Bioremediation of contaminated light-chestnut soils in southern Russia

E V Kalmykova¹, A I Belyaev², N Yu Petrov², A M Pugacheva² and M P Aksenov²

¹All-Russian Research Institute of Irrigated Agriculture, 9, Timiryazev Str., Volgograd, Russia
²Federal Scientific Centre of Agroecology Complex Meliorations and Protective Afforestation Russian Academy of Sciences, 97, University Ave., Volgograd, Russia

E-mail: aksenovmp@mail.ru

Abstract. The paper presents the results of studies of agrochemical and microbiological indicators of oil contaminated soils in southern Russia when using a biological preparation in the period from 2013 to 2019. Light-chestnut soils, tomato test culture, and biological preparation Biodux were chosen as objects of the study. The soil was contaminated with oil of various concentrations. Phytotoxicity of the soil was estimated by germination, the length of the above-ground part, the root, and the total biomass of plants. The study revealed that phytotoxicity and changes in the agrochemical indicators of light-chestnut soils in southern Russia are directly related to the concentration of applied oil. Thus, samples contaminated with 10 % oil show the most adverse effect on the studied parameters. Changes in the biological activity of soils contaminated with oil products primarily affected the growth, development and, ultimately, the yield of crops. The studied preparation Biodux formed resistance to stressful growing conditions in tomato crops and activated their growth and biological processes. Based on the data obtained, light-chestnut soils are not resistant to oil contamination in terms of biological indicators.

1. Introduction

Southern Russia is represented by the largest industrial and cultural region. Due to a significant concentration of industrial enterprises and plants, it is one of twenty regions with increased volumes of pollutant emissions, which cause acute environmental problems on this territory [1, 2].

The problem of soil contamination is no less acute than the situation with air and water pollution. The main reasons are: a large concentration of motor transport on the territory (population of about 1.3 million people own more than 450 thousand cars) and polluting enterprises: OOO Lukoil – Volgogradnefteperarabotka, OAO Volgograd Aluminum, VolgoGRES, OAO Khimprom and others.

Oil and oil products are common pollutants of all components of the natural environment, including soil. In Russia, damage from pollution by oil products in 2018 increased from 1.0 to 5.1 billion rubles compared to 2017 [3]. Soils of areas contaminated with oil lose fertility for many years and drop out of agricultural use [4, 5].

Oil contamination
causes deep and sometimes irreversible changes in morphological, physical, physicochemical, and microbiological properties of the soil. This leads to the loss of soil productivity, and often to complete degradation of landscapes, which negatively affects the growth and development of plants growing on these soils. [6–9].

Plants are the center for the formation of microbial communities, and therefore are considered as the main participants in bioremediation. When adapted to specific soil and climatic conditions, they exhibit rhizospheric and intracellular mechanisms for detoxification of pollutants, and therefore are of deep interest, both in scientific and applied terms [10–12].

In turn, due to its natural and economic conditions and resources, the southern part of Russia is a large producer of vegetable products and steadily provides 4–5 % of vegetables for the country or almost 30 % of the production volume in the Southern Federal District. In 2019, tomato crops occupied about 1.01 thousand hectares (4th position among the main vegetable crops) and the gross harvest amounted to 38.8 thousand tons.

Tomato (Latin Solánum lycopérsicum) is the most important agricultural crop cultivated not only in different climatic zones but also on different soils. It is very important to determine the limits of its resistance to various anthropogenic factors, in particular, to soil contamination with oil.

There are physical, chemical and thermal cleaning methods that are not only expensive and ineffective, but can also cause additional harm to the environment. Therefore, it is of high relevance to develop and apply new, effective, inexpensive and environmentally friendly methods for cleaning soils from oil products. Bioremediation was shown to have huge potential and competitive advantages, primarily due to the environmental safety and low cost [13]. Bioremediation is impossible without the use of biological products, which include viable cells of hydrocarbon-oxidizing microorganisms and their bacterial associations. The ability of microorganisms to transform or degrade oil hydrocarbons is well known and enables their use for bioremediation of contaminated areas. Analysis of literature data and patent search for existing biological preparations showed that in a number of cases their disadvantages include low halotolerance of microorganisms in their composition, a narrow range of pH and temperatures, as well as the lack of important data on the ability of microorganisms to produce bioemulsifiers, and on the efficiency of degradation of high concentrations of oil and oil products.

The aim of the study was to substantiate transformation of the main agrochemical parameters of light-chestnut soil and its phytotoxicity in tomato crops growing on oil contaminated soils in southern Russia.

2. Materials and methods
The experiments were carried out from 2013 to 2019 under the conditions of field stationary experiments. The object of the study was light-chestnut heavy loamy soils contaminated with oil during the cultivation of a vegetable test crop (tomato variety Hercules) and treatment with Biodux growth regulator. The plots for field experiments were bottomless boxes embedded in the soil to a depth of 0.30 m. The area of the plots amounted to 0.50 m², the experiment was repeated 4 times. The soil was artificially contaminated with commercial oil by pouring the plots from the surface in the amount of 1; 5; 10 % of the soil mass. Sowing of tomato seeds was carried out 5 days after oil introduction. The following plant parameters were analyzed: seed germination, survival and accumulation of plant biomass.

Experimental versions: 1. control (background soil); 2. treatment of tomato seeds and plants with Biodux growth regulator; 3. soil contaminated with oil (1; 5; 10 %); 4. treatment of seeds and plants with Biodux growth regulator + soil contamination with oil (1; 5; 10 %).

Biodux is a preparation based on a combination of biologically active polyunsaturated fatty acids of the fungus Mortierella alpina. The application rate of Biodux when soaking seeds for 60 minutes was 0.2 ml/kg (fluid consumption was 2 l/kg), when spraying in the budding phase, it amounted to 3 ml/ha and 400 l/ha, respectively [13, 14].

Agrochemical analyses of soils were carried out at the All-Russian Research Institute of Irrigated Agriculture using conventional methods: the content of humus and organic matter by Tyurin's method
in the modification of CRIASA in accordance with GOST 26213-91; pH of the salt suspension in the modification of CRIASA in accordance with GOST 26483-83; the content of mobile phosphorus and exchangeable potassium (GOST 26207-91), total nitrogen (GOST 26107-84). For the general characteristics of soils, the water extract was analyzed in a soil–water ratio of 1:5. The content of cations Ca, Mg, Na and anions Cl, SO$_4$,$^2$- , HCO$_3$ was determined.

To determine the total number of microorganisms assimilating organic forms of nitrogen, the study employed meat-and-peptone agar (MPA), mineral forms of nitrogen – starch-and-ammonia agar (SAA), and microscopic fungi – Czapek agar. Bacterial colonies were counted on MPA, and the colonies of bacteria, fungi, and actinomycetes were counted on SAA and Czapek agar. Cellulose-fermenting bacteria were counted on Hutchinson medium by sowing soil on the surface of an agar plate using the pellet method. The phytotoxic activity of microorganisms was determined by seed germination (GOST 19449-93).

Statistical processing of the analysis results was carried out by the analysis-of-variance method using programs for Microsoft Excel.

### 3. Results and discussion

Numerous studies show an extremely adverse effect of oil contaminated soils on the growth and development of plants. In this case, the main contributing factors are the toxic effect of oil hydrocarbons and changes in the physicochemical properties of the soil. The increase in hydrophobicity and other changes in the physical properties of the soil are due to the heavy fractions of hydrocarbons, and the direct toxic effect is due to the light fractions of oil hydrocarbons. In addition, oil enveloping of soil particles prevents the migration of mobile forms of phosphorus, nitrogen, potassium and other irreplaceable elements into the solution and thus can cause oppression of plants due to a lack of nutrients. Plants are in complex relationships with microorganisms that inhabit the soil. Oil contamination causes changes in plant–microbial complexes, primarily within the rhizosphere, where microbiota adapts to environmental conditions.

The study showed that oil contamination significantly changes the reaction of the soil medium. An increase in the degree of contamination led to a decrease in the acidity of the soil medium. The pH value increased from 6.1 in uncontaminated soil to 8.4 in contaminated soil, that is by 10 %. An increase in the degree of oil contamination caused alkalization of the soil and a sharp decrease in the hydrolytic acidity. Treatment of tomato seeds and plants with Biodux in uncontaminated soil contributed to a slight increase in the pH value – 6.4 % (Figure 1).

As a result of oil contamination, Biodux reduced soil alkalinization by 0.4 % (at 10 % soil contamination). A decrease in acidity and present alkalinity were associated with the replacement of hydrogen ions in the soil absorbing complex with sodium ions, the proportion of which in oil contaminated soils sharply increased by 0.486 % (100 g) relative to the version with uncontaminated soil.

The soil exhibited an increase in the ratio between the amount of carbon and nitrogen, the proportion of insoluble sediment in humus increased, which reduced the resistance of the soil-absorbing complex to adverse external effects and decreased fertility (Table 1).

In the humus composition of oil contaminated soils, the processes of mineralization of organic matter decreased, which affected the nitrogen regime of the soil and, ultimately, the yield of plants.

The study of the content of mineral forms of nitrogen in oil contaminated soil showed that the amount of N-NH$_4^+$ decreased more than twofold – from 3.7 mg/kg in the control version to 1.8 mg/kg at 10 % contamination with oil products. The use of Biodux on contaminated soil revealed a slight increase in this indicator relative to the values for the version with contaminated soil.

The content of the nitrate form of nitrogen was different. At 1 % oil contamination, the amount of N-NO$_3^-$ increased by 37.5 % relative to uncontaminated soil. At 5 % oil contamination, a decrease to 2.9 mg/kg of air-dry soil was observed, and at 10 % oil contamination, the nitrate form was not detected.
Figure 1. Effect of oil pollution on changes in soil composition (0.0–0.3 m depth), average for 2013–2019

Table 1. Effect of oil contamination on the agrochemical properties of soils (0.0–0.3 m depth), average for 2013–2019

| Experimental version | Carbon % | Nitrogen | Organic matter % | N-NO₃ mg/kg of air dry soil | N-NH₄⁺ mg/kg of air dry soil | P₂O₅ mg/kg of air dry soil | K₂O mg/kg of air dry soil |
|----------------------|----------|----------|------------------|---------------------------|----------------------------|---------------------------|---------------------------|
| Control (background soil) | 1.71 | 0.10 | 2.04 | 27.5 | 3.7 | 85 | 363 |
| Biodux | 1.68 | 0.10 | 2.06 | 28.0 | 3.6 | 84 | 361 |
| Oil contamination of soil, 1 % | 1.81 | 0.12 | 1.89 | 37.8 | 2.7 | 89 | 426 |
| Oil contamination of soil, 5 % | 1.89 | 0.13 | 1.61 | 2.9 | 2.3 | 92 | 495 |
| Oil contamination of soil, 10 % | 2.34 | 0.13 | 1.43 | not detected | | | |
| Biodux + oil contamination of soil, 1 % | 1.76 | 0.12 | 1.90 | 34.7 | 2.9 | 88 | 398 |
| Biodux + oil contamination of soil, 5 % | 1.82 | 0.13 | 1.65 | 3.2 | 2.4 | 90 | 445 |
| Biodux + oil contamination of soil, 10 % | 2.23 | 0.13 | 1.45 | 1.7 | 2.0 | 93 | 486 |

Oil contamination led to disruption of plant nutrition with basic microelements, for example, in the control samples, the content of nitrogen, phosphorus and potassium was lower than that in contaminated soil. This is due to the removal of trace elements by plants cultivated on the soils studied. In contaminated areas, nitrogen, phosphorus and potassium were not removed because oil contamination suppressed the growth and development of tomato test crops. Thus, an increase in the content of total nitrogen, mobile forms of phosphorus and potassium was observed.

The microflora is of particular relevance in removal of oil products from soils.

Microbiological activity is one of the ecologically significant soil parameters. Data on the composition of the main groups of microorganisms are important to assess the state of microbiocenosis and the potential ability of oil contaminated soil for self-recovery, and to observe the processes of destruction of pollutants.

Changes in the species composition of some groups of microorganisms revealed a clear relationship with the degree of oil contamination. A high degree of oil contamination can cause a complete suppression of the growth and development of microorganisms.

This type of pollution, significantly modifies the soil microbiota, and different groups of microorganisms respond differently: the number of some (nitrogen-fixing, ammonifying, denitrifying,
hydrocarbon-oxidizing, heterotrophs, spore-forming, bacteria, fungi, yeast, actinomycetes) increases, the number of others (nitro-decomposing, cellulose-fermenting) decreases, and some of them remain practically unchanged.

The study showed that the introduction of 1 and 5 % of oil products into the soil stimulated the development of almost all studied groups of soil microorganisms, with the exception of cellulose-fermenting ones (Table 2).

Complex reactions associated with the transformation of nitrogen were observed. The cycle of transformations of nitrogen-containing compounds in the soil was directly related to the development and biochemical activity of ammonifying, nitrifying, denitrifying and nitrogen-fixing microorganisms. The number of microorganisms that used organic forms of nitrogen (MPA) was initially (after 3 days) lower than that in the control. However, after 1 month the number of microorganisms was restored, and after 3 months it exceeded the values in the control. Similar results were observed when studying the effect of oil contamination on the quantitative composition of microorganisms that use mineral forms of nitrogen (SAA), and after 1 year the number of these groups of microorganisms in contaminated soils was higher than that in background soils.

Fungi proved to be the most resistant microorganisms to oil contamination of the soil. Treatment of tomato seeds and plants with a growth regulator had practically no effect on their population. At 1 % oil contamination, the number of fungi increased by 1.1 %, at 10 % oil contamination, the number of fungi increased 1.5 fold. Treatment of tomato seeds and plants with a growth regulator in contaminated soil hindered a further increase in the number of saprophytic fungi in the soil. This was due to the fact that an increase in the number of fungal primordia correlated with an increase in sporulation at high oil concentrations and exceeded the number of microorganisms in background soils.

Table 2. Effect of oil contamination on the number and composition of soil microflora in tomato crops, average for 2011–2019

| Experiment version                          | Microorganisms, MPA | Microorganisms, SAA | Fungi, Czapek agar | Actinomycetes | Cellulose-fermenting microorganisms |
|--------------------------------------------|---------------------|---------------------|-------------------|---------------|------------------------------------|
| Control (background soil)                  | 3.6                 | 2.3                 | 2.1               | 325.4         | 0.48                               |
| Biodux                                     | 3.9                 | 2.6                 | 2.2               | 386.7         | 0.52                               |
| Oil contamination of soil, 1 %             | 4.1                 | 2.8                 | 2.4               | 452.6         | 0.40                               |
| Oil contamination of soil, 5 %             | 4.5                 | 3.2                 | 2.7               | 375.3         | 0.36                               |
| Oil contamination of soil, 10 %            | 4.9                 | 3.5                 | 3.1               | 256.3         | 0.31                               |
| Biodux + oil contamination of soil, 1 %    | 4.3                 | 3.0                 | 2.3               | 412.5         | 0.44                               |
| Biodux + oil contamination of soil, 5 %    | 4.8                 | 3.5                 | 2.5               | 331.2         | 0.39                               |
| Biodux + oil contamination of soil, 10 %   | 5.2                 | 4.1                 | 2.8               | 297.2         | 0.35                               |
| LSD 0.5                                    | 0.12                | 0.2                 | 0.14              | 28.6          | 0.03                               |

A high oil contamination level inhibited hydrocarbons and depleted soil actinomycetes. An appropriate amount of actinomycetes in the soil is an indicator of the degree of oil contamination and can be used for biological monitoring.

Treatment of seeds with a biological preparation had an ambiguous effect on the number of microorganisms of various physiological groups, including cellulose-fermenting microorganisms (Figure 2).

Their number increased when the crops were treated with a biological preparation. This indicated the intensification of the processes of cellulose decomposition and, accordingly, the improvement of carbohydrate nutrition of plants under the impact of metabolites of the endomycorrhizal fungus, which are part of the biological preparation.

The study revealed a certain relationship between the microbial potential and the toxicity of oil
It was found that soil contamination with oil and oil products suppressed the growth and development of tomato plants. The main reasons for the oppressive development of plants or their death as a result of contamination can be the inappropriate supply of nutrients and water resources, as well as oxygen.

In oil contaminated soils, certain changes were observed 1.5 months after contamination in the form of linear dimensions of the roots and aboveground parts of plants (Figure 3).
Figure 3. Phytotoxicity of soil in tomato crops

Analysis of the data revealed the negative impact of oil contamination on the growth and development of tomato plants. Thus, the germination rate of tomato at 1% oil contamination decreased by 34.8%, at 5% oil contamination – by 47.7%, at 10% oil contamination – by 80.4%. Depending on the degree of oil contamination, the use of Biodux reduced the toxic effect on plants and increased germination by 5.6, 14.1 and 15.8%, respectively.

The length of the shoot and roots of tomato plants indicated the phytotoxic properties of oil contaminated soils. Depending on the degree of oil contamination, it suppressed the growth of green seedlings 1.32, 1.49 and 1.99 fold, while the length of the roots decreased 1.26, 1.59 and 2.87 fold compared with the control, respectively.

Treatment with Biodux on oil contaminated soil stimulated the growth of tomato plants. In this case, the shoot length increased 4.0, 4.8 and 7.7 fold compared to 1, 5 and 10% oil contamination, respectively.

Similar results were obtained when analyzing the root length. Similar results were observed for the total plant biomass. As the concentration of oil products increased, it decreased significantly.

Therefore, as mentioned above, the studied concentrations of oil products formed stressful conditions for plants and suppressed plant growth.

4. Conclusion
The results of the study showed that the toxicity and changes in the agrochemical properties of light-chestnut soils in southern Russia are directly related to the concentration of the applied oil. Thus, the samples contaminated with 10% oil show the most adverse effect on the studied parameters. When exposed to oil contamination, changes in the biological activity of the soil mainly affect the growth, development and, ultimately, the yield of vegetable crops. In many countries, the greening of
agriculture is becoming a priority during the development of agriculture. Solving of this problem implies the introduction of biological preparations based on microorganisms, which enables realization of the potential of plants in the plant-microbial system. The studied preparation Biodux formed resistance to stressful growing conditions in tomato plants and activated their growth and biological processes. Based on the data obtained, it should be noted that light-chestnut soils are not resistant to oil contamination in terms of biological indicators.

References

[1] Zaikina V N, Kasterina N G and Okolelova A A 2015 Ways of transformation of petroleum products in light chestnut soils Sci. almanac 8(10) 1193–1197
[2] Zaikina V N, Okolelova A A, Kasterina N G et al 2018 Transformation of oil products in the soils of the Volgograd agglomeration Izvestiya Vuzov-Prikladnaya Khimiya I Biotekhnologiya 8(1) 135–143
[3] Damage from oil contamination increased fivefold. Retrieved from: https://www.znak.com/2020-02-16/ucherb_ot_zagryazneniya_nefteproduktami_v_rossii_vyros_v_pyat_raz_do_5_mld (10/25/2020)
[4] Sharkova S Yu 2011 Changes in the chemical characteristics of soil affected by oil pollution Bull. of PGPU named after V.G. Belinsky 25 610–613
[5] Tumanyan A F, Tyutyuma N V, Bondarenko A N et al 2017 Effect of oil contamination on various types of soil Chem. Technol. Fuels Oils 53 369–376
[6] Buluktaev A A 2017 Phytoxicity and enzymatic activity of oil contaminated soils in Kalmykia Southern Russ.: ecol., devel. 12(4)
[7] Buluktaev A A and Sangadzhieva L Kh 2013 Resistance of light-chestnut soils of Kalmykia to oil contamination Bull. of the East Sib. State Univ. of Technol. and Manag. 3 173–180
[8] Ionescu (Ţopa) S, Mihăilescu S, Strat D and Gheorghe I F 2018 Effects of oil contamination on seed germination and seedling emergence toxicity Romanian Biotechnol. Letters
[9] Kalmykova E V, Petrov N Y, Tumanyan A F and Kalmykova O V 2020 Features of Southern Russian soils polluted by heavy metals Chem. Technol. Fuels Oils 56 73–79
[10] Dubrovskaya E V, Pozdnyakova N N, Muratova A Yu, Golubev S N, Bondarenkova A D and Turkovskaya O V 2019 Effect of oil contamination on plants in conditions of low humidity Ecobiotech. 2(3) 391–401
[11] Nazaryuk V M, Klenova M I and Kalimullina F R 2007 Role of mineral nutrition in increasing plant productivity and regulating the nutritional regime of soil contaminated with oil Agrochem. 7 64–73
[12] Tumanyan A F, Batovskqaya E K and Tyutyuma N V 2013 Effect of oil contamination on microbiological processes in soils Chemistry and Technology of Fuels and Oils 49(2) 186–180
[13] Ramazanov R R, Nazarenko D Yu and Pozharsky V G 2017 Safe solution to the problems of agroecosystems Plant protect. and quarant. 4 7–8
[14] Vinter M V, Dedyukhina E G and Krynitskaya A Yu 2011 Waste from biodiesel production as substrate for the cultivation of Mortierella alpina fungi Butlerov's quotes 26(9) 83–87