

## Studies on the Rehabilitation Ability of the Yakutian Permafrost Soil–Plant Model System under Oil Pollution

S. KH. LIFSHITS<sup>1</sup>, B. M. KERSHENGOLTS<sup>2</sup>, O. N. CHALAYA<sup>1</sup>, I. N. ZUEVA<sup>1</sup>, M. M. SHASHURIN<sup>2</sup> and YU. S. GLYAZNETSOVA<sup>1</sup>

<sup>1</sup>*Institute for Petroleum and Gas Problems, Siberian Branch of the Russian Academy of Sciences, Ul. Oktyabrskaya 1, Yakutsk 677891 (Russia)*

E-mail: [s.h.lifshits@ipng.ysn.ru](mailto:s.h.lifshits@ipng.ysn.ru)

<sup>2</sup>*Institute of Biological Problems of Cryolitozone, Siberian Branch of the Russian Academy of Sciences, Pr. Lenina 41, Yakutsk 677891 (Russia)*

(Received May 27, 2008; revised July 8, 2008)

### Abstract

Geochemical characteristics of Yakutian permafrost soils, soil enzymatic activity, as well as physiological, biochemical characteristics of the rough dandelion (*Taraxacum ceratophorum*) and relative resistance of the plant cell genome against the action of oil toxicants have been determined with oil (0.07–1.95 vol. %) entering into soil samples under the laboratory conditions. It is demonstrated that the permissible level of oil-pollution when soils are capable to rehabilitation amounts to ~1 g/kg of soil.

**Key words:** ecology, oil pollution biotransformation, soil-vegetation cover elements, natural geochemical background, acceptable oil pollution level

### INTRODUCTION

The intensive development of the oil-and-gas complex raises the risk of polluting the territories with oil and petroleum derivatives (PD). Having a high sorptive power, soils can accumulate oil and PD entering them due to spillage and leakage. When mixing man-caused hydrocarbons with the naturally occurring organic matter of soils there is a change of the characteristics of the natural geochemical background. Owing to the complex biocenotic system (first of all to the content of hydrolyzing and oxidizing enzymes including polyphenoloxidases and peroxidases), soils are themselves capable to utilize a part of the PD entered into a soil *via* biochemical degradation and oxidative destruction. As the result of these processes a part of the components of oil and PD is converted into organic substances such as low-molecular metabolites those are capable to be assimilated by plants.

It is known, that the biodegradation by soil microflora mainly affects relatively low-molecular hydrocarbons [1, 2]. When there is a low concentration of oil and PD components entered into the root layer of soil (rhizosphere), plants are capable to incorporate a part of chemical compounds into the metabolism. Moreover, due to the ability of the genetic complex of plant root cells to activate in the adaptive response mode the synthesis of antioxidants, DNA repair enzymes, oxidases, hydrolases and other species, the resistance of plants against oil pollution toxic components can increase [3–5]. However, in the case that the pollution level exceeds the allowable value, the soil-vegetation cover loses the ability of self-repair and a pressing need appears for artificial revegetation to carry out.

The acceptable level of the pollution by oil and PD varies depending on soil type, species composition of the vegetation cover, geological-and-geographical landscape and, first of all, to all appearance, on attaching the region un-

der study to either climatic zone. In order to estimate the allowable level of oil pollution for the permafrost soils of Yakutia, a multifactor camera experiment has been earlier carried out [6, 7].

## EXPERIMENTAL

For carrying out the experiment, we sampled sandy soil typical for the Central Yakutia (reference). The main characteristics of this soil are presented below:

Moisture, %/100 g of soil	0.75
Density, g/cm <sup>3</sup>	2.61
Fraction of particles (%) with diameter, mm:	
1.0–0.25	8.9
0.25–0.05	88.8
0.05–0.01	1.0
0.01–0.005	0.2
0.005–0.001	0.2
<0.001	0.9
Sum of particles <0.01 mm in diameter	1.3
Content of mobile species, mg/100 g of soil:	
N (NH <sub>4</sub> )	1.7
N (NO <sub>3</sub> )	2.0
P <sub>2</sub> O <sub>5</sub>	6.1
K <sub>2</sub> O	6.5

Equal amounts of soil (200 cm<sup>3</sup>) were placed into culture pans. Then we added oil taken from the Talakan petroleum deposit (to the extent of 0.07–1.95 vol. %) thereto and carefully mixed. The tilled soil was sowed with seeds of the rough dandelion (*Taraxacum ceratophorum* (Ledeb.)), 50 pieces in every of four replications. The physiological characteristics of plants were determined from the energy of germination (by the 7th day), seed germinability (by the 14th day) seeds and the survival rate of sprouts (by the 60th day, the experiment completion). The cytological and biochemical characteristics of the plant were determined basing on the activity of protective antioxidant systems those include superoxide dismutase (SOD), peroxidases, the pool of low-molecular antioxidants (LMAO), on the activity of the genetic system of dividing plant root cells in the processes of replication, DNA reparation (self-restoration DNA) directed on the translation (protein biosynthesis), as well as the genome resistance with respect to affecting environmental stress-factors [6].

The activity of peroxidase was determined according to the standard technique described in [8]. The activity of enzyme was registered basing on time-dependent accumulation of the oxidation product of aromatic amine *o*-dianisidine which forms a coloured product with the absorption maximum at the wavelength of 460 nm ( $\epsilon = 3 \cdot 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ ). The method for determining the total LMAO is based on the oxidation of these compounds by iron (III) chloride reduced in this process to yield iron (II) chloride. The amount of the latter was determined basing on the colouring intensity at  $\lambda = 490\text{--}510 \text{ nm}$  after adding *o*-phenantroline [9]. The determination of SOD activity was performed basing on the inactivation of superoxide radicals ( $\text{*O}_2^-$ ) by this enzyme [10]. The functioning activity of protein-synthesizing, DNA-replicating and repairing systems was determined with the use of a radioisotopic tracer technique [11].

After completing the experiments the samples of soils were dried up, sifted through 1 mm mesh sieve and divided into two parts. One part was used for determining the activity of soil enzymes such as polyphenoloxidase, catalase, urease and invertase taking part in utilizing some components of oil. Using colorimetric method we determined the activity of urease [12–14], polyphenoloxidase [13] and invertase [15]. The catalase activity was studied by means of gasometry [12, 16].

The second part of soils was investigated using the complex of geochemical methods for analysis [7] including the chloroform extraction, infrared spectroscopy (FT-IR Nicolet Protege 460 spectrometer, liquid adsorption chromatography and chromatography/mass spectrometry techniques. The GC/MS studies were carried out with the system including an Agilent 6890 gas chromatograph interfaced with an Agilent 5973N high-performance mass-selective detector. The chromatograph was supplied with a capillary quartz column of 30 m length, 0.25 mm in diameter impregnated with an HP-5MS phase. Helium was used as the carrier gas, the gas flow rate being of 1 mL/min. The evaporator temperature was of 320 °C. The temperature rise was programmed within the temperature range of 100–300 °C with a rate of 6 °C/min. The ionizing voltage was equal to 70 eV.

TABLE 1  
Variation of the bituminoid yield, the rough dandelion physiological parameters and the soil enzymatic activity with the amount of oil entered into soil

No.	Volume fraction of oil added, %	Bituminoid yield, mass %	Germination energy by 7th day, %	Germinability by 14th day, %	Survival rate by 60th day, %	Enzymatic activity			
						Invertase, mg <sub>gl</sub> /(g <sub>s</sub> ·h)	Urease, mg NH <sub>4</sub> <sup>+</sup> /(g <sub>s</sub> ·day)	Polyphenoloxidase, mg <sub>b/c</sub> /(10 g <sub>s</sub> ·h)	Catalase, mL O <sub>2</sub> /(g <sub>s</sub> ·min)
1	0	0.025±0.001	39±4	29±3	5±1	3.8±0.2	2.5±0.1	1.8±0.1	6.5±0.3
2	0.07±0.01	0.024±0.001	52±5	39±4	7±1	3.7±0.2	2.4±0.1	1.2±0.1	8.3±0.4
3	0.13±0.01	0.033±0.001	47±5	49±5	20±2	4.5±0.3	2.3±0.1	1.6±0.1	8.5±0.4
4	0.20±0.01	0.049±0.001	42±4	39±4	13±1	4.6±0.3	2.4±0.1	1.6±0.1	8.6±0.4
5	0.26±0.01	0.050±0.001	33±3	31±3	14±1	4.4±0.3	2.3±0.1	1.5±0.1	8.4±0.4
6	0.33±0.01	0.082±0.001	55±5	53±5	18±2	5.1±0.3	2.8±0.1	1.5±0.1	7.5±0.4
7	0.40±0.01	0.078±0.001	49±5	41±4	17±2	3.8±0.2	3.1±0.2	1.2±0.1	7.3±0.4
8	0.46±0.01	0.098±0.001	41±4	41±4	16±2	3.4±0.2	3.2±0.2	1.4±0.1	7.4±0.4
9	0.52±0.01	0.118±0.001	50±5	19±2	7±1	3.2±0.2	2.2±0.2	1.3±0.1	6.8±0.3
10	0.59±0.01	0.149±0.001	39±4	38±4	14±2	3.4±0.2	2.3±0.2	1.2±0.1	5.4±0.3
11	0.65±0.01	0.169±0.001	35±4	33±3	8±1	2.4±0.2	1.8±0.2	1.0±0.1	5.3±0.3
12	1.30±0.01	0.250±0.001	36±4	29±3	6±1	2.1±0.2	1.9±0.2	1.0±0.1	5.5±0.3
13	1.95±0.01	0.428±0.001	35±4	16±2	1±1	2.0±0.2	1.5±0.2	1.1±0.1	5.3±0.3

Note. gl – glucose, s – soil, b/q – benzoquinone.

## RESULTS AND DISCUSSION

The chloroform extracts (bituminoids) isolated from soil samples represent a complex mixture of organic compounds of natural and man-caused (oil pollution) origin. The studies on bituminoids with the use of complex geochemical analysis methods allows one to reveal oil-pollution against the background of naturally occurring organic matter of soils, to determine the character and level of oil-pollution as well as to investigate the processes of petroleum transformation under the influence of the elements of the soil-vegetation system.

The content of bituminoids in samples depending on oil amount of oil entered into a soil is presented in Table 1. There are physiological characteristics of plants and data concerning the activity soil enzymes, too. One can see that physiological characteristics of the dandelion and the activity of soil enzymes exhibit nonlinear dependence on the amount of petroleum entered therein; at the same time in a number of experiments petroleum takes a stimulating effect. The greatest stimulation effect as compared to the reference group is observed with entering oil to the extent of 0.40–0.46 vol. %. At a higher content of oil (up to 1.30 vol. %) its stimulation influence upon physiological characteristics of plants, including the increase in the survival rate level for sprouting plants (survival rate/energy of germination), still remains persistent. However, the activity of soil enzymes irreversibly decreases becoming lower than that for the reference group.

### Geochemical studies

The studies on the bituminoids of soil samples using a FT-IR spectroscopy technique has demonstrated that after oil entering the IR spectra of samples take on the hydrocarbonic character.

Bituminoids of modern deposits and oil-pollutants differ in the content of hydrocarbons and asphalt-resinous components. So, the asphalt-resinous components are prevalent comparing to hydrocarbons in the composition of the bituminoid from the reference soil sample, whereas in the composition of petroleum derivatives the fraction of hydrocarbons can exceed to a considerable extent the fraction of resins and asphaltenes [17, 18].

TABLE 2  
Group componental composition of bituminoids from soil samples

Sample	Volume fraction of oil added, %	Bituminoid yield, mass %	Pollution duration, days	Dandelion growing	Bituminoid composition, %		
					HC fraction	Resins	Asphaltenes
R	0	0.025±0.001		+	9.3±0.5	65.1±3.2	25.6±1.3
A	0.20±0.01	0.078±0.001	7	-	43.1±0.4	47.3±2.4	9.6±0.7
B	0.20±0.01	0.061±0.001	60	-	33.7±1.7	47.8±2.4	18.5±1.1
C	0.20±0.01	0.049±0.001	60	+	33.9±1.7	53.8±2.7	12.3±0.9

Data concerning the group componental composition obtained using a liquid adsorption chromatography technique indicate that the bituminoid from the reference soil sample (R) is characterized by a low content of hydrocarbons (9.3 %) and a higher content of resins and asphaltene (65.1 and 25.6 %, respectively). The data obtained are typical for organic matter of modern deposits (Table 2).

Data on the content of hydrocarbonic substances and asphalt-resinous components are also presented therein for the three soil samples (A, B, C), those were entered with an identical amount of oil (0.20 vol. %). The sample A was analyzed in 7 days, whereas the sample B was analyzed in 60 days after the pollution with oil, therewith the plants were not sowed. The sample of soil C was sowed with the seeds of the rough dandelion, the soil was analyzed after completing the experiment (in 60 days after the pollution) alongside with the other soil samples where the plants grew. It is seen that the sample A is characterized by the maximal yield of bituminoid, as well as also the highest content of hydrocarbonic substances in the bituminoid composition. For the sample B the yield of bituminoid and the content of hydrocarbonic fraction is by 22 % lower as compared to those for the sample A.

Thus, the hydrocarbonic fraction of oil pollution in petroleum-polluted soils is first of all transformed. This fact could be connected both with the processes of hydrocarbon biodegradation by soil microflora, and with the evaporation of hydrocarbons. The sample C is characterized by even lower yield of bituminoid (20 % lower as compared to the sample B), which, to all appearance, is caused by the transformation of an organic matter of oil nature by the elements of the soil-plant system, including the rhizosphere.

It should be noted that for soil samples with the sowing of plants where the petroleum entering does not exceed 0.46 vol. %, the fraction of hydrocarbonic substances in the composition of bituminoids varies within the range of 20–34 %. The further increase in soil pollution with oil results in a fast rise in the content of hydrocarbonic fraction. So, the samples with a maximum amount of oil entered (1.30–1.95 vol. %) the content of hydrocarbons in bituminoids ranges within 55.1–62.7 %, which is close to the composition of stripped oil. To all appearance, with entering oil at a rate of 0.46 vol. % (which corresponds to the pollution value of 0.098 mass % or ~1 g/kg of soil) the elements of the soil-plant system “manage” the pollution more efficiently than at higher amounts of oil added.

Basing on the data concerning the composition and the features of the distribution of individual hydrocarbons one can determine the hydrocarbon component of bituminoids. For this purpose we have performed the chromatography/mass spectrometry analysis of the hydrocarbon fractions of bituminoids. Figure 1, *a* demonstrates the mass fragmentograms profile ( $m/z$  57) of the sample R. One can see that homologues of normal (saturated) structure are prevailing in the composition (92.0 % of the overall identified alkanes), the maximum of *n*-alkanes is corresponding to the region of *n*-C<sub>31</sub>. The fraction is observed to be low (6.7 %), 2- and 3-methyl alkanes are present in trace amounts (1.3 %), whereas 12- and 13-methyl alkanes are absent. When entering oil into the soil the character of hydrocarbon distribution became different to a considerable extent (see Fig. 1, *b*).

So, for the sample A this character was almost identical to that of distribution for

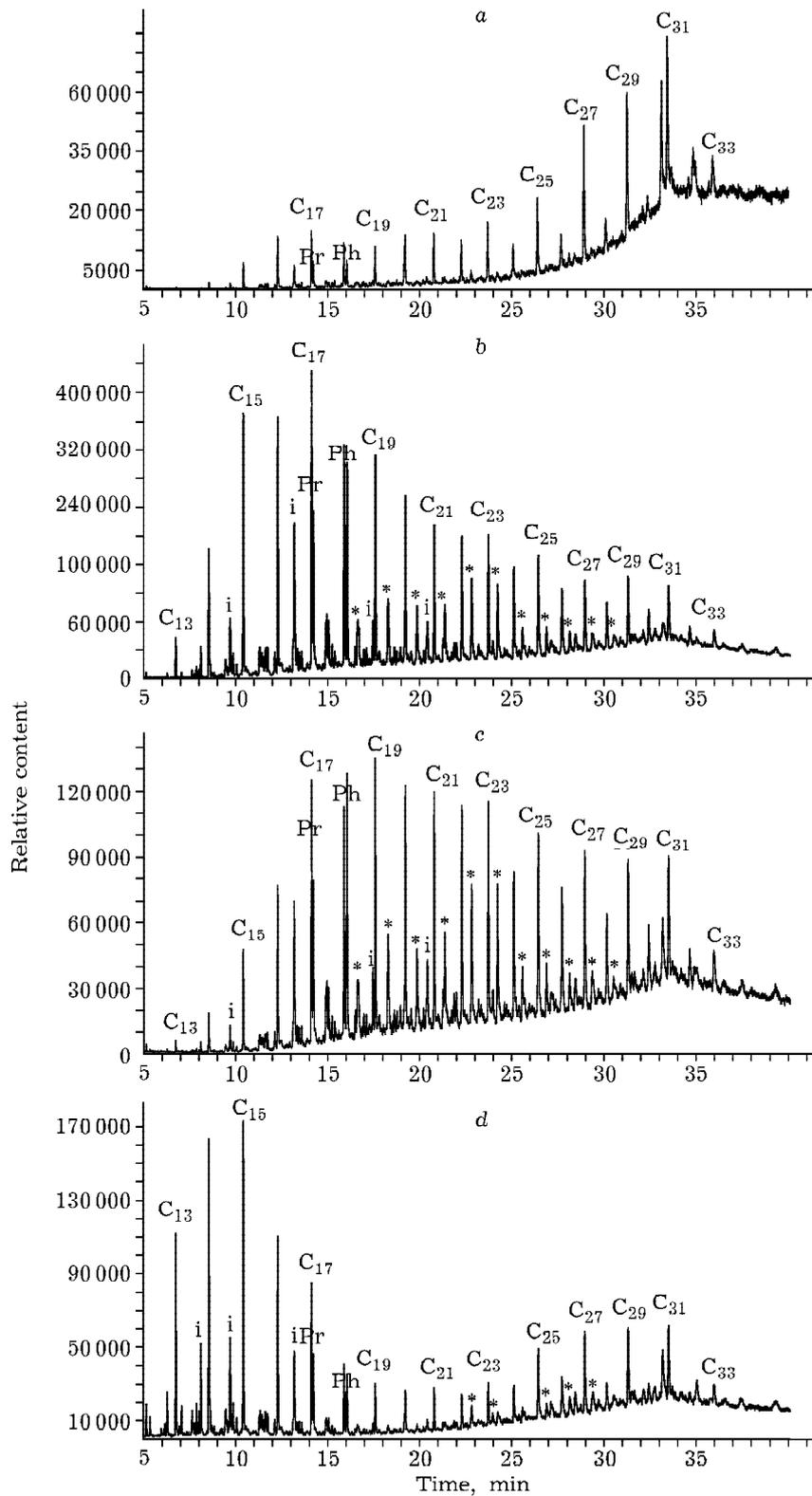


Fig. 1. Mass fragmentograms with  $m/z$  57 for hydrocarbonic fractions of bituminoids in the reference sample of soil R (a) and in the samples A, B, C with 0.2 vol. % of oil (b-d) added into soil. The samples R and C were grown with the rough dandelion, the samples A and B were with dandelion growing; the duration of pollution, day: 60 (c, d), 7 (b);  $C_{13}$ - $C_{33}$  - normal alkanes, Pr - pristane, Ph - phytane, i - isoprenoids, \* 12- and 13-methyl alkanes.

corresponding fraction of oil added [19]. Comparing to the reference soil sample (see Fig. 1, a) there is a reduction of the alkane fraction down to 55.7 %, a shift of their maxima towards a more low-molecular region of  $n\text{-C}_{17}$ , an increase in the fraction of isoprenoids up to 16.6 %, with that of 2- and 3-methyl alkanes increasing up to 11.1 %. At the same time an occurrence of 12- and 13-methyl alkanes (16.6 %) is observed. The comparative analysis of the mass fragmentograms for the samples A and B (see Fig. 1, b, c) demonstrates that the content of relatively low-molecular  $n$ -alkanes  $n\text{-C}_{12}\text{-}n\text{-C}_{18}$  in the sample B is almost twice lower in comparison with the sample A (11.1 and 21.2 %, respectively). It is unlikely that the decrease in the content of relatively low-molecular  $n$ -alkanes could be caused by their evaporation from the soil, since the boiling point values for the aforementioned hydrocarbons are ranging within 214–317 °C. To all appearance, the main loss of  $n$ -alkanes takes place due to the processes of biodegradation by soil microflora. Hence, while petroleum-polluting the soil in the case when the vegetation is absent, the biodegradation by soil microflora first of all affects low-molecular  $n$ -alkanes  $n\text{-C}_{12}\text{-}n\text{-C}_{18}$ . In this case the character of alkane hydrocarbons distribution is similar to that for the oil entered into soil. The soil sample C sowed with the seeds of the rough dandelion exhibits another distribution character for saturated hydrocarbons (see Fig. 1, d, c). The fraction of alkanes  $n\text{-C}_{18}\text{-}n\text{-C}_{25}$  in the hydrocarbon composition decreases from 29.3 % for the sample B to 14.8 % for the sample C. Light homologues from the series of 12- and 13-methyl alkanes disappear almost completely whereby their total fraction decreases twice. For the alkanes of normal structure amounting to 64.0 %, bimodal distribution is observed with the two maxima within a low-molecular region ( $n\text{-C}_{15}$ ) and a high-molecular one ( $n\text{-C}_{31}$ ). The fraction of isoprenoids amounts to 14.7 %, the fraction of 2- and 3-methyl alkanes being equal to 13.1 %, and that of 12- and 13-methyl alkanes amounting to 8.2 %. Similar character of the distribution for saturated hydrocarbons in the presence of vegetation cover is conserved until entering petroleum into the soil to the extent of 0.46 vol. %. In the case of adding oil into the soil to the extent of 0.59 vol. % and more the

fraction of alkanes with normal structure such as  $n\text{-C}_{18}\text{-}n\text{-C}_{25}$  and light homologues of 12- and 13-methyl alkanes is observed to increase in the composition of hydrocarbon fractions of bituminoids.

Data presented in Fig. 1 allow one to conclude that under the exposure to the elements of the soil-vegetation cover (including the rhizosphere) the soils polluted with oil become comparable in the composition and the distribution of saturated hydrocarbons with the soil organic matter of modern deposits, *i.e.* with the naturally occurring geochemical background.

### *Cytological and biochemical studies*

Table 3 displays cytological and biochemical characteristics determined for plantule cells of the rough dandelion depending on the amount of oil entered into soil. The antioxidant systems play the role of the first protection level of the genetic system against exogenous and endogenous toxicants. From the data presented in Table 3 one can see that the content of HMAO in plantule rootlet cells of the dandelion are insignificant for the reference sample ( $3.4 \mu\text{g-eq}_{\text{quer}}/\text{g}_{\text{pl}}$ ), whereas entering oil into soil results in 40–90 % reduction of this value. In this connection, within the framework of the present work we did not take into account this molecular system as an antioxidant protection system, thus the antioxidant protection factor ( $k_{\text{a,p}}$ ) was determined as half of the sum of SOD and peroxidase activity values.

As the amount of oil entered into soil increased, the SOD activity in dandelion rootlet cells all at first demonstrates a 2.5-fold increase (at 0.20 vol. % of oil), then it gradually decreases. For all variants of oil entering (except for 1.95 vol. %) the peroxidase activity is from 2.9 to 5.3 times higher compared to the reference. According to data presented in Table 3, entering oil into soil in all the cases all cases (except for the variant with entering 1.95 vol. % of oil) promotes a 1.7- to 2.7-fold increase in the total activation of the protective antioxidant enzymatic systems (SOD + peroxidase). When entering oil within the range of 0.13–0.20 vol. % a positive adaptation is observed that consists in the activation of the synthesis antioxidant enzymes resulting in an increase in the stabili-

TABLE 3  
Cytological and biochemical characteristics of the germ cells for the rough dandelion at different amount of oil entered into soil

No.	Volume fraction of oil added, %	LMAO content, $\mu\text{g-eq/g}$	Activity, $\mu\text{mol}/(\text{g}_g \cdot \text{min})$		Insertion rate		Mitotic index, %
			SOD	Peroxidase	$^3\text{H}$ thymidine, $\text{fmol}/(\text{g}_g \cdot \text{day})$	$^{14}\text{C}$ leucine, $\text{pmol}/(\text{g}_g \cdot \text{day})$	
1	0	3.40±0.03	1.91±0.02	1.21±0.01	376±4	272±3	1.9±0.2
2	0.07	1.91±0.02	1.70±0.02	3.52±0.04	292±3	156±2	2.5±0.3
3	0.13	0.32±0.03	2.92±0.03	4.93±0.05	269±3	158±2	3.1±0.3
4	0.20	2.01±0.02	4.81±0.04	3.60±0.04	185±2	152±2	4.2±0.4
5	0.26	1.43±0.01	2.53±0.03	4.21±0.04	1545±15	1132±11	2.6±0.3
6	0.33	0.84±0.01	1.33±0.01	5.24±0.05	422±4	247±2	2.9±0.3
7	0.39	1.01±0.01	1.12±0.01	5.93±0.06	586±6	296±3	3.2±0.3
8	0.46	1.02±0.01	1.33±0.01	5.19±0.05	226±2	148±1	1.7±0.2
9	0.52	1.33±0.01	0.95±0.01	4.93±0.05	250±3	133±1	3.9±0.4
10	0.59	1.92±0.02	1.32±0.01	4.19±0.05	248±2	126±1	4.6±0.5
11	0.65	1.01±0.01	1.01±0.01	6.44±0.06	811±8	467±5	3.1±0.3
12	1.30	0.84±0.01	1.12±0.01	5.32±0.05	605±6	401±4	3.0±0.3
13	1.95	0.10±0.01	0.20±0.01	0.30±0.01	n/d	n/d	n/d

Note. n/d – not determined ??? ввиду недостатка материала.

ty and productivity of plants. The second maximum (at 0.65 vol. % of oil), to all appearance, it is connected with the formation of third-type protective (defense)??? reactions in plant cells, which results in fast exhaustion of plasticity and energy resources of an organism at the following ontogenesis stages as well as to accelerated ageing [20–22]. According to the reaction of the enzymatic antioxidant systems of cells the permissible content of oil in soil for safe growth of the rough dandelion amounts to 780–980 mg/kg of soil (the volume fraction of oil being of 0.40–0.46 %).

The second protection level the genetic system against toxicants is presented by DNA reparation systems. With the use of an author's cytological-and-biochemical method for the estimation of genome resistance and its differential activity in the processes of DNA replication, translation and reparation [6, 21–23] we have investigate variations of their activity depending on the amount of oil entered into soil. Within the framework of this method, the rates of insertion of  $^3\text{H}$  thymidine into DNA molecules and of  $^{14}\text{C}$  leucine into proteins under synthesizing with the lifetime longer than 24 h in the meristematic cells of plant plantule rootlet as well as the mitotic index (MI) are registered. The activity of replication processes ( $k_{\text{repl}}$ ) is determined as the MI parameter normalized to the reference; the activity of translation processes ( $k_{\text{tr}}$ ) is determined as the parameter of  $^{14}\text{C}$  leucine insertion normalized to the reference; the activity of DNA reparation ( $k_{\text{rep}}$ ) is determined as  $[(^3\text{H thymidine})_N - (\text{MI})_N]$ . The genome resistance  $k_{\text{res.g}}$  within the framework of this method is determined from the expres-

$$k_{\text{res.g}} = k_{\text{a.p}} + k_{\text{rep}}/(k_{\text{repl}} + k_{\text{tr}} + k_{\text{rep}})$$

Basing on the cytological and biochemical characteristics obtained for the rough dandelion growing under the conditions of experimental oil pollution (see Table 3), we have determined values of  $k_{\text{repl}} + k_{\text{tr}} + k_{\text{rep}}$  and  $k_{\text{res.g}}$ . It is established that with entering the additions of oil into soil in amount of 0.07–1.30 vol. % all the variations of the studied characteristics of genome functional activity are of polymodal nonlinear character, which indicates the adaptive nature of this changes (Table 4).

TABLE 4

Genome resistance for the sprout root meristem of the rough dandelion and the characteristics of the genome functional activity depending on the amount of oil entered into soil (rel. units, normalized to the reference)

No.	Volume fraction of oil added, %	$k_{tr}$	$k_{repl}$	$k_{rep}$	$k_{res.g}$
1	0	1.00	1.00	1.00	1.00
2	0.07	0.57±0.03	1.32±0.07	0.46±0.02	1.13±0.06
3	0.13	0.58±0.03	1.63±0.08	0.08±0.04	1.31±0.07
4	0.20	0.56±0.03	2.21±0.11	0.04±0.02	0.82±0.04
5	0.26	4.15±0.21	1.37±0.07	3.74±0.18	1.68±0.08
6	0.33	0.91±0.05	1.53±0.08	0.59±0.03	1.34±0.07
7	0.39	1.08±0.05	1.68±0.08	0.88±0.04	1.66±0.08
8	0.46	0.54±0.03	0.89±0.05	0.71±0.04	1.55±0.08
9	0.52	0.49±0.03	2.05±0.10	0.37±0.02	0.66±0.03
10	0.59	0.46±0.02	2.42±0.12	0.00±0.01	0.35±0.02
11	0.65	1.71±0.09	1.63±0.08	1.53±0.08	1.67±0.08
12	1.30	1.47±0.07	1.58±0.08	0.89±0.05	1.53±0.08

One can see that the process rate for protein synthesis reaches maxima at the volume fraction of entered oil equal to 0.26 and 0.65 %, the processes rate for cellular division reaches maxima at 0.20 and 0.59 % of entered oil fraction, the processes rate for DNA reparation reaches maxima at 0.26 and 0.65 % of that. The genome resistance against the action of toxicants remain high enough at the volume fraction entered oil <0.46 %. The second maximum of genome resistance at 0.65–1.30 vol. % of entered oil, to all appearance, is already connected with other “negative” adaptation strategies of a plant organism with respect to the action of oil pollution, including also SOS reparations. Hence, according to the reaction of plant cell genome systems the permissible content of oil in soils is equal to 0.98 g, or approximately 1 g/kg of soil, which corresponds) to the volume fraction of entered oil amounting to 0.46 %.

## CONCLUSION

Basing on the obtained results of multifactor cameral experiment concerning the studies on the ability of the Yakutian permafrost soil to rehabilitation under oil-pollution one can draw the following conclusions.

1. The addition of oil into soil influences in a nonlinear manner the physiological and bio-

chemical parameters of plants growth as well as the enzymatic activity of soils. When the volume fraction of oil entered into soil is less than 0.40–0.46 % one can observe a cumulative stimulation effect with respect to the parameters such as the seed germinability, the survival rate of the rough dandelion plantules and the activity of the majority of soil enzymes under investigation. In all the cases (except for the variant with entering 1.96 vol. % of oil) a 1.7- to 2.7-fold increase is observed in the activation of the protection antioxidant enzymatic systems of plants. The content of oil in soil acceptable for safe growth of the dandelion amounts to 0.40–0.46 vol. %. All the studied characteristics of the functional activity obtained for the cell genome of the rough dandelion within the range of added oil amounts are of nonlinear character with a bimodal distribution of the activation parameters for the processes of protein synthesis, cellular division and DNA reparation. This fact allows one to assume that the species under investigation exhibit a number of adaptation strategies with respect to oil pollution. With entering oil in amounts less than 0.46 vol. % there is an increase observed in relative resistance of plantule cells genome against the action of oil toxicants. The second maximum of resistance at 0.65–1.30 vol. % of oil entered, to all appearance, is connected with

“negative” adaptation strategies of a plant organism with respect to the oil-pollution action including SOS reparation.

2. The investigation of soil bituminoids with the application of the geochemical complex of analytical methods has demonstrated that mainly the hydrocarbonic component of oil is transformed under the influence of soil and rhizospheric enzymes. Even small amounts of oil added (0.20 vol. %) into soil result in the change of the structure of bituminoids, providing them with hydrocarbonic character inherent in for stripped oil. In the absence of vegetation the biodegradation by soil microflora is observed for relatively low-molecular  $n$ -alkanes  $n$ -C<sub>12</sub>– $n$ -C<sub>18</sub>.

In the case of rough dandelion cultivation, the influence of rhizosphere enzymes first of all results in the transformation of alkanes  $n$ -C<sub>18</sub>– $n$ -C<sub>25</sub>, as well as of light of 12- and 13-methylalkanes. Thus, under the influence of the elements of the soil-vegetation cover the composition of soils is remediated to result in a naturally occurring geochemical background. Oil-pollution is transformed most actively at the volume fraction of entered oil less than 0.46 %.

The data obtained allow one to consider that the permissible level for oil-pollution of soils when they are capable for rehabilitation amounts to approximately 1 g of oil per 1 kg of soil.

## REFERENCES

- 1 Yu. I. Pikovskiy, Transformatsiya Tekhnogennykh Potokov Nefti v Pochvennykh Ekosistemakh, Vosstanovleniye Neftezagryaznennykh Pochvennykh Ekosistem (A Collection of Papers), Nauka, Moscow, 1988, pp. 7–22.
- 2 L. I. Svarovskaya, Sovremennye Dostizheniya v Issledovaniyakh Okruzhayushchey Sredy i Ekologii (A Collection of Papers), Tomsk, 2004, pp. 97–100.
- 3 F. Sh. Garifulin, Ekologicheskiye Usloviya i Fermentativnaya Aktivnost' Pochv, Ufa, 1979, pp. 85–94.
- 4 G. V. Rusanova, *Pochvoved.*, 2 (2000) 48.
- 5 E. G. Shadrina, Respubl. Nauch.-Prakt. Konf. “Ekologicheskaya Bezopasnost' Reki Leny. Monitoring i Tekhnogennye Katakлизmy” (Proceedings), Yakutsk, 2001, pp. 119–127.
- 6 A. N. Zhuravskaya, M. M. Shshurin, *Nauka i Obraz.*, 2 (2006) 29.
- 7 S. Kh. Lifshits, O. N. Chalaya, I. N. Zueva *et al.*, *Ibid.*, 4 (2006) 69.
- 8 V. S. Asatiani, *Fermentnye Metody Analiza*, Nauka, Moscow, 1969.
- 9 V. E. Arasimovich, A. I. Ermakov, M. I. Smirnova-Ikonnikova *et al.*, *Metody Biokhimicheskogo Issledobaniya Rasteniy*, Kolos, Leningrad, 1972, pp. 121–122.
- 10 N. G. Constantine, K. R. Stanley, *Plant Physiol.*, 59 (1977) 565.
- 11 L. A. Osterman, *Issledovaniya Biologicheskikh Makromolekul Elektrofokusirovaniyem, Immunoelktroforezom i Radioizotopnymi Metodami*, Nauka, Moscow, 1983.
- 12 A. Sh. Galstyan, *Itogi Nauchno-Issledovatel'skikh Rabot po Genezisu, Biokhimii, Melioratsii, Agrokhimii i Erozi Pochv*, Agrokhimiya, Erevan, 1983, pp. 41–50.
- 13 F. Kh. Khaziev, *Metody Pochvennoy Enzimologii*, Nauka, Moscow, 1990, pp. 171–197.
- 14 F. Kh. Khaziev, *Sistemno-Ekologicheskii Analiz Fermentativnoy Aktivnosti Pochv*, Nauka, Moscow, 1982, pp. 195–203.
- 15 F. Kh. Khaziev, Ya. M. Agafonova, A. E. Gulko, *Pochvoved.*, 11 (1988) 186.
- 16 F. Kh. Khaziev, *Fermentativnaya Aktivnost' Pochv*, Nauka, Moscow, 1976, pp. 20–56.
- 17 N. B. Vassoevich, *Priroda Organicheskogo Veshchestva Sovremennykh i Iskopyayemykh Osadkov*, Nauka, Moscow, 1973, pp. 11–59.
- 18 B. P. Tissot, D. H. Welte, *Petroleum Formation and Occurrence*, Springer-Verlag, Berlin, 1978.
- 19 O. N. Chalaya, I. N. Zueva, S. Kh. Lifshits, *et al.*, Konf. “Malotonnazhnaya Pererabotka Nefti i Gaza v Respublike Sakha (Yakutia)” (Proceedings), Yakutsk, 2001, pp. 165–170.
- 20 D. M. Grodzinskiy, *Nadezhnost' Rastitelnykh Sistem*, Naukova Dumka, Kiev, 1983, pp. 45–52.
- 21 A. N. Zhuravskaya, E. V. Filippov, B. M. Kershengolts, *Radiobiol., Radioekol.*, 40, 3 (2000) 254.
- 22 B. M. Kershengolts, *Nauka i Tekhnika v Yakutii*, 2 (2002) 11.
- 23 V. V. Sharoyko, G. V. Nureeva, A. N. Zhuravskaya, B. M. Kershengolts, *Sib. Ekol. Zh.*, 2 (2002) 127.