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SOIL BIOLOGY

Dynamics of Ecological and Biological Characteristics of Soddy-Podzolic Soils under Long-Term Oil Pollution

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Abstract—The dynamics of respiratory and enzyme activities and toxicological properties of loamy-sandy and loamy soddy-podzolic soils (Retisols) under the long-term influence of oil pollution were studied. The concentrations of the pollutant, at which the activity (the ability of self-purification) of the indigenous soil microflora is preserved, were determined. The dynamics of the decrease of oil product content and the time of elimination of the toxic effects on higher plants at the initial pollutant contents were revealed. The parameters of the respiratory and enzyme activities in the course of the 365-day experiment showed that the microbial community of the loamy-sandy soil was more sensitive to oil pollution. The phytotoxic characteristics of the oil-containing loamy-sandy and loamy soils did not correlate with their respiratory and enzyme activities. This fact testifies to some differences in the mechanisms of their influence on living organisms with different organizational levels and to the necessity of taking into account a complex of parameters when assessing the state of the soils under the long-term effects of oil and its products.

Keywords: oil, soddy-podzolic soils (Retisols), respiratory activity, enzyme activity, mineralization of oil products, phytotoxicity

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INTRODUCTION

The restoration of fertility and ecological properties of soils is crucially determined by the functioning of soil microbial community, changes in its activity, soil properties, and the level of anthropogenic impact.

The difficulties of studies related to the regularities of soil microbial cenoses functioning under oil pollution are related to the multicomponent nature of pollutants, heterogeneity of microbial communities, different directions of metabolic processes proceeding in soils, and changes in the properties and structure of soils differing in texture and at different levels of the pollutant impact.

The processes of oil decomposition in the soil can be better understood when determining the dynamic relationships between the respiratory enzyme activities and the phytotoxicity of technogenically polluted soils different in texture.

Numerous works showed that objective indices that permit prompt estimation of the biological state and toxicity of differently oil-polluted soils were indices of biological activity of the soil microbial cenosis [2, 5, 6, 8, 10, 15, 16]. The enzyme activity was shown to be a sensitive indicator for soil pollution, which reflects the intensity of decomposition of organic compounds [9, 11, 19, 20].

Oil and oil products are known to intensify the soil respiratory activity, which indicates the physiological activity of microorganisms in contaminated soils and characterizes the high rate of redox processes. With time, the intensity of CO_2 emission from soils is reduced, but it remains higher than the background one, testifying to a delay of hydrocarbon mineralization and other processes responsible for their decomposition—oxidation, co-oxidation, and condensation [5, 11].

The use of toxicological phytotests allows predicting the level and consequences of pollutant effects on plant objects and enables one to assess the soil fertility [15].

The objective of these investigations is the study of the dynamics of respiratory and enzyme activities and toxicological properties of oil-polluted loamy-sandy and loamy soddy-podzolic soils under the long-term impact of the pollutant.

OBJECTS AND METHODS

Two kinds of soddy-podzolic soils from the state forest fund were the objects for these studies:

– loamy-sandy soddy-podzolic soil under a pine forest on colluvium on a north-facing slope (1°) (Chuvash Republic). The organic matter content is medium,

Table 1. Physicochemical characteristics of the soddy-podzolic soils

Texture	Humus content, %	P mobile	K mobile	pH_{water}	Total N, %	Fractions (mm), content, %			
		mg/100 g				1.0–0.25	0.25–0.05	0.05–0.01	<0.01
Loamy-sandy	2.7	12.5	17.2	6.4	0.09	45.1	22.7	15.0	17.2
Loamy	6.4	13.1	23.1	5.9	0.25	3.0	21.3	40.2	35.7

which is characteristic of this soil type. The soil reaction is close to neutral; the phosphorus and potassium contents are referred to group III of provision.

—loamy soddy-podzolic soil under a goutweed–lime-tree forest with pine on colluvium on a northwestern slope (1°–3°) (Republic of Tatarstan). The organic matter content is high, which is characteristic of the humus horizons of forest podzolic soils. The reaction of the soil is weakly acid. The contents of mobile phosphorus and potassium are high (Table 1).

In the loamy-sandy soddy-podzolic soil, the total number of microorganisms and that of hydrocarbon-oxidizing microorganisms and actinomycetes in the soils were 3.9, 7.5, and 3.1 times higher, respectively. The number of cellulose-destroying microorganisms in the loamy-sandy soil was higher by 1.8 times and that of micromycetes by 2.8 times as compared to their numbers in the loamy soddy-podzolic soil.

The soils were air-dried, and plant roots and plant residues were removed. Then, the soil was sifted using a Winkler 1-mm sieve.

In the laboratory, the experiments were performed in plastic containers (180 × 140 × 90 mm) containing 1400 g of air-dried soils. The test variants with the assigned oil content were prepared in three replicates. Different quantities of sulfurous, paraffin, and tarry oils from the Yamashinskoe oil deposits (Republic of Tatarstan) were added to the soil samples.

Unpolluted soil was the control. The soils were moistened, and during the experiment, the moisture was maintained at 60% of the total moisture capacity; the incubation temperature was 20–24°C. Every week, the control and test soil samples were loosened.

Taking into account the sorption capacity, the test variants were used with the oil contents of 2.4, 4.8, 9.0, 13.0% in the loamy-sandy and 4.8, 7.1, 9.0, 13.0, 16.6, 20.0% in the loamy soddy-podzolic soils.

The total content of oil products in the soils was determined using IR spectrometry, a KN-2m analyzer, and the certified methodology [13]. The content of oil products (OP) was determined immediately after adding oil to the soils and on the 7th, 30th, 180th and 365th days of the experiment.

On day 7, 30, 180, and 365 after the incubation, the samples for the determination of potential respiratory and enzyme activities were taken.

The following parameters were determined by chromatography [7]: basal respiration (Vbasal), substrate-induced respiration (Vsir), coefficient of microbial respiration ($Q_R = V$basal$/V$sir), and the carbon content in microbial biomass (Cmicr) [1, 22]. The intensity of soil respiration was measured using a gas chromatographer "Khromatek Kristall 5000.2". A column was 3.0 m long with the internal diameter of 3 mm; an adsorbent was Hayesep N 80/100. A katharometer was used as a detector. The rate of respiration was expressed in μg CO_2/g of dry soil h.

The catalase, urease, and protease activities were determined using traditional methods [21].

Acute toxicity was determined every 6–12 days by the changes in the root length in wheat (*Triticum vulgare*) seedlings [12].

The results were processed statistically using the Microsoft Excel program.

RESULTS AND DISCUSSION

Respiratory activity. Soddy-podzolic soils have a low level of natural fertility. They are poor in organic matter and nutrients and have a low biological activity [3, 14].

The analysis of the respiratory activity of the test soils showed that in the unpolluted (control) loamy soddy-podzolic soil, it was 2.9–3.2 times higher than in the poorer loamy-sandy soil, and this correlates with the content of active "living" microbial biomass there. The latter was estimated by the content of Cmicr (Table 2). The calculated values of the coefficient of microbial respiration (Q_R) reflect the satisfactory state of the microbial pool in the soils [4].

The addition of oil to the soddy-podzolic soils has drastically changed the environment for the microorganisms. On the one hand, the soil became richer in organic matter, on the other, its structure and water–gas exchange have been altered and produced changes in the biological activity of the microbial cenosis due to the addition of toxic compounds to the soil.

Table 2. The initial respiratory activity of unpolluted (control) soils

Texture	Vbasal	Vsir	Cmicr, μg C/g	Q_R
	μg CO_2/(g h)			
Loamy-sandy	2.66	7.50	152.0	0.35
Loamy	8.62	21.76	437.7	0.40

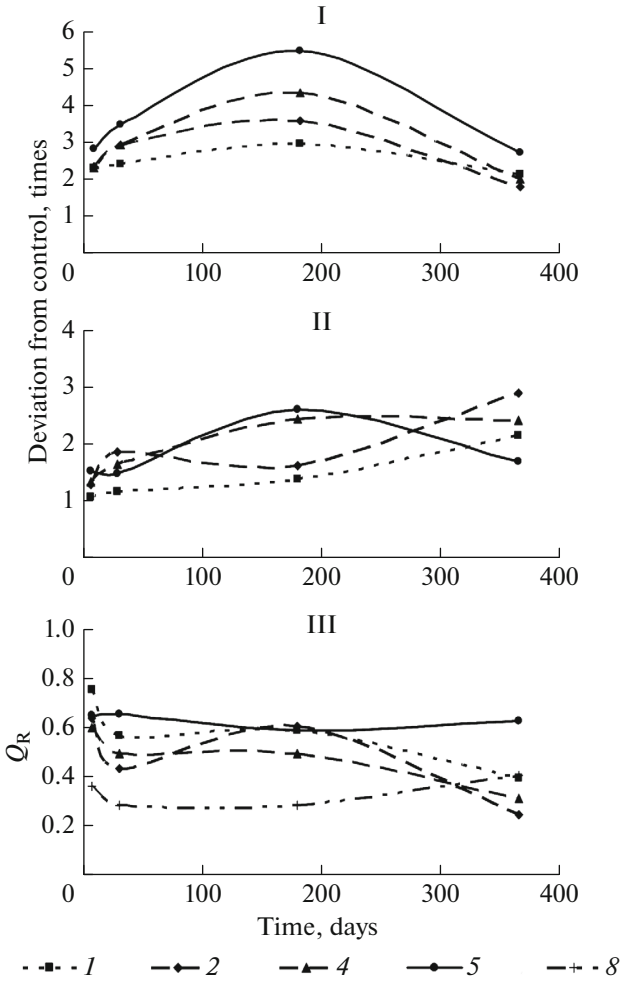

Fig. 1. Parameters of respiration in the loamy-sandy soddy-podzolic soil at different initial contents of oil. Here and hereafter, Arabic numerals designate the following oil concentrations (%): *1*–2.4, *2*–4.8, *3*–7.1, *4*–9.0, *5*–13.0, *6*–16.6, *7*–20.0, *8*–control. Designation here and in Fig. 2: I–*V*basal, II–*V*sir, III– Q_R.

The experimental data showed that the application of oil to the loamy-sandy soddy-podzolic soil increased the basal respiration. In the course of the experiment, the most intense CO_2 emission was recorded at the maximal concentration of the pollutant (Fig. 1, a). The peak of respiratory activity in the oil-containing soils was registered on the 180th day of incubation and was linearly related to the initial concentration of the organic substrate added. On the 365th day, *V*basal decreased to the values that were 1.8–3.1 times higher than those in the control soil.

In the variants with 2.4 and 4.8% of oil, the substrate-induced respiration of the loamy-sandy soddy-podzolic soil increased (Fig. 1, b). At the oil content of 9.0%, the intensity of CO_2 emission was stabilized at 12.6–13.2 μg CO_2/(g h); on the 180th day, in the variant with 13.0%, *V*sir increased to 13.5%; after 365 days of incubation, the accumulation of metabolites caused a decrease in the rate of CO_2 emission to 9.4 μg CO_2/(g h).

The toxic effect of oil at the 4.8% concentration did not manifest itself directly. Its higher concentration was responsible for a small peak on the 30th day of the experiment, which might be a response of the microbial complex to an increase in the content of available substrate in the absence of its inhibiting effect. In the variant with 9% of oil in the soil, a gradual adaptation of the community to the pollutant was observed. The level of metabolism at this oil concentration after the peak by the 180th day did not change. The decomposition of oil components and their metabolites proceeded at the rate that did not cause the accumulation of toxicants, which was observed in the period of 180–365 days in the variant with the initial oil concentration of 13%.

The addition of oil to the loamy-sandy soddy-podzolic soil decreased the stability of the microbial community; on the 30–180th day of the experiment, Q_R increased by 1.7–2.1 times (Fig. 1, c). In the course of adaptation and redox processes in the variants with 2.4–9.0% of oil by the 365th day, Q_R decreased to the values characteristic of the control soil.

In the variant with the initial oil content of 13.0% even after a year of incubation, the Q_R value was high, testifying to the unstable state of the soil biocenosis and disequilibrium of metabolic processes.

The dynamics of *V*basal in the loamy soddy-podzolic soil was specified by the initial oil concentration. At the oil content of 4.8–90%, *V*basal linearly decreased; by the end of the test, it was comparable with that in the control (Fig. 2, I. In the variants with 13–20% of oil in the soils, in the first 180 days at the high concentration of the nutrient substrate, the rate of *V*basal increased. Then, this value significantly decreased but it was greater than in the control variants.

Generally, the dynamics of substrate-induced respiration was similar to the pattern obtained for *V*basal. The temporal changes of *V*sir in the variants with 4.8–9.05% of oil had a linear dependence (Fig. 2, II). At the higher oil concentrations after the decrease in the microbial complex activity by the 180th day of incubation, the microbial activity increased with the subsequent reduction of *V*sir to the values characteristic of the control soil samples.

In the loamy soddy-podzolic soil (compared to the loamy-sandy soil), the Q_R values demonstrated the higher microbial activity and the higher resistance of the microbial complex to oil pollution. In the variants with the initial oil content of 4.8–9.0% on the 30th day, the Q_R values characterized the state of the soil biocenosis as a rather satisfactory (Fig. 2, III). In the variants with the higher oil content, by the 365th day of the test, the Q_R values evidenced a decrease in the stress impact on the soil microbial community.

Thus, the increase of the oil concentration in the loamy-sandy and loamy soddy-podzolic soils to 13 and 20.0%, respectively, did not decrease the rate of basal respiration. The parameters of substrate-induced respiration showed that the tested oil concentrations in the soils studied did not inhibit the activity of the soil microflora. The Q_R values showed that the anthropogenic impact on the loamy-sandy soddy-podzolic soil at the oil content of 2.4–9.0% became lower only by the 365th day of incubation. The disequilibrium of metabolic processes and stress effect of the pollutant at its concentration of 13% was also preserved in a year after the addition of oil to the soil. In the loamy soddy-podzolic soil, the activity and resistance of the microbial pool to oil pollution promoted a decrease in the stress and restoration of the microbial cenosis properties by the 30th day in the variants with 4.8–9.0% of oil in the soil. At the higher oil concentrations, by the 365th day of incubation, the Q_R values approached those reflecting the relatively good state of the soil.

Enzyme activity is determined by the texture of the soils studied. As the respiratory activity, the enzyme activity of the unpolluted loamy soddy-podzolic soil was several times higher than that of the loamy-sandy soil (Table 3).

A response of the soil microbial community was related to the initial oil concentration in the soils. The addition of oil to the loamy-sandy soddy-podzolic soil at the oil concentration of 2.4 and 4.8% increased the *catalase activity* by 22.4 and 10.9 times on the 7th day (Fig. 3, a). The increase in the time of soil incubation was accompanied by a decrease in the enzyme activity to the values that were higher than the activity of the unpolluted soil. In the variant with 9% of oil after 30 days of incubation, the catalase activity level was 1.6–3.0 times higher than that in the control samples. In the variant with 13% of oil in the soils, the longer incubation did not raise the catalase activity, which appears to be related to the inhibiting effect of the high concentration of oil components.

In all the experimental variants with the loamy-sandy soddy-podzolic soil, the level of catalase activity on the 7th day of incubation exceeded the activity of the unpolluted soil (Fig. 3, b). The maximum levels of catalase activity in the oil-containing soils, which exceeded the control ones by 4.2–6.7 times, were registered on the 30th and 180th days of the experiment. A weak inhibition of the catalase activity was observed

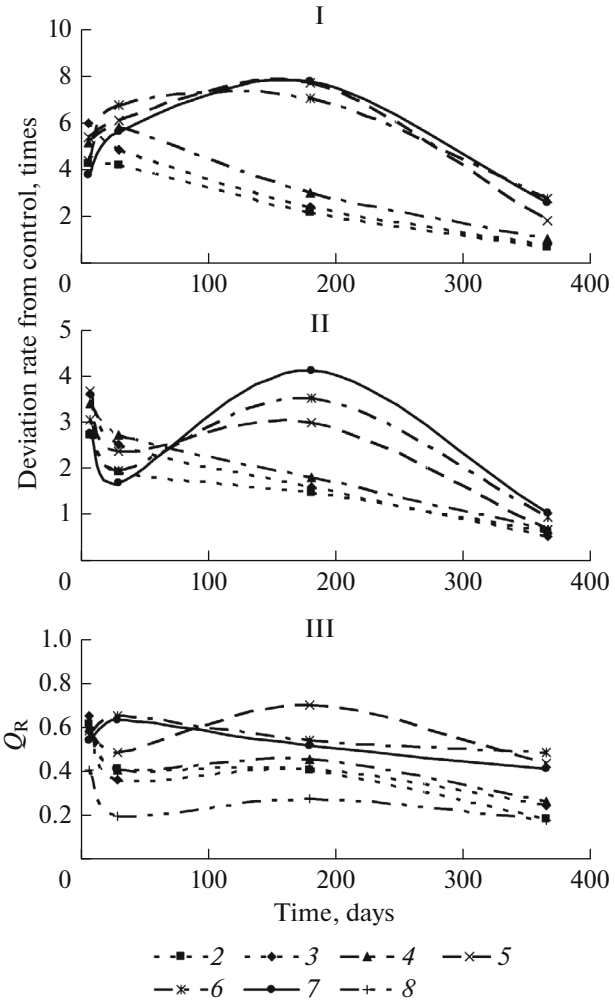

Fig. 2. Parameters of respiration in the loamy soddy-podzolic soil at different initial contents of oil.

on the 365th day of the experiment in the variants with the oil contents of 13.0 and 16.6%.

The urease activity of the loamy-sandy soddy-podzolic soil in all the variants was higher than in the control soil samples. At the same time, in the variants with 9.0 and 13.0% of oil concentration, the urease activity decreased on the 30th day of the experiment due to the long-term influence of pollutants on the soil microflora (Fig. 4, a). The depressed state of the soil microflora is related to the presence of toxic volatile fractions at high oil concentrations [17, 18]. In the variant with oil at 13% by the 365th day, the accumula-

Table 3. The enzyme activity of unpolluted (control) soils

Texture	Catalase, mg H_2O_2/g	Urease, μg N/(g h)	Protease, μg amino acids (g day)
Loamy-sandy	0.01	0.13	45.9
Loamy	0.81	0.59	340.8

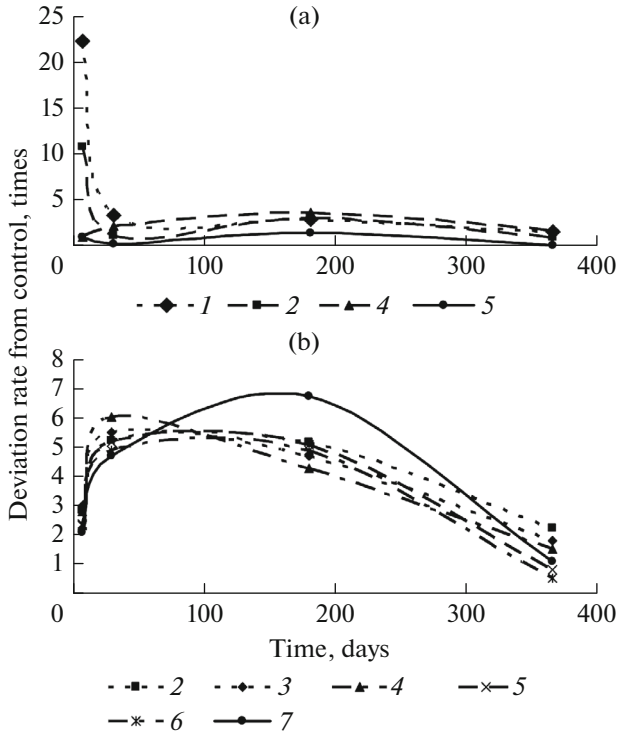

Fig. 3. Dynamics of catalase activity in the soddy-podzolic soils containing different oil amounts. Here and hereafter, a—loamy-sandy, b—loamy soil.

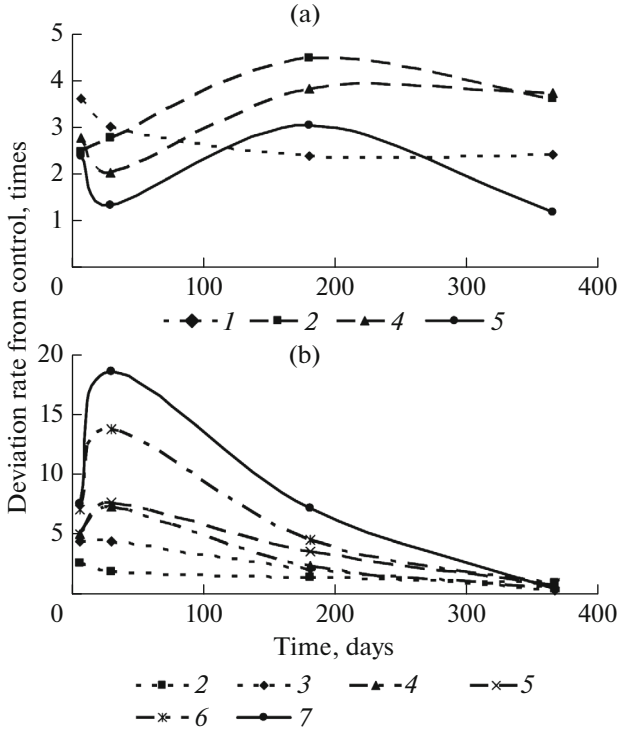

Fig. 4. Dynamics of urease activity in the loamy-sandy and loamy soddy-podzolic soils containing different oil amounts.

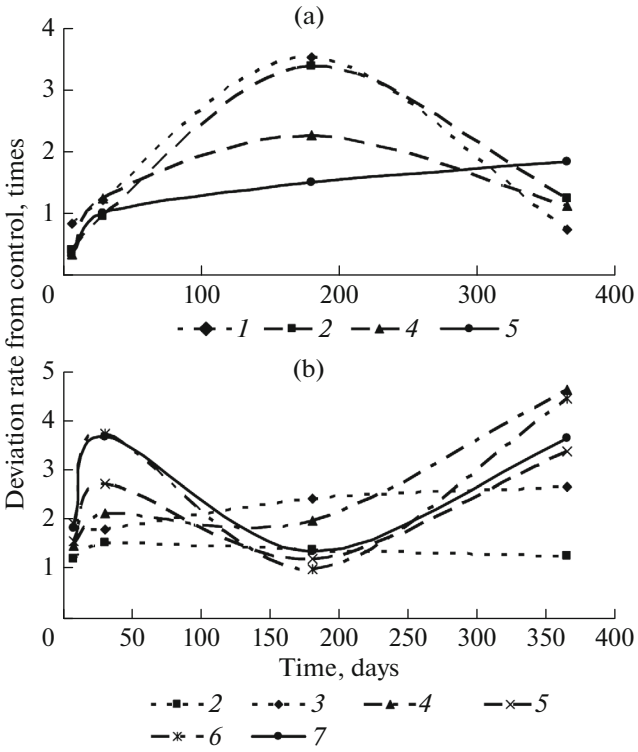

Fig. 5. Dynamics of protease activity in the loamy-sandy (a) and loamy (b) soddy-podzolic soils with different oil concentrations.

tion of oil metabolites and phytotoxins [10] caused a decrease in the urease activity to the control values.

Unlike the soil with lighter texture, the peak of the urease activity in the oil-polluted loamy soddy-podzolic soil at the oil content of 9.0–20.0% was registered on the 30th day of the experiment (Fig. 4, b). With the increasing time of incubation, the urease activity decreased, and on the 365th day, it amounted to 0.4–0.9 of the control values.

Protease activity. At the early stages of the experiment (7 days), a negative effect of oil on the loamy-sandy soddy-podzolic soil was manifested at all their initial concentrations (Fig. 5, a). On the 180th day, the level of urease activity was determined by the initial oil concentration and a decrease in the content of toxic light oil fractions in the soils. On the 7th and 180th days, the enzyme activity was inversely dependent on the initial oil concentration. On the 365th day of incubation, the protease activity decreased to the control values and lower at the oil concentration of 13%.

Under the addition of oil to the loamy soddy-podzolic soil, the influence of the initial oil concentration on the dynamics of protease was observed. In the first 30 days of incubation, the increase in oil content promoted the growth of protease activity (Fig. 5, b). The increase of soil exposition up to 180 days caused a decrease in the protease activity due to the accumulation of oil metabolites and/or phytotoxins in variants

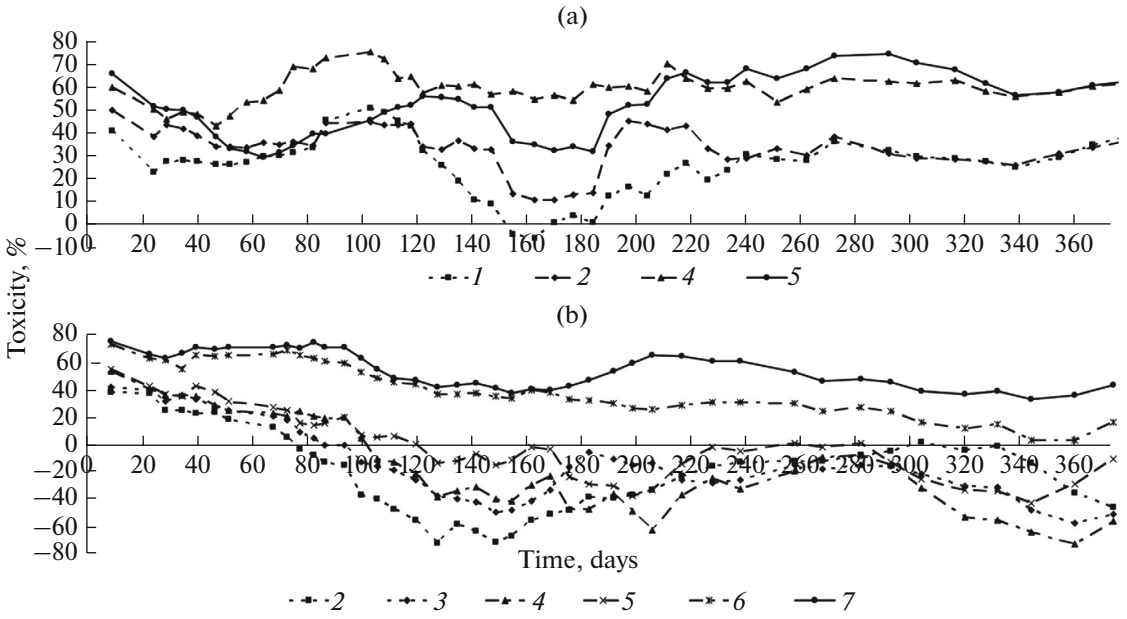

Fig. 6. Dynamics of phytotoxicity of the soddy-podzolic soils with different initial oil contents.

9.0–20.0%. The adaptation and biodegradation of toxic metabolites at long-term incubation are a reason for the secondary growth of the protease activity.

Thus, the application of oil to the loamy soddy-podzolic soil at the concentration of 20% and less increased the catalase activity. The inhibition of catalase activity was recorded on the 365th test day in the variants with 13.0 and 16.6% of oil. Oil at 9% concentration did not inhibit the catalase activity of the loamy-sandy soddy-podzolic soil. The addition of oil to the loamy-sandy and loamy soils at the concentrations of 13 and 20%, respectively, increased the urease activity. On the 365th day of the experiment, the urease activity of the loamy soddy-podzolic soil in all the variants decreased and amounted to 0.4–0.9 of the control values. The application of oil to the loamy-sandy soil at the concentrations of 4.8–13.0% inhibited the protease activity at the early stages of oil effects. Oil at the content of 20% did not inhibit the protease activity of the loamy soddy-podzolic soil.

Toxicity. The dynamics of the phytotoxic properties of oil-polluted loamy-sandy soddy-podzolic soils was non-monotonous. The maximal toxic level was registered in the variant with the application of 13% of oil on the 192nd and 203rd days (Fig. 6, a). The absence of acute toxic effect (the deviation of the root length from the control one was not more than 20%) was observed only in the variants with 2.4 and 4.8% of oil on the 129th–236th and 150th–190th days, respectively. The changes in the toxic properties of the loamy-sandy soddy-podzolic soils within the range of oil concentrations tested followed the general regularity—the oil concentration increased or decreased at approximately the same time periods and differed by the level of its influence on a test object related to the initial oil concentration. Probably, the changes in the phytoxicity were also dependent on the accumulation of metabolites in the soil that were formed periodically in the course of biological decomposition of oil components.

In the loamy soddy-podzolic soil, unlike the loamy-sandy soil, the acute toxic effect was gradually reduced within the range of oil concentrations of 4.8–13.0%. On the 76th–120th day, the toxic effect completely disappeared (Fig. 6, b). Later on, the oil components stimulated the growth of wheat roots that increased by 60–70%. In the variant with the initial oil content of 16.6%, the acute toxic effect was recorded on the 300th day of incubation, and in the variant with 20% of oil concentration, the phytotoxic effect was preserved to the end of the experiment.

Under the same initial content of oil pollutants, the soddy-podzolic soil of lighter texture was found to have a higher phytotoxic level. Within the range of concentrations tested, the oil-polluted loamy-sandy soddy-podzolic soil was characterized by non-monotonous changes in the phytotoxic properties that were also displayed on the 365th day of the test. In the loamy soddy-podzolic soil, at oil concentration of 4.8–13.0%, the acute toxic effect of oil disappeared on the 76th–120th days; at 16.6%, it was not observed on the 300th day of incubation. At the 20% initial oil content, the phytotoxic effect of oil was preserved to the end of the experiment.

Changes in the concentration of oil products in the soils. The analysis performed in 24 h after the oil application showed that the content of oil products in

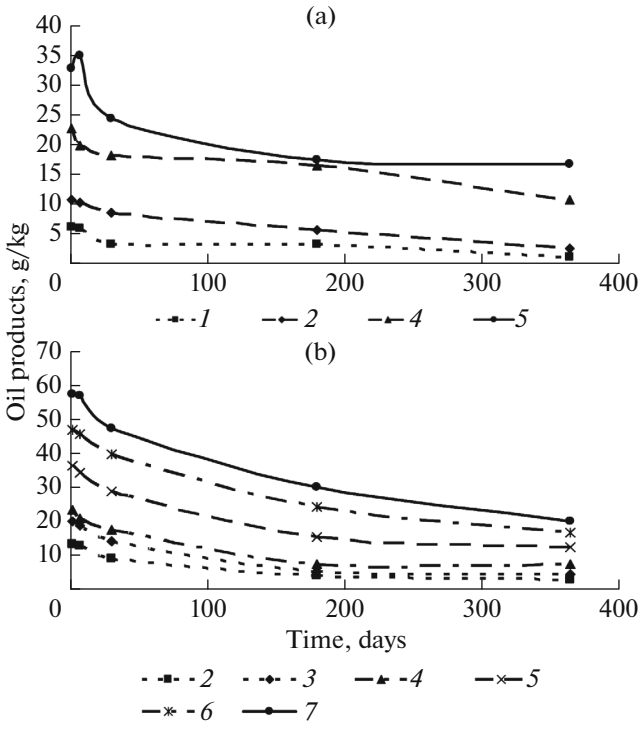

Fig. 7. The concentration of oil products in the soddy-podzolic soils under the different incubation times and different oil concentrations.

the test samples determined according to the certified methodology [13] amounted to 22–29% of the oil amount added to the soil.

The more intense decrease in oil content in the soil samples was found in the first 30 days of the test (Fig. 7). In the course of further incubation of the loamy-sandy soddy-podzolic soils, in the variants with oil content of 2.4–9.0%, the pollutant concentration decreased linearly. The changes in the rate of oil decomposition in the variant with 13% of oil in the loamy-sandy soil correlated to *V*sir (Fig. 1, b). The increase in the incubation time for the loamy soil caused a gradual oxidation of hydrocarbons there (Fig. 7, b).

It is worthy to note that by the 30th and 180th days of the test, the mineralization intensity of oil hydrocarbons in the loamy soddy-podzolic soil was 1.1–2.8 and 2.1–6.3 times higher, respectively, than that in the loamy-sandy soil. The increase of the initial oil concentration from 2.4 to 9.0% in the loamy-sandy soddy-podzolic soil was accompanied by the reduction in the intensity of oil oxidation (Table 4). By the end of the experiment in the loamy soddy-podzolic soil, the level of oil product mineralization was close to that in the variants with the application of oil at 4.8–7.1 and 9–20%.

Table 4. The efficiency of oil product mineralization (%) in the oil-polluted loamy-sandy and loamy soddy-podzolic soils by the end of the experiment

Soil texture	Initial oil content, %						
	2.4	4.8	7.1	9.0	13.0	16.6	20.0
Loamy-sandy	84	77	–	48	53	–	–
Loamy	–	77	78	67	66	65	65

CONCLUSIONS

The ecological and biological characteristics studied allowed determination of the oil concentrations at which the ingenious microflora of soddy-podzolic soils differing in texture remains active (the ability for self-purification). They also enabled one to reveal the dynamics of the decrease in oil product content and the time of elimination of toxic effects on higher plants at different initial contents of the pollutant.

The data on the respiratory and enzyme activities of the soddy-podzolic soils obtained in the course of the 365-day-long experiment showed that the microbial community of the loamy-sandy soddy-podzolic soil was more sensitive to oil pollution.

The phytotoxic characteristics of the oil-containing loamy-sandy and loamy soddy-podzolic soils do not correlate with their respiratory and enzyme activities. This indicates the difference in mechanisms of oil effects on living organisms of different organizational levels and the necessity to take into account a complex of characteristics when determining the state of soils under the long-term influence of oil and oil products.

The increase in the initial oil content caused a reduction in the intensity of oil product mineralization in the loamy-sandy soil. The higher oil content in the loamy soddy-podzolic soil affected the intensity of oil product mineralization to a lesser extent.

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