Effect of diesel fuel film on green algae *Ulva lactuca* L. and *Ulvaria obscura* (Kützing) Gayral ex Bliding of the Barents Sea

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Abstract. The effect of the film of diesel on green algae *Ulva lactuca* L. and *Ulvaria obscura* (Kützing) Gayral ex Bliding of the Barents Sea was studied. The *Ulvaria obscura* is widespread, the *Ulva lactuca* in recent years there has been a significant increase in the number and biomass in the area. The short-term (in the period of one tidal cycle) effect of the diesel fuel film on the physiological state of two species of green algae, and define the strength of adhesion of the film of diesel to the surface of the thallus were determined. Short-term exposure of algae by film of diesel in a period of one tidal cycle (1) reduced the rate of evaporation, (2) decreased the intensity of photosynthesis, (3) caused plasmolysis of the cells, especially in *U. lactuca*, (4) affected the development of spores and gametes and caused they mortality. The film of diesel adhered firmly to the algae surface and did not wash off even after 15 cycles. The data obtained indicate the danger of even a single spill of diesel into the littoral. In General, the damage to the population will depend on the stage of algae development and the duration of exposure.

1. Introduction

In the water area of ports, there is a large number of petroleum products in forms of a solution, emulsion, film and the deposited form. In contaminated areas, sensitive species of macroalgae are disappearing, and new algal communities, more resistant to toxicants but low biodiversity are emerging [1;2]. Some macroalgae eliminated rapidly, others, for example, some representatives of brown (*Fucus vesiculosus*) and green algae (*Ulva lactuca*), are well-tolerated to xenobiotics [3-5]. Usually, more sustainable are algae (1) with a dense cuticular layer, which prevents penetration of oil products inside cells [6], (2) with active mechanisms of repair and protection [7].

The most vulnerable are algae inhabit littoral zone, in the period of the tidal cycle they subject to significant changes in the environment: drying, a sudden shift in temperature (especially in winter), light, salinity etc. In the presence of oil spills, the film fraction cover algae during the low tide period.

Data on the influence of the oil products film on the physiological state of macroalgae inhabit the tidal zone extremely insufficient [8, 9]. And given that diesel is the most used product of oil refining, the impact of the spill of diesel on the littoral biocenoses remains unexplored.

The purpose of this work was to determine the impact of short-term (during the period of one tidal cycle) treatment the film of diesel on the physiological status of two species of green algae *Ulvaria obscura* and *Ulva lactuca* (the first species is widespread, the second species in recent years there has
been a significant increase in the number and biomass in the area [10]). The second purpose was to define the strength of adhesion of the film of diesel to the surface of the thallus.

2. Materials and methods

Green macroalgae *Ulvaria obscura* (Kützing) Gayral ex Bliding and *Ulva lactuca* L. were cut off from the littoral of Zelenetskaya Bay, Murmansk coast of the Barents Sea, in July 2018. The experiment was carried out in the laboratory condition with a temperature of 8-10°C, constant illumination by led lamps with an intensity of 100 W*m⁻². Before the beginning of the experiments, algae acclimated to laboratory conditions in jars with fresh seawater for seven days with daily change of water and its bubbling by an air compressor. The jars were covered with lids to reduce water evaporation.

The experiment simulated one tidal cycle with the effect of a film of diesel on algae: 6 hours algae stay on the air (low tide) and 8 hours in seawater (high tide). To form the film of diesel on the surface of the algae, a single thallus (≈20 g) placed in a Buchner funnel, through which the diesel spilt. Then the thallus was transferred to open Petri dishes, which were kept in the air in the laboratory for 6 hours (simulation of low tide), during which determined the rate of moisture loss. Then the thallus was placed for 8 hours in a jar of volume 1 litre with fresh seawater (simulating high tide), and after that determined the physiological state of algae as cells viability, the intensity of photosynthesis and inspiration. A control was thalli that were in the same conditions, but without the film of diesel on the surface.

The intensity of visible photosynthesis and respiration of algae determined by changes in the oxygen content of water during their incubation. For the measurement of photosynthesis algae placed in transparent jars and non-transparent jars for measurement of respiration. As a control, the change of oxygen content in jars without algae determined. The oxygen content in water before and after incubation of algae was determined using the oximeter HANNA HI 9141 (Germany). The intensity of photosynthesis and respiration calculated in µg O₂ per 1 g of wet algal weight per hour. The wet weight of algae determined after removal of droplet moisture from the surface by the filter paper. Light microscopy (Carl Zeiss Axio Imager D4 microscope) was used to define morphological changes in algae cells.

Investigation of the adhesive characteristics of the film of diesel onto the surface of the algae was carried out in the following manner: as described above, the thallus was precipitated by film and stay in 6 hours in the air condition in the laboratory. After that, step by step, it was placed in a jar with fresh seawater (200 ml) which were actively shaken for 3 min, then transferred to another jar with fresh seawater. A total of 15 water changes were carried out. The total hydrocarbons concentration (THC) in water accommodated fractions of diesel (WAF) was determined in seawater from each jar using the Fluorate 02-3M (Lumex).

Statistical data processing was performed using the software "Microsoft Excel". The figures show arithmetic means and standard deviations.

3. Results and discussion

The presence of the film of diesel on the surface of algae during the drying period prevented the evaporation of moisture, which for both species was less than 4%. Control algae of *U. obscura* lost 6% of moisture, *U. lactuca* - 10% (table 1).

Table 1. Changes of wet weight of *U. lactuca* and *U. obscura* in air conditions (% from initial wet weight).

| Duration, hours | 1       | 2       | 3       | 4       | 5       | 6       |
|-----------------|---------|---------|---------|---------|---------|---------|
| *U. obscura*    |         |         |         |         |         |         |
| Control         | 99.6    | 97.7    | 96.4    | 95.3    | 94.0*   | 92.8*   |
| Treatment       | 99.2    | 98.0    | 97.0    | 96.2    | 95.9*   | 95.7*   |
| *U. lactuca*    |         |         |         |         |         |         |
| Control         | 98.4    | 96.2    | 94.1*   | 92.2*   | 89.9*   | 87.6*   |
| Treatment       | 99.3    | 97.8    | 96.8*   | 95.8*   | 94.6*   | 93.5*   |
*statistically significant differences between indicated values (ANOVA, * p < 0.05)

The film of diesel caused the plasmolysis of the cells, especially in *U. lactuca* (figure 1). In the cells of fertile parts of thalli of both species developing spores and gametes lost motility and 48 hours later died off. Fertile areas acquired yellow colour (they usually are transparent-white).

![Figure 1](image)

**Figure 1.** Cells of *U. lactuca* and *U. obscura* in control and after exposure of the film of diesel (magnification 400x).

In the beginning, the intensity of photosynthesis of *U. lactuca* was lower than that of *U. obscura*. The level of respiration was high in both species. At the end of the experiment, in control, the intensity of photosynthesis of *U. lactuca* did not change, *U. obscura* increased. The respiratory rate of both species decreased. The film of diesel reduced the intensity of photosynthesis of *U. lactuca* by 1.5 times and by two times in *U. obscura*. Respiratory rate increased in *U. lactuca*, and *U. obscura* has not changed (figure 2).
Figure 2. Intercity of photosynthesis and respiration of *U. lactuca* (A) and *U. obscura* (B) after the treatment of a film of diesel.

The film of diesel has strong adhesion and remains on the surface of algae for a long time. Even after the 15th flush, about 0.2 mg l\(^{-1}\) of hydrocarbons entered the water, which corresponds to the excess of the maximum permissible concentration of oil products in water by more than 40 times (MPC for water is 0.005 mg l\(^{-1}\)). The most intensive wash out of hydrocarbons occurred during the first ten cycles (figure 3).

Figure 3. Total hydrocarbons concentration (THC) in water accommodated fraction (WAF) of diesel at washing off of a film of diesel from a surface of algae.

In oil spills water areas, on a water surface, there is a film of oil products which during low tide settles on algae growing in a littoral zone. The toxic effect of the film depends on the duration of exposure. The adhesion strength and, accordingly, the period of contact of the film and algae depends on the type of oil product and the film thickness [8], as well as on the kind of structure of the thallus of algae and their surface. Green algae *U. lactuca* and *U. obscura* have a lamellar structure and a smooth surface. As shown by our research, the film of diesel on their surface stored for a long time even with active flushing. With confidence may assume that species with a more complex organisation of thallus especially syphonocladal, such as Acrosyphonia, Cladophora, etc., the film of diesel will retain for an order of magnitude longer.

*Ulva lactuca* is resistant to dissolved forms of oil and able to accumulate toxicant but dies off with increasing concentration and time of exposure. It is possible that up to a certain period (specific feature) the cells able to control the toxicant inflow and to neutralise them [11].

In addition to toxic effects, in the low tide period, the film reduces the rate of evaporation of moisture from the surface of the thallus, thereby increasing the temperature of plants, and disrupts the
gas exchange of plants. At high tide, the film will also inhibit exchange with the environment by reducing gas exchange, nutrient uptake, and metabolite withdrawal [12, 13].

The intensity of photosynthesis of algae depends on the speed of gas exchange and internal physiological processes. Green algae mostly belong to C-3 plants [14], respectively, they cannot store carbon in intermediate compounds, as C4 plants. And although, for U. lactuca recently opened the possibility of switching to C4 metabolism [15], but the film of diesel will interfere with the process of restoring carbon stocks, which will cause a decrease in the intensity of photosynthesis. The products of oil refining lead to the inhibition of PS II and a reduction in the quantum yield of photosynthesis. Some researchers consider this process as a protective reaction of the species [16].

The impact of the film of diesel on evolving spores and gametes causes a decrease in their mobility and their further death. Formation of reproductive sites on thallus largely depends on external factors: temperature, availability of nutrients, drying [17] and periods syzygy tides. Thereby, the film of diesel attached to algae during this period is most dangerous, as toxicants will affect reproductive cells and early ontogenetic stages. All of this, accordingly, will harm the development of the population.

In chronic non-intensive pollution, changes in phytocenoses occur gradually over a long time. There is a gradual reduction of species composition, the appearance of a large number of ephemeral species, etc. For example, in Kamchatka in the most polluted habitats, there are only filamentous green algae. Among them, the most common are Urospora penicilliformis and Ulothrix pseudoflacca [18]. In Kola Bay, the most stable among green algae are species of the genera Ulva, Ulvaria, Ulotrix [19].

Our experimental studies have shown that the film of diesel stored for a long time on the surface of algae. And even with the short-term treatment, in green algae observed plasmolysis of cells, violation of the development of spores and gametes, reducing the intensity of photosynthesis. The damage to the population will depend on the stage of algae development and the duration of exposure.

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