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Oil Pollution Transformation and the Formation of Adaptive Plant Response in the Model Experiment with Permafrost Soils of Yakutia

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Abstract

Results are reported concerning a model experiment for studying the physiological characteristics of two plant species, the activity of soil enzymes, the intensity of oil pollution accumulation and oil composition depending on the amount of oil introduced into the permafrost soil. It was suggested that with increasing the intensity of oil pollution, the soil-plant system mechanisms from the antioxidant and DNA repairing systems to apoptosis and SOS reparations. Introducing the same amount of oil into the soil (0.16 %) in the experiments with growing plants without them demonstrated that the action of the soil microflora resulted in the biodegradation of about one-third (32.2 %) of the oil introduced. In the case of plant growth (wormwood and peppergrass) the transformation of oil-caused contamination was much more profound amounting to 60.0 and 66.7 %, respectively. In the presence of the plants the process of destruction, alongside with *n*-alkanes and 2- and 3-methylalkanes, involve a wide range of structural isomers, including 12- and 13-methylalkanes and isoprenoids. As a result, the hydrocarbon composition of bitumenoids demonstrates changes direction toward restoring the natural geochemical background. The results obtained in studying the adaptive capacity of plants and the pollution transformation efficiency allow one to find out the maximum permissible residual oil contamination level in the soil, which level amounted to 0.1 %, or 1 g/kg of soil.

Key words: transformation, biological degradation, oil contamination, adaptation, plants, soil-plant system, the maximum permissible residual oil contamination level in the soil

INTRODUCTION

Oil and oil product spills become nowadays the most common causes of environmental pollution. Under the permafrost conditions the biodegradation of oil pollution in soil induced by soil microbiota (enzymes) is very slow, which is resulted in the fact that an accident site requires for soil recultivation and restoration. In order to efficiently control the quality of the restoration work one needs information concerning the value of the permissible level of soil contamination by oil and oil products.

This value depends on climatic and geographic features of the area, on the landscape and a number of other factors. Small amounts of oil entering into the soil can even stimulate the growth of plants [1–4]. Simultaneously, plant growth accelerates the transformation of oil pollution, which promotes natural self-purification and restoration of soil [4]. This is, to all appearance, connected with the fact that biologically active substances (BAS) secreted by the roots of plants into the root zone (rhizosphere), influence upon the rate of biodegradation of oil directly and (or) through the activity of soil enzymes. Plant survival should depend not only on the amount of oil trapped in the soil, but also on the specificity of plant species, the tolerance inherent in either plant with respect to this type of pollution.

This work was aimed at studying the features of oil transformation in the frozen soils of Yakutia in the case of growth on it two kinds of wild plant species: peppergrass (*Lepidium ruderale* L.) and wormwood (*Artemisia vulgaris* L.); studying the effect of oil pollution on the formation of the adaptive potential of plant germs and the activity of soil enzymes, determination of the maximum permissible residual oil content in the soil under permafrost conditions.

EXPERIMENTAL

In order to perform laboratory experiments we took the samples of permafrost sod-meadow soil (Central Yakutia) with the following main characteristics:

Moisture, %/100 g of soil	0.75
Density, g/cm^3	2.61
Humus content, %	14.34
Fraction of particles, %, with a 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
Content of mobile forms, mg/100 g of soil:	
N (NH_4)	2.4
N (NO_3)	0
P_2O_5	13.25
K_2O	10.23

Soil in equal amounts (400 mg) were placed in culture pan to add therein the oil from the Talakan deposit with the following characteristics: density 842 kg/cm^3, viscosity 2.12 mm^2/s, the content (in the stripped oil) of hydrocarbons 84.3 %, resins 14.4 %, asphaltenes 1.3 %.

The amount of oil added ranged from 0.3 to 5.8 mL, which corresponds to the concentration range of 0.06–1.22 mass %. All the ingredients were thoroughly mixed. To the soil prepared were sown wormwood or peppergrass seeds (50 pcs) in quadruplicate. We determined the following physiological characteristics of plants: germinating power (by the 7th day), germinating capacity (by the 10th day), and the survival level of germs (by the 60th day, the end of the experiment).

Soil samples were dried after the experiment at a room temperature to obtain air-dry state sieved (1 mm mesh) and divided into two parts. One part was used to determine the activity of soil enzymes involved in utilizing some oil components (polyphenol oxidase, catalase, urease and invertase) using the methods described in [5]. The second part of the soil samples was investigated using a set of geochemical analytical methods in order to determine the level of residual oil pollution and to study the processes of its transformation under the influence of soil and vegetation cover. With this purpose, we performed a chloroform extraction of soil samples [6]. The chloroform extract (bitumenoid) isolated was studied by infrared spectroscopy (Protege 460 FT-IR spectrometer, Nicolet). The factional dividing the bitumenoid species into hydrocarbon, resinous and asphaltene-like components was performed using a liquid adsorption chromatography [6]. The hydrocarbon fraction was studied using gas a chromatography/mass spectrometry technique. The GC/MS investigation was performed with a system consisting of an Agilent 6890 gas chromatograph whose interface was equipped with an Agilent 5973N high resolution mass spectrometer. The chromatograph was equipped with a quartz capillary column 30 m long, 0.25 mm in diameter impregnated with HP-5MS phase. As a carrier gas we used helium with a flow rate amounting to 1 mL/min. The evaporator temperature was equal to 320 °C. Programming the temperature rise was performed from 100 to 300 °C at a rate of 6 °C/min. The ionizing voltage was equal to 70 eV.

RESULTS AND DISCUSSION

Table 1 presents data concerning the physiological characteristics of plants (germinating power and germinating capacity, germ survival level) depending on the amount of added oil. One can see that the relationships obtained demonstrate complex, nonlinear char-

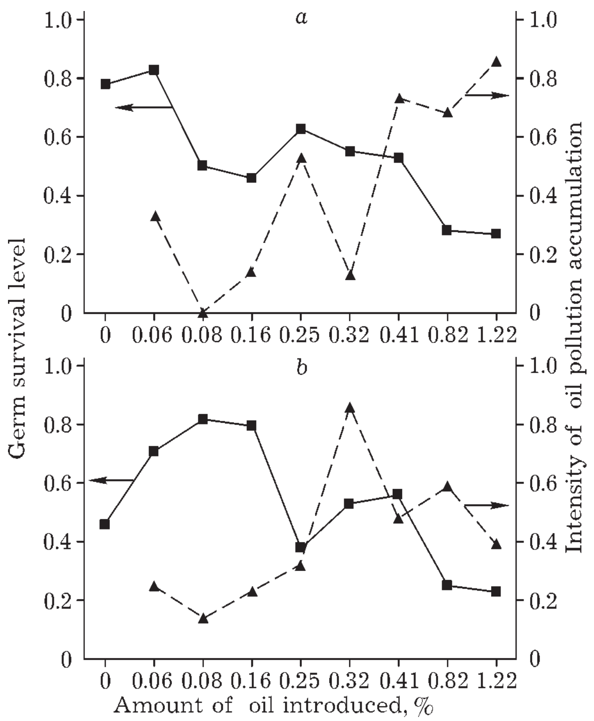

Fig. 1. Germ survival level and the intensity of oil pollution accumulation depending on the amount of oil introduced into soil for peppergrass (*a*) and wormwood (*b*).

acter to indicate an adaptive nature of these changes [7].

In order to assess the adaptation processes one consider not only absolute values of physiological characteristics to be important but also relationships between them, thus we introduced such an indicator as survival coefficient (the ratio between germ survival level to their germinating capacity).

Figure 1 demonstrates that for both species the germ survival level depending on the amount of oil added is of bimodal appearance with a trend to decreasing. Each of the two areas maxima on these curves is, to all appearance, corresponding to either survival strategy of plants. When small amounts of oil are entered into the soil – up to 0.16 % for peppergrass and 0.25 % for wormwood (the region of the first maximum) – the germ survival level is comparable to or even higher than that for the plants grown in non-contaminated soil. Perhaps, any antioxidant and DNA reparation protective systems are switched on at this stage of adaptation, with increasing the adaptive capacity of the plants [4, 7].

Within the region of the second maximum, where the amount of oil added is higher, the germ survival level though increases after a minimum, but it does not reach former maximum values. For the adaptation (survival of the population) under these conditions, the plants seem to turn on the following mechanism for the "defense" associated with starting the apoptosis and accelerated cell division, which results in a significant depletion of energy resources and functionality of the plant organism [4, 7].

Further increasing the amount of added oil (0.82 % and more) results in the fact that, despite the efforts of plant populations to adapt to a toxic environment by increasing the germinating power and germinating capacity (see Table 1), the most of germs appear inviable. To

TABLE 1

Physiological characteristics of plants depending on the amount of oil entered into soil

Oil content in soil, mass %	Peppergrass			Wormwood		
	Germinating power, %	Germinaton level, %	Germ survival level, %	Germinating power, %	Germinaton level, %	Germ survival level, %
0	42±4	46±5	36±4	70±7	70±7	32±3
0.06±0.01	32±3	36±4	30±3	66±7	70±7	50±5
0.08±0.01	42±4	40±4	20±2	54±5	56±6	46±5
0.16±0.01	24±2	26±3	12±1	50±5	52±5	42±4
0.25±0.01	38±4	38±4	24±2	22±2	26±3	10±1
0.32±0.01	20±2	22±2	12±1	28±3	34±3	18±2
0.41±0.01	28±3	30±3	16±2	22±2	32±3	18±2
0.82±0.01	10±1	14±2	4±1	24±2	24±2	6±1
1.22±0.01	26±3	30±3	8±1	28±3	44±4	10±1

TABLE 2

Activity of soil enzymes

Oil content in soil, mass %	Plant growing	Peppergrass /Wormwood			
		Invertase, $mg_{gl}/(g_s \cdot h)$	Urease, mg NH_4^+ /($g_s \cdot$ 24 h)	Catalase, mL $O_2/(g_s \cdot min)$	Polyphenol oxidase, $mg_{b/q}/(10g_s \cdot h)$
0	–	2.7±0.2/2.6±0.2	2.6±0.1/2.6±0.1	1.0±0.1/1.0±0.1	2.1±0.1/2.1±0.1
0	+	3.8±0.2/2.5±0.2	2.7±0.1/2.2±0.1	3.3±0.2/3.5±0.2	1.9±0.1/1.8±0.1
0.06±0.01	+	4.4±0.3/4.5±0.3	2.4±0.1/2.3±0.1	2.3±0.1/2.0±0.1	1.2±0.1/1.6±0.1
0.08±0.01	+	3.7±0.2/3.4±0.2	2.5±0.1/2.3±0.1	2.0±0.1/1.2±0.1	1.6±0.1/1.5±0.1
0.16±0.01	–	2.9±0.2/2.8±0.2	2.8±0.1/2.8±0.1	2.2±0.1/2.2±0.1	1.5±0.1/1.5±0.1
0.16±0.01	+	5.1±0.3/3.8±0.2	3.1±0.2/2.5±0.1	2.8±0.1/1.5±0.1	1.5±0.1/1.2±0.1
0.25±0.01	+	4.7±0.3/3.2±0.2	3.2±0.2/2.2±0.1	2.0±0.1/1.5±0.1	1.2±0.1/1.4±0.1
0.32±0.01	+	4.4±0.3/3.4±0.2	2.9±0.2/2.3±0.2	3.0±0.2/1.8±0.1	1.2±0.1/1.3±0.1
0.41±0.01	+	3.2±0.2/2.4±0.2	2.7±0.2/1.9±0.2	2.2±0.1/0.5±0.1	1.5±0.1/1.2±0.1
0.82±0.01	+	3.0±0.2/2.1±0.2	2.2±0.2/2.0±0.2	1.8±0.1/1.2±0.1	1.4±0.1/1.0±0.1
1.22±0.01	+	2.5±0.2/2.0±0.2	2.0±0.2/1.5±0.2	1.0±0.1/1.0±0.1	1.4±0.1/1.0±0.1

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

all appearance, at this stage a mechanism SOS reparations is observed to switch on [4, 7].

Changing the activity of soil enzymes under study with the amount of oil added also exhibits a complex, nonlinear character. With an equal amount of oil introduced into soil the activity of soil enzymes depends on the growth of plants and their species (Table 2). It is known that the enzymatic activity of soil is composed not only of the enzyme activity of soil microflora, but also of the activities of enzymes excreted by plant roots into the basal area in order to prepare potential nutritional substrates for absorption [1]. Just this determines how the activity of soil enzymes depends on plant species. For polyphenol oxidase, especially in the case of wormwood growing, there was a fairly uniform decrease in activity observed with increasing the levels of pollution. The activity of invertase (peppergrass), urease (wormwood) and, especially, catalase for both species was greatly influenced by the fact of plant growth itself. The activity of these enzymes also varied to a great amount with increasing the amount of oil added to soil, passing through maximums, and decreased at the highest levels of pollution. The formation of either adaptation strategy in the organisms of plants, with no doubt, exerts an effect on the activity of soil enzymes, however, no distinct correlations with the physiological characteristics of plants were found.

The nonlinear behaviour of plant physiological characteristics and soil enzymatic activity observed depending on the amount of oil

TABLE 3

Characteristics of bitumenoids inherent in soil samples

Oil content in soil, mass %	Peppergrass/Wormwood			
	Bitumenoid yield, mass %	Group componential composition of bitumenoid, %		
		Hydrocarbons	Total resins	Asphaltenes
0.06±0.01	0.021±0.001/0.016±0.001	39.1±2.0/51.4±2.5	49.1±2.5/46.1±2.3	11.8±0.6/2.4±0.1
0.08±0.01	0.020±0.001/0.019±0.001	46.6±2.3/57.3±2.8	49.7±2.5/38.7±2.9	3.8±0.2/4.0±0.2
0.16±0.01	0.030±0.001/0.036±0.001	47.2±2.4/50.7±2.5	51.0±2.6/44.3±2.2	1.8±0.1/5.1±0.3
0.25±0.01	0.080±0.001/0.066±0.001	58.7±2.9/60.6±3.0	39.6±2.0/37.0±2.9	1.8±0.1/2.4±0.1
0.32±0.01	0.088±0.001/0.120±0.001	58.7±2.9/72.6±3.6	40.2±2.0/25.7±2.4	1.1±0.1/1.8±0.1
0.41±0.01	0.157±0.001/0.165±0.001	71.5±3.6/71.0±3.5	28.2±1.4/28.3±2.4	0.4±0.1/0.7±0.1
0.82±0.01	0.436±0.001/0.408±0.001	77.0±3.9/78.7±3.9	22.7±1.1/21.1±2.1	0.3±0.1/0.3±0.1
1.22±0.01	0.778±0.001/0.579±0.001	79.3±4.0/78.1±3.9	20.5±1.0/21.7±2.1	0.2±0.1/0.2±0.1

added seems to indicate that the soil-plant system in the adaptation process behaves as an open and self-organizing system with successive switching on all the possible mechanisms of protection as a response to increasing a stress factor such as increased concentration of oil introduced into soil.

The chloroform extract of soil samples characterizes a bituminous component of soil organic matter. We used in the experiment permafrost sod-meadow soil, whose yield of bitumenoid appeared more than 20 times lower than that for samples contaminated with oil (Tables 3 and 4). Therefore, for this experiment, it could be assumed that the yield of bitumenoid or chloroform extract resulted from soil contaminated with oil indicates the level of oil pollution.

From the data presented in Table 3 one can see that, with increasing the amounts of oil added to soil the contamination level demonstrate an increase. Let us introduce an index for the intensity of oil pollution accumulation defined as a ratio between an increment of oil pollution level and the increment of the added oil amount (dimensionless parameter, since both values are expressed in the same units, mass %). The accumulation of oil pollution depending on the amount of oil added (see Fig. 1) is presented by multimodal curve. The first maximum on the curve of oil pollution accumulation intensity in the case of wormwood growing is noted with adding the amount of oil, equal to 0.32 %, which corresponds to the residual contamination of the soil at the end of the experiment (0.12 %), whereas in the case of peppergrass growing the mentioned values are equal to 0.25 and 0.08 %, respectively. It should be noted that in the case of the same oil additions, as mentioned above, an increase in the germ survival level of appropriate plants was observed after passing by the minimum.

TABLE 4

Characteristics of the hydrocarbon fractions of bitumenoids contained in soil samples A–E

Parameters	A	B	C (wormwood)	D (peppergrass)	E
Plant growing	–	–	+	+	–
Duration of the experiment, days	7	60	60	60	–
Oil amount added, mass %	0.16	0.16	0.16	0.16	0
Bitumenoid yield, mass %	0.090	0.061	0.036	0.030	0.001
Bitumenoid composition, %:					
hydrocarbons	64.4	65.2	50.7	47.2	9.4
resins	31.4	30.5	44.3	51.0	74.2
asphaltenes	4.1	4.3	5.0	1.8	16.4
Group composition of alkane hydrocarbons, % with respect to the sum of identified species:					
n-alkanes	50.5	38.8	69.1	68.0	87.9
isoprenoids	15.0	31.9	17.0	16.6	6.6
2- and 3-methylalkanes	22.5	12.2	10.2	9.3	5.5
12- and 13-methylalkanes	12.0	17.1	3.7	6.1	–
Isoprenoids/*n*-alkanes	0.3	0.8	0.3	0.2	0.1
Σi. c.–n-C_{20}/Σn-C_{21}–f. c.	1.0	1.2	0.5	0.5	0.2
Maximum of the distribution of *n*-alkanes	n-C_{17}	n-C_{15}	n-$C_{14,15}$, n-$C_{29,31}$	n-$C_{15,16}$, n-$C_{29,31}$	n-C_{31}
Odd/oven ratio*	1.3	1.3	2.1	1.9	5.2
(i-C_{19} + i-C_{20})/(n-C_{17} + n-C_{18})	1.0	3.3	1.4	1.7	1.1

Note. i-C_{19} – pristane, i-C_{20} – phytane.

*Ratio between *n*-alkanes with odd number of C atoms and *n*-alkanes even with even number of C atoms.

Thus, the adaptation strategy realized by plants with lower amounts of oil introduced into the soil, including oil transformation by soil microbial and phytogenous enzymes as well as and activation of antioxidant and DNA repairing systems [4, 7], to all appearance, becomes inefficient with the growth of oil pollution. As a consequence, the next adaptation strategy started, which consists in the activation of apoptosis and stimulation of cell division.

The results concerning the studies on the group componential bitumen composition (see Table 3) demonstrated that with increasing the amount of oil introduced into soil the percentage of hydrocarbon fractions in their composition increases, whereas the percentage of asphalt-resinous components is reduced. This is probably connected with the fact that the enzymes of soil-plant system, including the rhizosphere, no longer cope with the transformation of petroleum hydrocarbons, thus the bitumenoids become close in composition to stripped oil. A high percentage of hydrocarbon fractions in bitumenoids were observed in the case of the growth of both species with the addition of oil in the amount of 0.25 % and more.

Table 4 presents the results of studying the individual hydrocarbon fractions from the bitumenoid samples of soil wherein the same amount of oil (0.16 %) was introduced. The soil samples A and B were not used for seeding the plants, whereas the soil samples C and D were seeded with wormwood and peppergrass, respectively. Sample E represents the initial soil wherewith the experiment was conducted. The soil sample A was analyzed in 7 days after starting the experiment; for the remaining samples the duration of the experiment was equal to 60 days. For the soil sample A the comparison of the amount of oil added with the yield of the extract allows us to determine the amount of petroleum hydrocarbons evaporated during the experiment with the assumption that the processes of destruction had not time to exert a significant effect on the composition and content of bitumenoid. The amount of evaporated hydrocarbons was equal to 43.7 %.

For the soil sample B the yield of bitumenoid was 32.2 % lower comparing to the sample A. When assumed that the most volatile petroleum hydrocarbons have time to evaporate within 7 days, the difference in the content of bitumen, to all appearance, reflects the activity of proper soil microflora in the process of oil pollution biodegradation. In the case of plant growth (samples C and D) the yield of bitumenoids and the content of hydrocarbon fractions were found to be lower, whereas the amount of asphalt-resinous components was higher comparing to the control experiment, with no seeding the plants (sample B). In the experiment with wormwood, additionally 27.8 % of oil pollution was transformed under the action of germs, whereas in the case of peppergrass this value amounted to 34.5 %. In these cases, the content of hydrocarbon fractions in bitumenoids decreased by 22.2 % (for wormwood) and 27.6 % (for peppergrass) comparing to sample B. Thus, the growth of plants promotes the processes of oil pollution transformation occurring in soil those are accompanied by the destruction of hydrocarbons.

This is indicated also by the character of the IR spectra of bitumenoids (Fig. 2). The spectra of samples C and D demonstrate absorption bands inherent in the natural geochemical background (sample A). They are a doublet within the range of 720–730 cm^{-1} caused by the absorption of long methylene chains, and intense absorption band (within 1710–1735 cm^{-1}) of the

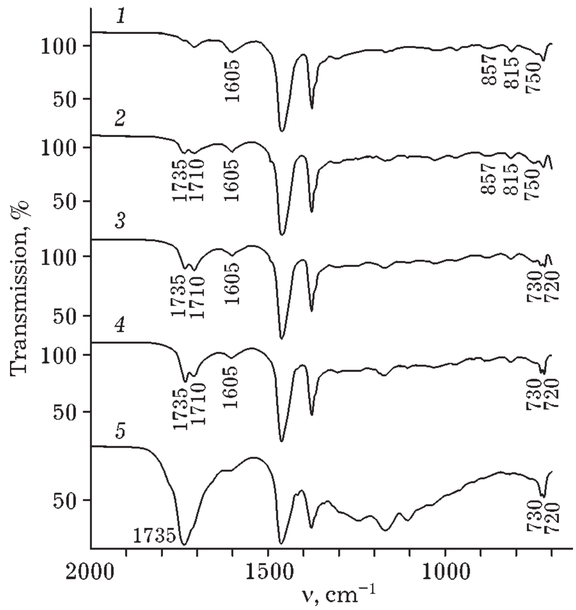

Fig. 2. IR spectra of bitumenoids for soil samples: *1–5* – samples A–E, respectively.

carbonyl groups of carboxylic acids, ketones and esters inherent in the organic matter of modern deposits. The character of the spectra of A and B samples is typical with respect to oil-contaminated soil, as it is indicated by a less intense absorption of carbonyl groups and a higher intense absorption for aromatic structures (1605, 887, 815, 750 cm^{-1}).

Using the method of gas chromatography-mass spectrometry, we studied individual composition of hydrocarbon fractions. In order to estimate the biodegradation level of oil, researchers often use a coefficient equal to ratio of the total pristane and phytane content to the amount of normal alkanes n-C_{17} and n-C_{18} eluted near to them ((i-C_{19} + i-C_{20})/(n-C_{17} + n-C_{18})) [8]. This ratio for the soil sample B was 3.3 times higher comparing to the sample A (see Table 4), which indicates a high biodegradation level for the oil pollution influenced by soil microorganisms. For the soil samples C and D, where plants grow, this ratio increased only by 1.4 and 1.7 times, respectively, whereas the number biodegraded oil was equal to 60.0 and 66.7 % comparing to the sample B (32.2 %).

Thus, in the case of plant growth this coefficient, to all appearance, does not reflect completely a real level of biodegradation. Under the action of proper soil microflora, the biodegradation mainly occurred with respect to n-alkanes and 2- and 3-methylalkanes (see Table 4). The amount of 2- and 3-methylalkanes exhibited an almost twice decrease. The content of n-alkanes with respect to the amount of identified hydrocarbons exhibited a decrease from 50.5 to 38.8 %. As far as the hydrocarbon fraction of the sample B is concerned, an almost two-fold increase in the fraction of isoprenoids was observed against the background of decreasing the content of n-alkanes and 2- and 3-methylalkanes comparing to the sample A, which indicates a greater stability of isoprenoids in the process of biodegradation.

In the case of plant growing (samples C and D), the prevailing biodegradation among n-alkanes was observed for n-C_{17}–n-C_{22}: their content was twice reduced. As a result, the ratio between relatively low molecular mass alkanes and relatively high molecular mass ones (Σi. c.–n-C_{20}/Σn-C_{21}–f. c.) decreased down to 0.5 as compared to 1.0 for the sample A. It should be noted that the ratio between the content of isoprenoids and n-alkanes in samples C and D is much lower comparing to the sample B (see Table 4). This could indicate the fact that in the presence of plants, isoprenoids are also involved in the biodegradation processes alongside with n-alkanes. Among the hydrocarbons identified, the content of 2- and 3-methylalkanes exhibited a two-fold decrease. The feature of the Vendian-Cambrian oil species of the Nepa-Botuoba oil and gas field including the oil of the Talakan deposit consists in a significant content of relict hydrocarbons such as 12- and 13-methylalkanes. Plant growing stimulated the transformation of these hydrocarbons, resulting in the fact that the content of 12- and 13-methylalkanes decreased twice in the presence peppergrass and demonstrated a 3.2-fold decrease in the case of wormwood.

Thus, as far as oil contaminated soil is concerned, growing the plants causes the soil remediation to accelerate. In this case, not only the pollution level is reduced, but also the range of hydrocarbons affected by biodegradation is extended. Figure 3 demonstrates mass fragmentograms of the hydrocarbon fractions of bitumenoids for the samples of soil those were introduced with the same amount of oil (0.16 %); the scanning was performed with respect to the ion with m/z = 57, inherent in alkanes. This figure also demonstrates mass fragmentograms (m/z 57) for the hydrocarbon fraction of the initial sample E which characterizes natural geochemical background. It is seen that only for the samples C and D, where plants grew, the distribution of individual hydrocarbons exhibits peculiarities inherent in organic matter of modern deposits alongside with the features inherent in oil pollution:

– An increased content of n-alkanes and decreasing the percentage of 2- and 3-methylalkanes.

– The presence of an additional maximum within high molecular mass region at n-C_{29-31} inherent in the distribution of n-alkanes in modern deposits.

– A higher value of the ratio between n-alkanes with odd number of C atoms and n-alkanes even with even number (2.1 and 1.9 for wormwood and peppergrass, respectively) comparing to that for oil (about 1.0).

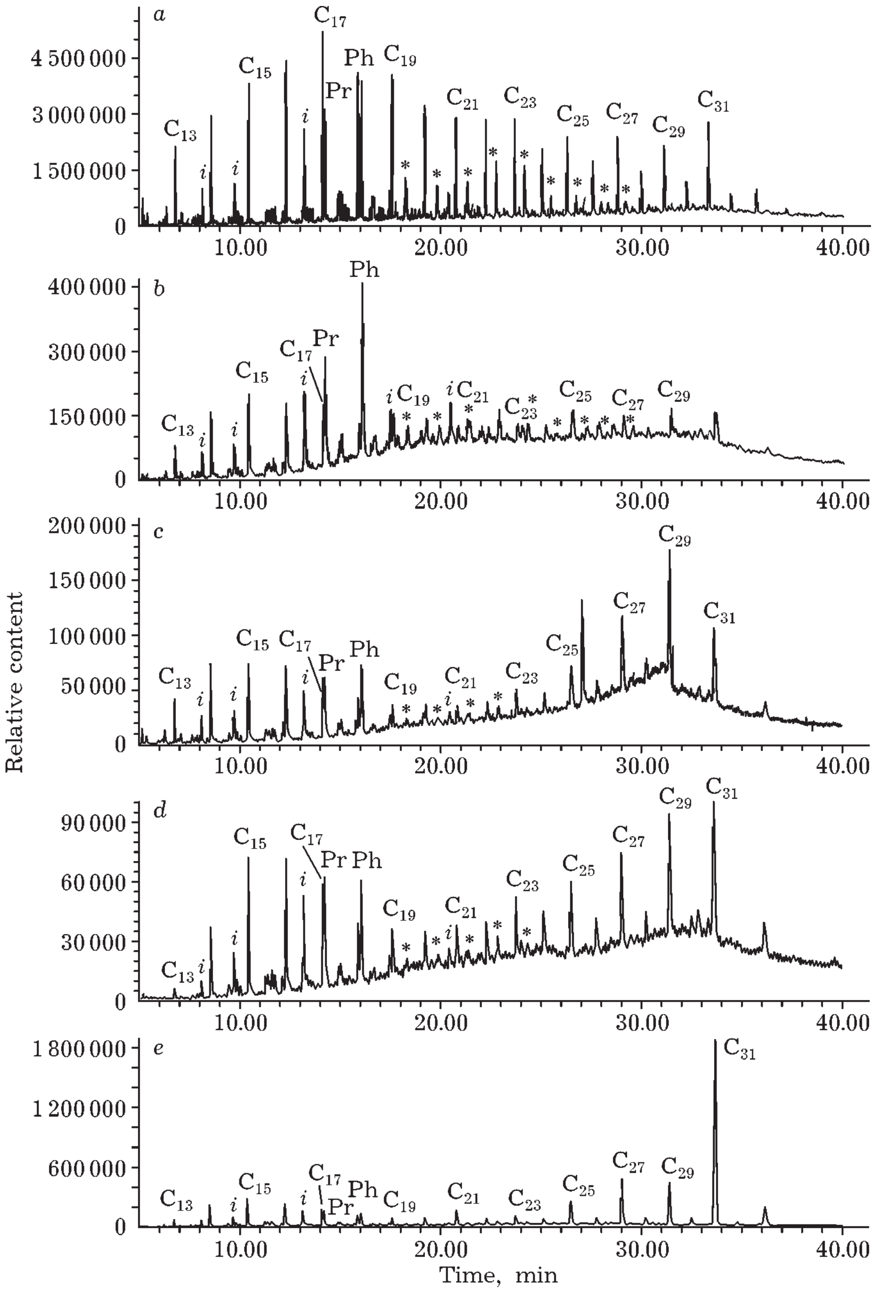

Fig. 3. Mass fragmentograms (m/z 57) for hydrocarbon fractions of soil samples: a–e – samples A–E, respectively; C_{13}–C_{31} – normal alkanes; Pr – pristane, Ph – phytane, i – isoprenoids; *12- and 13-methylalkanes.

– Significant decreasing the content of relict 12- and 13-methylalkanes, non-inherent to the organic matter of modern deposits.

With increasing the amount of oil added to the soil where the plants grow, the distribution of individual hydrocarbons gradually changed, losing some characteristics inherent inorganic matter of modern deposits to exhibit the character of degraded oil (at high pollution values these characteristics exhibited the character of oil itself). So, with adding of oil in the amount of 0.25 % (in the case of peppergrass) and 0.32 % (in the case of wormwood) the maximum in the distribution of n-alkanes was shifted towards a low-molecular mass region of n-C_{13}–n-C_{13-15} the ratio between odd n-alkanes and even ones became close to 1.0 (1.0 and 1.3, respectively), the amount of relatively low molecular mass n-alkanes exhibited an increase (Σi. c.$-n$-$C_{20}/\Sigma n$-C_{21} amounted to 1.7 and 0.9, respectively), the percentage of 12- and 13-methylalkanes increased.

These data indicate the fact that the plants are no longer able to participate efficiently in the process of petroleum hydrocarbons degradation. Therefore, to the concentrations of residual oil pollution equal to 0.080 and 0.120 % for peppergrass and wormwood, respectively, corresponding to the mentioned oil additions should be taken as maximum permissible value for these plants. It can be seen that wormwood is characterized by a greater resistance with respect to oil contaminated soil as compared to the peppergrass. The values of permissible oil content in the soil determined with taking into account distribution patterns for individual hydrocarbons are in a good agreement with the data concerning the studies on the physiological characteristics of plants, as well as with data concerning the yield and composition of bitumenoids. For permafrost soil, the maximum permissible residual oil content in the soil could be taken on average amounting to 0.1 %, or 1 g/kg of soil.

CONCLUSION

Studying the behaviour of plants in the model experiment with the permafrost sod-meadow soil of Yakutia contaminated with oil demonstrated that among the physiological characteristics of the plants the most informative for understanding the processes of adaptation with respect to oil pollution conditions one could consider the value of survival coefficient, defined as a ratio between the germ survival level to the germinating capacity. Its changes depending on the amount of oil introduced into the soil for both plants are bimodal with the trend to decrease, whereon for the curves one could two fields of maxima, each of which seems to correspond to either strategy for plant adaptation with respect to increasing the intensity of a stressor.

The comparison of the results of experiments with plant growth and without it, with the same amount oil (0.16 %) added into the soil, demonstrated that in the presence of plants results in a more profound transformation of oil contamination. The plant growth accelerated the degradation of petroleum hydrocarbons, which resulted in decreasing the oil pollution by 60.0 % (for wormwood) and 66.7 % (for peppergrass) comparing to the initial sample, where the biodegradation of oil pollution occurred only under the influence of soil microorganisms (32.2 %). The investigation of the patterns in the distribution of individual hydrocarbons demonstrated that the presence of plants, alongside with increasing the transformation level of oil pollution resulted in the extending the range of alkane structural isomers subjected to biodegradation (alongside with n-alkanes and 2- and 3-methylalkanes, additionally 12- and 13-methylalkanes and isoprenoids experienced transformation). As the result, the hydrocarbon composition of bitumen demonstrates changing toward restoring the natural geochemical background.

Adding the oil in the amounts of 0.25 and 0.32 % (for peppergrass and wormwood, respectively) resulted in abrupt increasing the intensity of accumulation for oil contaminated soil. As far as the pollution composition is concerned, the percentage of hydrocarbon fractions became close to that of stripped oil, whereas the distribution of individual alkanes became close to that inherent in oil. To all appearance, the levels of oil pollution of oil corresponding to the mentioned additions, amounting to 0.080 % for peppergrass and 0.120 % for wormwood (*i. e.*, about 0.1 %, or 1 g/kg of soil) should be recognized as maximum permissible values for frozen soil.

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