

UDC [[574.635:582.26/.27]:665.7](268.45.04)

**THE ROLE OF ALGAE MACROPHYTE
IN BIOREMEDIATION OF PETROLEUM PRODUCTS
OF THE KOLA BAY OF THE BARENTS SEA**

© 2021 **G. M. Voskoboinikov¹, S. V. Malavenda¹, and L. O. Metelkova²**

¹Murmansk Marine Biological Institute of the Russian Academy of Sciences, Murmansk, Russian Federation

²Saint Petersburg Federal Research Center of the Russian Academy of Sciences, Saint Petersburg,
Russian Federation

E-mail: grvosk@mail.ru

Received by the Editor 07.02.2021; after reviewing 11.05.2021;
accepted for publication 01.09.2021; published online 20.09.2021.

The contribution of macroalgae to the removal process of diesel fuel from the Kola Bay of the Barents Sea was estimated. The calculations were based on the results of: 1) recent expeditionary observations of the reserves, spreading, and biomass of algae macrophyte of the phyla Chlorophyta and Rhodophyta, as well as the class Phaeophyceae of the phylum Ochrophyta, inhabiting three bay areas; 2) laboratory research of the ability of macroalgae *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. distichus*, *F. serratus*, *Saccharina latissima*, *Palmaria palmata*, and *Ulvaria obscura* to neutralize the toxic effect of diesel fuel. As shown, the total contribution of the algae studied into bioremediation of diesel fuel in the bay was of 312 kg·day⁻¹. The differences in the absorption capacity of algae macrophyte were revealed. This process was most efficiently carried out by *S. latissima*; the minimum efficiency of participation in bioremediation was determined for *U. obscura*. It was concluded that the existing littoral and sublittoral thickets of marine macroalgae of the Kola Bay can be considered as the key element in the implementation of the preventive, daily cleaning of coastal water from the petroleum products. The inclusion in the calculations of data on the ability of other representatives of the bay phytobenthos to neutralize diesel fuel may increase the role of algae macrophyte in cleaning the coastal marine areas from the petroleum products. As concluded, the thickets of brown algae can be considered as the key component of repair and homeostasis in coastal ecosystems. The destruction of even a part of algae natural communities can change the ecosystem balance.

Keywords: macrophytes, ecosystem of the Kola Bay, sustainability, diesel fuel, bioremediation

The Kola Bay of the Barents Sea has a length of 57 km and occupies one of the leading places in the Arctic for the transportation and transshipment of petroleum products (hereinafter PPs). The complexes for the processing of hydrocarbon raw materials have already been built, and several ones are under construction on the bay coast. The bay is considered one of the most contaminated sea water bodies on the Kola Peninsula. The contamination problem is aggravated by the fact that most of the year the water temperature in the bay is slightly above 0 °C; at a given temperature, the natural decomposition of PPs is greatly slowed down. Microorganisms, *inter alia* microalgae, play an important role in water purification from PPs (Il'inskii, 1995 ; Peretruxhina et al., 2006 ; Semenova et al., 2009 ; Atlas, 1978 ; Wrabel & Peckol, 2000), but their contribution to bioremediation decreases during the polar night.

In the Kola Bay, the content of PPs in water (background concentration) varies from $0.04 \text{ mg}\cdot\text{L}^{-1}$ (about 1 threshold limit value, TLV) to $2.3 \text{ mg}\cdot\text{L}^{-1}$ (46 TLV), with $1 \text{ TLV} = 0.05 \text{ mg}\cdot\text{L}^{-1}$. The background content of PPs in the bays of the Murmansk coast in the summer-autumn period ranges 3 to 8 TLV. There were no bays completely clean of PPs. In a number of bays in winter and spring, the content of PPs decreased down to 1–2 TLV since the traffic of ships, mainly fishing, increases in spring and decreases in late autumn (Voskoboinikov et al., 2017 ; Kola Bay and Oil, 2018). The highest concentration of PPs in the bay was noted in its southern area (in the area of an oil terminal, a commercial port, and Atomflot piers), as well as in the middle area (in the area of transshipment complexes). This is due to the existing sources of contamination: operating piers, shipping, refueling of ships, and refueling from tankers on the roadstead. During oil spills, concentration of PPs in water reaches $25\text{--}50 \text{ mg}\cdot\text{L}^{-1}$ (500–1000 TLV) and higher values.

In recent years, we have proposed to use algae macrophyte in the remediation of the Barents Sea water from PPs. It has been shown that many species of macrophytes are resistant to the effect of PPs. The tolerance range differs even in species with similar taxonomy and structure; however, all macroalgae studied by us and other authors earlier (12 species) demonstrated the ability to accumulate PPs from seawater on the thallus surface, to destruct them using epiphytic hydrocarbon-oxidizing bacteria, and to absorb and neutralize PPs subsequently in plant tissues. As revealed, a decrease in the content of PPs in water goes parallel with its increase in algae thalli (Voskoboinikov et al., 2017, 2018).

The studies carried out made it possible to form an evidence base confirming the ability of macroalgae, which differ in structure and taxonomy, to absorb and destruct PPs (Voskoboinikov et al., 2017, 2018, 2020 ; Pilatti et al., 2016 ; Pugovkin et al., 2016 ; Ryzhik et al., 2019). On the basis of the symbiotic association of macroalgae and hydrocarbon-oxidizing bacteria, biotechnologies of plantations-biofilters have been created for purifying coastal water areas from PPs. Assumptions were repeatedly put forward about the role of coastal phytocenoses in the seawater bioremediation of various toxicants (Mironov, 1985 ; Patin, 2017b); however, neither specific data nor calculations based on field or laboratory experiments were provided.

To a great extent, this can be explained by the lack of work on assessing macroalgae reserve and species diversity in the water areas described. Thus, studies of algae reserves on the Murmansk coast, which were carried out in 1980s–1990s (Makarov, 1998 ; Peltikhina, 2005), with attention paid to the Kola Bay phytobenthos, concerned kelps only. The work presented is the first one, where calculations of the participation of macrophytobenthos in bioremediation of PPs for the bay are carried out. The calculations are based on the results of recent expeditionary observations of the distribution and biomass of the dominant phytobenthos representatives – *Fucus vesiculosus*, *F. distichus*, *F. serratus*, *Ascophyllum nodosum*, *Saccharina latissima*, *Palmaria palmata*, and *Ulvaria obscura* – in three Kola Bay areas. Preliminary studies have shown that the species analyzed have a fairly wide tolerance range to PPs and a relatively high coefficient of absorption of the toxicant (Voskoboinikov et al., 2017, 2018, 2020 ; Ryzhik et al., 2019). In parallel with field observations, laboratory analysis of the neutralization capacity of diesel fuel (hereinafter DF) in the above-mentioned algae species was carried out, and their total role in bioremediation of DF for the Kola Bay was assessed.

The authors of the work presented hope that this research will contribute to understanding the role of coastal phytocenoses in the seawater bioremediation of petroleum products not only in the Kola Bay of the Barents Sea, but in other coastal areas of the World Ocean as well.

MATERIAL AND METHODS

Laboratory experiments on the effect of PPs on algae. The objects of study were brown algae (Phaeophyceae) (fucoids *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. distichus*, and *F. serratus*, as well as kelp *Saccharina latissima*), green alga (Chlorophyta) *Ulvaria obscura*, and red alga (Rhodophyta) *Palmaria palmata*. Thalli approximately equal in size and mass were sampled in Zelenetskaya Bay ($69^{\circ}07'09''\text{N}$, $36^{\circ}05'35''\text{E}$) of the Barents Sea (Fig. 1a). *S. latissima* was sampled in the sublittoral zone from a depth of approximately 3 m, and other species – in the littoral zone. Algae were cleared of fouling and placed in 1.3-L glass containers with seawater, except for *S. latissima*: the experiments with this species were carried out in 3-L vessels. Seawater with a salinity of 33 ‰ was taken from the spots algae inhabit and filtered through a cotton-gauze filter; then, summer diesel fuel was added: $6.5 \text{ mg}\cdot\text{L}^{-1}$, which is 130 TLV for water in terms of the gross content of PPs. Seawater and DF were not sterilized. The experiment was carried out in a temperature-controlled box at $+7\dots+8^{\circ}\text{C}$, irradiance of $16\text{--}18 \text{ W}\cdot\text{m}^{-2}$, and constant water aeration.

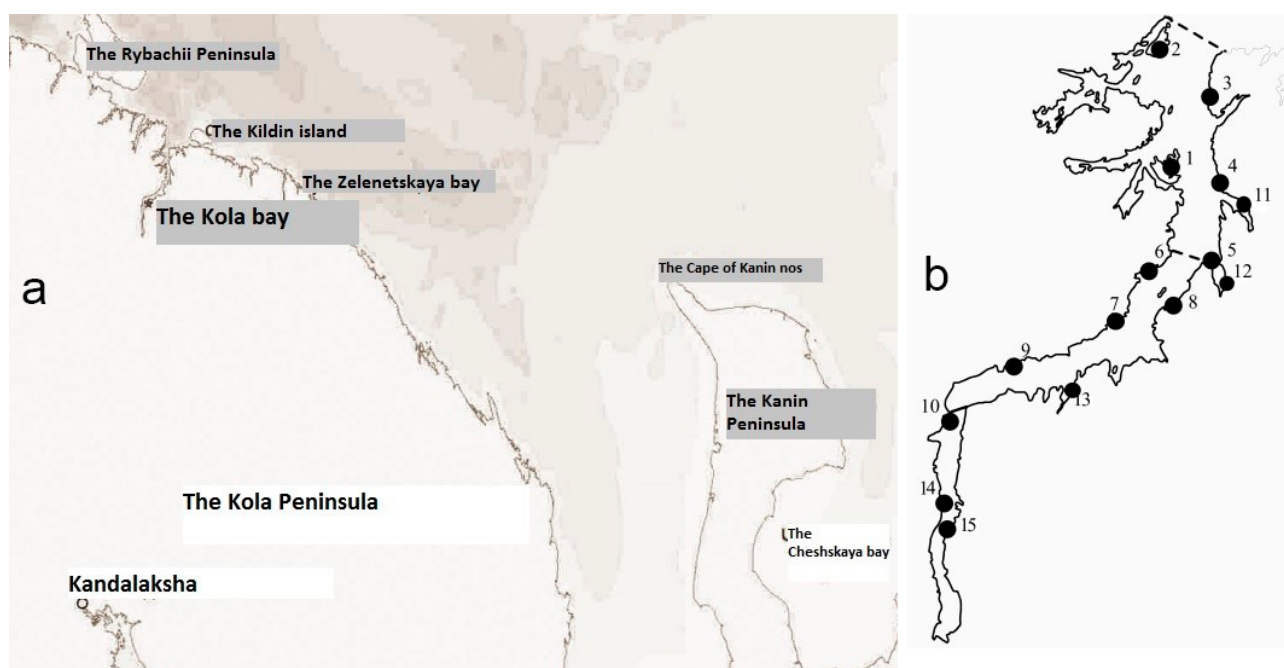


Fig. 1. Work area: a – the Kola Bay and Zelenetskaya Bay of the Barents Sea; b – sampling stations (1–15) in the Kola Bay

The experiment with the determination of algae morphological and functional state lasted for 10 days. The gross content of PPs in water and algae was detected by gas chromatography – mass spectrometry at the beginning and after 5 days of the experiment. Sample preparation and instrumental analysis were carried out by the previously described method (Voskoboinikov et al., 2018). To assess the background content of PPs, samples of water and algae from Zelenetskaya Bay were analyzed. Calculations of the content of DF in algae were carried out on a dry weight basis, taking into account the coefficient of the ratio of wet/dry mass: for *Saccharina* – 7/1; fucoids – 5/1; and *Palmaria* and *Ulvaria* – 4/1. During the experiment, changes in the state and morphology of the algae thallus were monitored visually and by light-optical methods under a Mikmed-6 microscope.

Assessment of algae biomass and reserves in the Kola Bay. Macrophyte reserves were accessed by the traditional method (Peltikhina, 2005). The material was sampled during MMBI RAS expeditions in 2009–2019 (with partial financial support of the Russian Geographical Society). Inspection of a significant part of the bay coastline made it possible to describe macrophytobenthos distribution and to determine the size of the fucoid zone for assessing the reserves.

To calculate the biomass of macrophytobenthos and separate algae species, quantitative samples were taken by the method of sample plots' layout along the transect perpendicular to the water edge. In total, 15 transects were laid along the entire bay; in the southern and middle areas, work was carried out every two years (Fig. 1b). The area of the frame during sampling in the littoral zone was 0.25 m², in the sublittoral zone – 1.0 m². On each section, stations were performed on the upper, middle, and lower horizons of the littoral zone (about 3.0, 1.5, and 0.5 m above sea level, respectively); in the sublittoral zone – at depths of 5, 10, and 15 m. Three samples were taken at each station. The width of the species zone was estimated on average for an area of relatively uniform vegetation of at least 500 m in length. The dominant species and the projective bottom cover with algae were assessed visually. Sampling in the sublittoral zone was carried out using scuba-diving equipment. The extent of littoral areas with certain types of communities was estimated using the MapViewer 8.0 software. The topographic base was the contour of the coastline in the nautical charts for the Kola Bay, digitized by MMBI RAS specialists.

RESULTS

Macrophyte distribution, reserves, and biomass in the Kola Bay. Four fucoid species investigated are unevenly distributed in the Kola Bay littoral zone.

Fucus vesiculosus was found everywhere from the Tuloma River estuary to the bay mouth; the species uses various substrates for attachment, *inter alia* hydraulic structures. The maximum reserves were recorded in the littoral zone of the middle area. In most of the littoral zone, *F. vesiculosus* biomass was about 1 kg·m⁻², but due to the large area occupied by this species its reserves were very large (Table 1). In a number of spots of the littoral zone of the middle area, the biomass reached (9.4 ± 0.3) kg·m⁻². The total reserves in the bay are 2315.0 tons of wet weight, which is 463.0 tons of dry weight.

Fucus distichus grows in the middle and lower horizons of the littoral zone of the entire Kola Bay. The highest biomass – up to (6.9 ± 3.4) kg·m⁻² – was recorded on boulder beaches in the middle and northern bay areas, but its reserves are concentrated in the southern area, where the species covers vast sandy beaches. On steep cliffs, the biomass is not more than 1 kg·m⁻². The total reserves in the bay are 2017.5 tons of wet weight, which is 403.5 tons in terms of dry weight.

Ascophyllum nodosum lives mainly on boulder beaches, which are protected or weakly protected from wave effect; its thickets achieve significant development on the islands on the leeward side. In the northern area, the biomass reaches (4.2 ± 0.8) kg·m⁻². The total reserves in the bay are 371.4 tons of wet weight, which corresponds to 74 tons in terms of dry weight.

Fucus serratus is distributed mainly in the northern bay area; on Ekaterininsky Island, a high biomass – (6.1 ± 1.5) kg·m⁻² – was recorded. *F. serratus* total reserves are 114 tons of wet weight only, which is 22.7 tons in terms of dry mass.

Saccharina latissima forms thickets in the sublittoral at a depth of 4–6 m in the northern area and is found in small numbers in the middle area. The reserves are 450 tons of wet weight, which corresponds to 64.3 tons of dry weight.

Palmaria palmata is confined to a coarse substrate; it is recorded mainly in the middle and lower horizons of the littoral in the middle and southern bay areas. The reserves are 155.3 tons of wet weight, which is 38.8 tons of dry weight.

Ulvaria obscura is present fragmentarily in the littoral zone in three bay areas. The species occupies a variety of substrates: from stones to wooden structures, metal boards, and ships abandoned on the littoral. The reserves amount to 5.38 tons of wet weight, which corresponds to 1.35 tons of dry weight.

Table 1. Specific rate of diesel fuel absorption by algae macrophyte and estimated volumes of diesel fuel absorption by algae in different areas of the Kola Bay

Species	Specific rate of diesel fuel absorption, $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$	Volume of diesel fuel absorption, $\text{kg}\cdot\text{day}^{-1}$			
		Northern bay area	Middle bay area	Southern bay area	The bay
<i>F. vesiculosus</i>	202.0	12.4	64.2	17.2	93.8
<i>F. distichus</i>	169.0	13.8	22.8	31.6	68.2
<i>A. nodosum</i>	44.0	0.8	2.2	0.8	3.8
<i>F. serratus</i>	123.0	3.0	0	0	3.0
Fucoids in total	538.0	30.0	89.2	49.6	168.8
<i>S. latissima</i>	1752.0	138.0			138.0
<i>P. palmata</i>	146.4		4.4		4.4
<i>U. obscura</i>	25.4		0.17		0.17
In total		168.6	92	51.9	312.5

Potential contribution of macrophytes to the sorption of PPs: result of the experiments.

The content of PPs in macrophytes in the habitat (at a concentration of PPs in water of $0.2 \text{ mg}\cdot\text{L}^{-1}$, which is 4 TLV) was as follows: *F. vesiculosus* – $25 \mu\text{g}\cdot\text{g}^{-1}$, *F. distichus* – 24, *F. serratus* – 28, *A. nodosum* – 18, *S. latissima* – 1980, *P. palmata* – 124, and *U. obscura* – $1.4 \mu\text{g}\cdot\text{g}^{-1}$. Visual and light-optical observations revealed no changes in *Fucus* thalli after 5 and 10 days in seawater with diesel fuel at a concentration of $6.5 \text{ mg}\cdot\text{L}^{-1}$ (130 TLV). In the thallus color, there were no changes; in the cells, there were no plasmolysis, changes in the plastid color, and vacuolization, which were described earlier when studying the effect of other damaging factors on algae (Ryzhik et al., 2019).

After 5 days of the experiment, the content of DF in 4 fucoid species, *Saccharina*, *Palmaria*, and *Ulvaria* was as follows: in *F. vesiculosus* – $1036 \mu\text{g}\cdot\text{g}^{-1}$, *F. distichus* – 870, *F. serratus* – 641, *A. nodosum* – 236, *S. latissima* – 10,740, *P. palmata* – 856, and *U. obscura* – $128 \mu\text{g}\cdot\text{g}^{-1}$ of dry weight. Table 1 shows the mean data on DF absorption by the algae species studied per 24 hours and the volume of DF absorbed in different Kola Bay areas. The results of calculations of the total daily contribution of the algae investigated to the bay remediation of PPs are shown as well, taking into account their total reserves determined.

DISCUSSION

In the experiments carried out, all macrophytes showed resistance to DF at a concentration of $6.5 \text{ mg}\cdot\text{L}^{-1}$ (130 TLV). The algae had no signs of damage after 10 days under experimental conditions.

Analysis of the content of PPs in macrophytes sampled for experiments (at a concentration of PPs in the water in the habitat of $0.2 \text{ mg}\cdot\text{L}^{-1}$, which is 4 TLV) showed that algae differ in their ability

to absorb PPs. Among three *Fucus* species, these differences are insignificant: from 24 $\mu\text{g}\cdot\text{g}^{-1}$ in *F. distichus* to 28 $\mu\text{g}\cdot\text{g}^{-1}$ in *F. serratus*; *U. obscura* absorbed PPs 6 times less (1.4 $\mu\text{g}\cdot\text{g}^{-1}$), and *Palmaria*, on the contrary, 5 times more. *Saccharina* accumulated DF at a given concentration in water 80 times more than fucoids. At higher concentration of PPs in water, the level of DF absorption by algae changes. The maximum absorption level is shown by *Saccharina*: 1752 $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$; the species absorbs 3.3 times more than all fucoids algae in total. At relatively high concentrations of DF in water, *Palmaria* is inferior in terms of DF absorption not only to *Saccharina*, but also to two *Fucus* species (Table 1).

The main sources of contamination in the Kola Bay are concentrated in its southern and middle areas. There, handling of hydrocarbon raw materials is carried out; partially, the works are carried out in the middle area from large-capacity tankers on the roadstead. Moreover, ship-repair enterprises, docks, and oil terminals operate. PPs, that got into the water because of spills, mainly end up in the littoral zone (Kola Bay and Oil, 2018 ; Patin, 2017b), *i. e.* in the spots of *Fucus* and *Palmaria* growth, which are a natural biofilter in this situation. Out of algae studied, *Fucus* is the most resistant to PPs. Previously, we noted the ability of *F. vesiculosus* to survive for a long time under conditions of constant contamination: on the littoral zone on stones in a layer of fuel oil (“chocolate mousse”) and near oil terminals and ports. The algae retained their vital functions, but the indicators of their functional activity were at a lower level compared to those of *F. vesiculosus* specimens from clean habitats (Voskoboinikov et al., 2017). Other *Fucus* species demonstrate a lower resistance to contamination; nevertheless, they also survive for a long time when the surface is covered with a film of PPs and are involved in the detoxification (Stepanyan & Voskoboinikov, 2006). A significant ability to accumulate and neutralize PPs was recorded in *P. palmata* as well.

Comparing the volumes of DF potentially absorbed by algae, it should be noted that the primary role in bioremediation is played by fucoids. Out of species studied, *Ulvaria obscura* has the lowest values in terms of reserves and absorption capacity: 5.38 tons of wet weight and 25.4 $\mu\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$, respectively.

Thus, the algae macrophyte studied are capable of neutralizing 312 kg of DF *per* 24 hours. According to the modern classification, this volume of PPs corresponds to the mean local oil spill in seawater (Patin, 2017a). The efficiency of algae participation in bioremediation is affected by several factors: 1) temperature, light, hydrodynamics, and salinity, determining algae physiological state, quantitative and qualitative composition, and hydrocarbon-oxidizing activity of microorganisms – algae epiphytes; 2) the composition of PPs. In our work, we were unable to take into account the contribution of a large group of algae – the bay inhabitants, representatives of various taxonomic groups – to the bioremediation of the Kola Bay due to the lack of data on their ability to neutralize PPs. The inclusion of this data can significantly increase the importance of algae macrophyte in the bioremediation of coastal waters, although it is undoubted even now.

Conclusion. The ecosystem of the Kola Bay has withstood chronic petroleum contamination for over a hundred years due to a combination of several circumstances. In the bay, most of the coastline is occupied by boulder beaches, where communities of *Fucus* algae with a very high biomass have formed. These communities provide constant water purification from petroleum products, take on the main burden of neutralizing not only PPs dissolved in water, but also “film” forms of toxicants. In the sublittoral zone, kelps develop on separate boulders and purify water from dispersed PPs. The efficiency of the kelp participation in bioremediation is largely ensured by the significant area of contact surface with PPs and, possibly, by the high abundance of epiphytic hydrocarbon-oxidizing bacteria on the thallus surface.

In the Kola Bay water, hydrocarbon-oxidizing microorganisms are constantly present, which process PPs. It can be considered established that there is a natural mechanism for the utilization of petroleum and PPs in natural coastal ecosystems. Undoubtedly, there are lethal doses of PPs for macrophytes, which make this system sensitive to large oil spills or to the effect of its refined products. Taking into account the long period of formation of petroleum hydrocarbons in the Earth's crust, we can say that the use by bacteria of such a high-calorie product as hydrocarbons as a source of energy was a very likely event. The revealed ability of brown, red, and green algae to absorb hydrocarbons is surprising since it is unusual for them, unlike for other autotrophs, to use carbon sources alternative to carbon dioxide in the metabolism. This is based on the ability of macrophytes and hydrocarbon-oxidizing bacteria to form symbiotic associations.

The study presented has shown that the role of algae macrophyte is not limited to the creation of primary production and to regulation of the content of carbon dioxide and oxygen. The thickets of brown algae can be considered as the key component of the repair and homeostasis system in coastal ecosystems, the existence of which was not previously suspected. It should be understood that the destruction of even a part of algae natural communities can change the ecosystem balance. In this regard, the designers and builders of new enterprises on the Kola Bay coast should be warned against backfilling the littoral zone with soil in order to expand the coastal area. The littoral zone is the main habitat of *Fucus* algae, which are a natural biofilter largely ensuring the purity of the Kola Bay water.

The work was carried out with the financial support of the RFBR, project No. 18-05-80058 "New technologies of bioremediation and the role of phytocenoses in purification of coastal waters of the Arctic seas in case of emergencies connected with storage and transportation of hydrocarbons", program "Dangerous phenomena".

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РОЛЬ ВОДРОСЛЕЙ-МАКРОФИТОВ В БИОРЕМЕДИАЦИИ ОТ НЕФТЕПРОДУКТОВ КОЛЬСКОГО ЗАЛИВА БАРЕНЦЕВА МОРЯ

Г. М. Воскобойников¹, С. В. Малавенда¹, Л. О. Метелькова²

¹Мурманский морской биологический институт Российской академии наук,
Мурманск, Российская Федерация

²Санкт-Петербургский Федеральный исследовательский центр Российской академии наук,
Санкт-Петербург, Российская Федерация
E-mail: grvosk@mail.ru

Выполнена оценка вклада макроводорослей в очистку от дизельного топлива Кольского залива Баренцева моря. В основу расчётов положены результаты: 1) экспедиционных наблюдений последних лет о запасах, распределении и биомассе водорослей-макрофитов отделов

Chlorophyta и Rhodophyta и класса Phaeophyceae из отдела Ochrophyta, обитающих в трёх частях залива; 2) лабораторных исследований по способности макроводорослей *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. distichus*, *F. serratus*, *Saccharina latissima*, *Palmaria palmata* и *Ulvaria obscura* к нейтрализации токсического действия дизельного топлива. Показано, что общий вклад у исследованных водорослей в биоремедиацию от дизельного топлива в заливе составляет 312 кг в сутки. Выявлены различия в поглощающей способности у водорослей-макрофитов. Так, наиболее эффективно процесс осуществляет *S. latissima*, минимальная эффективность участия в биоремедиации определена у *U. obscura*. Сделан вывод о том, что имеющиеся литоральные и сублиторальные заросли морских макроводорослей Кольского залива являются важным элементом в процессе профилактической, повседневной очистки вод залива от нефтепродуктов. Включение в расчёты данных о способности к нейтрализации дизельного топлива у других представителей фитобентоса залива может увеличить роль водорослей-макрофитов в очистке прибрежных морских акваторий от нефтепродуктов. Сделан вывод, что заросли бурых водорослей — важная составляющая системы репарации и гомеостаза в прибрежных экосистемах. Уничтожение даже части природных сообществ водорослей может изменить баланс, существующий в экосистеме.

Ключевые слова: макрофиты, экосистема Кольского залива, устойчивость, дизельное топливо, биоремедиация