Increase in Plant Survival in the Processes of Phytoremediation of Oil-Contaminated Soils of the Permafrost Zone

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Abstract. Oil and oil products are among the most common soil contaminants. The phytoremediation method is widely used to restore oil-contaminated soils. Permafrost conditions are an additional stress factor for plant growth. The experimental results showed that the dependences of the physiological characteristics of plants on the amount of added oil are nonlinear, which indicates the adaptive nature of these changes. Small amounts of oil (0.16-0.25%) even stimulate plant growth. However, oil additions in excess of 0.82% already inhibit plant growth. The study of increasing plant survival in permafrost soils in a toxic environment (oil pollution) showed that positive results had been achieved by soaking seeds in solutions of biologically active substances. Seed soaking increased the resistance of plants even at the stage of seed treatment, which contributes to their survival in a toxic oil-polluted environment. For example, it is possible to recommend a sowing of Vicia sativa seeds that have been pre-treated by soaking in salicylic acid solutions of different concentrations or humate for increase the effectiveness of measures to clean up areas from oil pollution.

1. Introduction
The ever-increasing demand for oil and oil products inevitably leads to an increase in the number of accidents, spills and leaks. Currently, oil and oil products have become one of the most common pollutants of soil and bottom sediments. The safest from the point of view of ecology is soil remediation by means of bioremediation, when the transformation of pollution is mainly carried out due to microorganisms-oil destructors [1-4]. In many cases, at the final stage of bioremediation work, perennial herbaceous plants that are tolerant to oil pollution are planted in the soil [5]. It should be noted that the use of phytoremediation methods for the restoration of technologically disturbed lands has found widespread use in the environmental sphere [6], also for cleaning lends from oil pollution [7-10]. The root system of plants is capable of releasing various metabolites, oxidative and hydrolytic enzymes into the soil (rhizosphere), which, apparently, contribute to the oxidative destruction of oil pollution [11]. As studies have shown [12], in the case of soil pollution with oil, it is under the influence of plants that biodegradation processes cover a wider range of petroleum hydrocarbons, as a result of which the composition of soil bitumoids most closely approximates the natural background. Northern ecosystems are the most vulnerable, it is more difficult and slower to recover. The cold climate is an additional stress factor for plant growth and survival. This research laboratory experiments were carried out with goal.
studying the possibility of increasing plant survival in oil-contaminated soils of the permafrost zone.

2. Methods and objects
For laboratory experiments, the meadow soil of Central Yakutia was selected, to which oil from the Talakan field was added. In one series of experiments, the share of added oil was from 0.06 to 1.22%, in another - 5 and 10%. In the first series of experiments, seeds of *Artemisia vulgaris* L. and *Lepidium ruderale* L. were sown in the contaminated soil. In the second series - the seeds of *Vicia sativa*. Before sowing, *Vicia sativa* seeds were treated in solutions of biologically active substances (solutions of the Baikal EM-1 preparation, humates, salicylic acid 10⁻³ and 10⁻⁴ mol/l). The physiological characteristics of plants were determined by the germination energy (on the 7th day), germination seeds (on the 10th day) and the survival of seedlings (on the 60th day, the end of the experiment). The level of oil pollution was detected by the yield of chloroform extract (ChE). The processes of transformation of oil pollution were studied using liquid-adsorption chromatography, IR-Fourier spectroscopy and chromatomass spectrometry.

3. Results and discussions
Figure 1 shows how the physiological characteristics of plants change with the amount of added oil in the first series of experiments. It can see that the dependencies are of a complex nonlinear nature, indicating the adaptive nature of these changes.

![Figure 1. Dependence of the physiological characteristics of plants on amount of added oil.](image)

Let us introduce such an indicator as the plant survival rate, i.e. the ratio of the survival of seedlings to their germination (K_survival). The dependence of this coefficient on the amount of added oil makes it possible to perform the primary characterization of plant survival strategies under conditions of an increasing stress factor (oil pollution of soils). It can be seen (Figure 2) that the dependence of the K_survival on the amount of oil added to the soil is bimodal with a downward trend. When small amounts of oil are introduced into the soil, up to 0.16% for the *Lepidium ruderale* L. and 0.25% for *Artemisia vulgaris* L. (the area of the first maximum), the K_survival is close or even higher than that for plants growing in clean soil. Thus, low concentrations of oil in the soil can even stimulate plant growth.

When share of added oil was above 0.82%, a very low K_survival of seedlings was observed against the background of a sufficiently high germination. As a result, a second series of experiments was carried out to study the possibility of increasing plant survival in toxic environments (oil-contaminated soil). Currently, a new direction is successfully developing to protect plants from diseases (phytopathogens) and unfavorable environmental conditions, the essence of which is to enhance the immune status of plants due to the effect on them of various biologically active substances and preparations of biological and abiotic origin [13-14].

The introduction with irrigation of such drugs as "Baikal EM-1", humates are used not only to increase the productivity of agricultural crops, but also to enhance the activity of microorganisms-oil
Presowing treatment of seeds with salicylic acid solutions, as well as in combination with biological products and micronutrient fertilizers, increases plant resistance to phytopathogens and unfavorable environmental conditions [16-17].

Figure 2. Dependence of the $K_{\text{survival}}$ of plants on amount of oil added to the soil.

*Vicia sativa* is a herbaceous plant tolerant to oil pollution and is often used for phytoremediation of disturbed lands [5]. This plant was sown in oil-contaminated soil in the second series of experiments. Table 1 shows the conditions for preliminary soaking of seeds, the physiological characteristics of plants *Vicia sativa* during the experiment and the coefficient of destruction of oil pollution. Oil pollution of soil samples on the fourth day after the start of the experiment was taken as the initial level of oil pollution (no plants were sown on these soil samples). As the weighing of soil samples has shown, during this time the main part of the volatile components of oil has time to evaporate, and the processes of biodegradation of oil pollution under the influence of its own soil microflora can be neglected.

Despite significant additions of oil to the soil (5% and 10%), pretreatment of seeds made it possible to increase the survival of *Vicia sativa* on oil-contaminated soil. A positive effect was achieved due to the soaking of seeds in salicylic acid solutions of different concentrations or humate solution, while the greatest stimulating effect was noted with a higher oil addition to the soil, 10%.

The isolated chloroform extracts were studied by liquid adsorption chromatography and FTIR spectroscopy. The research results showed that, despite the course of biodegradation of oil pollution, they retained an oil character. This was evidenced by the high content of hydrocarbon fractions in the chloroform extracts (74.3 - 79.0%) and rather low - resinous components (20.3 - 24.6%). For comparison, the fractions of hydrocarbons and resins in bitumoid of the control sample were 25.2% and 51.3% respectively. Chromato-mass spectrometric studies also have shown a close distribution of individual hydrocarbons in the bitumoids of the contaminated soils to the oil distribution.

It should be noted that an increase in the degree of destruction of oil pollution correlates well with the survival of seedlings ($K_{\text{corr}} = 0.9$) (Table 1). That is the more plants grew on oil-polluted soil, the more hydrocarbon pollutants were destroyed. This confirms the supposed role of plants in accelerating the processes of biodegradation of petroleum hydrocarbons.

The results of the experiments showed that the greatest effect in increasing the resistance of *Vicia sativa* to oil pollution in terms of the survival of seedlings and the degree of destruction of oil pollution was detected in the case of soaking seeds in solutions of $10^{-4}$M salicylic acid with a 5% oil addition, and $10^{-3}$M - at 10% addition. When treating seeds with a solution of potassium humate, a slightly smaller effect was recorded. The most noticeable result from the pretreatment of seeds in solutions of these preparations was with a 10% addition of oil to the soil. The preparation "Baikal EM-1" had a small positive effect only with a 5% oil addition.
It should be noted that the main direction of use of the preparation "Baikal EM-1" and humates is an increase in agricultural productivity of plants, an increase in their resistance to adverse environmental conditions. The preparation "Baikal EM-1" is a microbiological fertilizer. It contains a large number of microorganisms, including hydrocarbon-oxidizing ones. Apparently, the best result from the use of this preparation in conditions of oil pollution can be achieved when it is applied with irrigation directly into the soil. The positive effect in the form of destruction of oil pollution and the state of the microbial community from the introduction of the preparation "Baikal EM-1" into oil-contaminated soil is indicated in [18].

Table 1. Results of experiments on soaking seeds of *Vicia sativa* (p<0.05).

| Experimental conditions | Days | Share of added oil, % | Germination, % | Survival, % | ChE yield, % | K\textsubscript{destr}, % |
|-------------------------|------|-----------------------|----------------|-------------|--------------|-----------------|
| Without plants          | 4    | 5.0±0.1               | 3.735±0.001    |             |              |                 |
| Water                   | 60   | 5.0±0.1               | 3.452±0.001    | 7.6±0.1     |              |                 |
| Salicylic acid solution | 10^{-3} mol / l | 60 | 5.0±0.1 | 68±3 | 66±3 | 2.874±0.001 | 23.0±0.1 |
| Salicylic acid solution | 10^{-4} mol / l | 60 | 5.0±0.1 | 68±3 | 66±3 | 2.997±0.001 | 19.7±0.1 |
| Humate solution          | 60   | 5.0±0.1               | 66±3           | 65±3        | 3.047±0.001  | 18.4±0.1       |
| "Baikal EM-1" solution  | 4    | 10.0±0.1              | 7.013±0.001    |             |              |                 |
| Water                   | 60   | 10.0±0.1              | 6.714±0.001    | 4.3±0.1     |              |                 |
| Salicylic acid solution | 10^{-3} mol / l | 60 | 10.0±0.1 | 25±1 | 23±1 | 6.354±0.001 | 9.4±0.1 |
| Salicylic acid solution | 10^{-4} mol / l | 60 | 10.0±0.1 | 50±2 | 43±2 | 6.157±0.001 | 12.2±0.1 |
| Humate solution          | 60   | 10.0±0.1              | 40±2           | 39±2        | 6.249±0.001  | 10.9±0.1       |
| "Baikal EM-1"           | 60   | 10.0±0.1              | 56±3           | 40±2        | 6.238±0.001  | 11.1±0.1       |

The protective effect of humic substances is explained not only by the formation of non-toxic complexes with ecotoxicants that are inactive in the soil and inaccessible to living organisms, but also by adaptogenic activity, which consists in an increase in the resistance of living organisms to stress loads, in particular, to chemical stress [14]. Humic substances are assumed to be able to accelerate the processes of anaerobic decomposition of organic pollutants due to the redox mediator properties, humic substances.

Salicylic acid in high concentrations is toxic to plants. At the same time, its low content in the liquid phase of the physiological substance of plants is an important indicator connected with the manifestation of plant immune responses [19, 20]. For example, a direct correlation was found between the concentration of salicylic acid in a plant and the level of its resistance to diseases [21]. The mechanism of action of salicylic acid is to inhibit catalase, which leads to the accumulation of H\textsubscript{2}O\textsubscript{2}.

An increase in the peroxide content serves as a signal for the activation of peroxidase and "genes of systemic acquired resistance" [21], which, in turn, suggests its noticeable effect on the activation of the processes of oxidative destruction of oil pollution. Thus, salicylic acid in low concentrations (10^{-3} - 10^{-4} M) is apparently capable of inducing prooxidant processes with the participation of H\textsubscript{2}O\textsubscript{2}, leading,
first, to an increase in the resistance of the plant organism to environmental stress factors even at the stage of soaking seeds. Secondly, H$_2$O$_2$ and peroxidase secreted into the rhizosphere enhance the processes of oxidative destruction of oil pollution.

4. Conclusions
The adaptive nature of changes in the physiological characteristics of plants, depending on the amount of oil added to the soil, is shown using the example of the Artemisia vulgaris L. and the Lepidium ruderale L. Small additions of oil to the soil, up to 0.16 - 0.25%, can even increase the survival of plants. However, oil additions in excess of 0.82% are already detrimental to plant growth.

The results of experiments to study the possibility of increasing plant survival in oil-contaminated soil using the example of Vicia sativa showed that a positive effect can be achieved with preliminary soaking of seeds in solutions of biologically active substances. The best result in terms of plant survival was found in the case of seeds soaking in solutions of salicylic acid of different concentrations or humate. With a 5% addition of oil, a 10$^{-4}$M salicylic acid solution was optimal for soaking, and with a 10% addition, - 10$^{-3}$M. Soaking the seeds in a solution of potassium humate gave a slightly lower result. The preparation “Baikal EM-1” showed a small positive effect only with a 5% addition of oil to the soil. The positive result from soaking seeds in potassium humate is apparently associated with its adaptogenic activity. Salicylic acid in low concentrations (10$^{-3}$ - 10$^{-4}$ M) is apparently capable of inducing prooxidant processes with the participation of H$_2$O$_2$, leading, first, to an increase in the resistance of the plant organism to environmental stress factors even at the stage of seed soaking. Secondly, H$_2$O$_2$ and peroxidase secreted into the rhizosphere enhance the processes of oxidative destruction of oil pollution.

In all experiments, the degree of destruction of oil pollution correlated well (K_{corr} = 0.9) with the survival of Vicia sativa.

Using the example of Vicia sativa, to increase the effectiveness of measures to clean up areas from oil pollution, it is possible to recommend sowing seeds that have been pre-treated by soaking in salicylic acid solutions of different concentrations or humate.

5. References
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