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INFLUENCE OF OIL ON THE NUMBER OF DIFFERENT GROUPS OF MICROORGANISMS INVOLVED IN THE NITROGEN CYCLE

Abstract. In oil-polluted ecosystems, nitrogen is one of the main biogenic elements. Maintenance of its level in the environment provided by the active activity of various microorganisms participating in its cycle. In this connection, the problem of the influence of oil pollution on the transformation of nitrogen in the soil is of interest, which closely related to practical issues of biodegradation. In all periods of the study, the number of azo-fixing microorganisms and oligonitrophils in polluted soil was higher than that of uncontaminated soil. An increase in the intensity of nitrogen fixation in contaminated soil may be due to an increase in the content of readily available organic substances – alcohols, organic acids, amino acids and other compounds. It correlates with an increase in the number of groups of anaerobic nitrogen fixers of the genus Clostridium. It is possible that nitrogen-fixing microorganisms can use metabolic products of hydrocarbon oxidizing microorganisms.
Key words: microorganism, nitrogen, soil, oil, pollution, gasoline, bacterium.

Introduction. All periods of the study, the number of azo-fixing microorganisms and oligonitrophils in polluted soil was higher than that of uncontaminated soil. An increase in the intensity of nitrogen fixation in contaminated soil may be due to an increase in the content of readily available organic substances - alcohols, organic acids, amino acids and other compounds. It correlates with an increase in the number of groups of anaerobic nitrogen fixers of the genus Clostridium. It is possible that nitrogen-fixing microorganisms can use metabolic products of hydrocarbon oxidizing microorganisms. An increase of nitrogen-fixing microorganisms in oil-polluted soil noted by a number of researchers [1]. According to some data, oil in various concentrations stimulates the development of oligonitrophils and nitrogen-fixing microorganisms both in gray forest soil and in leached chernozem during the whole period of research. The intensification of nitrogen fixation in oil-contaminated soil explained by the low partial pressure of oxygen. It is likely that an increase in the number of nitrogen fixing microorganisms is a kind of "compensatory" mechanism necessary to preserve nitrogen compounds in the soil. The most sensitive to oil pollution are nitrifying bacteria, which are an agronomically important group of microorganisms. Their abundance fell with increasing oil concentration and did not return to the background level after a certain time and when contaminated soils, fertilizer complex, activated sludge and other stimulants used for reclamation. The decrease in the number of nitrifying microorganisms in contaminated soil is due to the fact of that an unfavorable air regime created in the soil. However, under the same conditions, an increase in the activity of ammonifying and aerobic nitrogen fixators observed. Nitrifiers in normal soil do not meet a large amount of easily digestible organic matter. Hydrocarbon oils themselves are relatively easily digestible organic compounds. On the other hand, studies showed that biodegradation of oil in the process of self-purification accompanied by an increase in the content of organic substances. Hydrocarbons used in certain doses that do not exceed the phytotoxicity of the soils, as a "stabilizer" of the conversion of nitrogen, an inhibitor of the nitrification process, to prevent nitrogen loss. Microorganisms carrying out the process of denitrification are widely distributed in nature. In general, denitrifying agents represent a rather indefinite biological group of bacteria capable of using oxidized nitrogen compounds in addition to oxygen as an electron acceptor. Most of them belong to the genera *Pseudomonas, Achromobacter, Micobacterium*, etc. The number of denitrifying microorganisms in contaminated soils during self-purification is much higher than in uncontaminated ones. A number of researchers note an increase in the number of denitrifying agents in oil-contaminated soils [2]. Developing in polluted soils of denitrifying agents promoted by excess of organic mass, alkaline reaction of the environment and micro-ecological factor - low oxidation-reduction potential. Strengthening the process of denitrification is an undesirable phenomenon in contaminated soil, since the soil depleted with nitrogen, which under the conditions of a sharp increase in the biomass of microorganisms serves as a factor limiting the rate of self-purification. On the other hand, involving products of partial oxidation of hydrocarbons in the oxidative metabolism in the conjugate reduction of nitrates, denitrifying agents participate in biodegradation processes.

According to other researchers [3], the total number of denitrifiers under the influence of oil, basically, do not change. In some cases, their numbers increase, especially when using organic and mineral fertilizers for reclamation. This is probably due to the creation of anaerobic conditions due to oil clogging pores in contaminated soil.

As the results of studies [4] show, soil contamination with oil products also affects its nutritional status. Due to the decrease in the number of nitrifying microorganisms, the processes of nitrification weakened and there is a complete absence of nitrates in the soil. Suppression of the nitrification process in the soil probably leads to the accumulation of ammonia nitrogen.

It is known, that a certain part of the nitrogen in the ammonia form, which formed in the process of nitrification goes directly into the medium and is used by microorganisms as a nitrogen feed. The oil fractions present in the soil are physicochemical and biodegradable. The volatilization of hydrocarbons, a decrease in their concentration, the appearance of new intermediate decomposition products, imposes its imprint on the biological activity of the soil. There is a transition from more toxic at the first moment fractions to less toxic and vice versa.

It showed that, while adding in soil contaminated with oil bacteria of the genus Azotobacter applied to various species, the rate of soil self-purification increased. Bacteria are able to assimilate petroleum hydrocarbons as the sole source of carbon and energy both in the presence of bound nitrogen and nitrogen fixation. The activating influence of *Azotobacter chroococcum* on the growth of hydrocarbon oxidizing bacteria, which are part of the drug Devoroyl, was revealed.

Materials and methods of research. Microbiological examination of oil contaminated sites was carried out according to generally accepted microbiological practices. In laboratory experiments typical medium loamy sierozems with different contents of oil product soils were used. The object of our research was Kumkol oil, diesel fuel, fuel oil, oil sludge and various grades of gasoline (AI-80, AI-85, AI-90, AI-96).

Results and its discussion

In laboratory conditions on a Voroshilov-Dianova liquid medium with 2.0% oil as a hydrocarbon source, the biodegrading ability of association 1 consisting of strains of *Micrococcus varians* B1Ag16G, *Micrococcus luteus* B1Ag8G, *Micrococcus roseus* B1Ag6G, *Bacillus subtillis* G311/1, *Azotobacter chroococcum* GR11, *Az. chroococcum* GR21, *Az. chroococcum* GR35, *Az. chroococcum* GR149, association 2, consisting of strains *Micrococcus varians* B1Ag16G, *Micrococcus luteus* B1Ag8G, *Micrococcus roseus* B1Ag6G, *Bacillus subtillis* G311/1. As a result of the conducted studies, it was found that the highest degree of biodegradation of oil was noted in the variant using association 1, where the content of hydrocarbons in 28 days decreased by 52.7%, which is 20.7% higher than in the variant using association 2, in accordance with figures 1, 2.

Thus, established that the introduction of heterotrophic microorganisms and nitrogen fixers in the association increases the degree of biodegradation of oil by 38.5% in comparison with the variant where only heterotrophic microorganisms are used.

In the laboratory conditions, the possibility of using such cultivated plants as kidney beans (*Faesolis sp.*) and oats (*Avena sp.*) used as phyto-test objects. The choice of these cultures determined by the rapid germination and large biomass of seedlings.

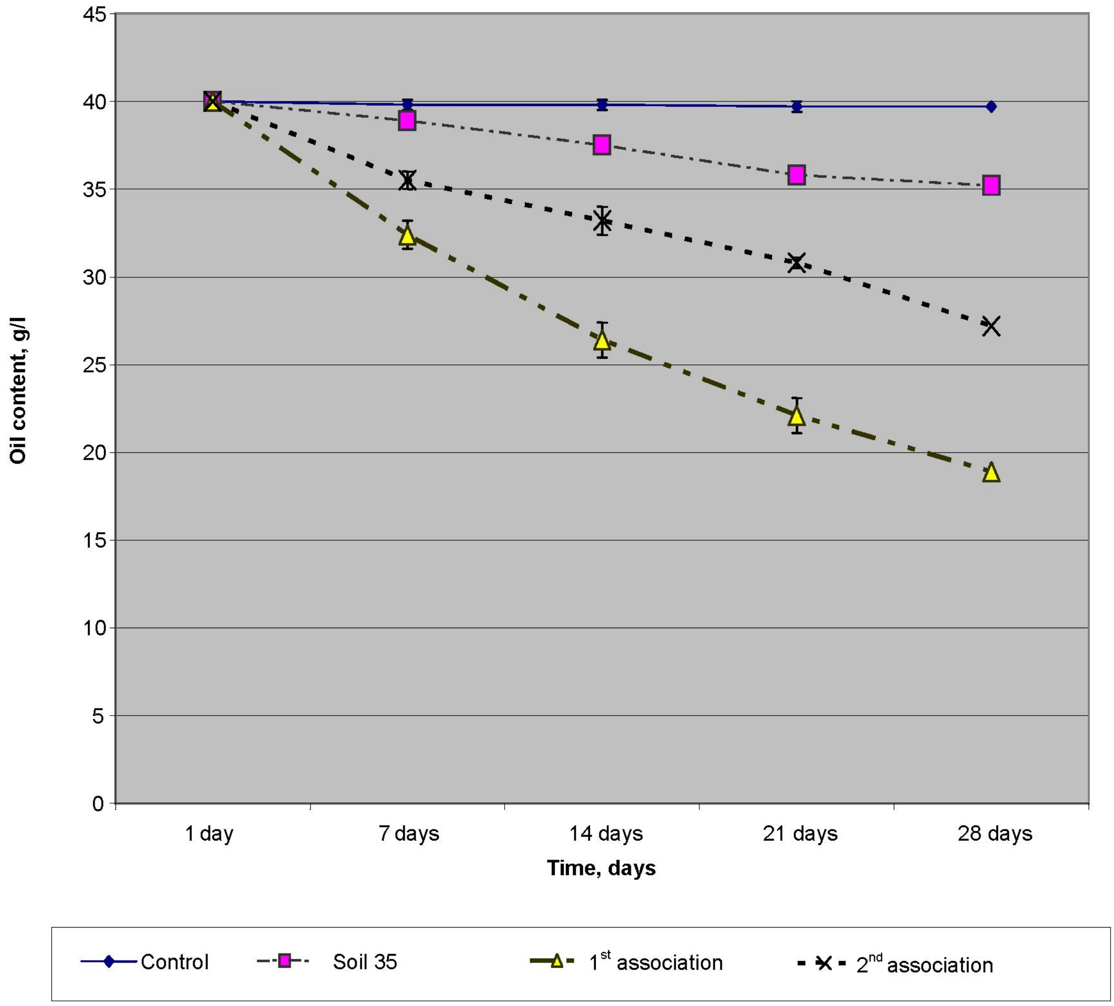

Figure 1 – Reduction of the concentration of oil products under the influence of the association of microorganisms

It found that plants react more sharply to soil contamination with light fractions of petroleum products, while it found that irrespective of the brand of gasoline, its content in the soil to 0.1% causes complete loss of seeds.

Less toxic activity showed diesel fuel and oil. Established that, their concentration in soil to 0.1% provides stimulating effect on plant grow, all experimental plants increased control samples on 1.75±0.5 cm, and on biomass 0.2±0.05g.

In assessing the phytotoxicity of soils under laboratory conditions, where the following variants were tested:

– control - soil containing 2% of Kumkol oil;

– Variant 1 – initial soil with 2% oil and additional 1% aqueous solution of ammophos;

– Variant 2 – initial soil with 2% oil + 1% aqueous solution of ammophos + association 2, consisting of hydrocarbon-oxidizing bacteria;

– Variant 3 – initial soil with 2% oil + 1% superphosphate;

– Variant 4 – initial soil with 2% oil + 1% aqueous solution of superphosphate + association 1, consisting of hydrocarbon-oxidizing and nitrogen-fixing microorganisms.

After 30 days of incubation oat seeds introduced into the soil. Analysis of the results of the experiment on10 days showed that in the control variant, all the seeds were lost. In variants 1 and 3, the seed germination energy was 8.0 and 9.0%, respectively, but the seedlings looked depressed, chlorosis of the leaves noted, and a part of the seedlings died on day 2-3. In variants 2 and 4, the seed germination energy was 42.0% and 65.0%, respectively. In both cases, seedlings appeared unfriendly, but on the 4-5 days they had a bright color. In variant 4, the plants were ahead of the control samples by 1.7 ± 0.5 cm.

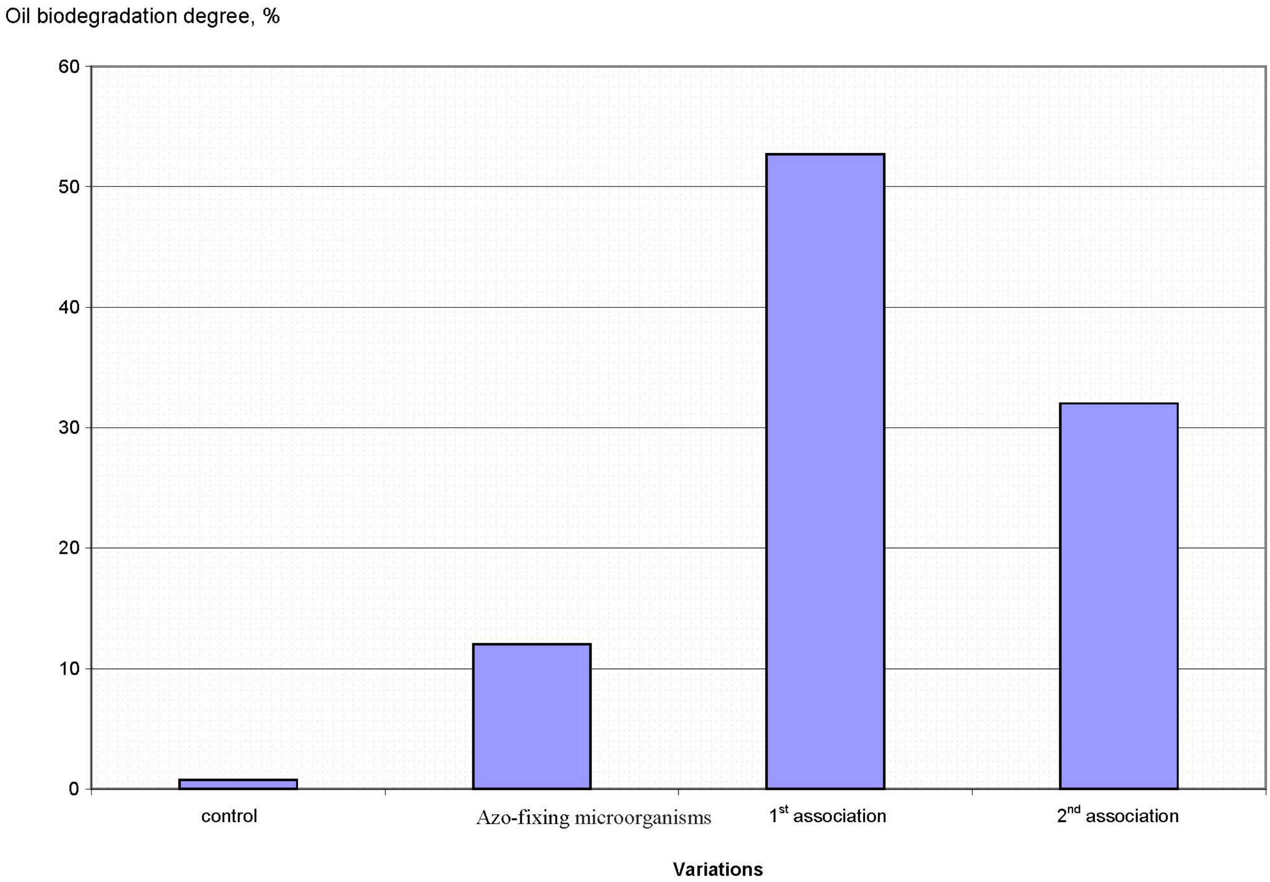

Figure 2 – Degree of biodegradation of oil
by spontaneous microflora and associations of microorganisms

Similar results observed in experiments with beans, where the same experimental scheme used. In this case, the advance in height of plants in variant 4, comparing with variant 2, was 4.1±0.3 cm.

When studying the reaction of plants to soil contamination with gasoline, it found that, regardless of the brand of gasoline, its concentrations of more than 0.5% exert an acute effect on the energy of germination of both beans and oats. Seeds mold and rot. Reducing the concentration of gasoline to 0.1% did not reduce the toxic effect on germination of seeds. The introduction of gasoline grades AI-80, 85, 92 at a concentration of 0.01; 0.02; 0.03% in the soil had a weak stimulating effect on the growth and development of plants. All seeds in experimental variants sprouted, while the height of sprouts exceeded the control samples by 4.2±0.5 cm in the case of bean seeds and 2.3±0.3 cm in variants with oat seeds on 8-10 days.

However, a further increase in the concentration of gasoline of these grades to 0.05% caused a decrease in the growth of seedlings by 0.8±0.2 cm and a decrease in seed germination energy by 10.0 ± 1% in versions with beans, by 0.5±0.03 and 15.0±1% in variants with oat seeds. As the result of laboratory tests, it found that AI-96 gasoline has an acute effect on the growth and development of experimental plants, even at a concentration of 0.01%.

Microfield experience in the development of technology for reclamation of accidental soil contamination by non-volatile fractions of petroleum products carried out at the site of the TSC located in Petro Kazakhstan Oil Products. In the experiment, the processes of destruction of hydrocarbons by nitrogen-fixing and hydrocarbon-oxidizing microorganisms studied. As a bio-test, which characterizes the degree of reclamation, also as a possible active recultivation agent, oats and beans sowed in the experimental plots in two series of experiments.

It showed that a further decrease in the concentration of gasoline or a small amount thereof has an insignificant effect on plant growth.

To simulate accidental contamination, a gas condensate consisting of 60% heavy and 40% volatile hydrocarbons (aliphatic, aromatic and alicyclic) applied to the soil prior to laying the test on the soil, before penetrating it to a depth of 20 cm. Before applying the oil to the soil and after application, the content of organic substances and separately oil the next: the amount of organic substances in the soil before adding oil equal to 1569 mg/kg, and oil products – 1408 mg/kg, and after the application of petroleum products, the amount of organic substances was 5907 mg/kg, oil – 4259 mg/kg.

Samples for analysis selected in 3-fold replication before filling the soil with gas condensate, 3 days after pouring the soil, during the laying of the experiment and during the harvesting period of oats and beans. Total recultivation period lasted for 60 days.

The scheme of the experiment consisted of three series. In the first series, spontaneous microflora tested as bio-destructors of oil products. In the second series – UOM oats and beans. In the third series, nitrogen-fixing microorganisms introduced. The area of the plots in the variants of the experiment is 2 m^2. The repetition of the experiment is threefold.

Scheme of experience:

1. Control (loosening + watering + plants).
2. Variant 1 (UOM + plants + 1% ammophos).
3. Variant 2 (nitrogen fixing microorganisms + plants + 1% superphosphate).
4. Clean soil without contamination (loosening + watering + plants).

Бактериальные препараты и биогенные вещества, согласно схеме опыта, вносились в соответствующие варианты и повторности опыта и лопатой перемешивались со слоем грунта на глубину проникновения газового конденсата.

Bacterial preparations and nutrients, according to the experiment scheme, introduced into the corresponding variants and replicates of the experiment and shoveled with the soil layer to the depth of penetration of the gas condensate.

The results of the experiment indicate the inactive destruction of petroleum products by hydrocarbon oxidizing microorganisms. The most intensive decomposition of hydrocarbons noted in the variant where nitrogen-fixing microorganisms introduced together with superphosphate. The content of oil products reduced, and compared to the initial content, their quantity decreased by 98.3-99.4%. At the same time, the introduction of sawdust, together with mineral fertilizers, reduced the content of petroleum products.

The sowing of oats and beans as test cultures against the background of the action of UOM and nitrogen-fixing microorganisms showed that as the quantity of oil products in the soil increases, the yield naturally decreases. It noted the decrease in the activity of oxidation of oil products under the cover of plants of oats and beans in comparison with the variant without plants. More active destruction of petroleum products in a variant without plants can be explained by the lack of competition introduced into the soil by oil-oxidizing microorganisms from the soil microflora destroyed as a result of hydrocarbon contamination, whereas under the plants of oats and beans the possibility of competition from the epiphytic microflora increased.

An analysis of the phytotoxicity level of the soil in the studied variants of the experiment showed that the control variant, where agrotechnical measures not taken, was characterized by minimum seed germination and maximum phytotoxicity. The lowest level of toxicity noted in the second variant with the minimum starting dose of mineral fertilizers (table). A high level of toxicity in oil contaminated soil can

Level of plant phytotoxicity in variants of the experiment

Experiment variant	Similarity, %	Weight of 1st sprout, g	Presence of signs of chlorosis
Control	54.7±5.8	8.9±0.07	Present
Variant 1	74.7±5.3	9.2±0.08	Present
Variant 2	72.0±4.9	10.1±0.08	Absence
Pure soil	86.6±5.7	10.5±0.10	Absence

In this case, the absence of chlorosis in plants in this variant of visual signs is an indirect indicator that the soil of this variant, despite the minimal amount of nitrogen fertilizers used, contained a sufficient amount of nitrogen, possibly due to the active flow of nitrogen fixation processes.
be caused by the accumulation at the early stages of microbiological destruction of a large number of petroleum acids and other products of primary oil degradation, which are highly toxic, both for plants and for most microorganisms.

Thus, the compilation of a scheme for remediation measures, and in particular the calculation of doses of nitrogen fertilizers should begin with an analysis of the level of activity of nitrogen-fixing microflora in a specific oil contaminated soil. In the case of a high level of nitrogen fixation, the use of high initial doses of nitrogen mineral fertilizers is inadvisable, because of providing an initial reduction in the content of petroleum products in the soil, they subsequently lead to a sharp suppression of the rate of biodegradation of oil in the soil and an increase in the phytotoxicity level of the soil, which may negatively affect the effectiveness of subsequent phytomelioration.

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МҰНАЙДЫҢ АЗОТ ЦИКЛЫНА ҚАТЫСАТЫН ӘРТҮРЛІ МИКРОАҒЗАЛАР САНЫНА ӘСЕРІ

Аннотация. Мұнаймен ластанған экожүйелерде азот негізгі биогенді элементтердің бірі болып табылады. Қоршаған ортадағы оның деңгейін қамтамасыз ету оның цикліне қатысатын әртүрлі микроағзалардың белсенділігі арқылы қамтамасыз етіледі. Осыған байланысты, мұнаймен ластанудың топырақтағы азоттың қайта өңделуіне әсер ету мәселесі қызығушылық тудырады, ол биодеградацияның практикалық мәселелерімен тығыз байланысты болып келеді. Зерттеудің барлық кезеңдерінде, ластанған топырақта азотбекітуші микроағзалар мен олигонитрофилдер саны ластанбаған топыраққа қарағанда жоғары болды. Ластанған топырақтағы азотты бекітетін қарқындылықтың жоғарылауы оның қолжетімді құрамындағы спирттер, органикалық қышқылдар, аминқышқылдар және басқа да қосылыстары бар органикалық заттарға негізделеді. Мұны *Clostridium* түріндегі анаэробты азотбекітуші топтары санының артуымен байланыстырады. Азотты микроағзалар, көмірсутекті тотығу микроағзаларының метаболикалық өнімдерін пайдалана алады.

Түйін сөздер: микроағзалар, азот, топырақ, мұнай, ластану, жанармай, бактерия.

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ВЛИЯНИЕ НЕФТИ НА ЧИСЛЕННОСТЬ РАЗЛИЧНЫХ ГРУПП МИКРООРГАНИЗМОВ, УЧАСТВУЮЩИХ В КРУГОВОРОТЕ АЗОТА

Аннотация. В нефтезагрязненных экосистемах азот является одним из основных биогенных элементов. Поддержание его уровня в окружающей среде обеспечивается активной деятельностью различных микроорганизмов, принимающих участие в его круговороте. В связи с этим представляет интерес проблема влияния нефтяного загрязнения на превращения азота в почве, что тесно связано с практическими вопросами биодеградации. Во все периоды исследования численность азофиксирующих микроорганизмов и олигонитрофилов в загрязненной почве оказалась выше, чем незагрязненной. Увеличение интенсивности азотфиксациии в загрязненной почве может быть обусловлено увеличением в ней содержания легкодоступного органического вещества – спиртов, органических кислот, аминокислот и других соединений. Оно коррелирует с увеличением численности групп анаэробных азотфиксаторов рода *Clostridium.* Возможно, что азотфиксирующие микроорганизмы могут использовать продукты метаболизма углеводородокисляющих микроорганизмов.

Ключевые слова: микроорганизм, азот, почва, нефть, загрязнение, бензин, бактерия.

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