meat Peptone Agar at a temperature of 28 °C for 24 hours. Then a suspension was prepared in physiological solution according to a turbidity standard of 10 U (sensu State Scientific Research Institution of Standardization and Control). Further on, the suspension was added to investigated soil samples at the rate of 1 ml per 100 g of material. We used the following soil sample options, which contained various combinations of sorbent and bacteria:

1. Control: an initial sample of the studied soil

2. S1: 84 g of sorbent

3. S1B: a suspension of bacteria and 84 g of sorbent

4. S2B: a suspension of bacteria and 168 g of sorbent

5. S2: 168 g of sorbent

6. B: suspension of bacteria.

To study the phytotoxicity of the studied soil samples, we used the so-called *seedling method* (FR 1.39.2006.02264), which makes it possible to assess the dynamics of the toxic effect of petroleum products and the change in soil toxicity when bacteria and sorbent are added to the samples. Radish seeds (*Raphanus sativus*) were used as a test culture; 10 seeds were placed on filter paper in Petri dishes and moistened with prepared soil extracts of control and experimental soil samples. As they were drying, the seeds were re-moistened. The first measurements of the root length and aerial part of the seedlings were performed on the 4th day, and the numbers of germinated seeds in the control and experimental samples were counted. The second measurement of the vegetative parts of the plants was conducted on the 12th day of the experiment. According to the proposed methodology, the following parameters were determined:

1. Mean value of the lengths of the aerial and root parts of germinated seeds;

2. Seed germination energy;

3. Toxicity index of evaluated factor.

Germination energy (B) was calculated using the formula:

$$B = \frac{a}{b} \times 100\% , \quad (1)$$

where *a* is a number of germinated seeds;

B is a total number of seeds in the experiment.

On the basis of seed germination energy values, we calculated the toxicity index of the evaluated factor (TIF):

$$\mathrm{TIF} = \mathrm{TF_e} / \mathrm{TF_c} , \quad (2)$$

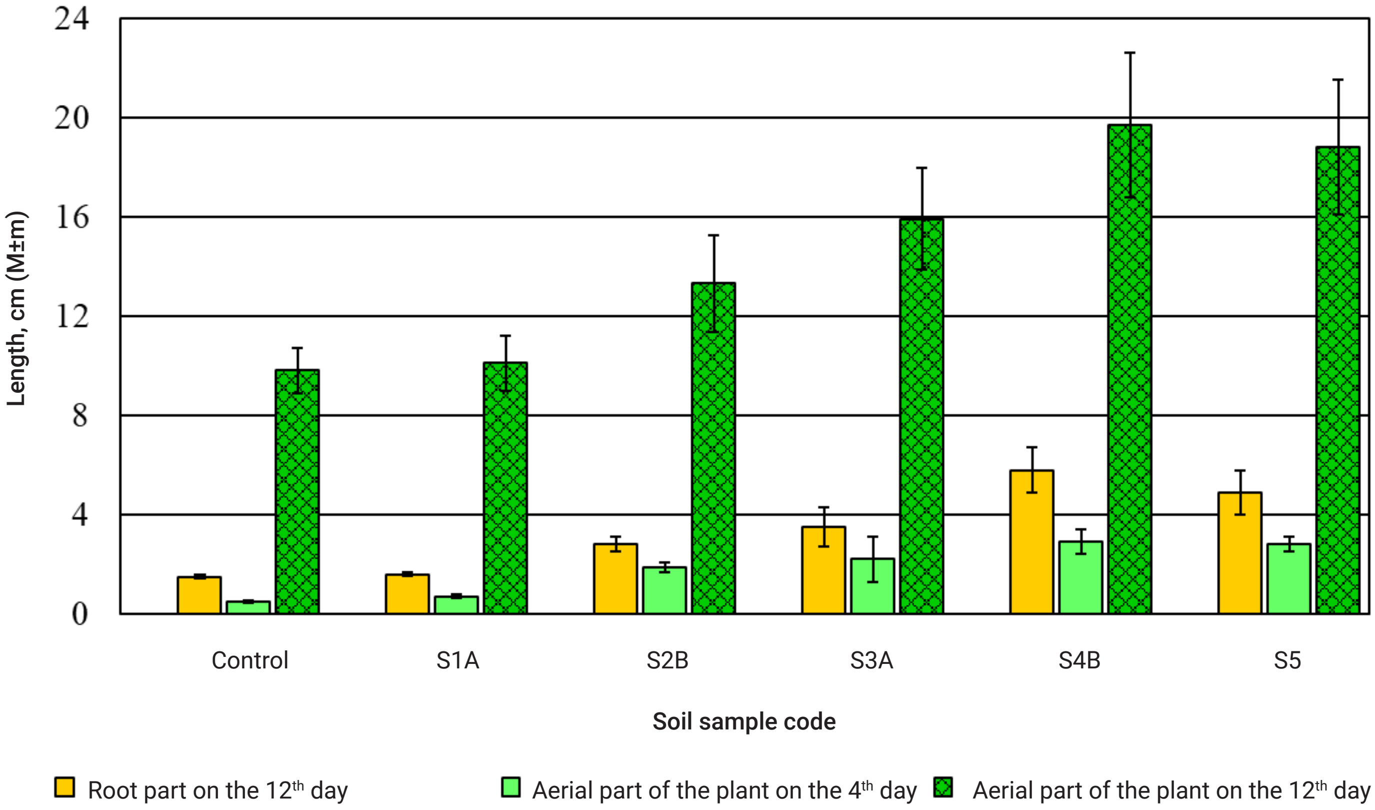

Figure 1. **The mean values of the lengths of the root and aerial parts in germinated seeds of *Raphanus sativus*, treated with soil extracts (2-day exposure)**

where TF_e is the value of registered test function in the experiment;

TF_c is the value of registered test function in the control (65 %).

According to the assessment scale of soil toxicity, the following soi pollution grades were established.

1. Stimulation: TIF > 1,1;

2. Norm: TIF = 0.91–1.10;

3. Low toxicity: TIF = 0.71–0.90;

4. Medium toxicity: TIF = 0.5–0.7;

5. High toxicity (LD50): TIF < 0.5;

6. Extremely high toxicity: TIF = 0.

To test the statistical significance of obtained results, our studies were carried out in five replicates. Statistical processing of the results was conducted using the Statistica 6.0 software package (for Windows; Stat Soft, Inc., USA), Statgraph (Version 2.6; Coulter), Microsoft Excel 2003 (for Windows XP). The results were considered statistically significant at $p \leq 0.05$.

Results and Discussion

We established that when the seeds of R. *sativus* were treated with both control and experimental soil extracts, the root part in seedlings was still absent on the 4th day from the onset of the experiment. The aerial parts of the seedlings, treated with experimental samples of soil extracts, grew more intensively than in the control sample, and the strongest stimulation of plant growth was observed when treated with a sample of soil extract S2 (Figure 1). On the 12th day from the onset of the experiment, the root part was formed by all radish seedlings and the highest growth intensity was exhibited when they were treated with soil extracts S2 and B. Similar results were obtained for the aerial parts of the test object.

We were interested to evaluate the effect of the duration of the presence of *B. pumilus* and bentonite, separately and in combination, on the phytotoxicity of petroleum-contaminated soils. With this goal

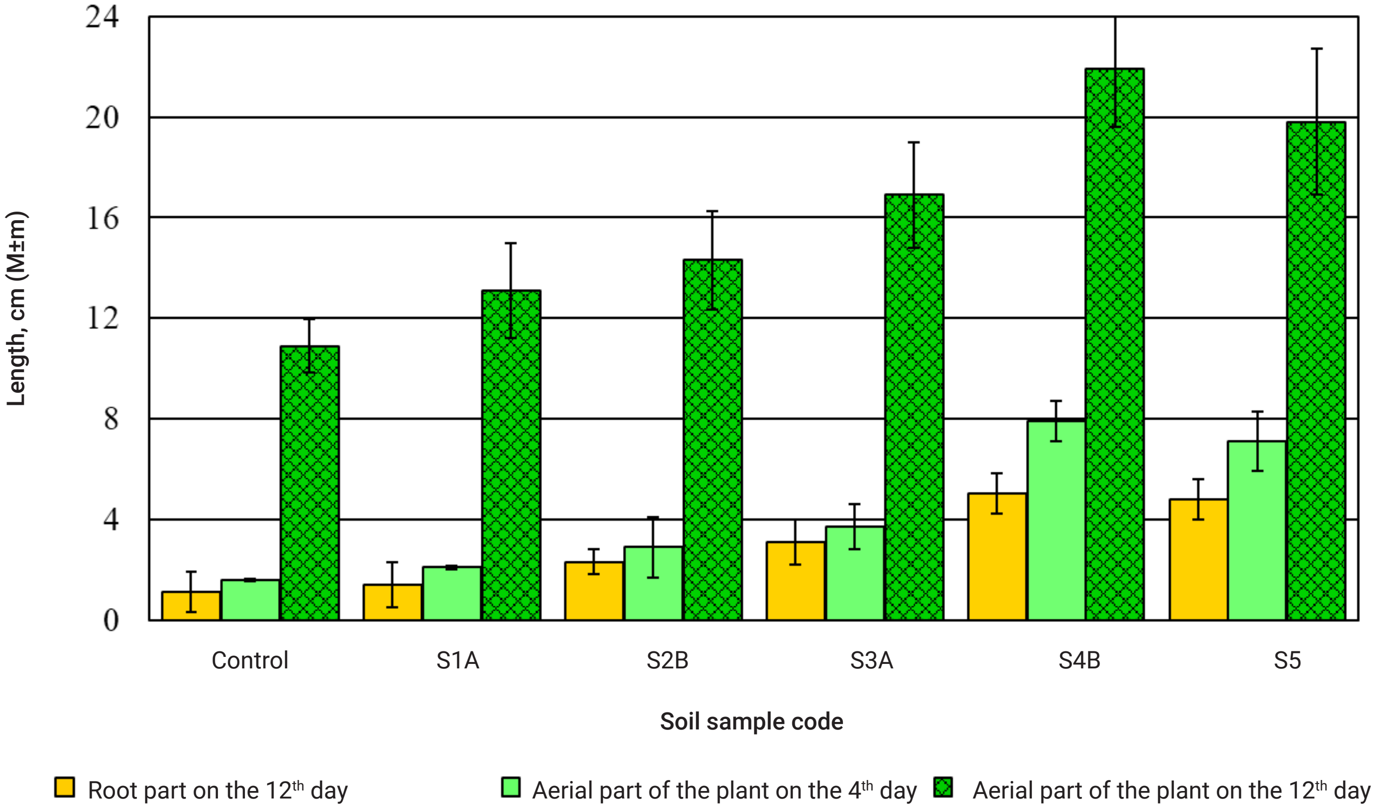

Figure 2. **The mean values of the lengths of the root and aerial parts in germinated seeds of *Raphanus sativus*, treated with soil extracts (30-day exposure)**

in mind, test samples of petroleum-contaminated soils were left for 30 days at room temperature. Further studies were conducted in a similar way. We established that on the 4th day from the onset of the experiment, the root part of seedlings was still absent when the seeds were treated with soil extracts of all samples of petroleum-contaminated soils, and the aerial part was most intensively formed when exposed to soil extracts from the samples S2 and B (Figure 2). On the 12th day from the beginning of the experiment, the formation of the root part of *R. sativus* was significantly higher than the analogous values in the previous experiment, and the strongest stimulating effect was discovered for samples S4B and S5. The aerial part of *R. sativus* by the 12th day was also formed more intensively in comparison with the values of the previous experiment, and the best results were also obtained when the seeds were treated with soil extracts of samples S2 and B.

Evaluation of seed germination energy in *R. sativus* showed that its values were in the range of 20–80 % and depended on the amount of the applied sorbent and the time of the presence of *B. pumilus* and bentonite in the samples of petroleum-contaminated soils (Figure 3). Whenever *B. pumilus* bacteria and sorbent were introduced 30 days before the start of the experiment, soil extracts from such samples contributed to a higher germination energy of *R. sativus* seeds.

It was established that the toxicity of soil extracts samples depended on the duration of the presence of *B. pumilus* bacteria, bentonite and their combination in petroleum-contaminated soil samples (Figure 4). It was revealed that the presence of the studied components in samples of petroleum-contaminated soils for only 2 days did not have a significant effect on the toxicity of soil extracts: S2B, S2, and B exhibited an average degree of toxicity, whereas S1 and S1B had high toxicity, similar to the control sample.

The presence of *B. pumilus*, bentonite, and their complexes in petroleum-contaminated soil samples for 30 days contributed to an intensive reducting in their toxicity indicators. For example, both control sample and experimental sample S1 were characterized by low toxicity, TIF values for samples S1B and S2B corresponded to the norm, and the soil extracts

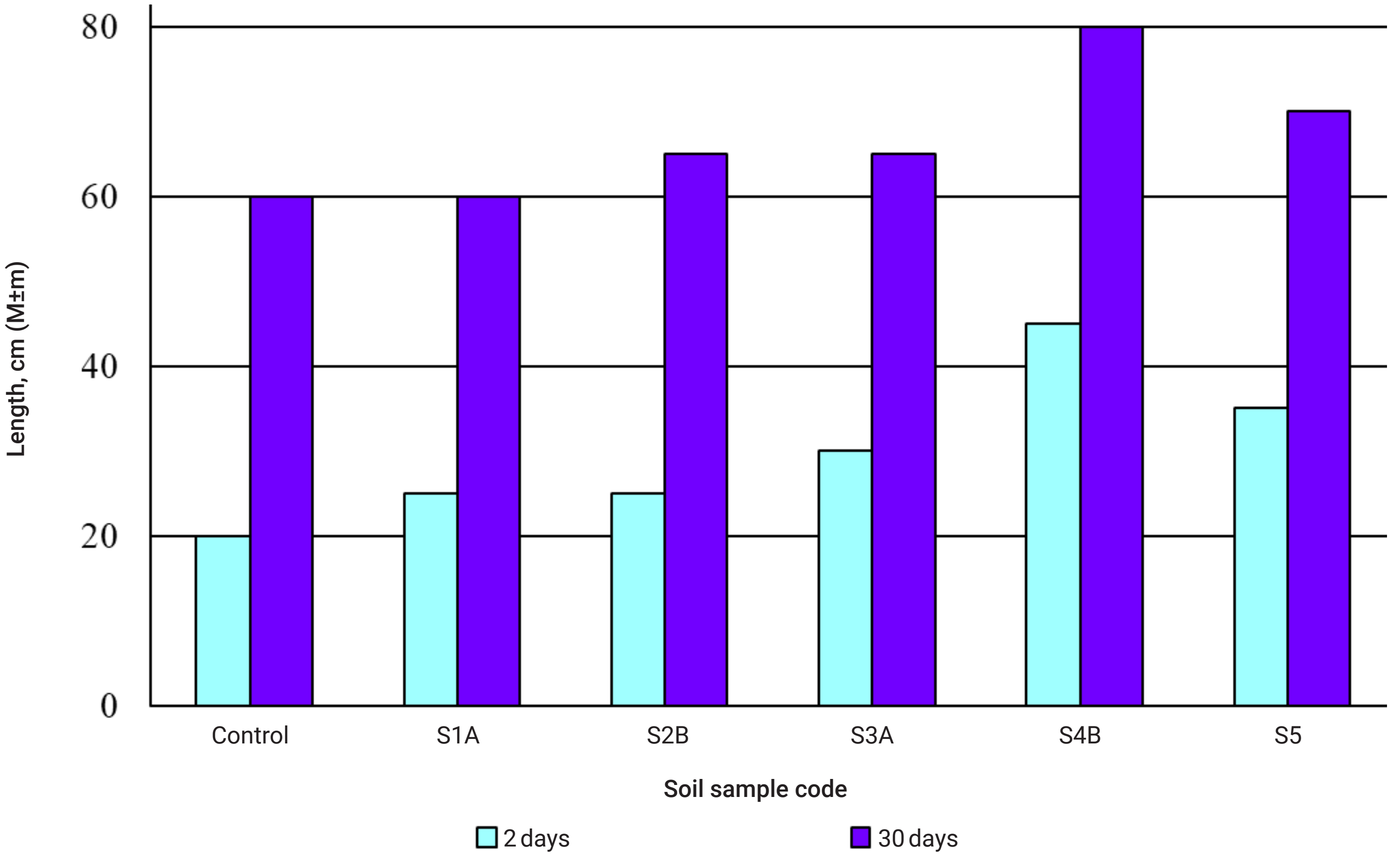

Figure 3. **Seed germination energy in samples with introduced bacteria and sorbent**

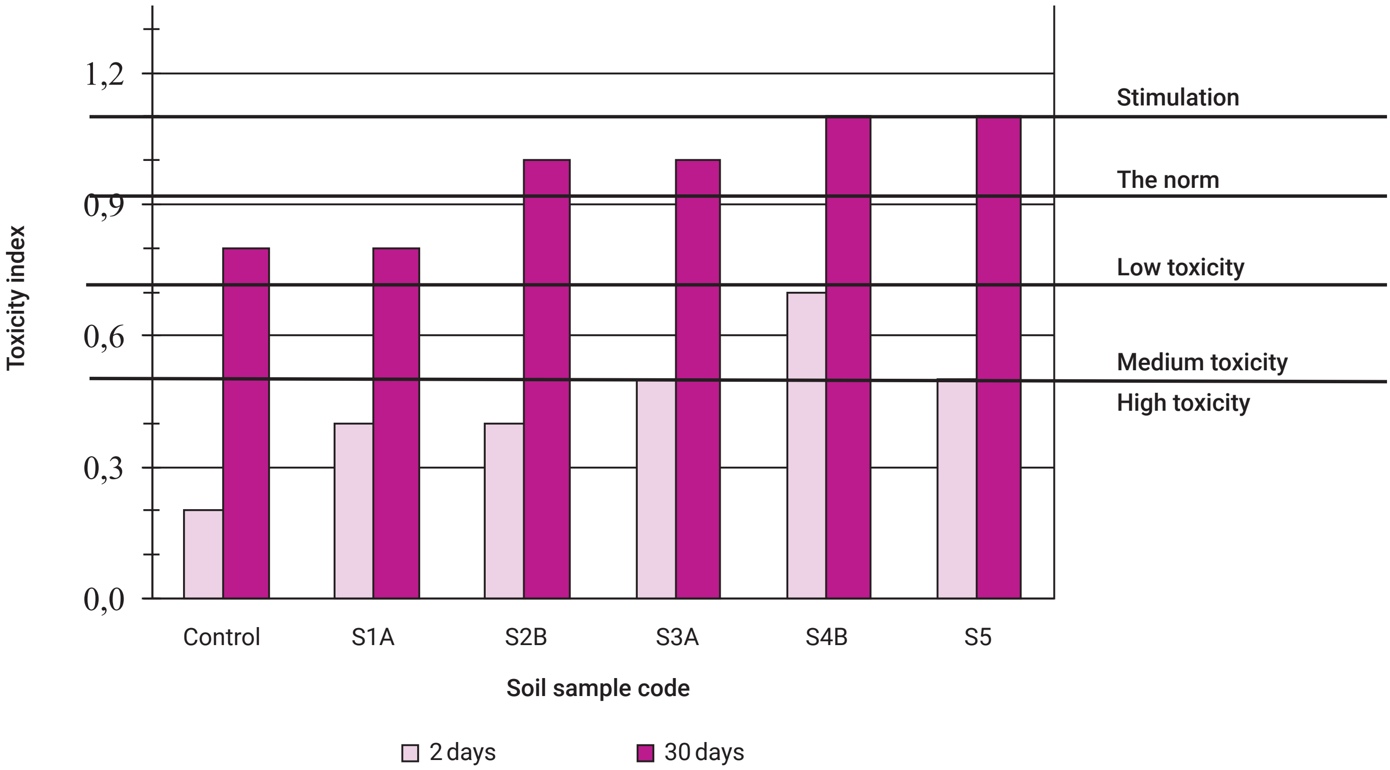

Figure 4. **The value of TIF in samples with introduced bacteria and sorbent**

from samples S2 and B stimulated the growth of *R. sativus*. The reduction in toxicity indicators in the control sample occurred, probably, as a result of the processes of abiotic transformation of petroleum products.

Conclusion

The introduction of the *B. pumilus* bacterial strain, bentonite, and their combination, into the samples of petroleum-contaminated soils led to a decrease in soil phytotoxicity, the level of which depended on the duration of the presence of the studied components and the amount of introduced sorbent. The best results in reducing toxicity were obtained for the variant of petroleum-contaminated soil S2, which contained 168 g of sorbent, and B, which contained solely the *B. pumilus* strain. The obtained results made it possible to consider the strain of saprophytic bacterium, *B. pumilus,* and the natural sorbent, bentonite, effective components in the development of biological products for the restoration of petroleum-contaminated soils.

References

1. Pikovsky P.I. Natural and technogenic flows of hydrocarbons in the environment. Moscow: *Moscow State University Publishers*, 2019. 208 p. (In Russ.).

2. Taleghani N.D., Tyagi M. Impacts of major offshore oil spill incidents on petroleum industry and regional economy. *Journal of Energy Resources Technology*. 2017;139:022913. DOI: 10.1115/1.4035426

3. Sangadzhieva L.Kh., Davaeva Ts.D., Buluktaev A.A. Influence of petroleum pollution on phytotoxicity of light chestnut soils in Kalmykia. *Kalmyk University Bulletin*. 2018;1:44-47. Available at: https://cyberleninka.ru/article/n/vliyanie-neftyanogo-zagryazneniya-na-fitotoksichnost-svetlo-kashtanovyh-pochv-kalmykii-1 (In Russ.).

4. Buluktaev A.A. Phytotoxicity of petroleum-contaminated soils in arid territories (under the conditions of a model experiment). *Russian Journal of Ecosystem Ecology*. 2019;4(3):147-156. (In Russ.). DOI: 10.21685/2500-0578-2019-3-5

5. Zabolotskikh V.V., Tankikh S.N., Vasiliev A.V. Technological aprproaches to detoxify and restore contaminated land. *Bulletin of Samara Scientific Center of the Russian Academy of Sciences*. 2018; 20(3-5): 341-351. (In Russ.). Available at: https://ter-arkhiv.ru/1990-5378/article/view/88182

6. Kholodenko V.P., Chugunov V.A., Zhigletsova S.K., Rodin V.B., Ermolenko Z.M., Fomchenkov V.M., Irkhina I.A., Kobelev V.S. Volkov V.Ya. Development of biotechnological methods for the elimination of petroleum pollution of the environment. *Russian Journal of Chemistry*. 2001; 40(5-6): 135-141. Available at: http://ged.chem.msu.ru/rus/journals/jvho/2001-5-6/135.pdf (In Russ.).

7. Atlas R.M., Hazen T.C. Oil biodegradation and bioremediation: A tale of the two worst spills in US history. *Environmental Science & Technology*.2011;45(16):6709-6715. DOI: 10.1021/es2013227

8. Korshunova T.Yu., Chetverikov S.P., Bakaeva M.D., Kuzina E.V., Rafikova G.F., Chetverikova D.V., Loginov O.N. Microorganisms in the elimination of the consequences of petroleum pollution. *Applied Biochemistry and Microbiology*. 2019; 55(4): 38-349. (In Russ.). DOI: 10.1134/S055510 9919 0 400 93

9. Zaitseva T.A., Rudakova L.V., Kombarova M.M. Microorganisms as oil destructors. *Scientific Studies and Innovations*. 2010; 4(4): 59-63. (In Russ.).

10. Varjani S.J. Microbial degradation of petroleum hydrocarbons. *Bioresource Technology*. 2017;223: 277-286. DOI: 10.1016/j.biortech.2016.10.037

11. Gogoleva O.A., Nemtseva N.V. Hydrocarbon-oxidizing microorganisms of natural ecosystems. *Bulletin of Orenburg Scientific Center of Urals Division of RAS*. 2012; 2: 2-8. Available at: https://cyberleninka.ru/article/n/uglevodorodokislyayuschie-mikroorganizmy-prirodnyh-ekosistem/viewer (In Russ.).

12. Ivanchikov E.A., Bubeev A.T., Baldaev N.S., Tsyrenov V.Zh. Prerequisites for the creation of artificial consortia of microorganisms for the disposal of pollutants of various origins.

Sciences of Europe. 2018; 32: 14-21. Available at: https://cyberleninka.ru/article/n/predposylki-sozdaniya-iskusstvennyh-konsortsiumov-mikroorganizmov-dlya-utilizatsii-zagryaznyayuschih-veschestv-razlichnoy-prirody (date of application 26.12.2021). (In Russ.).

13. Kuppusamy S., Thavamani P., Venkateswarlu K., Lee Y.B., Naidu R., Megharaj M. Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. *Chemosphere*. 2017;168: 944-968. (In Russ.). DOI: 10.1016/j.chemosphere.2016.10.115

14. Xiong B., Zhang Y., Hou Y., Arp H.P.H., Reid B.J., Cai C., Enhanced biodegradation of PAHs in historically contaminated soil by *M. gilvum* inoculated biochar. *Chemosphere*. 2017;182: 316-324. DOI:10.1016/j.chemosphere.2017.05.020

15. Nechaeva O.V., Kameneva V.V., Bychkov A.R., Tsapok D.A., Uspanova D.M., Shurshalova N.F. Development of a biological product based on natural sorbents for the rehabilitation of petroleum-contaminated soils. *Proceedings of VI International Scientific and Practical Conference, “Natural Protected Areas: Past, Present, Future”*. Khvalynsk, 2019. 262-266. Available at: https://www.elibrary.ru/item.asp?id=41493398&pff=1 (In Russ.).

16. Dementyeva N.A., Nechaeva O.V., Kameneva V.V. Study of the microbial composition of sludge sediments and the selection of the most promising strains of bacterial destructors. *Scientific Publications of Kvalynsk National Park*. Khvalynsk, 2017. 103-107. (In Russ.).

17. Uspanova D.M., Nechaeva O.V., Shurshalova N.F., Kameneva V.V., Bychkov A.R. Studying the effectiveness of using the saprophytic strain of *Bacillus pumilus* for utilizing the xenobiotics of hazard classes I and II. *Bulletin of Saratov University. New Series. Chemistry. Biology. Ecology.* 2020; 20(2): 200-206. (In Russ.). DOI: 10.18500/1816-9775-2020-20-2-200-206

18. Annagiev, M.Kh., Mamedov, U.A. Study of the texture characteristics of bentonites from the Gyzyl-Dare deposit (Azerbaijan). *Relevant Issues in Humanities and Natural Sciences*. 2010; 2: 10-13. (In Russ.).

19. Koshelev A.V., Vedeneeva N.V., Zamatyrina V.A., Tikhomirova E.I., Skidanov E.V. Development of technology for producing sorbents based on bentonite clays for water purification systems. *Water and Ecology: Problems and Solutions.* 2018; 2(74):32-39. (In Russ.). DOI: 10.23968/2305–3488.2018.20.2.32–39