Research article

Physiological changes and rate of resistance of *Acrosiphonia arcta (Dillwyn) Gain* upon exposure to diesel fuel

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ABSTRACT

The changes in the morpho-physiological state of green alga *Acrosiphonia arcta* upon exposure to diesel fuel (DF) at concentrations of 20; 100; 1,000; 2,000; 3,000 of maximum permissible concentrations (MPC) were studied. The main physiological stress markers, such as enzymes of the antioxidant system (AOS), non-enzymatic anti-oxidants (carotenoids) and free amino acids (as components of plant metabolome) were measured. In general, all concentrations of the petroleum product used changed the activity of the antioxidant system, changed the intensity of physiological processes (photosynthesis, free amino acid synthesis) and also affected the structure of microbiomes inhabiting the surface of algae.

It was shown that the concentration of DF within 1 mg/l (20 MPC) was not lethal as plants were able to maintain physiological activity and the observed changes were reversible.

Although DF exposure caused decreases in superoxide dismutase (SOD) activity, proline concentration and photosynthetic rate, increases in catalase activity and pigment concentration were observed. After the effects of stress disappeared, most physiological parameters were restored, except for carotenoid content.

Higher DF concentrations (100 MPC and higher) caused injury to cell structures and damage to the pigment apparatus. The restoration of functions after the termination of exposure to stress was not achieved. Epiphytic bacterial communities actively responded both to the introduction of a toxicant and to the changing physiological parameters of algae by the change in the numbers of cultured heterotrophic bacteria. The results of this study showed that the concentration of petroleum products in the water decreased to values not exceeding MPC in the presence of algae in the environment.

1. Introduction

Increased traffic load in the Arctic region (Trump et al., 2018) is often accompanied by emergency situations and leads to permanent pollution, including those caused by oil products (Ferrando et al., 2015; Lindeberg et al., 2018). The pollutants in the coastal zone mainly affect the primary producers, i.e. macrophyte algae, which, due to the peculiarities of their vital activity, cannot avoid the adverse impact of hydrocarbons. Petroleum products that enter aquatic environments cause mechanical damage to algae because they stick to thalli, and also lead to inhibition of their physiological processes, which causes the death of organisms. The loss of key primary producer species from phytocenoses can significantly reduce species diversity and the volume of primary products supplied to the ocean (O'Brien, Dixon, 1976; Stekoll and Deysher, 2000; Stepanyan,

Voskoboynikov, 2006; Lewis, Pryor, 2013; Ryzhik et al., 2019, Mala-venda, 2019).

Acrosiphonia arcta (Dillwyn) Gain is one of the first settlers involved in the preparation of substrate for its colonization by perennial dominant species, fucus algae, for example. Accordingly, it contributes to the formation of perennial biotic communities (Malavenda et al., 2017). Disappearance of *Acrosiphonia* from communities may result in a slowdown in the processes of succession and in irreversible transformations in phytocenoses.

The ecology of *A. arcta*, its morpho-physiological traits, stages of early development and life cycle are well-studied (King, Shramm, 1976; Latala, 1990, Karsten et al., 1991, Sussman, Dewreed, 2001; 2005, Roleda et al., 2010, Sussman, Scrosati, 2011). There is much less research on the effect of hydrocarbons and other pollutants on different *Acrosiphonia* species.

We can single out the research by Kusk (1980), conducted on three Acrosiphonia species, namely *A. sonderi*, *A. arcta* and *A. centralis*, which showed a decrease in photosynthetic activity in all the studied species upon exposure to petroleum hydrocarbons (PH). Also noteworthy is the work on the absorption of polycyclic aromatic hydrocarbons by *A. coalita* (Christensen, 2007).

Taking into account the prominent role of *A. arcta* in marine coastal ecosystems, it is especially important to determine the degree of its resistance to various concentrations of petroleum hydrocarbons in the habitat. Diesel fuel (DF) is one of the most common petroleum products (PP) that pollutes aquatic systems. Therefore, the study of its effect on living organisms is of great interest (Patin 2008; Bausch, 2010; Voskoboinikov et al., 2020).

The ability of the organism to resist the effects of toxicants is an important resistance index. This is manifested in the change in the algal cells' metabolic activity (activity of enzymes of the antioxidant system, concentration of photosynthetic pigments, photosynthetic rate, concentrations of metabolomes), as well as in a modification of the populations of microorganisms inhabiting the surface of algae. Similar transformations occur jointly and interdependently, and allow us to assess the tolerance (or resistance) of the organism to toxic effects.

The objective of this work was to evaluate the resistance of *A. arcta* to the effects of petroleum products by evaluating the changes in the activities of metabolic processes following petroleum exposure.

2. Materials and methods

In July–August of 2020, *the experimental work* was performed at the seasonal biostation of the Murmansk Marine Biological Institute of the Russian Academy of Sciences located in the settlement of Dalniye Zelentsy.

The experiment consisted of two stages: the first stage was to determine the degree of impact of different concentrations of diesel fuel (DF) on the morpho-functional state of *A. arcta*. The second stage was to assess the possibility of restoration of physiological processes in algae after the transfer of the experimental samples from the water contaminated with PP to clean water.

Algal thalli were collected from the littoral zone of the Zelenetskaya Bay and placed in the laboratory environment, i.e. in a thermal control room with lighting of 150 W/m^2 (24L: 0D), at a water temperature of 8–10 °C and with continuous aeration of the vessels with algae. The experiment was conducted during the polar day (continuous daylight). In order to preserve the biological rhythms of algae in the laboratory environment, the 24:0 light schedule was used. The algae were acclimated to laboratory conditions for 3 days. Then, some plants were placed into the control vessels (with pure seawater, salinity of 33 ‰) and some into the experimental vessels (seawater with salinity of 33 ‰) with the addition of summer diesel fuel. The diesel fuel concentrations were 0.86, 4.3, 43, 86, 129 mg/l, which corresponded to 20, 100, 1000, 2000 and 3000 of maximum permissible concentrations (MPC).

The water in the Kola Bay contains an average of 0.05–0.17 mg/dm^3 of oil products. Concentrations from 20 to 100 MPC correspond to the maximum which was periodically registered in the waters of the littoral zone of the Kola Bay from 2014 till 2016. Concentrations of 1000–3000 MPC can be registered after accidental oil spills.

The first stage of the experiment lasted for 10 days. Photosynthetic rate and the number of bacteria were recorded on the 3rd, 6th and 10th days of the experiment. Enzymatic activity, concentrations of pigments and free amino acids were recorded on Day 0 and on the 10th day of the experiment. The total concentrations of PP in waters of each treatment and controls were recorded on Day 0 and the 10th day of the exposure.

The second experimental stage lasted for 5 days and was aimed at assessing the possibility of restoration for algae after the impact of oil products. For that purpose, the thalli of *A. arcta* were relocated from the vessels with DF into the vessels with pure seawater. The physiological states of algae (cell morphology, photosynthetic rate, pigment concentration, enzyme activity) at the beginning and at the end of the experiment were compared.

The morphology of algal cells was studied using the Micmed–6 Microscope (LOMO JSC, St. Petersburg, Russia) at magnification x400.

Photosynthetic rate (PR) was measured using the Winkler titration method and the change in the oxygen content in water during the incubation period of thalli was calculated (mcg O$_2$ per 1 g of thallus wet weight per hour). Algae in the vessels without petroleum products served as the control. The measurements were taken three times and the values averaged.

Catalase activity (CAT) was determined according to the spectrophotometric method (Ryzhik et al., 2019) and superoxide dismutase (SOD) activity was assessed according to Giannopolitis (Giannopolitis, Ries, 1977). Enzyme activity (CAT and SOD) was calculated per 1 g of dry weight. The measurements were taken three times and the values averaged.

Content of photosynthetic pigments (carotenoids and chlorophylls (A and B) was measured according to the spectrophotometric method with the JENWAY 6305 UV/VIS Spectrophotometer (Japan) (Jeffrey, Humphrey, 1975; Seely et al., 1972). The pigment content was expressed as mg/g of wet weight of algae. The measurements were taken three times and the values averaged.

Free amino acid (FAA) content was determined by the capillary electrophoresis method with preliminary derivatization of amino acids using phenylisothiocyanate (PITC) (Ryzhik et al., 2021). The measurements were taken three times and the values averaged.

Wet weight of plants was measured using scales (BJT-310, Gosmeter, Russia, Accuracy Class II) after having removed moisture droplets from the surface with the help of filter paper.

Dry matter content. After wet weight determination, the thalli were dried (wet weight) were dried in a drying oven (Snol, E5CN) for 24 h to a constant weight at 105 °C and re-weighed. The dry matter content was determined as the portion of dry weight to wet weight.

Microbiological studies. In order to calculate the number of cultured heterotrophic bacteria, the limiting dilution method was used with utilization of liquid media. The modified ZoBell 2216E medium was used for saprotrophic bacteria (SB). Marine mineral media, with the addition of summer DF, as the only source of carbon and energy, was used for hydrocarbon oxidizing bacteria (HOB). The most probable number of bacteria was determined using the McCredie tables. The number of bacteria was recalculated per 1 g of wet weight of algae (Ryzhik et al., 2019). The measurements were taken three times and the values averaged.

Measurement of total hydrocarbon concentration (THC). The THCs in WAF (Water Accommodated Fraction) of DF treatments were determined using the liquid analyser "Fluorat-02" (Lumex, Russia) according to the standard procedure described by Bratskaya et al. (2006). The analysis is based on the fluorometric method for measuring the mass concentration of organic and inorganic substances in the spectral region of 250–900 nm. The THC was measured at the beginning (day 1) and end (day 6) of the experiment. The measurements were taken three times and the values averaged.

2.1. Statistical analysis of results

Reliability of differences between the variants was calculated for the initial data using Student's t-test and a probability of 95% (p ≤ 0.05). In order to assess the significance of the impact of DF treatments, one-way analysis of variance (ANOVA) was used. Pearson correlation coefficient was calculated in order to determine the presence of a linear correlation between the studied parameters. Reliability of biomass differences was assessed by Student's paired test. In order to process and analyze the obtained data, the statistical software package of (Microsoft Excel 2010) was used.

3. Results

3.1. Cell morphology

At the first stage of the experiment, the cell structure in the control did not change. Chloroplasts were evenly distributed in the entire volume of cells and had a bright green color. Plasmolysis, structural damage of chloroplasts and a change in colour of the cytoplasm were observed in the DF-treated cells. The nature of the changes depended on the amount of the toxicant: minimal plasmolysis, intact chloroplasts and transparent cytoplasm were observed in the treatments with 20–100 MPC. The maximum changes were observed in the cells upon exposure to a concentration of 1,000 MPC or more, namely, plasmolysis was observed, the cytoplasm acquired a granular structure and the chloroplasts were destroyed.

In the second part of the experiment, the algal cells restored their structure after their relocation from the water containing DF (20 MPC) into pure water. In the rest of the treatments, following relocation, plasmolysis and damaged structure of chloroplasts were observed in the cells. The chloroplasts that maintained their green colour had a granular structure and were distributed in the entire volume of a cell.

3.2. Physiological state of cells

3.2.1. Activity of enzymes of the antioxidant system (AOS)

The activity of SOD after 10 days of exposure to 20 MPC DF did not differ from the enzyme activity in the control treatment. In the treatments with exposure of 100 and 1,000 MPC, the SOD activity indices were 30–40% higher on average than in the controls. At higher concentrations of DF, the SOD activity index did not differ from the controls (Figure 1a).

Catalase activity in the 20 MPC DF-treated plants was 30% higher compared with that of the control plants. It was 15% higher at 100 MPC and it decreased by 30% at 1,000 MPC. At higher concentrations of DF (2,000 and 3,000 MPC), the enzyme activity exceeded the index in the controls by 10% on average (Figure 1 a).
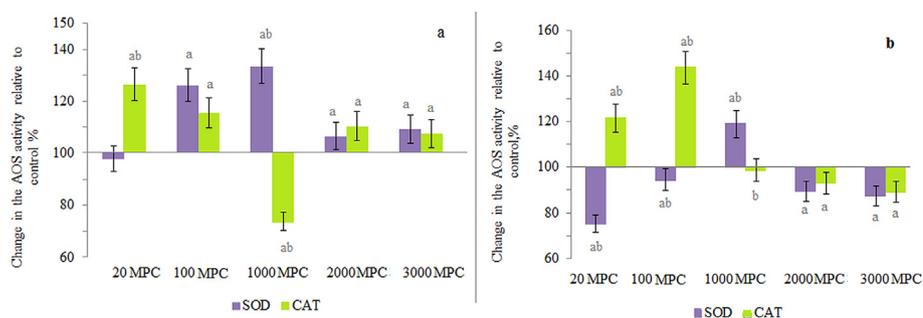
In the second part of the experiment, after the algae had been transferred to and held in clean water, the following changes in enzymatic activity were determined.

SOD activity on exposure to DF at 20 MPC was about 25% lower relative to the control whereas at 100 MPC it was similar to that of the control. Upon exposure to DF at concentration of 1,000 MPC, SOD was 20% higher and at higher concentrations it was 10% lower than the control (Figure 1b).
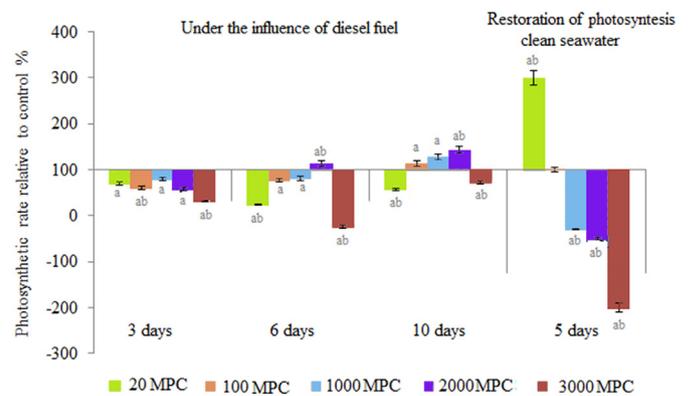
Catalase activity after the exposure to DF at concentrations of 20 and 100 MPC was 20% and 40% higher than the control, respectively. At 1,000 MPC, it did not differ from the control but at higher DF concentrations, it was 10–15% lower than the control (Figure 1b).

3.2.2. Photosynthetic rate (PR) and concentration of photosynthetic pigments

During the experiment, multidirectional changes in the photosynthetic rate were observed (Figure 2).

Figure 2. *Change in photosynthetic rate (PR) relative to the controls in A. arcta treated with different concentrations of diesel fuel (DF) during the first and second stages of the experiment. The graph shows the averaged data when calculating the concentration of pigments as a percentage of the control (n = 3) with indication of the standard deviation (a - difference from the control, b - difference between the treatments (p≤0,05)).*

On the 3rd day of the experiment, the PR was 30–70% lower in all treatments than in the controls. On the 6th day of the experiment, there was a further decrease in PR in the treatments with 20 MPC and 3,000 MPC. However, in the treatment with 100–2,000 MPC, a gradual increase in PR was observed.

On the 10th day, PR remained lower than that of the control in the treatments with 20 MPC and 3,000 MPC. In the other DF treatments this parameter was 15–40% higher than in the control (Figure 2).

After the DF-treated plants had been relocated into clean water (the second part of the experiment), PR increased significantly compared with that of the control. Following 20 MPC treatment, the observed PR was 300% higher than that of the control. Upon exposure to 100 MPC, PR did not differ from that of the control, however, after the algae had been exposed to higher concentrations, their PR decreased by 30–200% (Figure 2). In these treatments, respiration in algae prevailed over their photosynthesis.

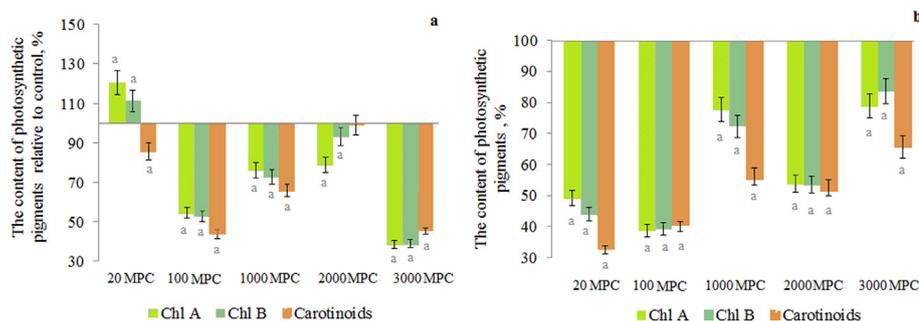#### 3.2.3. Content of photosynthetic pigments

On the 10th day of the experiment, the chlorophyll content decreased compared to the control in all treatments except for 20 MPC. The most significant changes occurred in the variants with 100 and 3,000 MPC (Figure 3A).

After the algae had been transferred to and held in clean water, the content of all analyzed pigments in DF-treated plants was lower than that of the controls (Figure 3B).

3.2.4. Free amino acids

In the course of the experiment, the qualitative composition of amino acids in the DF-treated samples did not change compared to the controls.

In *A. arcta*, 21 amino acids were found in both DF-treated and control samples, 17 of which are proteinogenic. Regarding the non-

Figure 1. Change in the activity of AOS enzymes (superoxide dismutase (SOD) and catalase (CAT)) relative to the controls in *Acrosiphonia arcta* following treatment with different concentrations of diesel fuel (DF) during the first and second stages of the experiment: (a) 10 days of the experiment with DF, (b) restoration of enzymatic activity, after relocating the plants into clean seawater. The graph shows the averaged data when calculating the concentration of pigments as a percentage of the control (n = 3) with indication of the standard deviation (a - difference from the control, b - difference between the treatments (p≤0,05)).

Figure 3. *Changes in content of photosynthetic pigments compared to the controls in A. arcta following treatment with different concentrations of diesel fuel relative to the control during the first and second stages of the experiment (A) – 10 days of the experiment under the impact of diesel fuel (B) - restoration of indices after the plant relocation into clean seawater. The graph shows the averaged data when calculating the concentration of pigments as a percentage of the control (n = 3) with indication of the standard deviation (**a - difference from the control, b - difference between the treatments (p≤0,05)).*

proteinogenic amino acids, the composition of FAA pool included ornithine, hydroxyproline, sarcosine and taurine. Proline, glutamic (glutamate) and aspartic (aspartate) acids and alanine were dominant in the FAA composition.

After exposure to DF, amino acid content changed, i.e. the amount of FAA, individual amino acids and their ratios. The degree of change depended on the DF dose.
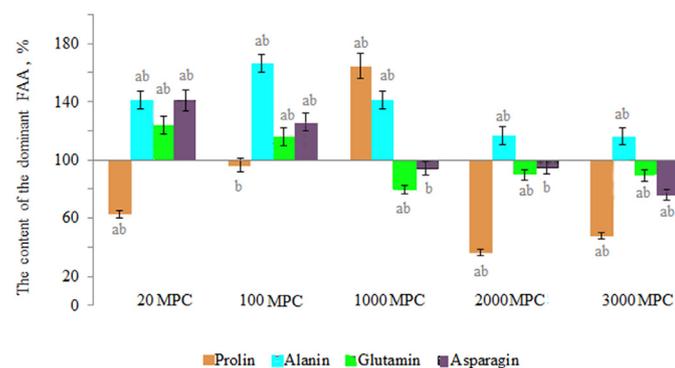
Following 10 days of exposure to DF at concentration of 20 and 100 MPC, the content of proline decreased whereas the content of alanine, glutamate and aspartate increased compared with the control. Upon exposure to DF doses of 1,000 MPC, the content of proline and alanine was higher, and the content of glutamate and aspartate was lower than that of the control. The content of proline, glutamate and asparate in algae following the 2,000 and 3,000 MPC treatments was lower, and the content of alanine was higher compared with that of the controls (Figure 4).

3.3. Epiphytic saprotrophic and hydrocarbon-oxidizing bacteria

The number of saprotrophic bacteria on the surface of *A. arcta* increased significantly in all the treatments, including the controls (Figure 5a).

On the third day, the greatest increase in the number of bacteria was observed in the treatments with 100 and 2,000 MPC. The number of bacteria increased by 1,450 and 870%, respectively, compared to the controls (8.6 and 5.1 million cells/g). The number decreased in the vessels with DF of 1,000 MPC, which amounted to approximately 50% of the controls (290,000 versus 590,000 cells/g).

On the 6th day of the experiment, the number of cultured epiphytic bacteria increased in all treatments relative to the controls. At 100 and at 1,000 MPC of DF the number of bacteria on the surface of algae increased by 127.7 and 132.8%, respectively, and amounted to 19.24 and 25

million cells/g. At 2,000 MPC, the number of bacteria was significantly higher and amounted to about 225 million cells/g of algae, i.e. 1,192 % of the controls.

By the 10th day of the study, in all treatments except for the one with 100 MPC, compared to the sixth day, the number of bacteria decreased relative to the control. In the treatment with 100 MPC of DF, the number of bacteria increased by 307%. Considering that the number of bacteria increased both in the experimental vessels and in the control ones, the differences between the experiment and the control were, in total, minimal on the 10th day.

The number of HOB also increased in the course of the study, both in the DF-treated vessels and in the controls (Figure 5b).

No HOB was observed in algae in natural conditions (Day 0). In the course of the study, their number increased in all treatments. HOB in the control vessels appeared on the sixth day. By the end of the study (10 days), their number increased and even became comparable with the number values observed in the vessels with 100 and 1,000 MPC (0.7, 0.45, 0.55 thousand cells/g, respectively). At the same time, on the 3rd day, in the treatments with 100 and 1,000 MPC, their number increased to 9 and 29% of the control, respectively (Figure 5b). At 2,000 MPC, the number of bacteria increased to 214% of the control.

The maximum number of cultured HOB was observed on the sixth day in the 1,000 MPC of DF treatments (1,327% of the control value, amounting to 25 thousand cells/g). On the 10th day, the HOB number in this treatment decreased to 87% of the control. Also on day 10, in the treatment with 2,000 MPC, there was an abrupt increase in the number of hydrocarbon-oxidizing bacteria (HOB) up to 1,225% of the control value (5.7 thousand cells/g).

It should be noted that the portion of HOB relative to the total number of saprotrophs remained fairly low throughout the entire study and varied from 0.0001 to 0.1%, reaching the maximum values at 1,000 MPC on the 6th day and 2,000 MPC on the 10th day of the experiment.
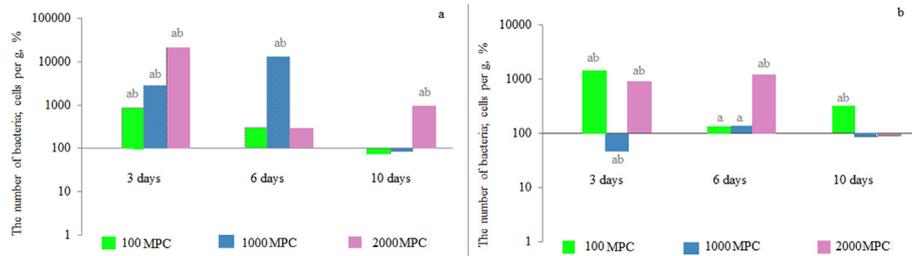
3.4. Changes in the concentrations of petroleum products in water

In order to determine the possible role of algae in purification of the environment from petroleum hydrocarbons, we selected 2 concentrations of DF at which algae remained viable. They were 20 and 100 MPC.

It was shown that in 10 days the concentration of DF in water at 20 and 100 MPC decreased by 16 and 7%, respectively, in the DF-treated vessels without algae (Figure 6). The concentration of petroleum products in the presence of algae decreased by 65% (at 20 MPC) and 77% (at 100 MPC) of the initial values.

4. Discussion

Exposure to a petroleum toxicant is most clearly reflected in the state of the antioxidant system, which promptly responds to emerging stress. Later, the changes affect the state of the photosynthetic system and nitrogen metabolism. It is reflected not only in the internal conditions of the organism, but also in the external ones. Algae are not gnotobionts as their surfaces may be inhabited by many microorganisms such as

Figure 4. Dominant FAA content in A. arcta following treatment with different concentrations of diesel fuel during the first stage of the experiment. The data are expressed as the FAA content as a percentage of the control with indication of standard deviation (a - difference from the control, b - difference between the treatments (p≤0,05)).

Figure 5. Change in the number of cultured epiphytic saprotrophic bacteria *(a) and of cultured epiphytic hydrocarbon-oxidizing bacteria (**HOB**) (b) at different concentrations of DF in the study. The graph shows the change in the number of bacteria as a percentage of the control (a - difference from the control, b - difference between the treatments (p≤0,05))*.
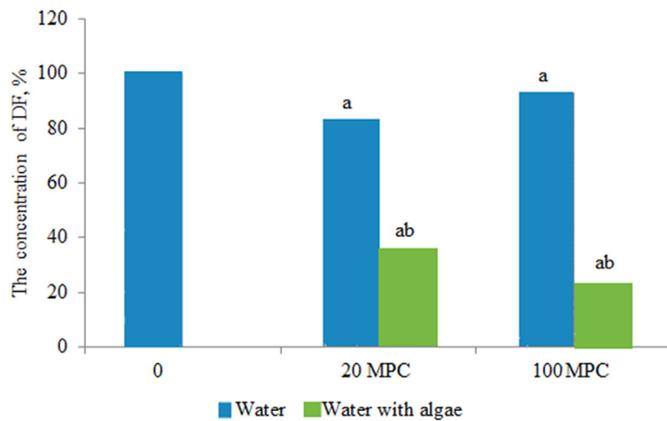
Figure 6. Change in the concentration of DF in water after 10 days of the experiment. The graph shows the change in the concentration of DF as a percentage of the initial concentration (a - difference from the control, b - difference between 20 MPC and 100 MPC (p≤0,05)).

protozoa, fungi, microscopic algae and bacteria. Bacterial communities are an indicator of oil pollution. The microbiome structure changes following exposure to a petroleum toxicant and, indirectly, the state of the macrophyte itself can also change (Dittami et al., 2014). It is advisable to consider all physiological processes in the complex response because that is the way to most precisely assess the overall picture.

This study has shown differences in the physiological state of *A. arcta* after 10 days of exposure to different concentrations of DF. Plants withstand pollution levels up to 100 MPC, actively participating in the degradation of DF. When stress effects are eliminated, the plants restore their functions (for example, photosynthetic rate). Upon exposure to 1,000 MPC, vital processes slow down, and at higher concentrations of DF in the environment, suppression of the organism's protective systems and plant death are observed.

The adaptations that help algae survive petroleum exposure are implemented through internal mechanisms. First, the antioxidant defence system is activated, which is responsible for neutralizing reactive oxygen species (ROS) formed under the impact of a toxicant. Also, the stress reactions are launched, which include synthesis of substances responsible for the increase in resistance (amino acids, polyphenols, etc.). When the toxicant ingress into the environment takes place, ROS begin to form in the cells and trigger the activation of SOD that converts them into less toxic hydrogen peroxide. Similar changes in SOD activity were observed for *Chlorella vulgaris* (Calderón-Delgado et al., 2019), *Phaeodactylum tricornutum* (Wang et al., 2008) and *Ulvaria obscura* (Salakhov et al., 2020). SOD suppression at a diesel fuel concentration of 5 mg/l (which corresponds to our 100 MPC) was shown by other authors in experiments on *Chlorella sp.* (Ramadass et al., 2016, 2017).

An increase in the concentration of hydrogen peroxide stimulates catalase production, which was observed in our experiment upon exposure to DF at concentration up to 100 MPC. In *Palmaria palmata*, catalase activity increased at petroleum product concentrations of 60 MPC (Voskoboinikov et al., 2018). Higher concentrations of DF caused inhibition of enzyme activity that may be associated with an excess of peroxide formed. The inhibited activity of this enzyme by the formed hydrogen peroxide was noted in a number of works (Xu et al., 2013, Moacir A. Torres et al., 2008; Calderón-Delgado et al., 2019). Against the backdrop of a decrease in catalase activity, the mechanisms of synthesis activation of other ROS neutralization systems, such as carotenoids, glutathione, etc., may increase. The dependence of the state of AOS enzymes (SOD, catalase, peroxidase) on DF concentration was also shown in microalgae (*Pseudokirchneriella subcapitata* and Chlorella sp. MM3.). At the same time, a clear inverse correlation between the oil product concentration and the formation of free radicals and the synthesis of antioxidants was observed, i.e. the higher the petroleum concentration was, the more intensively the enzymatic activity was suppressed (Ramadass et al., 2016, 2017). The authors note the following dependence of the enzymatic system - POX ≥ SOD > CAT (Ramadass et al., 2017).

Another system that directly responds to the introduction of a petroleum toxicant is the photosynthetic system. Some of its components are directly involved in the photosynthetic process and others (carotenoids) perform, among other things, an antioxidant function.

In the first part of this study, a substantial decrease in the photosynthetic rate (PR) was observed at all tested concentrations of DF. Restoration of PR occurred upon exposure to low concentrations of DF (20 MPC) only.

It was previously shown that photosynthesis in *A. sonderi* was most intensively inhibited by oil during the first 4 h of incubation and the inhibition decreased within the next 8 h (Kusk, 1980). It is assumed that inhibition is caused by volatile aromatic substances that are components of the oil. Studies by Shiels and his colleagues (Shiels et al., 1973) demonstrated a 13-fold decrease in the PR in *Enteromorpha intestinalis* upon a 4-day exposure to an oil emulsion at concentration of 200–250 MPC. The authors also mentioned the inhibition of PR in *Ulva fenestrata* upon exposure to petroleum products at a concentration of 150 MPC. A similar effect of hydrocarbons on *Enteromorpha* was shown in studies by O.V. Stepanyan (Stepanyan, Voskoboynikov, 2006), as well as on *Coccotylus truncatus* and *Saccharina latissima* (Hsiao et al., 1978). During the experiments on the effect of a DF film on the green alga *Ulva lactuca,* it was shown that a short-term exposure to the film (up to 6 h) resulted in cellular plasmolysis, impaired development of spores and gametes and in a decrease in the photosynthetic rate (Ryzhik and Makarov, 2019). Photosynthetic rate, chlorophyll fluorescence (Fv/Fm) and total chlorophyll of *Ulva lactuca* decreased with increasing toxicant (PAH) concentration (Maghraby, Hassan, 2021). The photosynthetic rate changes (activation or suppression) were shown to depend on petroleum concentration in the number of algal species, such as *Nereocystis luetkeana, Fucus distichus*. In addition, the suppression effects remained even after petroleum exposure ended (Antrim et al., 1995; Wegeberg et al., 2020).

The response reaction of *Acrosiphonia* was similar to the results of studies with other species of green algae. In general, an increase in the DF

concentration in the environment led to irreversible disturbances in the functioning of the photosynthetic apparatus and the death of algae.

Photosynthetic pigments, in particular carotenoids, can act as antioxidants that neutralize toxicants through the formation of chelate compounds (Britton, 2008; Petrussa et al., 2013; Pilatti et al., 2016). A number of studies have shown an increase in the concentration of pigments in response to exposure to DF (Havaux, 1998). In contrast, a decrease in the chlorophyll-A concentration in response to all petroleum concentrations tested was shown in studies of unicellular algae (*Pseudokirchneriella subcapitata* and Chlorella sp. MM3) (Ramadass et al., 2017). Also, a study with *Hypnea musciformis* carotenoids demonstrated that chlorophyll concentration increased in 30 min and 12 h and decreased in 1 and 24 h of DF exposure (Ramlov et al., 2014). Similar changes in these algae were reported after their exposure to various petroleum concentrations (Ramlov et al., 2019). In our current study, no increase in the concentration of carotenoids in the presence of DF was observed. In most cases, their content was reduced, as DF damages the pigment complex of algae. This may be due either to damage to the photosynthetic apparatus or to a short duration of the second part of this. Restoration took a longer time.

During this study, no direct relationship was shown between the photosynthetic rate and the concentration of pigments (which changes more slowly than the photosynthetic rate).

DF can affect the protein synthesis system. For example, when studying the effect of a DF emulsion on the microalgae Nannochloropsis (Monallantus) salina Hibberd, it was shown that the total amino acid content decreased. In higher aquatic plants, it was shown that, under conditions of petroleum pollution, the total amount of free amino acids increased by 16% in clasping-leaved pondweed, by 36% in common reed, by 28% in Canadian elodea. The increase in the amino acid content was mainly due to dicarboxylic amino acids (mostly glutamate and aspartate), proline and monocarboxylic amino acids with hydrophobic radicals (Sachkova (Fuchedzhi) et al., 2005). However, the composition of the cellular pool of amino acids remained unchanged (Mohammady et al., 2005). When studying *Platymonas helgolandica*, it was shown that the proline concentration increased upon exposure to water soluble fractions of oil (Li et al., 2021). This amino acid accumulation can contribute to the osmotic pressure regulation in cells and ensure protection of enzymes, biological membranes and polyribosomes by forming stable complexes with free radicals, which otherwise may be toxic. During stress, proline is involved in maintaining the NAD(P)+/NAD(P)H ratio at the levels typical of normal growing conditions (Hare and Cress, 1997; Torres et al., 2008).

An increase in the content of free amino acids may also be the result of the inhibitory effect of petroleum on protein synthesis. The effect of inhibition of macromolecular synthesis upon exposure to petroleum was revealed in the studies with the culture of unicellular algae *Scenedesmus armatus* (Zachleder, Tukaj, 1993). An increase in FAA concentration in the intercellular space can be one of the adaptive defence mechanisms. As such, FAA can act not only as neutralizers of reactive oxygen species and stabilizers of enzymes, membranes and subcellular structures, but can also influence the intake and withdrawal of substances from cells, participate in the synthesis of enzymes of the antioxidant system (catalase, SOD, glutathione, etc.) and regulate the cytoplasmic pH (Rai, 2002; Trovato et al., 2008; Hildebrandt, 2018).

The survival of algae under petroleum pollution conditions is possible due to physiological rearrangements and the development of their adaptations to the toxicant exposure. Survival is also due to a decrease in the concentration of petroleum products, which occurs by mechanisms of physico-chemical destruction. These mechanisms are activated when DF gets into the water. There, its gradual decomposition (transformation) immediately begins due to evaporation, exposure to light, etc (Patin 2008). This is also evidenced by the results of our study, during which, in 10 days, a decrease in the concentration of petroleum by 7–16% was recorded in the water without algae. Mainly, physico-chemical degradation occurs as a decrease in the length of the alkyl chain (Voskoboinikov et al., 2018; 2020). These become the light fractions that are the

most toxic to plants, as they penetrate through cell walls and plasma membranes more easily (Patin, 2008). They accumulate in the medium by the 7th day (Voskoboinikov et al., 2020). The main changes in algae organisms occur precisely during the appearance of the light fractions of petroleum products. Light fractions at low concentrations of hydrocarbons may appear earlier in the environment, so plants react to them earlier and faster. The effects of high concentrations of petroleum product are prolonged.

Apart from physico-chemical mechanisms, biodegradation processes occur in the environment at the same time. They are related, among other things, to the activity of microorganisms capable of including hydrocarbons in their metabolism.

A complex of microorganisms is constantly present on the surface of algae which includes both epiphytic bacteria integrated into the surface structures of algae, and microorganisms inhabiting the mucous layer or the water layer in contact with the surface of plants (Goecke et al., 2010; Unnithan et al., 2014; Kouzuma, Watanabe, 2015; Ramanan et al., 2015; Müller et al., 2016). In the course of this study, intensive development of heterotrophic bacteria, including hydrocarbon-oxidizing ones, was observed in all treatments.

It was previously shown that almost all oil fractions undergo microbial destruction. However, the rate and completeness of their destruction depend, among other things, on the composition of the petroleum product (Walker et al., 1976, Oudot, 1984; Yemashova et al., 2007; Kappell et al., 2014). Microorganisms are capable of destroying n-alkanes with chain lengths of up to 44 carbon atoms. Alkanes and isoalkanes with short chains underwent the most complete microbial destruction (60–80%), and aromatic compounds with four and five cycles (30–45%, 0–30%, respectively) turned out to be the most resistant (Oudot, 1984). In contrast, most bacteria do not grow well on substrates with 5–10 carbon atoms. In this study, the bacterial population peaks varied over time depending of the petroleum concentrations. They probably peaked during periods of maximum amounts of petroleum hydrocarbons (PH) available for microbial destruction because the rate of PP decomposition can vary significantly at different concentrations. A decrease in the HOB population at a concentration of 1,000 MPC on the 10th day of the experiment may be related to a decrease in the amount of both the hydrocarbons that are available for bacterial destruction and the total PH in the environment, which is evidenced by the experimental results (Figure 6). On the 10th day large quantities of available hydrocarbons remained at 2,000 MPC.

On the other hand, considering the closeness of the experimental systems and the increase in the number of cultured bacteria, including those in the controls, we can assume the algae themselves influence the development of the microorganisms inhabiting their surface (Ramanan et al., 2016; Müller et al., 2016; Wolter et al., 2021). Antagonistic relations between different groups of microorganisms can also play a role (Egan et al., 2000). Besides, macrophytes can be sources of metabolic byproducts including hydrocarbons (phytane and prystane) (Dean, Whitehead, 1961; Mironov, 1985). As they accumulate in the system, they will substantially stimulate the development of bacteria. This may be evidenced in this study by a decrease in the ratio of the number of bacteria (including HOB) relative to the control.

5. Conclusion

DF inhibits synthesis of pigments, disrupts synthesis of free amino acids, decreases photosynthetic rate and changes the activities of the antioxidant system. Under the influence of DF, the ratio of saprotrophic/hydrocarbon-oxidizing bacteria changes increasing the proportion of the latter.

Depending on the DF concentration and time, the processes we observed can be reversible. The concentration up to 20 MPC is not lethal for the studied macrophyte. 100 MPC is a boundary concentration. The response of algae depends on the exposure time. DF concentrations above 1,000 MPC were lethal.

Acrosiphonia arcta together with HOB take part in the degradation of DF. The concentration of DT in the environment decreases in their presence. In other words, this species of algae is able to contribute to the bioremediation of the aquatic environment from diesel fuel at concentrations not exceeding 100 MPC.

Declarations

Author contribution statement

I.V. Ryzhik: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

D.V. Pugovkin; M.P. Klindukh; G.M.Voskoboynikov: Performed the experiments; Analyzed and interpreted the data.

D.O. Salakhov: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

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Additional information

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