Effect of growing conditions on the physiological and biochemical status of remediating plants

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Abstract. This study evaluated the effects of oil pollution, low humidity, and plant co-cultivation in kastanozem on Sorghum bicolor L. Moench and Medicago falcata L. Under the influence of oil pollution, the accumulation of sorghum biomass and the content of its photosynthetic pigments decreased. At the same time, an increase in the concentration of protein in the roots of this plant was not accompanied by an increase in the peroxidase activity. The reduced humidity did not have a negative impact on sorghum. The pollution also reduced the yellow medick biomass and the content of its photosynthetic pigments and proteins in leaves. At the same time, an increase in the protein content in the roots was accompanied by an increase in the peroxidase activity. The low humidity promoted an increase in the concentration of protein in the leaves and an increase in the peroxidase activity in the roots. The plant co-cultivation reflected positively on the sorghum state and negatively on the alfalfa state. The data obtained can be used by phytoremediation technology for kastanozem cleaning up from oil hydrocarbons and rehabilitation of soil biological activities in arid regions.

1. Introduction
The Volga region is one of the regions in which oil production, transportation and processing are actively carried out. This activity is often accompanied by the environmental pollution and especially the soil. In the Volga region, kastanozem is widespread, the remediation of which is complicated by arid conditions. Sorghum bicolor L. Moench and Medicago falcata L. are known remediating plants for oil-contaminated soils in arid conditions [1, 2]. The manifestation of the remediation potential of plants strongly depends on their physiological and biochemical status. In this regard, the aim of the work was to assess the effects of oil pollution, low humidity, biochar treatment and plant co-cultivation on the sorghum and yellow medick status.

2. Materials and methods
The experiment was carried out with two plant species sorghum (Sorghum bicolor L. Moench) (S) and yellow medick (Medicago falcata L.) (F) for 70 days using kastanozem, typical soil for the arid areas of the Saratov Volga region. The soil contained 14.4 and 10.7 mg kg−1 of N–NH4+ and N–NO3−, respectively, 253.3 mg kg−1 of P2O5, the total moisture capacity was 39%, pH was 7.2. Plants were grown in 1 L pots separately and together. Oil (15 g kg−1) (O) was added to the soil 5 days before planting. Watering was carried out daily up to 50% of the total moisture capacity. Plants were cultivated
in a growth room with a 14 h light period at 23-25 °C (light intensity, 8000 lux) and a 10 h dark period. Ammonium nitrate was added at a concentration of 0.4 g kg\(^{-1}\) 3 and 6 weeks after sowing. Drought (D) was simulated after 30 days of the experiment by reducing irrigation to 30% of the total moisture capacity for 40 days. Wood biochar in (B) a concentration of 1% (w/w) was used as a remediation technique.

Plant biomass, content of photosynthetic pigments (chlorophylls a and b, carotenoids) [3], protein production [4], and intracellular peroxidase activity [5] were analyzed. The enzyme activity was determined spectrophotometrically with respect to 2,7-diaminofluorene at 600 nm in 50 mM K-Na-phosphate buffer (pH 6.0) and was expressed in μmol of oxidized substrate min\(^{-1}\) (mg of protein)\(^{-1}\). All experiments were analyzed in three replicates. Data obtained were processed by calculating the means and standard deviations using Microsoft Excel 2007 (USA).

3. Results and discussion
The use of a grass-legume mix for remediation of soils contaminated with hydrocarbons is described [6]. Plants are usually selected based on their phytoremediation potential and suitability for specific soil and climatic conditions. Effects of oil pollution, low humidity and co-cultivation of sorghum and yellow medick on their physiological and biochemical status were assessed in this study.

Oil pollution had a pronounced negative effect on the accumulation of sorghum biomass: the weight of roots decreased by about 4 times both in monoculture and when grown together with yellow medick, and shoots by 7.5 and 9 times, respectively (figure 1a). A decrease in the accumulation of plant biomass under the influence of oil pollution was also noted by other researchers; at the same time, pollution had a greater negative effect on the aboveground part [7, 8]. Low humidity did not affect this parameter. It was found that yellow medick stimulated the accumulation of sorghum biomass: the weight of roots in monoculture has decreased by almost seven times, and that of shoots - by more than 20 times. The reduced humidity did not affect the aboveground biomass, but root mass was stimulated by 70% in clean soil in monoculture. It can be assumed that by increasing the root mass, the plant improved its water supply. In the literature, a similar effect was found at low humidity in plum seedlings [9]: an increase in the ratio of the mass of roots and shoots was noted, which can be interpreted as a strategy for maximizing the absorbing root surface and increasing the rate of absorption of water and nutrients. Co-cultivation with sorghum significantly inhibited yellow medick. This was especially noticeable in clean soil: at normal humidity, the mass of both roots and shoots decreased by almost half, and at low humidity, more than 4 times. When plants were grown together, some authors also noted a decrease in their biomass compared to monocultures, attributing this to their competition for light [10]. Probably, smaller yellow medick plants were at a disadvantage over larger sorghum plants.
The content of photosynthetic pigments in plant chloroplasts depends on the stage of plant development and external conditions. The toxic effect of oil on plants at the physiological and biochemical levels is characterized by the destruction of chlorophylls and carotenoids [7]. Drought also inhibits plant photosynthesis, causing changes and damage to the photosynthetic apparatus [11], leading to a violation of the ratio of chlorophylls a and b in favor of the latter. The studied plants have different photosynthesis types: C4 in the case of sorghum and C3 in the case of yellow medick, in connection with which the reaction of their photosynthetic apparatus to stress conditions was especially interesting.

Oil pollution reduced the concentration of all studied pigments in the leaves of the sorghum monoculture at normal humidity by 30-40% (figure 2). In this case, the ratio of chlorophylls a and b increased in favor of the latter by more than 40%. Drought did not have a pronounced effect on the photosynthetic apparatus of the sorghum monoculture. The presence of alfalfa not only compensated, but also increased the pigment content (this was especially noticeable at normal humidity).

In general, the content of pigments in yellow medick leaves was significantly higher than that of sorghum, which is typical for C3 plants (figure 3). Oil pollution had a pronounced negative effect both at optimal and low humidity, reducing the level of chlorophylls and carotenoids by almost half. The low humidity conditions did not affect the photosynthetic apparatus of yellow medick. Co-cultivation yellow medick with sorghum insignificantly increased the content of all pigments in clean soil at low humidity; under other conditions, no effect was found.
Figure 2. Effects of oil pollution, soil moisture, and plant co-cultivation on the content of chlorophyll a (a), chlorophyll b (b), and carotenoids (c) in sorghum leaves.

It is known that proteins in plant cells perform structural, biocatalytic, regulatory, and protective functions. Stress conditions can affect the rate and nature of protein synthesis in plant tissues in different ways. Thus, in the tissues of alfalfa under arid conditions, a decrease in protein production was observed [12]. According to [13], plants require increased production of amino acids and proteins to detoxify oil pollution.

Figure 3. Effects of oil pollution, soil moisture, and plant co-cultivation on the content of chlorophyll a (a), chlorophyll b (b), and carotenoids (c) in yellow medick leaves.

In the leaves of sorghum, no influence of external factors on the protein content was revealed (figure 4a). In the roots, protein production increased by about two times in monoculture under oil pollution, and by 2.2-4.5 times when co-cultivated with yellow medick.

In yellow medick leaves, protein production decreased under oil pollution in all variants, while low humidity contributed to its increase (almost three times in clean soil) (figure 4b). In the roots, the
opposite tendency was observed: an increase in the protein concentration was observed with pollution more pronounced in monoculture; drought did not have a noticeable effect on this parameter.

![Figure 4. Effects of oil pollution, soil moisture, and plant co-cultivation on protein production in leaves and roots of sorghum (a) and yellow medick (b).](image)

Plant peroxidases are involved in various important physiological processes of plant growth and development throughout their life cycle, and are also one of the main antioxidant enzymes [14]. The ability of plants to detoxify ROS may correlate with their drought tolerance [15]. In addition, there is a point of view according to which plants are able to carry out enzymatic degradation of organic toxicants to a greater extent using peroxidases [16]. Thus, under pollution conditions, peroxidases protect plants from oxidative stress and can participate in the degradation of organic pollutant components that enter the plant. In this regard, we detected the activity of intracellular peroxidases in the leaves and roots of sorghum and yellow medick.

No pronounced effect on the peroxidase activity of the tested factors was found in sorghum leaves (figure 5a). In the roots, both pollution and low humidity led to a decrease in the activity. Such tendency was found both in monoculture and in mix with yellow medick, and in the latter this parameter was lower.

In yellow medick leaves, under the influence of pollution, the peroxidase activity increased (especially noticeably in monoculture under normal humidity conditions) (figure 5b). In the roots, stress conditions led to an increase in the activity (especially noticeable at low humidity). In a mixed culture, the activity was lower, but the same trend was kept.

4. Conclusion

Thus, this study highlights physiological and biochemical responses of *Sorghum bicolor* L. Moench and *Medicago falcata* L. to oil pollution, soil moisture reduction, and plant co-cultivation, which can be used by phytoremediation technology to clean up kastanozems from oil hydrocarbons and restore the soil biological activities in arid regions.
Figure 5. Effects of oil pollution, soil moisture, and plant co-cultivation on peroxidase activity (according to DAF) in leaves and roots of sorghum (a) and yellow medick (b).

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