Phytotoxicity of gaseous limit and aromatic hydrocarbons in relation to some agricultural plants

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Abstract. The authors of the article determine the phytotoxicity of aromatic hydrocarbons in various concentrations in relation to some agricultural plants and the ability of these plants to accumulate hydrocarbons. This is done for the first time on the basis of field studies of plantings at different distances from the enterprise for the production of carbon black, which is a source of gaseous saturated and aromatic hydrocarbons, as well as after exposure of these mixtures to methane, phenol, benzene and toluene. The objects of study were Phaseolus vulgaris, Hordeum vulgare and soft spring wheat called "Memory of Aziev". Statistically significant changes in the pigment complex of plants, expressed in a decrease in the content of pigments and carotenoids, an increase in the ratio of b and a chlorophyll under the action of hydrocarbons were revealed. The activity of the polyphenol oxidase enzyme tended to initially increase, in response to pollution, and to a subsequent decrease under the action of higher concentrations and high toxicity of the gas. Plant peroxidase activity increased 1.5–7 times with an increase in the concentration of toxin in comparison with the control analogues. According to the degree of phytotoxicity, the studied gases are arranged in the following sequence: phenol > mixture of aromatic hydrocarbons > benzene > toluene > methane. Phaseolus vulgaris turned out to be the most sensitive to the action of hydrocarbons, and also capable of active absorption of hydrocarbons.

1. Introduction

Contaminants inadvertently precipitated or absorbed by agricultural plants pose a constant threat to both the life of the plants and the health of the consumer [1, 2]. Increased atmospheric pollution with marginal and aromatic hydrocarbons is observed near large industrial cities, chemical and petrochemical enterprises, and fuel and energy enterprises. At the same time, quite often private gardens and agricultural landings are located near enterprises or directly in large cities. A number of researchers conclude that aromatic hydrocarbons are highly toxic: oxidative stress, changes in protein activity in living, including in plants, organisms [2-5]. Increased hydrocarbons are found in fruit and vegetables grown near roads and in urban areas [6]. At the same time, research on the direct accumulation of atmospheric marginal and aromatic hydrocarbons preferential for the urban and industrial environment, as well as their physiological and biological effects on agricultural plants, has not been met: the bulk of the research is devoted to the accumulation of toxins (kerosene) through soils [7]. All this determined the purpose of the research: to study the physiological and biochemical characteristics of some agricultural plants in the zone of industrial hydrocarbon pollution, including the consequences of their exposure to hydrocarbons.
2. Materials and methods of research

The objects of study were the following agricultural plants: beans (Phaseolus vulgaris), barley (Hordeum vulgare) and soft spring wheat of the “Memory of Aziev” variety.

Field studies were conducted in four zones located at different distances from the carbon black plant (the city of Omsk, Russia). To obtain carbon black, petrochemical products are used; when burned, under the action of high temperature, decomposition of hydrocarbons occurs. In this case, large quantities of such toxic components as benzopyrene, methane, phenol, toluene, naphthalene, and benzene are formed in the reactors. The results of the analysis of the quality of atmospheric air at the border of the sanitary protection zone of the enterprise revealed an average excess of the maximum permissible concentrations over the summer period: for phenol – by 2 times, benzene - 1.5 times, toluene - 1.05 times. The first zone was located directly on the territory of the enterprise, the second - in its sanitary protection zone, the third defended by 7 km. All zones are located in the direction of the prevailing winds. The fourth zone was the control, background, and was outside the city of Omsk (24 km in the opposite direction of the winds). Sampling was carried out in the morning from all zones simultaneously.

Soil analysis was carried out in all gas-dynamic zones, indicating agrochemical and mechanical uniformity of soils of all areas represented by black soil, heavy loam [8].

Additionally, experimental exposures of plants to methane, phenol, benzene, and toluene in various concentrations were carried out. Fumigation was carried out with the help of glass chambers with a volume of 20 liters, in which the specified gas concentrations obtained using calibration gas mixtures were introduced. Control plants were under similar conditions in the chambers without introducing hydrocarbons. The exposure was carried out for 3 and 10 hours.

Further, the plants were investigated for the content of phenol in the leaves, the amount of photosynthesizing pigments, and the activity of oxidative enzymes: peroxidase and polyphenol oxidase. The content of phenols in the leaves was determined by titration with indigocarmine according to Levental method; enzyme activity was determined by iodometric method; the amount of a and b pigments, as well as of carotenoids was determined using a spectrophotometer [8].

All studies were carried out in plants for 5 years, in triplicate. Statistical data processing included Student criterion, correlation and analysis of data variance.

3. Results and discussion

Planting on the source territory and in its sanitary protection zone had a lighter colour and a smaller size compared to plants in other areas.

Near the source, there is a significant decrease in the content of chlorophylls and carotenoids in the leaves of plants. The content of chlorophyll in beans and wheat decreased by an average of 30.7% (ŋ = 0.96; p <0.001), carotenoids - by 19.5% (ŋ = 0.45; p <0.05); in barley, there was no significant decrease in the amount of pigments. During the growing season, changes in the pigment complex of plants in contaminated areas are increasing. An increase in the ratio of components of green pigments in beans and wheat in polluted areas was also noted, which indicates a sufficiently more significant decrease in b chlorophyll in comparison with a chlorophyll. Apparently, this is due to the fact that when exposed to an aggressive environment, the free form of chlorophyll is damaged more strongly than the form associated with protein.

The activity of polyphenol oxidase and peroxidase plants in gassed areas increases. The most pronounced increase in polyphenol oxidase activity was found in barley – by 1.25-2.8 times from control plants, and the most stable breed is beans (increase by 2-12%) (ŋ = 0.34-0.87; p <0.01-0.001). Peroxidase activity increases to a somewhat lesser extent in all plants, by about one magnitude (10-70% of the background zone plants). Increasing the activity of enzymes in these conditions can be considered as a protective reaction of plants, expressed in increasing the energy production necessary for life support in unfavourable environments.

Studies of the content of phenols in the leaves of plants revealed the ability of agricultural plants to absorb hydrocarbons in large quantities. The content of phenols in the leaves of beans in polluted areas
increased by 1.4-1.6 times ($\eta = 0.70; p <0.001$). The difference between the experimental and control plants is significant with $p <0.001$ in the third gas-dynamic zone. In barley and wheat, a statistically significant increase in the concentration of phenols was observed only in the most polluted areas. In the leaves of barley, an increase of 1.3-1.45 times was observed, and of wheat - an increase of 1.21-1.29 times in comparison with control analogues ($p <0.05-0.001$) [8].

Damage to plants growing on the enterprise’s territory and in the sanitary protection zone at a distance of 1 km from the source turned out to be very similar. Also, the concentrations of hydrocarbons in the atmospheric air of these zones are about the same. This may be due to the fact that gaseous hydrocarbons are deposited at some distance from the source, a multiple of the size of the exhaust pipes. The third gas-dynamic zone is separated from the emission source at a distance of 7 km, no excess of the maximum permissible concentrations has been identified here, the gas content of the zone is similar to the urban environment. Nevertheless, deviations between plantings of this zone and background zone are present.

Correlation analysis revealed a high negative relationship between the concentration of phenols in ambient air and the pigment content in the leaves of plants ($r = 0.7; p <0.001$); a positive relationship was found between the concentration of this gas and the activity of polyphenol oxidase ($r = 0.48; p <0.05$).

Hydrocarbons were exposed to concentrations of 20, 40 and 80 mg/m$^3$, as preliminary experiments with different gas concentrations revealed that concentrations of less than 20 mg/m$^3$ do not have a physiological effect on the studied plants, and at 80 mg/m$^3$, external damage to plants is observed.

External manifestations of damage were observed only when exposed to phenol in the maximum concentration on the beans. The leaves of the plant acquired a dark oily colour, while the lamina plate had a slightly twisted appearance. The rest of the plant species remained virtually unchanged; in barley, a small part of the leaves had a dark marginal colour.

Damage to the pigment complex was expressed in a decrease in the number of pigments - directly proportional to the concentration of the toxicant. Figure 1 shows a picture of the decrease in the number of pigments and carotenoids in beans - as the most sensitive to pollution.
Figure 1. Pigment complex of plants (mg/g of air dry mass) under the influence of hydrocarbons of various concentrations: Chrolophyll content: exposure for 3 hours; for 5 hours. Carotenoids content: ----- exposure for 3 hours; --- for 5 hours.

The pigment complex of other plants also underwent changes, although not as pronounced ($p < 0.05 – 0.01$). Phenol had the most toxic effect on plants, methane was the least toxic. Short-term exposure to methane at a concentration of 20 mg/m$^3$ did not lead to statistically significant changes in the pigment complex. One can also note a steady decrease by 5–15% in the content of chlorophylls and carotenoids with an increase in the time of exposure to hydrocarbons.

Selective results of the study of the activity of enzymes polyphenol oxidase and peroxidase after exposure to hydrocarbons are presented in figure 2.

Hydrocarbon exposure caused an increase in the activity of plant polyphenol oxidase, only phenol and benzene in high concentrations reduced the enzyme activity in beans by 10–40% ($p < 0.01 – 0.001$) (figure 2). The remaining plants showed an increase in enzyme activity in direct proportion to the gas concentration of 1.1–3 times. The decrease in the activity of polyphenol oxidase is associated with deeper disorders and, accordingly, a decrease in the physiological activity of the plant organism.

The peroxidase enzyme reaction had a similar pattern with polyphenol oxidase and was manifested by an increase in enzyme activity, but to a stronger degree. So, after exposure to phenol at maximum concentration, peroxidase activity in beans has increased by 7 times, in barley – by 5 times, in wheat – by 2 times.
The activity of enzymes polyphenol oxidase and peroxidase (ml 0.01 N J) plants after exposure to hydrocarbons.

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All hydrocarbons in significant quantities were absorbed by plants. The content of phenols in the leaves of beans has increased by an average of 1.6 times, compared with the control analogues, barley by an average of 1.48 times and wheat by 1.4 times [8].

4. Conclusion
The high content of marginal and aromatic hydrocarbons in the atmospheric air undoubtedly worsens the vital activity of agricultural plants, and also causes undesirable accumulation of hydrocarbons in their tissues. Plants in polluted areas have a depressed appearance, smaller sizes, and yield decreases. Under the action of hydrocarbons, a statistically significant decrease in the amount of pigments and carotenoids in the leaves occurs. The reaction of polyphenol oxidase and peroxidase enzymes manifests itself in an initial increase in the activity of functional adaptive reactions, then, with an increase in the concentration and toxicity of the gas, there is a decrease in the activity of enzymes, inhibition of metabolic processes.
All the studied hydrocarbons, in accordance with their toxicity, can be distributed as follows: phenol > mixture of aromatic hydrocarbons > benzene > toluene > methane.

Phaseolus vulgaris turned out to be the most sensitive plants to the action of gas among the other studied, at the same time, this species is also capable of maximizing the accumulation of atmospheric phenolic compounds.

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