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ACTIVATION OF THE PLANT BIOCHEMICAL PROTECTION MECHANISMS FROM OIL POLLUTION AND PARA-AMINOBENZOIC ACID

ABSTRACT. This article explores the topic of the influence of oil pollution on the biochemical plant protection systems. The tests were carried out on the species of the narrow-leaved catoptric (Typha angustifolia L.) and hoary sedge (Carex canescens L.) collected from an oil spill in Kalchinskoe field of the Tyumen region. We also used cultivated oat (Avena sativa L.) which was grown in the oil contaminated soil and watered with oil contaminated water from Kalchinskoe field.

The article states that the morpho-physiological and biochemical features of the cultivated oat growing in the oil contaminated soil are negatively affected. Such negative effect was smaller in the cultivated oat watered with oil contaminated water. The narrow-leaved catoptric is the least sensitive to oil pollution and the hoary sedge is the most sensitive.

Oil pollution causes development of lipid peroxidation products such as Schiff bases and diethenoid conjugates in the plant cells. The damaging effect is neutralized by biochemical protection systems of the plants (such as carotenoid, flavonoid and phenolic). Para-aminobenzoic acid partially eliminates the negative effect of oil. It leads to increase in the concentration of chlorophyll, carotenoid and flavonoids. Phenols are the most effective substances in the biochemical plant protection system against oxidative stress.

KEY WORDS. Para-aminobenzoic acid, oil pollution, biochemical plant protection system, lipid peroxidation.

Introduction. In the oil industry the most dangerous from the ecological perspective accidents are those which are accompanied by large spills of oil as a result of drilling or pipeline damage.

High concentrations of oil act as inhibitors influencing the growth and development of plants. On the level of an organism the effects of oil are seen in the morphological and physiological disorders of certain plants [1]. Under the influence of hydrocarbons we can observe death of the plant cover, deceleration of the plants growth, chlorosis and dehydration tendency, disorders of the breathing and photosynthetic functions, change in the structure of chloroplasts [2].

In cases of oil contamination oil hydrocarbons damage cell membranes, free oxygen atoms appear which initiate lipid destruction – peroxidation [3]. The main products of lipid peroxidation are diene conjugates, malondialdehyde and Schiff bases. It is known that under normal living conditions there is always a certain level of lipid peroxidation in the cell induced by development of free oxygen. Lipid peroxidation

in a cell stays on the same level due to a multi-level antioxidant protection system. Thus, balance of the two parts of this system – peroxidation on the one hand and antioxidant activity on the other – is a condition necessary to support normal living activity of the cell [4-5].

Phenolic compounds display an adaptogenic and stimulating effect. They play an active role in various physiological processes – photosynthesis, breathing, growth and protective reactions of plants [6]. Flavonoids performing protective functions belong to the class of phenolic compounds. Flavonoids are restorative agents that, together with other natural compounds (carotenoids, ascorbic acid), are able to protect cells from oxidative stress [7].

The main pigments of higher plants and green algae are chlorophyll A and B. A chlorophyll molecule is capable of photochemical transformation of the energy of an excited electron into chemical energy by way of oxidation-reduction reactions [6]. The process of photosynthesis, as a very sensitive one, serves for bioindication of environmental pollution. During contamination the amount of chlorophyll in the plant cells decreases [8]. Carotenoids, in addition to chlorophylls, are another pigment of photosynthesis. Apart from participation in the process of photosynthesis, carotenoids act as antioxidants protecting photosystem of plants from oxidative stress [9].

As an activator of protective mechanisms we used para-aminobenzoic acid (PABA). By regulating ferment activity PABA increases an organism's adaptability to the unfavorable environment i.e. is an adaptogen [10].

The goal of our research was analysis of the activation of biochemical protective mechanisms of plants and changes of their physiological indicators under the conditions of polluted soil and water in Kalchinskoe oil field.

Materials and methods. Kalchinskoe field is situated in Uvat district of Tyumen region. It is being developed by the company "TNK-Uvat". In the middle of May 2008 on the territory of Kalchinskoe field there was an oil spill from the infield pipeline. Oil got into the soil and drain ditch going along the communication lines. Technical (oil-contaminated soil was covered with sand) and chemical (oil was gathered with the help of sorbents) remediation of the place of the accident was performed.

In order to estimate the oil contamination degree on Kalchinskoe field we took samples of water, soil and plants of dominant species. Depending on the territorial contamination degree there were singled out four sampling points: O1 (test 1) — at the place of the oil spill; O2 (test 2) – 20 m away from the place of the spill; O3 (test 3) - 35 m away from the place of the spill; and K (control) – samples from the background territory.

Chemical analysis of the Kalchinskoe field soil samples revealed surpassing of the maximum allowed concentrations of iron, petroleum products and cuprum in the area of maximum contamination.

We have chosen plants of the species dominant on the territory in question ss test-objects: *Typha angustifolia L., Carex canescens L.* and *Avena sativa L.* used in grass mixtures for remediation.

Analysis of soil phytotoxicity of Kalchinskoe field was carried out in tests on *Avena sativa L.* (cultivated oat). The seeds were grown in plastic cups filled with the tested soil. Each test variant had four replications: control – soil from Kalchinskoye field from the background territory (K); test 1 – soil from the oil spill (O1); test 2 – soil from 20 m away from the oil spill (O2); test 3 – soil from 35 m away from the oil spill (O3); test 4 – soil from the background territory +0.001% solution of para-aminobenzoic acid (O4); and test 5 – combined impact of the oil spill and PABA: soil from the oil spill + 0.001% solution of para-aminobenzoic acid (O5). The experiment lasted 28 days.

We checked such parameters of all the tested plants as content of flavonoids, phenolic compounds, substances resulting from lipid peroxidation (diene conjugates and Schiff bases), and photosynthetic pigments.

Research findings and their discussion. The *Typha angustifolia L.* from Kalchinskoye oil field displayed increased concentration of chlorophyll A and carotenoids in all the tested variants; increase in chlorophyll B was observed only in the plants collected 20 m away from the place of the accident. Chlorophyll A is the main photosynthetic pigment but under the influence of stress factors the plant has to increase production of organic substances which is possible due to increase in the concentration of auxiliary Chlorophyll B.

Analysis of the content of phenolic compounds in the *Typha angustifolia L.* from Kalchinskoye oil field (fig. 1) has shown their increase in all the tested items which indicates activization of the plants protection system under the influence of oil pollution.

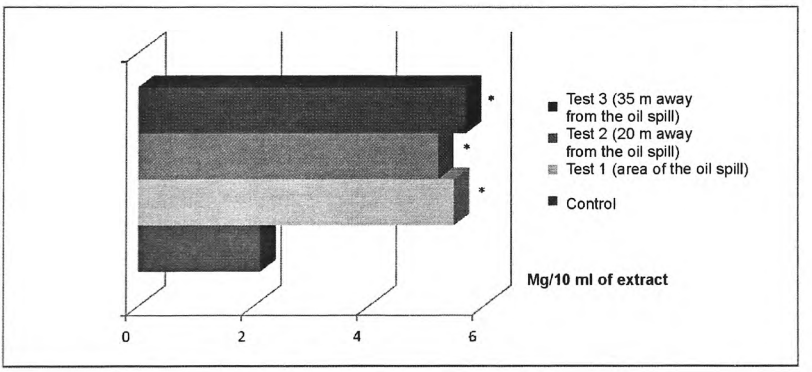

Fig. 1 Content of phenolic compounds in the *Typha angustifolia L.* from Kalchinskoye oil field

Note: * statistically significant differences between control and test variant ($P<0.05$).

Flavonoid content in *Typha angustifolia L.* decreased in the plants gathered in the oil spill area which indicates a suppressed condition of the flavonoid system occurring as a result of high petroleum concentration. The value in question increased in the plants in the area 20 m away from the oil spill which means activation of the bioprotection system of the plants. In the test variant with the plants collected in the area 35 m away from the oil spill the content of flavonoids reached control level which implies normalization of the antioxidant protection system.

The content of the primary products of lipid peroxidation (diene conjugates) in the *Carex canescens L.* from Kalchinskoye oil field (fig. 2) increased in relation to the control level which proves the damaging effect of the oil spill. Concentration of Schiff bases did not differ much from the control level under the influence of the oil spill. This fact may be explained by activation of the plants protection system.

The *Carex canescens L.* from the area 20 m away from the spill had a decreased concentration of chlorophylls. The plants from the area 35 m away from the spill had an increased chlorophyll and carotenoid content. Carotenoids actively eliminate excess of the active forms of oxygen protecting pigments and unsaturated fatty acids in lipids from oxidative damage.

The content of phenolic compounds in *Carex canescens L.* increased in all the tested variants which indicated activation of the biochemical protection system of the plants.

Flavonoid content increased in the *Carex canescens L.* species gathered in the area of the oil spill and 20 m away from it which also implies activation of the plant protection system.

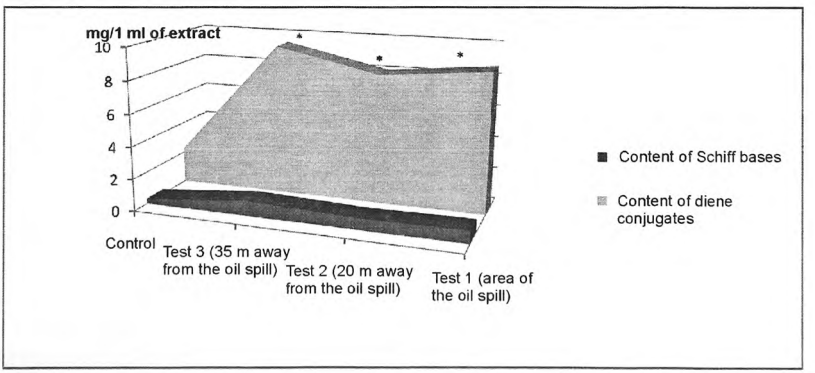

Fig. 2 Content of Schiff bases and diene conjugates in the *Carex canescens L.* from Kalchinskoye oil field

Note: * statistically significant differences between control and test variant ($P<0.05$).

Thus, the study of the biochemical systems of the plants gathered from Kalchinskoye field demonstrated the damaging effect of petroleum which is expressed in the increased

quantities of the products of lipid peroxidation: diene conjugates and Schiff bases. Moreover, we have revealed activation of the biochemical cell protection systems of the plants which is revealed in the increase in the content of carotenoids, phenols and flavonoids. According to our study, phenolic compounds proved to be the most effective in the protection of the plant cells. In all the tested variants we observed increase in their concentration both in *Typha angustifolia L.* and *Carex canescens L.*

The content of the products of lipid peroxidation (diene conjugates and Schiff bases) in the cultivated oat grown in the soil from Kalchinskoye field increased in all the tested variants which indicates damage in the plant cells. The smallest concentration of the lipid peroxidation products was observed in the case with a combined effect of petroleum and PABA which means PABA protective capacity.

The content of all the studied photosynthetic pigments decreased in all the tested variants but for carotenoids concentration under the combined influence of petroleum and PABA which proves PABA protective capacity.

Analysis of the content of phenolic compounds in cultivated oat revealed their decrease in all the tested variants with the exception of the one with PABA.

Analysis of the content of flavonoids in the cultivated oat grown in Kalchinskoye field revealed their decrease in the case involving the soil from the area of the oil spill which indicates suppressed condition of the plants. Flavonoid content decreases in the variant with the use of PABA. In the test variant employing both petroleum and PABA this value also decreases as petroleum products concentration is very high (4333.3 mg/kg of soil); and the remedial agent is unable to repair the damage completely. In the variant with the soil taken 35 m away from the oil spill and treated with PABA the content of flavonoids increased which means activation of the work of the biochemical protection systems.

Judging by the results of the experiments we can state a damaging effect of petroleum on the cells of the cultivated oat which is revealed in the increase in the concentration of lipid peroxidation products. PABA partially relieves the suppressing effect produced by oil resulting in a relatively low, in comparison with the other tested variants, concentration of diene conjugates and Schiff bases. The cultivated oat grown in the soil from Kalchinskoye field had decreased concentrations of antioxidants (carotenoids, phenols and flavonoids) almost in all the test variants. The oat does not belong to the plants growing naturally on the territory of the field as a consequence it is worse adapted to oil pollution. The use of oat in remediation mixtures is connected with its ability to accumulate contaminating substances; however, at that the plant is suppressed which is revealed in the decrease in the concentrations of the substances responsible for its cells biochemical protection.

We have calculated the correlation dependence between the content of lipid peroxidation products and concentration of antioxidants and revealed species-specificity in the work of the biochemical protection systems which is expressed in the activation of various protection systems in the plants of different species. Phenols have been proved the most effective substances in the mechanisms of the cell protection from oxidative stress.

Conclusions:

1. While growing cultivated oat on the soil of Kalchinskoye oil field we have noted suppression of its morphophysiological and biochemical parameters; PABA partially reduced the damaging impact of petroleum. While growing oat in the oil-polluted water the revealed regularities were not as strong.

2. From all the plants growing on the territory of Kalchinskoye oil field *Typha angustifolia L.*turned out to be the least sensitive to oil pollution; *Carex canescens L.* was more sensitive.

3. Oil contamination leads to development of lipid peroxidation products (diene conjugates and Schiff bases) in the plant cells, whose negative effect is neutralized by the system of biochemical (carotenoid, flavonoid and phenolic) plant protection.

4. We have also established PABA protective action in the oil contaminated environment which is revealed in the growing concentration of chlorophylls, carotenoids and flavonoids.

REFERENCES

1. Moskovchenko, D.V. *Neftegazodobycha i okruzhajushhaja sreda: jekologo-geograficheskij analiz Tjumenskoj oblasti* [Oil and Gas Production and Environment: Ecological and Geographical Analysis of Tyumen Region] Monograph. Novosibirsk, 1998. 112 p. (in Russian).

2. Kadakina, I.V. *Toksicheskoe dejstvie VRF Holmogorskoj i Shaimskoj nefti na morfofiziologicheskie pokazateli rjaski maloj* [Toxic Effect of VRF of Kholmogorskoy and Shaimskoi Oil on Morphophysiological Indices of Lemna Minor]. Tyumen, 1991. 55 p. (in Russian).

3. Petuhova, G.A. *Jekologo-geneticheskie posledstvija vozdejstvija neftjanogo zagrjaznenija na organizmy* (dokt. diss.) [Ecological and Genetic Consequences of Oil Pollution on Living Organisms (Diss. Doct)]. Tyumen, 2007. 526 p. (in Russian).

4. Baraboj, V.A. Stress mechanisms and Peroxidation of Lipids. *Uspehi sovremennoj biologii — Successes of Contemporary Biology.* 1991. Vol. 111. Issue 6. P. 923-932 (in Russian).

5. Anbar, M., Neta, P. Reactivity of the hydroxyl radical in aqueous solutions. *Intern. J. Appl. Rediat. Isot.* Vol. 18. 1967. Pp. 495-523.

6. Medvedev, R.R. *Fiziologija rastenij* [Physiology of Plants] Textbook. St. Petersburg: St.-Petersburg State University publ., 2004. 336 p. (in Russian).

7. Zagoskina, N.V. Polyphenols and their Role in Plant Protection from Stress Factors. *Novye i netradicionnye rastenija i perspektivy ih ispol'zovanija (*New and Non-Traditional Plants and Perspectives of their Usage. Materials of VI International Symposium). 2005. Vol. 3. P. 300-302 (in Russian).

8. *Bioindikacija zagrjaznenij nazemnyh jekosistem* [Bioindication of Contaminations of Terrestrial Ecosystems]. Ed. by R. Shubert. M.: Mir, 1988. 350 p. (in Russian).

9. Filimonova, M.V. *Vlijanie jekologicheskih faktorov na sintez nizkomolekuljarnyh antioksidantov i nakoplenie mikrojelementov v lekarstvennyh rastenijah podzony srednej tajgi (v predelah Hanty-Mansijskogo avtonomnogo okruga)* (avtoref. diss. kand.) [Influence of Ecological Factors on Synthesis of Low-Molecular Antioxidants and Accumulation of Microelements in Medicinal Plants of Middle Taiga Sub-Zone (within Khanty-Mansy Autonomous Region) (Cand. Diss. Thesis]. Surgut, 2006. 23 p. (in Russian).

10. Stroeva, O.G. Biological Properties of Para-Aminobenzoic Acid. Ontogeny. *Ontogenez — Ontogeny.* 2000. Vol. 31. № 4. Pp. 259-283 (in Russian).