Sirena Limited Liability Company

Approved by

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PROJECT REPORT

CLEANUP OF ARCTIC MARINE ENVIRONMENT USING BIOLOGICAL FILTRATION POTENTIAL OF BROWN ALGAE

This pilot project was implemented under UNEP/GEF project “Russian Federation - Support to National Action Plan for Protection of Arctic Marine Environment”

Project Director:

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EXECUTIVE SUMMARY

The pilot project *Cleanup of Arctic Marine Environment Using Biological Filtration Potential of Brown Algae* demonstrates an innovative method of cleanup of marine environment from oil pollution. This method is based on biological filtration potential of a symbiotic association of brown algae and hydrocarbon oxidizing bacteria. The new technology has been implemented at the experimental plantation of algae in Olenya Bay of Barrens Sea. Two main sources of oil pollution in this basin are *Nerpa* ship repair yard, which has a submarine dismantling dock, and naval ships anchored in the open-sea part of the bay.

The innovative method of seawater cleanup was tested between November 2007 and October 2008. The experimental plantation consisted of engineering structures with horizontal cable ropes stretched on the water surface, which provided the substrate for *Fucus vesiculosus.* These ropes supported 5-meter-long vertical slings, which served as substrate for *Laminaria saccharina* thalluses and epiphyte hydrocarbon oxidizing bacteria, at the depths of 0.5-5 meters. The floating structure had the area of 0.5 ha and was attached to artificial anchors at the depth of 15-25 meters. Several major anthropogenic oil spills occured in Olenja Bay during the project implementation period. Fucus algae at the experimental plantation directly contacted with oil film for a long time, serving as slick bars and cleaning water surface.

Simultaneously with the tests performed *in situ* at the plantation, several experiments were conducted in high seas and in the laboratory of biological station of Murmansk Institute of Marine Biology, located in Dalnie Zelentsy village on the coast of Barents Sea. These experiments demonstrated the potential of fucus algae to clean seawater from oil pollution.

Project results can be briefly summarized by the following conclusions:

1) The proposed plantation design and biological filtering method can be implemented all year round.
2) Sanitary algae plantation contains oil pollution and adsorbs oil products. The absorbed oil products are metabolized and neutralized by symbiotic association of algae and epiphyte bacteria.
3) The activity of hydrocarbon oxidizing bacteria (epiphytes of brown algae) increases in the presence of oil products, which is an important factor to consider during construction of the plantation.
4) An original finding of this project is identification of five species of dominant epiphyte bacteria, which neutralized oil products on the surface of algae.

5) Individual modules of sanitary algae plantation can be effectively used for containment of oil films and sustainable development of aquaculture in Barents Sea.

6) Based on *in-situ* measurements and laboratory experiments, we showed that one hectare of biofiltering plantation might neutralize about 100 kg of oil products per week.

7) Valuable bioactive substances can be extracted from laminaria harvested at the sanitary algae plantation.

8) Although we designed the plantation for Barents Sea, the proposed method can be implemented in other seas after modifications that account for specific regional abiotic and biotic factors.

Based on the results of this pilot project, project authors filed a patent application “A method of purification of coastal seawater from oil films and oil products, dispersed in the surface layer” and obtained the patent № 2007106573/13 (007130). The results of this pilot project are extensively illustrated by photographs.

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I. INTRODUCTION

I.1. Project goal and alternative solutions

During a few last decades, Russian seas have been extensively used for transportation of gas condensate, oil and products of oil refining industry. These economic activities were accompanied by intense pollution of littoral area, which negatively affected marine biota.

The problem of pollution of marine environment was scrutinized in the *Marine Doctrine of the Russian Federation until 2020.* In particular, Paragraph 3 *Provision of safety of marine activities* of Chapter *Principles of national marine policy* formulated the goal to "reconcile the economic need to increase offshore extraction of hydrocarbons and other natural resources with environmental objectives: preservation, reproduction and extraction of marine biological resources".

The 39th Session of Executive Council of UNESCO International Commission on Oceans held in Paris with participation of Russian Delegation on 21-28.07.2008 formulated four priority areas of its activities. One of these was "environmentally responsible management of oceans and littoral areas for sustainable reproduction of marine resources".

Oil pollution poses the greatest risks for marine biota in littoral areas, where the concentrations of oil products are maximal. Littoral areas provide spawning and early development grounds for many species of fish, invertebrates and algae (Patin 1997).

Currently existing oil pollution containment options are subdivided into mechanical and chemical measures. Slick bars are a good example of mechanical containment. Chemical methods require application of chemicals for decomposition and coagulation of oil products. Unfortunately, these chemicals themselves negatively affect littoral biota.

Sirena Ltd. organized a task force for development of innovative biotechnological method for cleanup of oil pollution. This task force has comprised the leading experts in marine biology, biotechnology and ecology, who proposed to use brown algae as a natural barrier for containment of marine oil pollution.

The proposed method (a sanitary algae plantation) combines the advantages of mechanical and chemical containment of oil spills. On the one hand, this plantation prevents spreading of oil pollution and functions as a slick bar. On the other hand, biological association of algae with epiphyte hydrocarbon oxidizing bacteria absorbs and metabolizes oil products (OP).

The experts identified the following compelling reasons for implementation of this pilot project:

1. Oil pollution poses a real threat to the littoral areas of Arctic seas.
2. Literature and original research data documented possible role of macrophyte algae and hydrocarbon oxidizing microorganisms (HOM) in purification of seawater from OP pollution.
3. The researchers thoroughly studied biology of fucus algae, laminaria and HOM.
4. Sirena Ltd. (Project Consultant) has elaborated theoretical models and acquired practical experience in cultivation of algae in Arctic seas.
5. Sirena Ltd. established a project task force that invited qualified experts in marine biology, biotechnology, hydrology, engineering and ecology, who had experience of implementation of R&D programs in related fields.

This report presents the results of field tests and approbation of the proposed method at the project site (workstation) in Barents Sea. All project activities were performed under the Terms of Reference specified in the Contract № CS-NPA-Arctic-02/2007 of 29.08.2007 between Project Contractor (Russian National Pollution Abatement Facility) and Project Consultant (Sirena Ltd.). This Contract was implemented in the framework of a larger project "Russian Federation - Support to National Action Plan for Protection of Arctic Marine Environment".

The purpose of the pilot project presented in this paper was to demonstrate an economically efficient method of purification of Arctic seas from oil pollution. The property of brown algae to absorb oil pollution may be gainfully utilized for cleanup of Arctic seas. Application of this method may greatly reduce negative consequences of economic activities of the Russian Federation in Arctic seas.

Project Consultant put forward the following work plan to attain the project objective:

Step 1: Preliminary activities and preparations for construction of the experimental plantation.

Task 1: Selection of the project site in Barents Sea near the coast of Kola Peninsula; preparations for construction of the experimental plantation.

Task 2: Detailed examination of the prospective project site, hydrological and hydrochemical tests.

Step II: Construction of the experimental plantation.
Task 3: Construction of plantation of brown algae at the selected project site.

Step III: Implementation of melioration measures and monitoring of plantation performance and the state of marine environment near the plantation.
Task 4: Implementation of melioration measures and monitoring of plantation performance and the state of marine environment near the plantation.

Step IV: Evaluation of project results.
Task 5: Harvesting of laminaria and preparations for its utilization and processing.
Task 6: Development of technology of utilization of polluted algae; processing of clean algae for production of commercial products.
Task 7: Evaluation of project results.
Task 8: Drafting of guidelines and recommendations for construction of a typical sanitary algae plantation for cleanup of marine environment.

I.2 List of project implementing agencies and persons

G. M. Voskoboinikov	Project Director, Doctor of biology, expert in marine biology and biotechnology
G. G. Matishov	Member of Russian Academy of Sciences, Project Consultant
V. V. Ilyinsky	Doctor of biology, expert in marine biology
S. E. Zavalko	Candidate of biology, expert in hydrology and ecology
V. A. Korobkov	Candidate of engineering, expert in ecology and marine construction
Y. V. Kuranov	Candidate of economics, expert in economics
M. V. Makarov	Candidate of biology, expert in hydrobiogy, aquaculture and plant physiology
A. A. Metelsky	Junior research associate, expert in algology
A. A. Nikolsky	Candidate of economics, expert in economics
D. V. Pugovkin	Junior research associate, expert in marine microbiology
I. V. Ryzhik	Candidate of biology, expert in algology
E. Y. Zubova	Biotechnology engineer
O. M. Makarova	Biotechnology engineer

The following organizations participated in project implementation at various stages:

Murmansk Institute of Marine Biology of Russian Academy of Sciences;

Nerpa Ship Repair Yard - a Chief Affiliate of *Zvezdochka* Federal State Unitary Enterprise, Snezhnogorsk;

Moscow State University;

Saint-Petersburg Academy of Chemistry and Pharmaceutics;

Biotechnocom Ltd., Saint-Petersburg;

Nord-Service Ltd., Murmansk;

Murmansnab Ltd., Murmansk;

Biophys Ltd., Snezhnogorsk.

1.3 List of abbreviations

AS	Anion surfactants
BAA	Biologically active additives
CFC	Colony-forming cells
CMS	Chromatographic mass-spectrometry
DF	Diesel fuel
GLF	General Life Function
HM	Heavy metals
HOB	Hydrocarbon oxidizing bacteria
HOM	Hydrocarbon oxidizing microorganisms
MAC	Maximum allowable concentration
OHC	Oil hydrocarbons
OP	Oil products
PAHC	Polyaromatic hydrocarbons
PCB	Polychlorinated biphenyls
SAP	Sanitary algae plantation
SRY	Ship-repair yard
WS	Workstation

II. METHODS OF ONSITE AND LABORATORY RESEARCH

II.1 Project methodology and equipment

II.1.1 Project methodology

Project site selection was based on general evaluation of background situation, visual survey, analysis of published data, engineering and environmental surveys.

Engineering and environmental surveys involved internationally recognized methods and calibrated equipment, standard oceanographic, hydrochemical, hydrobiological and microbiological methods and good laboratory practices.

Evaluation of background environmental situation in Olenya Bay of Kola Peninsula included the following activities:

1. Sampling and analysis of bottom sediments, algae, surface and bottom waters.
2. Onsite and laboratory measurements of the following indicators of water pollution:
    - Summary fraction;
    - Sum of phenols;
    - Anion surfactants.

3. Measurements of hydrochemical parameters of bottom water (pH, Eh, available oxygen, temperature).
4. Measurements of concentrations of water pollutants:
    - Heavy metals (Fe, Mn, Ni, Co, Zn, Cd, Cu, Pb, Cr, Hg, As, Sr);
    - PAHC;
    - PCB.

Environmental monitoring methods were developed using the experience of regional environmental studies of Sirena Ltd., Murmansk Institute of Marine Biology of Russian Academy of Sciences, and Federal State Unitary Enterprise “VSEGEI” in Barents Sea, Kara Sea, Lake Ladoga, Neva Bay and Gulf of Finland.

Fieldwork methods

The samples of bottom sediments from the open-sea, littoral and sublittoral parts of Olenya Bay were taken by a compact Peterson scoop from a research vessel during high tide. We also took the samples of bottom sediments during low tide and on shore from small prospect holes. All samples of bottom sediments were collected from the upper sediment layer.

The samples of bottom sediments were analyzed for geochemical and microbiological parameters and organic pollutants. Geochemical measurements involved analyses of heavy metals: Fe, Mn, Ni, Co, Hg, As, Pb, Zn, Cu. For such analyses, bottom sediments were collected in plastic bags. For microbiological analysis, the samples were collected in the upper layer of sediments and kept in porcelain cups or Petri dishes with diameter 100 mm, for preservation of natural humidity. For detection of organic pollutants (hydrocarbons, PCB, PAHC, phenols), the samples were collected in plastic bags; the weight of each sample was between 0.25 and 0.5 kg.

Water samples for hydrochemical analyses were collected by a special fluoroplastic bathometer at the depth of +1m above the sea bottom. These samples of bottom water were analyzed for:

- Heavy metals (sample volume 250 ml);
- Oil products (sample volume 500 ml);
- Phenols (sample volume 200 ml);
- AS (sample volume 100 ml);
- PAHC (sample volume 1 l);
- PCB (sample volume 1 l).

Water samples for detection of oil products, phenols, AS, PAHC and PCB were collected exclusively in glass bottles. Water samples for detection of heavy metals were immediately preserved in aquafortis. Acidity levels were controlled by multipurpose indicator paper.

We used the following instruments to measure temperature, pH, Eh, and dissolved oxygen in the samples of bottom water:

- Microprocessor pH-meter-ionomer I-500 to measure pH;
- ORP HI 98201 HANNA instruments to measure Eh;
- Thermo-oxymeter AQUA-OXY to measure temperature and dissolved oxygen.

Several water pollutants (oil products, phenols, AS) were directly measured *in situ* using the analyzer “Fluorate 2M” and the methods developed by NPF “Lumex”, which are commonly used in many certified Russian labs.

Analytical methods

Ecological nature of this research required *approximate-quantitative spectral measurements* of 48 elements: SiO_2 (0.01), Al_2O_3 (0.01), MgO (0.01), CaO (0.01), Fe_2O_3 (0.01), K_2O (1), Na_2O_3 (0.01), P_2O_5 (0.08), Sr (0.01), Ba (0.01), Ti_2O_3 (0.001), MnO (0.001)[*], V (5), Cr (10), Co (0.5), Ni (3), Zr (10), Hf (10), Nb (10), Ta (100), Sc (1), Ce (100), La (20), Y (1), Yb (0.5), U (300), Th (100), Be (0.3), Li (10), W (10), Mo (2), Sn (2), Cu (0.3), Pb (5), Zn (20), Cd (5), Bi (1), Ag (0.03), In (2), Ge (2), Ga (2), Tl (2), As (100), Sb (20), Te (100), B (10)[**].

Insuffecient detection capacity of this method for several important heavy metals required additional measurements with increased accuracy and sensitivity.

X-ray diffraction analysis was used for detection of Sr (1), U (2), Pb (3), As (9)[**].

Atomic adsorption analysis was used for detection of Cr, Co, Ni, Cd, Cu, Zn, Hg (cold steam technology).

Gamma-spectrometry analysis was used for detection of isotopes ^{226}Ra (8), ^{232}Tr (5.6), ^{40}K(37), ^{137}Cs (2.8), ^{60}Co (3.8).[***]

Infra-red analysis was used to calculate sum of oil hydrocarbons in bottom sediments and soil.

Chromatographic mass-spectrometry (CMS) was used to detect hydrocarbon type content of oil hydrocarbons and to study chemical mechanisms of decomposition of OHC.

Chromatographic and spectrophotometric detection was used to detect 3,4 benzpyrene (0.001), PCB (0.010) and phenolic index (0.002). [****]

Evaluation of background environmental situation also required *collection of algae samples*. Algae samples were collected in the middle horizon of littoral using a 0.25-m^2 frame. We identified species composition and measured the mean biomass of dominant species. Projective overlay was determined visually. We measured length, mass and number of dichotomous branchings of fucus plants. The number of dichotomous divisions (branchings) of fucus plants depends upon their age. This property was used to group the plants into several age groups.

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[*] The numbers in brackets indicate the lower detection limit,% by mass.
[**] The numbers in brackets indicate the lower detection limit, ppm.
[***] The numbers in brackets indicate the lower detection limit in Becquerels.
[****] The numbers in brackets indicate the lower detection limit, mg/kg.

The levels of bioaccumulated pollutants in algae were measured in the following way: the plants were washed off with sea water, then overgrown organisms and debris were rasped away and the plants were dried in filter paper at 20-22ºC and packed in special containers. Fresh algae plants were preserved for selected analyzes at the temperatures under 7ºC no longer than 24 hours.

Measurement of oil products in algae

Freshly collected algae plants were cleaned with cotton soaked in hexane, after which the cotton was extracted with hexane. Cleaned algae were minced and extracted with hexane in the ultrasonic bath. Algae extracts were dried up in nitrogen flow to 100-microliter volume and analyzed by gas chromatography and CMS.

Hydrodynamic studies

Measurements of water movement near the experimental plantation

Water movement is by far the most important environmental factor, which determines structural and biological production parameters of macrophyte plants in littoral areas. Hydrodynamics of algae environment greatly influences their growth. Intense water movement stimulates growth of algae. This effect was observed at the level of individual thalluses (Conover 1968; Hailov 1988; Oliger and Santelices 1981) and algae populations (Zavalko 1983; Voskoboinikov et al 2007).

Water mobility determines thalline morphology to a great extent (Hailov et al 1988; Zavalko 1993), which in turn influences the character of water flow around algae plants. Besides, hydrodynamics of water flow greatly influences biochemical composition of macrophyte tissue, total content of bioorganic substances, and distribution of assimilated substances across the thalline profile (Hailov et al 1984; Zavalko 1993; Voskoboinikov 2006).

Materials and methods of hydrodynamic measurements

The importance of water movement for algae activity and consequently for their water purification properties requires special studies of water movement on the experimental plantation. Hydrodynamic measurements of algae environment pose several specific requirements to the instruments and materials:

1. Characteristic dimensions of water flow meter or gauge should correspond to the typical scale of turbulent vortexes around the plants;
2. Both turbulent and laminar components of water flow should be measured;
3. Water flows should be measured in all directions; rapid changes of flow speed are very important;
4. The number of measurements should provide a reliable and realistic description of character of water flow.
5. Water flow should be measured synchronously at different scales.

The method of plaster structures (Muus 1968) meets all the above requirements. It requires application of plaster balls of different sizes. Plaster dissolution process provides an adequate physical model of 'output' component of algae metabolism in water flow. This method takes into account laminar and turbulent components of water flow, as well as the flows moving in opposite directions. We modified this method to obtain information synchronously at different scales: hundreds of meters, meters, decimeters, centimeters and millimeters. It is important that plaster dissolution most comprehensively describes general hydrodynamics of algae environment, which determines their metabolism and biological production.

We used standard plaster balls with 31 mm diameter for hydrodynamic measurements at the experimental plantation. All balls were weighted and labeled before their immersion. Each ball was fastened by eight ropes with lengths between 7 and 10 meters. These ropes were vertically placed at different points across the plantation, and attached to the horizontal cable ropes stretched on the water surface. In these experiments, we measured hydrodynamics of the upper layer of water at the depths up to one meter in the greatest detail. The first five plaster balls were tied to each rope at the depths of 20, 40, 60, 80 and 100 cm. Additional balls were tied at the depths of 2, 3, 4 meters, et cetera. There were eight ropes of different depths: four 10-m ropes

(#1, #2, #3, #4); two 8-m ropes (#5, #6) and two 7-m ropes (#7, #8). We chose these lengths to adequately describe water movement across the vertical profile of the plantation (total depth of the plantation assembly was 4 m) and water movement underneath the plantation. The depth of water underneath the plantation varied from 30 m in the open sea part to 7 m in the coastal part of the plantation.

Microbiological studies

Sampling and preparation of samples for analyses

Macrophyte samples of thalluses had to be freed from accretions. Fragments of middle sections of thalluses were cleaned by sterile cotton buds. These cotton buds were shaken in a vortex in 5 ml of water. Using this desorption method, we registered the greatest bacterial counts.

Water samples for microbiological studies were drawn with a sample thief with a detachable sterile flack. No water samples were drawn within one meter from macrophytes to avoid their influence on microbial population of seawater.

Bacterial counts

Total bacterial counts were measured by epiluminescent microscopy with acridine orange dye. Heterotroph bacteria were counted by limiting dilution method in liquid media. We used modified Zobell solution for saprotroph bacteria and marine mineral media for carbon oxidizing bacteria. To model hydrocarbon pollution, we added two drops of sterile summer grade diesel fuel in each 4.5 ml test tube (Ilyinsky 2000). This was the only source of hydrocarbons in the laboratory experiments. Bacterial counts were determined by Mac-Credy maximum likelihood tables (Egorov 1976).

Genetic analysis

Species composition of extracted bacterial cultures was determined by genetic analysis 16S rRNA in Federal State Unitary Enterprise "NIIGenetica".

II.1.2 Project equipment

The following instruments were used during oceanographic, hydrochemical and biological research, collection of samples and measurements of environmental pollution levels.

- Compact Peterson scoop;
- SEA-BIRD ELECTRONICS STD probe SEACAT SBE 19plus (USA);
- OTE PVC 5-liter bathometer (USA);
- 0.5-l microbathometer;
- KFK-2 photocalorimeter;
- HACH all-purpose digital titrator;
- Laminar box;
- Autoclave;
- Thermostatic shaking bath;
- Microburette;
- Kamovsky vacuum pump;
- I-500 microprocessor pH-meter-ionometer;
- ORP HI 98201 HANNA instruments;
- Fluorate-2M analyzer;
- Specord spectrophotometer;
- AQUA-OXY thermo-oxymeter;
- Finnigan DSQ II Focus chromatographic mass-spectrometer;
- Laboratory glassware;

- Reagents;
- Refrigerator;
- Tubes, canisters, sample storage containers;
- Exposition vials of various sizes;
- Frame for collection of algae samples;
- Special plastic bags for sampling;
- Diving equipment for sampling;
- Video camera and digital still cameras.

III. RESULTS

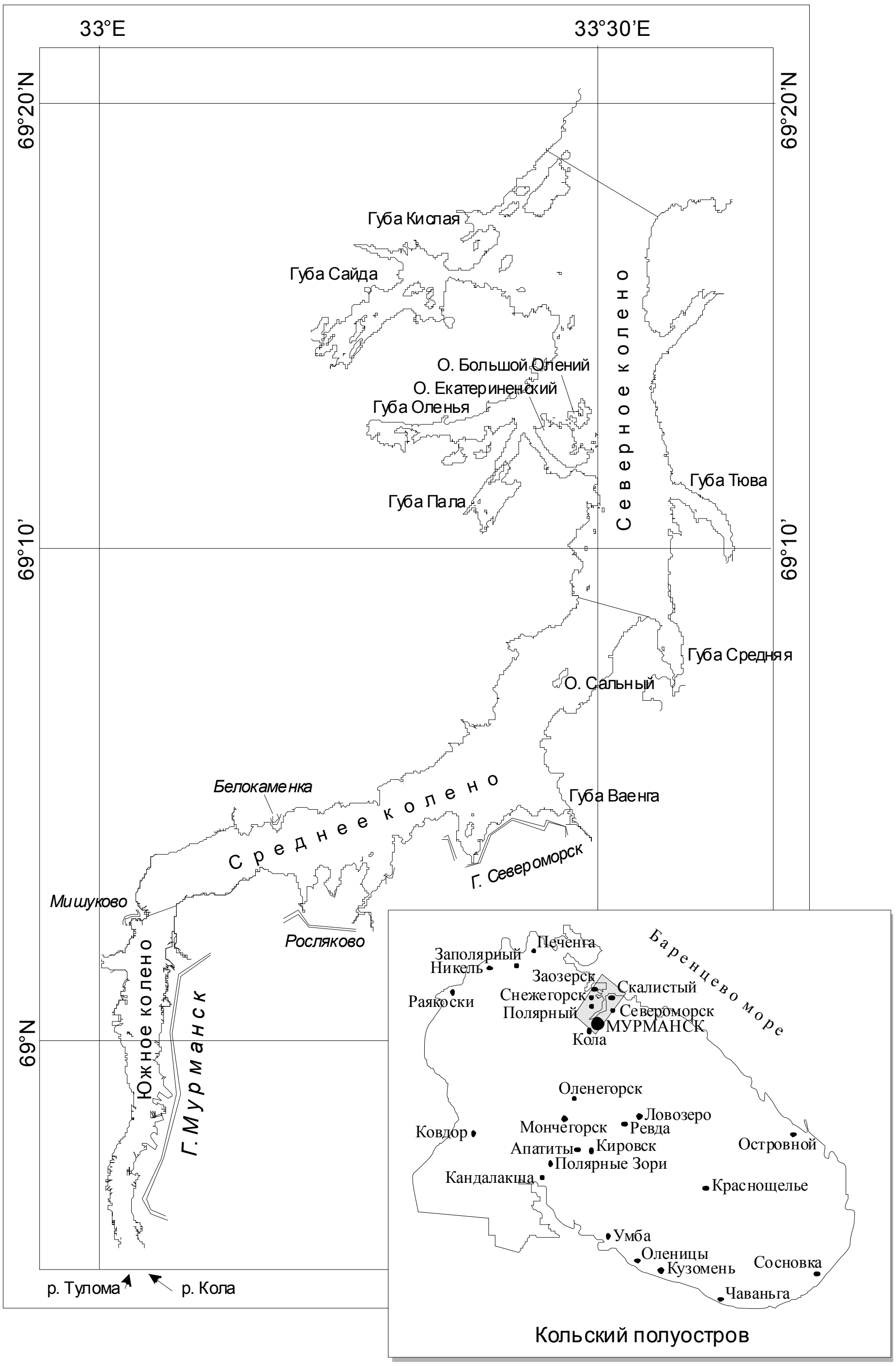
III.1 Selection of project site and preparations for construction of biofiltering plantation

III.1.1 Olenya Bay as a prospective project site

The bays of Murmansk coast of Barents Sea are currently being gradually turned into industrial sites for loading, processing and transportation of gas condensate, oil and oil refining products (Fig. 1). Three new fuel terminals are to be constructed in this area (in Lavna river mouth, Belokamenka and Mishukovo villages). One may expect increase in oil pollution near the oil processing facilities and transportation terminals, especially near a big gas-processing terminal scheduled for construction in Teriberskaya Bay. This terminal will process and transfer gas condensate from Shtokmanovskoye gas-field, and serve as the terminal point for Shtokmanovskoye gas condensate pipeline. Intensification of overseas transportation will cause considerable environmental pollution of the littoral area. Marine oil pollution will endanger aquatic life. The Project Consultant has developed a method of construction of slick bar zones utilizing sanitary algae plantation (SAP) technology. This method was proposed to contain marine oil pollution around industrial sites, involved in development of oil and gas deposits, transportation, processing, and storage of oil, gas condensate and oil products.

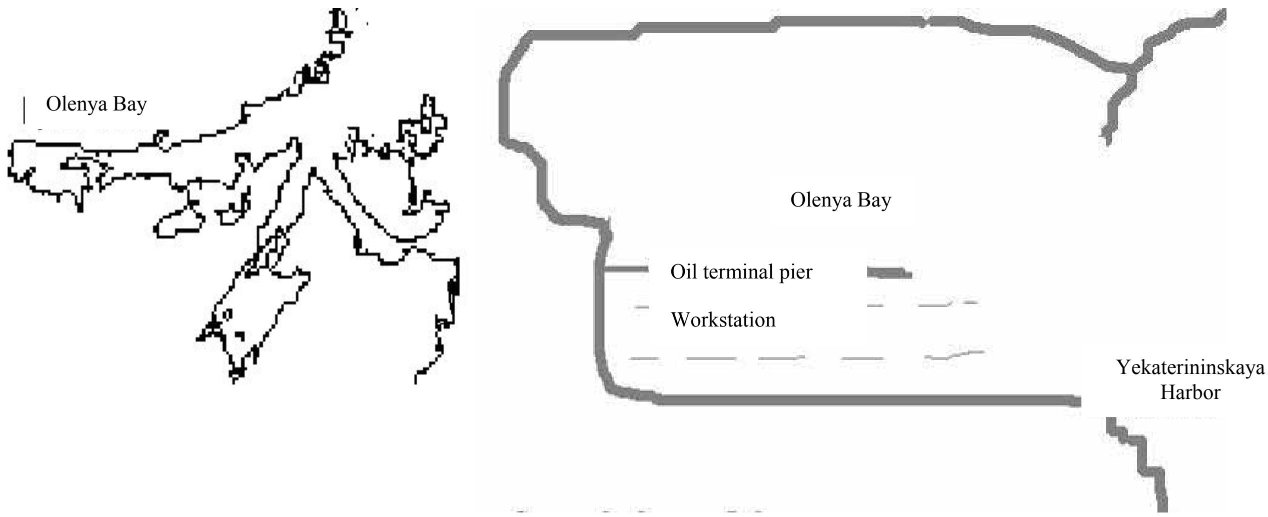
Embayment of coasts of Barents Sea and other northern seas by fiords and bays presents opportunities for construction of SAP and utilization of their biofiltering potential for oil pollution abatement.

Fig.1 Map of Kola Bay



Project site selection was influenced by the following reasons. Olenya Bay of Kola Bay of Barents Sea hosts *Nerpa* ship-repair yard (SRY). This enterprise is a chief affiliate of Federal State Unitary Enterprise *Zvezdochka*, which repairs, dismantles and disposes of nuclear submarines (Fig. 2a,b). The project task force obtained the required approvals for construction of SAP after consultations with the management of SRY *Nerpa* and Murmansk regional environmental authorities.

Fig. 2a Olenya Bay with SAP site



The following considerations were taken in account during project site selection:

- Visual survey of the seashore;
- Location of construction waste or metallic structures on the sea bottom;
- Analysis of characteristics of sea bottom, terrain, aquatic biota, environmental situation, and shipping activity.

The project site is situated between the oil terminal pier and seashore opposite Nezametnaya Harbor, in the latitude of $69^0 12'$ N and longitude of $33^0 20'$E.

The roadstead warships in Yekaterininskaya Harbor near the mouth of Olenya Bay regularly pollute the project site with oil products. According to the environmental service of SRY *Nerpa*, oil film frequently covers Olenya Bay.

WS faces open sea part of the bay on the north. The west fringe of WS is located approximately 100 m away from the shore. The south fringe of WS runs along the flat seashore, which is covered with shrub. Supralittoral and littoral parts of the shore are covered with medium-sized boulders and gravel. Fucus algae naturally grow in the littoral.

The diving survey of the prospective project site showed that the depth increases from 6-8 m in the west part of WS to 20 m in its east (open sea) part. Sea bottom underneath WS is covered with sand and muck. There are no large boulders, attached macrophyte plants, any construction waste or metal structures on the bottom.

The visual survey showed that the intensity of water surface oil pollution was 1-2 points. It means that 60-80% of the surface is covered with oil film. Water surface is gray with rare specks. The divers observed occasional 30-50 cm^2 oily spots on the bottom. Oil film covered some boulders and littoral algae plants.

Fig. 2b Biofiltering plantation and sources of oil pollution in Olenya Bay. 1- oil terminal pier of SRY *Nerpa*, 2 - navy ships moored in Yekaterininskaya Harbor in the mounth of Olenya Bay.

To summarize this chapter, the choice of the project site was based on the following considerations (refer to paragraphs III.1.2- III.1.8.):

1) Constant presence of sources of oil pollution: the ships moored at the oil terminal pier of SRY *Nerpa* and anchored in Yekaterininskaya Harbor in the mounth of Olenya Bay; submarine dismantling yard of SRY *Nerpa*.

2) Absence of intense navigation through the prospective project site.

3) Presence of fucus algae in the littoral, which indicated vegetation of fucus algae in this area.

4) Minimal depth at the prospective project site was 6 meters.

5) Construction of SAP on the proposed project site was approved by SRY *Nerpa*, Murmansk Environmental Authority, Murmansk Regional Agency on Technical and Environmental Surveillance (Rostechnadzor) and Murmansk Marine Inspectorate.

III.1.2 Analysis of littoral plant association

We identified six species of algae (with total projective overlay up to 70%) near the prospective project site. These species are listed below in the descending order of their abundance. The dominant species belong to Phaeophyta family: *Fucus vesiculosus* (90%), *F. distichus, Ascophyllum nodosum, Pylaiellla litoralis;* Chlorophyta*: Enteromorpha intestinalis;* Rhodophyta*: Palmaria palmata.* Species composition of macrophytes in Olenya Bay is generally less diverse than that in most other bays of Barents Sea. Average age and mass of *Fucus vesiculosus* plants in Olenya Bay are much lower than those typically observed elsewhere in Barents Sea.

III.1.3 Microbiological research

The concentrations of eutrophic and oligotrophic bacteria in Olenya Bay are higher than typically observed in northern latitudes. These concentrations are 5.05-5.70 million bacterial clumps per milliliter in water and 4.01-4.26 million cells per centimeter of mactophyte surface.

Microbiological research of taxonomertic composition of hydrocarbon oxidizing bacteria in Olenya Bay identified the following seven species of HOB: Pseudomonas, Proteus, Micrococcus, Arthrobacter, Corynebacterium, Mycobacterium, and Rhodococcus (Table 1).

Table 1. Genuses of hydrocarbon oxidizing microorganisms

Genus	Description of bacterial clump in Chapek medium
Micrococcus	Roundish, yellowish
Rhodococcus	Roundish, smooth, bulging, glossy, film-forming
Proteus	Roundish, bulging, with characteristic smell, trailing growth
Corynebacterium	Smooth, semi-transparent, matte surface
Mycobacterium	Smooth, bulging, glossy

Pseudomonas	Roundish, smooth, mucous
Arthrobacter	Roundish, bulging, glossy, soft

III. 1.4 Analysis of water samples

Water temperature in the surface layer near the project site was 6.7-6.8ºC between October 15 and October 20. Water temperatures at different depths were 6.6, 6.4, and 6.1ºC at the depths 6, 12 and 20 meters, respectively. Snezhnogorsk weather station reported that the annual maximum water temperature of 7.1-7.2ºC was observed between September 26 and October 5; and the annual minimum water temperature of –1.5ºC was observed between February and March. These temperatures were measured ½ mile away from SAP.

We also measured water salinity in the surface and bottom layers at the project site between October 15 and October 20. Water salinity varied between 32.5 points at the depths of 6-12 meters and 33.5 points at the depth of 20 meters in the open-sea part of Olenya Bay. Snezhnogorsk weather station reports that water salinity usually falls down to 27.5-29 points in the summer season.

Water clarity during October 15 and October 20 near the project site was up to 16 meters. The divers told us that seasonal minimum of clarity is observed in spring and summer, which is the snow-melting season; but even then water clarity rarely falls below 10 meters.

We could not calculate directly the period of water renewal. Literature sources reported that this period was roughly 5 days in Kola Bay in 2009.

Wind velocity and gurgitation in Olenya Bay reach annual maximum between November and February, according to the reports of Snezhnogorsk weather station. The experimental plantation was constructed in the landlocked portion of Olenya Bay, and gurgitation there was much smaller than in the open-sea part of the bay.

Biofiltering properties of the experimental plantation greatly depend upon the hydrodynamic regime near the plantation. This regime was thoroughly studied during the experiment.

III.1.5 Hydrodynamic regime

We studied hydrodynamic regime after construction of the plantation, because the presence of many fucus plants on the water surface significantly changed this regime, compared to the original situation.

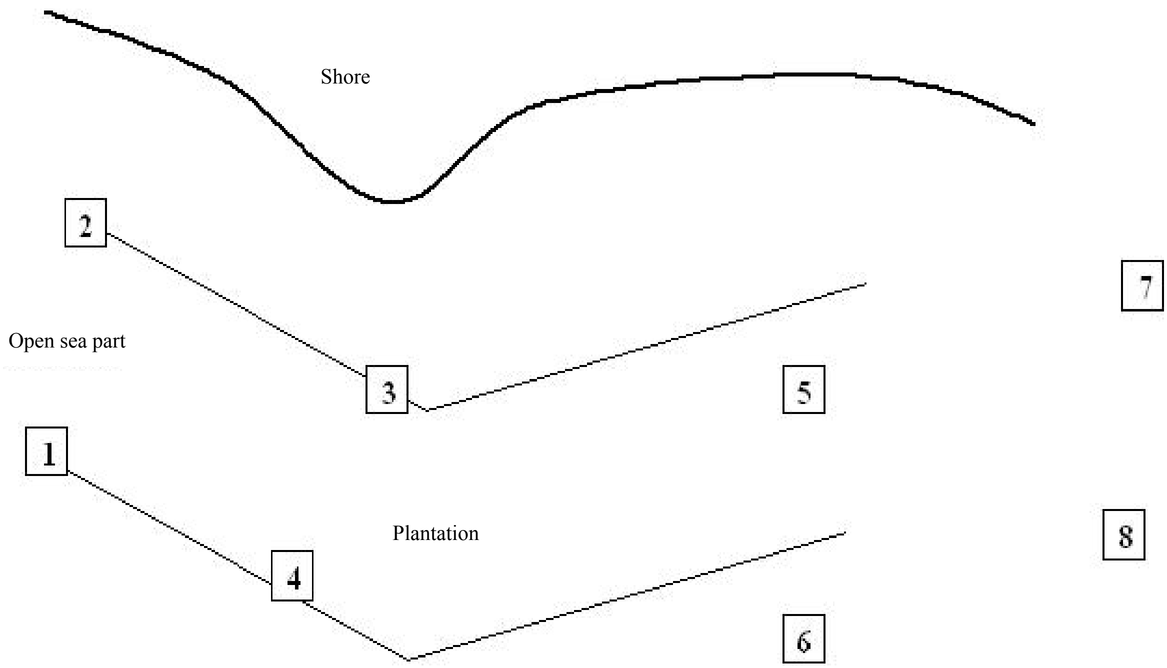
Placement of plaster balls at the plantation

Plaster balls were mounted on vertical strings tied to the horizontal cable ropes (Figure 3 and Table 2). We used the strings of three different lengths. The length of strings 1, 2, 3 and 4 was ten meters; the length of strings 5 and 6 was eight meters; and the length of strings 7 and 8 was seven meters. These lengths were measured between the attachment point at the horizontal cable rope and the weight at the bottom end of each string.

Table 2. Positions of plaster balls on vertical strings

Depth of ball from the cable rope, m	Ball №							
	String 1	String 2	String 3	String 4	String 5	String 6	String 7	String 8
0	1	16	31	46	61	74	87	99
0.2	2	17	32	47	62	75	88	100
0.4	3	18	33	48	63	76	89	101
0.6	4	19	34	49	64	77	90	102
0.8	5	20	35	50	65	78	91	103
1	6	21	36	51	66	79	92	104
2	7	22	37	52	67	80	93	105
3	8	23	38	53	68	81	94	106
4	9	24	39	54	69	82	95	107
5	10	25	40	55	70	83	96	108
6	11	26	41	56	71	84	97	109
7	12	27	42	57	72	85	98	110
8	13	28	43	58	73	86		
9	14	29	44	59				
10	15	30	45	60				

Fig. 3 Location of vertical strings across the plantation



All plaster balls were immersed in the water for 23 hours. After the exposition, the balls were washed off in distilled water, dried up and weighed. The intensity of plaster dissolution was estimated with the following equation:

$V^{Ca} = (W_1 – W_2) / t$, grams of $CaSO_4$ per hour,

where W_1, and W_2 are initial and final weights of the ball, and t is exposition time.

Water movement (mov_S^{Ca}) was expressed as the ratio of intensity of plaster dissolution and the area of ball surface:

$\mathbf{mov_S^{Ca}} = (W_1 – W_2) / (t \cdot S)$, grams of $CaSO_4$ per hour per square centimeter,

where S is the area of ball surface (cm^2).

mov_S^{Ca} was determined with a calibration regression equation, estimated by Mr. S. A. Kavardakov:

$\mathbf{mov_S^{Ca}} = 0.0267 (V^{Ca})^{1.0673}$, grams of $CaSO_4$ per hour per square centimeter.

In the original method proposed by Muus (1968), linear velocity of water flow was estimated from the intensity of plaster dissolution. Calibration equation was estimated by measurements of this speed in standard vessels with fixed velocity of linear water flow. Calibration was performed under a variety of water temperatures. Muus reported the following dependency between the linear velocity of water flow and the intensity of plaster dissolution at the water temperature of 5.5ºC:

$$V = 257018\ (mov_S^{Ca})^{1.8263}$$

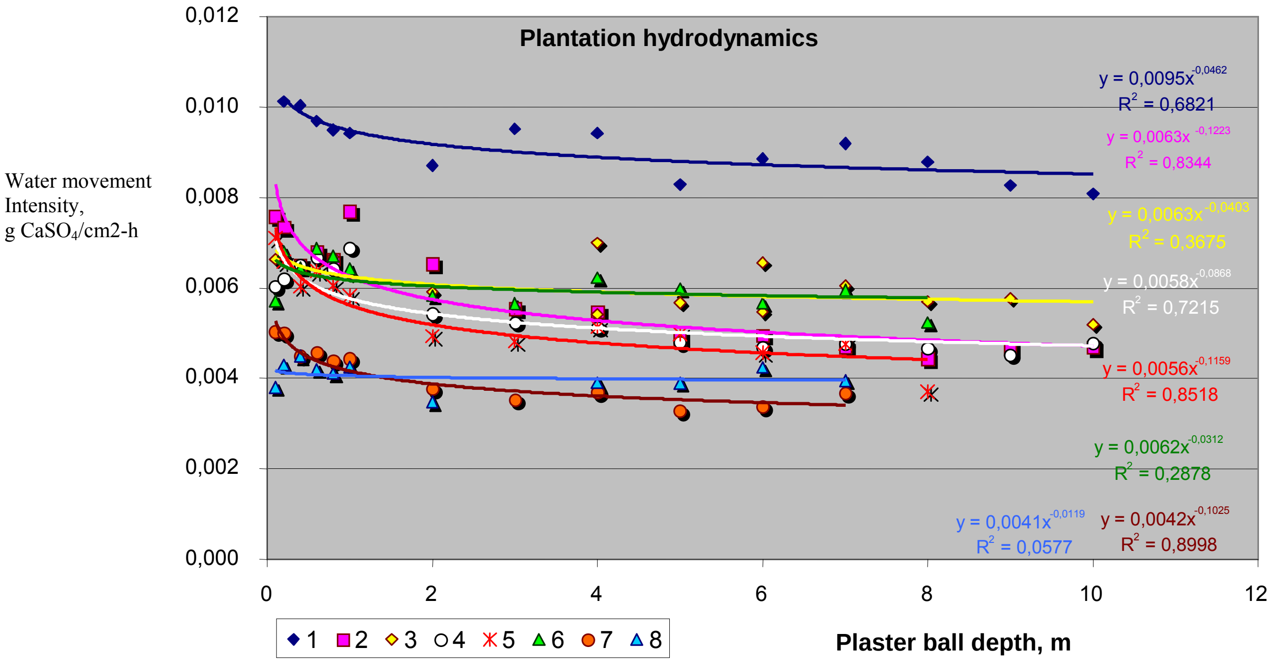
Results and discussion

Water movement throughout the plantation was highly nonuniform. The mobility of the upper one-meter layer was significantly greater in all measurement points at the plantation. This result agrees with previous research in various seas. More precise measurements indicated exponential increase in water mobility even in the upper 10-cm layer (Hailov et al 1988). Such hydrodinamical measurements are quite important for assessment of functional properties of the experimental plantation, because the sorbtion properties of the plantation depend upon the abundance of *F. vesiculosus*, which mostly grows in the upper 10-cm layer, which has the greatest water mobility.

On the whole, hydrodynamic activity of the water space occupied by the plantation decreased from its open-sea part (where the water mobility was maximal) towards its coastal part. The results of measurements of relative intensities of dissolution of plaster balls agreed with the results of other measurements in Barents Sea and elsewhere. We concluded that the plantation was situated in a rather calm area, where the intensity of water movement is close to the mean values typically observed in Barents Sea.

Figure 4 shows the distribution of linear velocities of water flow across the plantation. Overall character of this distribution agrees with the above conclusion.

Fig. 4 Plantation hydrodynamics



III.1.6 Hydrochemical regime

Dissolved oxygen

Average concentration of dissolved oxygen in the surface layer was 8.4 mg/l, or 91% of the saturation limit.

Hydrogen ion exponent (pH)

Hydrogen ion exponent (pH) at the workstation was equal to 8.1-8.2. The seawater in Olenya Bay belongs to K-Na-Cl type with 31.91% mineralization. Mean concentrations of chemicals in the water at the workstation are reported in Table 3.

Table 3. Mean concentrations of chemicals in the water at the workstation

Chemicals	Mean concentration at the WS, g/l	Standard values for seawater, g/l
K+Na	10.00	11.100
Ca	0.37	0.420
Mg	1.27	1.300
Cl	18.08	19.350
SO_4	2.14	2.700
HCO_3	0.14	–
CO_3	0.01	0.070
∑	31.91	34.94

Biogenic matter

Concentrations of *phosphates* were 32 mkg/l in the coastal part of the plantation and 29 mkg/l in its open-sea part. We did not observe any vertical trends in phosphate concentrations.

Silicon concentrations were 305-320 mkg/l in the surface layer and 200-210 mkg/l in the bottom layer. There were no significant variations in silicon concentrations across the plantation.

Mean concentrations of *nitrate* ions were uniform across the plantation and across the vertical profile (40 mkg/l).

Concentrations of *nitrite* ions were 1.2 mkg/l in the surface layer and 2.0 mkg/l in the bottom layer.

Mean concentrations of *ammonia* ions were 240 mkg/l in the surface layer and 200 mkg/l in the bottom layer.

Hydrochemical measurements showed that surface and bottom waters were characterized by weakly alkaline properties. The value of pH did not change much and remained above 8.0. Bottom water in the sublittoral part was more alkaline than average across the plantation, which might be caused by the role of saline waters.

The measurements of oxidation-reduction potential at the three points near the bottom showed that full tide water was characterized by fairly high positive Eh potential (+152 … +225 mV).

III.1.7 Geochemistry of bottom waters

The main objective of geochemical analysis was to identify anthropogenic pollutants in the bottom waters. We measured concentrations of Cu, Zn, Cr, Co, Ni, Cd, Hg, As, benz(a)pyrene, PCB, OP, phenols and AS in the bottom layer. The bottom water is less mobile and therefore more susceptible to anthropogenic pollution.

The levels of several HM (copper, zinc, arsenic) slightly exceeded the applicable sanitary standards - Russian Maximum Allowable Concentrations (MAC), see Table 4. According to the published literature sources, copper concentrations exceed MAC everywhere in Kola Bay.

Table 4. Average levels of heavy metals in the bottom layer

Chemical element	Average concentration, mg/l	MAC for fishing waters, mg/l
Cd	0.00023	0.005
Ni	0.0014	0.01
Co	0.0006*	0.01
Pb	0.0046	0.1
Cu	0.0021 (2.1)	0.001
Zn	0.0116 (1.2)	0.01
Cr	0.0012	0.02
As	0.024 (2.4)	0.01

*) This value is the lower detection limit. The numbers in brackets are the ratios of average concentration and MAC.

III.1.8 Oil pollution near the project site

Average concentration of OHC is about 0.03 mg/l and does not exceed MAC (Table 5).

Table 5. Concentrations of selected organic pollutants near the workstation

	PAHC (benz(a)pyrene), ng/l	PCB, mkg/l	OHC, mg/l	Phenol, mkg/l	AS, mkg/l
Mean level	2.54	<0.010	0.028	2.18	11
Relative error	50%	-	20%	20%	30%

The concentrations of PAHC and PCB in the surface and bottom water are quite low (below the detection limit). At the same time, concentrations of phenol exceed MAC by a factor of 1.6-1.9. These measurements agree with the regional trends reported in literature.

The mean concentration of AS in the surface and bottom water does not exceed MAC.

OHC levels in the bottom sediments vary from 12 mkg/kg in the open-sea part to 20 mkg/kg in the coastal part. Such levels of pollution are rated as “relatively clean”.

The mean concentration of benz(a)pyrene in the bottom sediments is 0.009 mg/kg and does not exceed MAC. The most polluted areas of Kola Bay are characterized by much higher concentrations of benz(a)pyrene (up to 58 mg/kg).

PCB levels in the bottom sediments remained below the detection limit (0.01 mg/kg of soil).

The mean concentration of phenol in the bottom sediments at the workstation was 0.059 mg/kg. The local maximum of phenol concentrations was recorded in the coastal part of the workstation (0.128 mg/kg).

The levels of OHC in algae near the workstation are higher than in the water (Table 6). At the same time, these levels are lower than typically observed in algae in the most polluted areas of Barents Sea (Murmansk seaport and the pier in Teriberka village).

Table 6. Concentrations of organic pollutants in *Fucus vesiculosis* near the workstation, arithmetic mean of six measurements for PCB and OHC

Pollutant	PAHC (benz(a)pyrene)	PCB	OHC
mg/kg of wet weight	-	0.021	2.134

Analysis of macrophyte algae did not show any bioaccumulation of benz(a)pyrene. The concentrations of this pollutant in algae were below the detection threshold (0.0005 mg/kg). The concentrations of PCB in macrophytes were also below the detection threshold.

The samples of macrophyte algae were analyzed for chlorinated organic pesticides (the sum of hexachlorinated cyclohexanes), DDT and its metabolites, hectochlorine and aldrin. No traces of these substances have been found. We concluded that the concentrations of these pollutants were below the detection limits.

The following conclusions could be drawn on the basis of our studies of seawater, bottom sediments and littoral algae.

The water in Olenya Bay belongs to marine water type. The concentrations of copper, zinc and arsenic slightly exceed corresponding MAC standards. At the same time, the concentrations of these metals are lower than in other bays of Kola Bay. The most likely sources of pollution include SRY *Nerpa*, warships and navy bases in Ekaterininskaya Harbor. It is there that maximum concentrations of heavy metals in seawater were detected. Average concentrations of copper, zinc, arsenic and nickel in the water samples taken in Ekaterininskaya Harbor were greater than MAC by 14, 16, 2.3 and 1.2 times, respectively. The levels of other analyzed pollutants were below the applicable hygienic standards.

High levels of OHC in fucus algae in the littoral area and relatively low concentrations of OHC in the water indicated long-term bioaccumulation of these pollutants. Such bioaccumulation could take place over the lifetime of algae thalluses (4-6 years). We conclude that macrophytes accumulate pollution over time and can serve as indicators of anthropogenic pollution. Their bioaccumulation capacity may facilitate natural self-purification of water reservoirs.

According to Snezhnogorsk environmental service and the interviewed local residents, continuous oil film could be observed on the water surface in Olenya Bay several days per year.

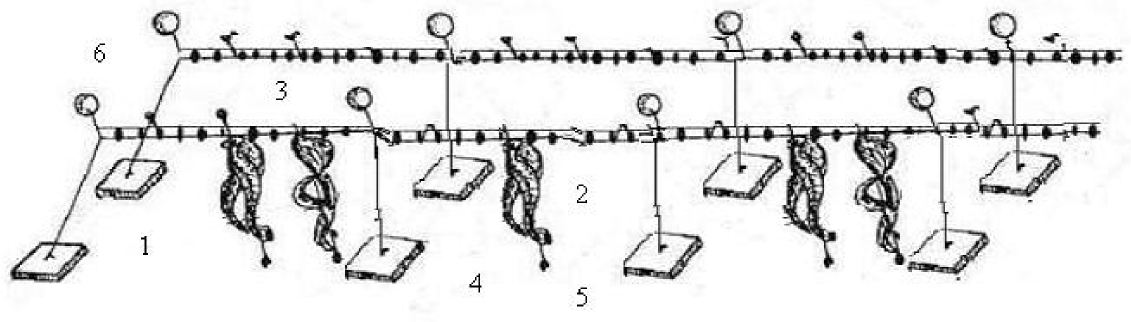
We hypothesize that oil products and heavy metals come into Olenya Bay during utilization of submarines. This multistage process includes towing, cutting, slitting, towing and disposal of submarine parts. The oil terminal pier is another source of oil products, located in the coastal part of the bay. Oil tankers regularly moor there and deliver diesel fuel for communal needs of Snezhnogorsk and SRY *Nerpa*, which is the biggest enterprise in the town.

Warships constantly pollute the bay, because they are moored in the open-sea part of Olenya Bay and in Yekaterininskaya Harbor. East winds drive oil film from Yekaterininskaya Harbor to Olenya Bay.

III.2 Construction of experimental plantation

III.2.1 Assembling of plantation carcass

Fig. 5 Plantation design. 1- concrete one-ton blocks. 2 – vertical catenary nylon ropes. 3 – horizontal 20-meter nylon cable ropes. These ropes carried entwined fucus thalluses. 4 – vertical 5-meter slings with entwined laminaria thalluses or spores. 5 – lead weights keeping the slings in vertical position. 6 – floats.

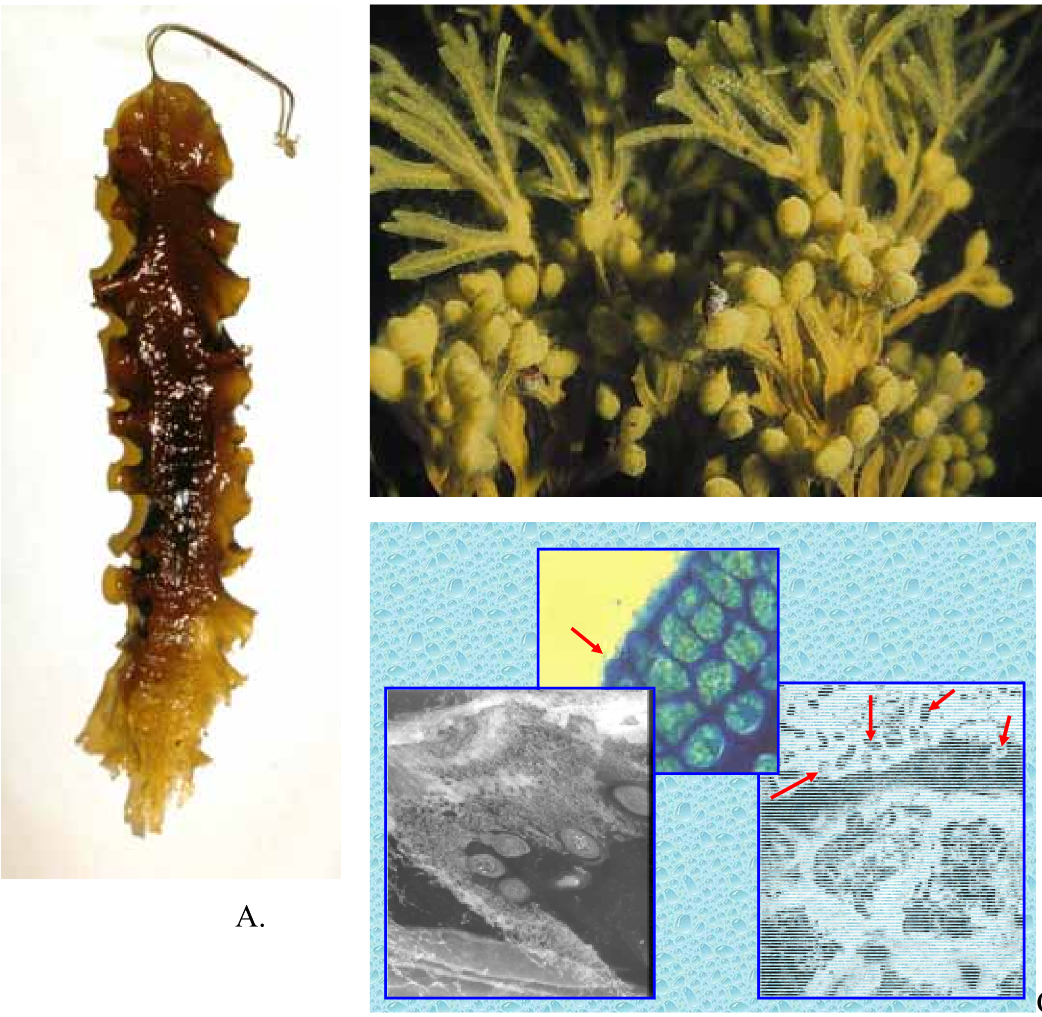


Reinforced concrete anchors were lined up in the two parallel 200-meter lines along the workstation fringes; the distance between each pair of anchors was 25 meters. Anchor depths varied from 6-8 meters in the coastal part to 20-24 meters in the open-sea part of the plantation. Four anchors fixed the poles with signal lights at the four corners of the plantation. Two separate reinforced concrete anchors secured the platform with the monitoring instruments in the center of the plantation. There were 22 anchors total. A rope with a marking float was attached to each anchor to mark its position on the water surface. These marking ropes had 5-cm diameter.

III.2.2 Preparation and mounting of horizontal cable ropes with fucus

We collected *Fucus vesiculosis* thalluses aged between 3 and 5 years in the littoral area of Zelenetskaya Zapadnaya Bay. These thalluses were entwined in the horizontal cable ropes so that each 10-cm portion of the rope bore 3-5 entwined thalluses.

Fig. 6 Symbiotic association of algae and bacteria. A - *Laminaria saccharina*; B - *Fucus vesiculosus* L.; C - bacteria, which live on algae surface; some of them oxidize hydrocarbons.



A. B. C.

All assembling works were performed at the temperatures between +2 and +5ºC. We entwined 750-1000 fucus thalluses in each cable rope. An estimated 12000-16000 fucus thalluses were entwined during plantation setup.

To speed up colonization of fucus thalluses by microorganisms, they were soaked in the barrels with seawater, enriched with natural mix of HOM cultures, OP-destructing microorganisms and nutrients.

Ten cable ropes with entwined fucus thalluses were delivered to the plantation in the barrels with seawater. These ropes were then secured on the water surface in horizontal position by previously installed vertical load-carrying ropes with floats.

III.2.3 Preparation and mounting of vertical slings with laminaria

Nylon slings with 5-m length and 5-6 mm diameter were used for cultivation of laminaria. These slings were washed off in seawater in special pervious bags and soaked in the barrels with seawater and prepared suspension of laminaria spores for 24 hours. Special object-plates were inserted in these barrels to observe the process of adhesion and development of laminaria spores. After 24-hour exposition, we found out that some spores not only adhered to the object-plates but also sprung. Therefore, we had every reason to believe that laminaria spores adhered in the same way to the surface of nylon slings and sprung there.

Fig. 7 Entwining of fucus thalluses into the nylon substrate. Each of the 25-m cable ropes bore about 1000 thalluses.

The nylon slings with laminaria spores were transported to the plantation in the barrels with sea water. These slings were fastened on the horizontal cable ropes one meter apart from each other. We tied metal weights to each sling to keep them in vertical position. This prevented crossing and entangling of the slings during stormy weather. A total of 300 vertical slings were seeded with laminaria spores and fastened on 12 horizontal cable ropes. Additional floats were then tied to the horizontal ropes to keep them afloat.

Although we entwined fucus thalluses in the ropes, we did not entwine young laminaria sporophytes in the same way for two reasons:

1) There are very few young laminaria sporophytes in the fall, and they are hard to find. Young sporophytes do not develop in the fall because of very short daylight period and frequent storms.
2) Frequent storms reduce the probability of survival of young sporophytes.

This is why we chose spore seeding instead of sporophyte entwining procedure. This option adequately modeled massive natural seeding of spores in the fall season. Voskoboinikov et al (2005) have previously implemented and described the procedure of artificial seeding of laminaria spores. Yet, we entwined young laminaria sporophytes into some vertical slings six months later, during the spring season.

Fig. 8 Horizontal cable ropes with entwined fucus thalluses are mounted on the plantation

III.3 Melioration measures and monitoring of experimental plantation and marine environment

III.3.1 Melioration measures

We implemented two types of melioration measures during cultivation of algae. The first type is routinely scheduled measures, performed regularly once in 2-4 weeks. The second type includes emergency measures - repairs of plantation elements in case of their breakdown.

Fig. 9 Biofiltering plantation in Olenya Bay in April of 2008. On the background: the warships anchored in the mouth of Olenya Bay regularly pollute the bay with oil products.

Melioration activities were undertaken during the whole period of project implementation. These measures included tightening of horizontal ropes, which was especially important in spring and summer, when biomass of algae and encrusting organisms rapidly increased. To create the required buoyancy and keep the ropes afloat, we fastened about 100 additional bobbers and floats during project implementation. We also liquidated occasional crossovers of cable ropes after storms to prevent wear and tear of algae. Some fucus algae were torn out of their substrate after winter storms. We patched such fragments with new plants. It was important to remove encrusting plants, because biofouling hindered development of laminaria sprouts.

III.3.2 Monitoring

III.3.2.1 Monitoring of changes in water quality near the plantation during the experiment

We observed the same seasonal changes as in other bays of Barents Sea during monitoring of seasonal dynamics of water temperature, clarity, and chemical composition. The only difference was related to water salinity. During the spring and summer period, water salinity of the surface layer in the central part of the bay dropped to 33.2 points. At the same time, water salinity in the coastal part of the bay dropped to 29.5 points, because of seasonal increase in freshwater yield.

We measured hydrodynamic characteristics of water movement at the experimental plantation with plaster balls immediately after the construction of the plantation. We repeated these measurements in June.

These measurements showed nonlinear increase in water mobility near the surface. The same trend was observed in all measurement points across the plantation.

Hydrodynamic activity in the upper one-meter layer of water was generally more intense than in any subsequent layer underneath it. Since water movement accelerates metabolism of macrophytes, most of the biomass of the experimental plantation concentrated in the upper one-meter layer. This seemed to be a reasonable design choice.

Seasonality of water mobility has changed after construction of the plantation. For example, before construction, water mobility in June was the same as in November. After construction, water mobility in June had become lower than that in November, as we had expected.

Reduced water mobility after construction of the experimental plantation led to greater accumulation of oil products by algae. Another reason for greater accumulation of oil products was presence of the floating workshop *Shaber* in the coastal part of the plantation. This part of the plantation also had higher concentrations of suspended solids.

Fig. 10 Preparation for monitoring of hydrodynamic regime using plaster balls

We carefully monitored the concentrations of oil products. In the fall and winter seasons, OP levels exceeded MAC only slightly. In the end of April, however, OP levels exceeded MAC more than five-fold. We attributed this increase to more intense navigation during this time of the year. In the summer, we frequently observed continuous oil film on the water surface in Olenya Bay, which was attributed to massive discharges of oil products in the bay. Even though east winds drove oil film from Yekaterininskaya Harbor to the bay as usual, this was not the main source of oil pollution that summer. The main source was a stranded ship on the coast of Bezymiannaya Harbor, which spilt more than one ton of oil products in Olenya Bay.

Visual observations and analysis of photographs of the plantation during June-August proved that most oil products tended to concentrate in the open-sea part of the plantation. The outer cable rope was covered with oil products of various stages of degradation: from oil film to oil foam. Only relatively small oily spots usually surrounded the inner cable rope.

Table 7 reports concentrations of OHC near the workstation during summer. Immediately after the oil spill of 23.06.08, OHC concentration exceeded MAC hundredfold.

Table 7. Oil hydrocarbons in the water at the workstation

Date of analysis	20.11.07	21.12.07	25.02.08	23.04.08	23.06.08	30.08.09	MAC
OHC concentration, mg/l	0.03	0.09	0.12	0.16	8.0	7.2	0.05

The measurements of hydrodynamic regime by plaster balls method showed that tidal currents caused periodical changes in the direction of water flow through SAP. We registered 28 such changes during 14-day period of observations. The oil spot periodically advanced on the plantation and retrieved back during such tidal movements. Oil products were trapped in the coastal part of the plantation during such tidal movements and settled on fucus plants. The reversible back and forth currents redistributed OP and facilitated biological digestion of OHC in the less polluted parts of the plantation.

The complex interactions of SAP with the marine environment depend upon weather conditions, which greatly influence hydrodynamic regime and tidal currents. Tidal currents in Kola Bay have half-day periodicity. Table 8 shows changes in the velocity of water movement inside SAP, measured between the tides, when the rip was less than one point and the wind-induced water setup was relatively small.

Table 8. Velocity of water movement at different depths and distances from the entrance section of SAP

SAP part	Probe depth, m	Distance between the entrance section of SAP and the probe (m) and water flow velocity (cm/s)			
		0	**5**	**10**	**15**
1	***2***	***3***	***4***	***5***	***6***
Inner (coastal)	0.2	32.5	27.5	26.6	16.1
	3.0	19.5	19.6	15.0	13.0
	7.0	14.5	22.8	14.7	9.2
	10.0	14.3	17.3	-	-
Outer (open-sea)	0.2	58.5	23.8	28.3	12.2
	3.0	52.3	17.6	20.2	9.3
	7.0	45.2	14.8	22.2	10.5
	10.0	38.8	14.9	-	-

The direction of currents at the workstation changes periodically. The tidal and wind components of water currents usually have opposite directions. During strong wind, intense rip and high water, the interactions between the marine environment and SAP become quite complex and cannot be described by one-dimensional model. The transition to the turbulent state is characterized by emergence of upward currents and variations of current direction with depth. Such transition increases the stress on algae. Turbulent currents make the thalluses move in oscillatory manner in horizontal and vertical planes, instead of simply being deflected by linear current. These changes intensify transport of OP and equalize concentrations of pollutants across SAP.

III.3.2.2 Analysis of changes in plantation biota during the experiment

III.3.2.2.1 *Fucus vesiculosis*

Morphological and functional characteristics

Algae do not grow during winter season. In winter, their respiration dominates over photosynthesis. Photosynthetic rate in winter is so small that it can be registered only by isotopic method. When the daylight period becomes longer in February, photosynthetic activity increases and fucus begins to grow. Photosynthetic rate in February was greater than that in October and November, when the plantation was originally constructed. We monitored the dynamics of algae growth during the project implementation period. In spring, average length of fucus thalluses increased by 9 mm during 60-day period (March and April). Increase in plant length was accompanied by development of new apexes. Average weight of fucus plants increased by 0.3 g during the same period.

Plantation growth of algae was more intense than natural growth in the littoral area. During May and June, the average weight of individual plant at the experimental plantation increased more than fivefold, while the average weight of the plants growing in the littoral increased only threefold. At the same time, the length of fucus plants increased by 20-40%. Each plant developed between one and four new dichotomous branchings. Some plants developed even six or seven branchings. The increases in length of plantation and littoral plants were approximately the same; no statistically significant differences have been observed. At the same time, plantation plants had more lateral branches than littoral plants. The degree of stooling was significantly greater for cultivated plants. This phenomenon may explain the difference in weight between plantation and littoral plants with equal length.

In August and September, the mean weight of cultivated fucus plants aged 3-5 years reached 16 g.

Microbiological studies

Bacterial counts in the water and on the surface of fucus plants remained the same during winter and spring (December-April) as in October, when the plantation was constructed. In June, bacterial counts increased significantly. Saprotrophic bacterial counts on fucus, which was taken from the outer ('dirty') cable rope, were 10-100 times greater than those for the inner ('clean') cable rope. The counts of HOB living on 'dirty' fucus were 100-1000 times greater than those for 'clean' fucus. The same relative differences in bacterial counts were observed in August.

Table 9. The dynamics of populations of OHC-oxidizing plankton and epiphyte bacteria on the surface of plantation fucus plants during the experiment

Sampling date		20.11.07	21.12.07	25.02.08	23.04.08	23.06.08	30.08.08
The counts of OHC-oxidizing bacteria	Plankton, (bc/ml)*	4000	2000	2000	30000	68000	74000
	Epiphytes (bc/cm^2)	6000	5000	3500	5000	72000	79000

*) bc=bacterial clumps

Biochemical research

Between November and April, the content of alginate in algae decreased from 22% to 10% and the content of fucoidane decreased from 13% to 4%. Between April and June, we did not observe any accumulation of polysaccharides in algae. In August-September, the content of carbohydrates in algae tissue increased to 20% (alginate) and 16% (fucoidane). All percentages are calculated for dry weight. The content of polysaccharides in plantation algae was similar to that in one-year-old algae plants growing in natural conditions. The dynamics of changes in polysaccharide content agrees with the theory of morphological, functional and biochemical changes (Titlyanov et al 1987; Voskoboinikov 2006). This theory put forward the concept of General Life Function (GLF) of the plant. In August, GLF directs morphological, functional and biochemical changes in the plant to help it survive during the dark winter period, when respiration dominates over photosynthesis. In particular, the plant accumulates carbohydrates. In winter, the plant consumes accumulated carbohydrates during respiration. In spring and summer, GLF directs the plant to grow its thallus, and carbohydrate consumption makes up for energy loss during photosynthesis.

Measurement of levels of oil products

The levels of OHC in algae, measured in December, February and April, did not show any statistically significant seasonal differences. Rapid increase in concentrations of oil products in fucus algae was detected in June, after a major influx of OHC in Olenya Bay.

Fig. 11 Fucus plantation cleans up surface waters from oil pollution

Visual inspection of the plantation showed that the surface of fucus plants in June was covered with oil film. When we lifted the cable rope, oil products partly flew down, and partly remained on the surface of fucus plants. When we touched the plants, our hands got smeared with oil film. The plants had a specific smell.

We measured the levels of OHC in ‘dirty’ algae collected from the outer cable rope of the plantation. Total content of OHC was 6956 mg/kg, of which 3238 mg/kg was in the plant tissue and 3718 mg/kg was on the plant surface.

Fucus plants, entwined in the horizontal cable ropes in the coastal part of the plantation, contained less OHC. They accumulated 3420 mg/kg of OHC. Such measurements were repeated in August and September (Table 10).

Table 10. OHC levels in fucus algae during the experiment

Sampling date		20.11.07	21.12.07	25.02.08	23.04.08	23.06.08	30.08.09
OHC content in fucus algae	Outer cable rope	2.134	1.80	1.76	2.24	6956	6700
	Inner cable rope	2.134	1.72	1.74	2.0	3420	1180

The samples of fucus algae were delivered to Mendeleev Metrology Institute in Saint-Petersburg (VNIIM) for chromatographic analyses. These analyses proved the ability of fucus algae to biologically accumulate hydrocarbon fractions of oil products. During such analyses, we compared OHC levels in the tissue of fucus plants collected from the outer cable rope of the experimental plantation with OHC levels in the tissue of fucus plants collected in relatively clean open-sea part of the littoral of Olenya Bay. Table 11 summarizes the results of these measurements.

Table 11. Assimilation of OHC by *Fucus vesiculosis* algae. After sampling, fucus plants were stored in a refrigerator at 6ºC and relative humidity 98%

	Sampling date and location	OHC concentration in the surface and in the tissue of fucus plants		
		Total content, mg/kg	Content on the plant surface, mg/kg	Content in plant tissue mg/kg
1	Sample 1.Clean area (Olenya Bay, 24.06.08),	Traces	0	<50
2	Sample 2. The experimental plantation, one day after sampling (25.06.08)	6956	3228	3718
3	Sample 2. The experimental plantation, seven days after sampling (02.07.08)	1084	832	252
4	Sample 2. The experimental plantation, 14 days after sampling (09.07.08)	807	25	782

These measurements showed that total OHC levels on the plant surface decrease non-linearly with time elapsed from the sampling day. The rate of assimilation of OHC by fucus plants decreases as the total mass of OHC accumulated on the thalline surface decreases. This rate also decreases as the plant tissue gets saturated with the products of OHC metabolism. It is likely that fucus plants can be saturated with OHC only to a certain limit, but metabolism of assimilated OHC eventually restores the capacity of algae to resorb OHC.

The data reported in Table 10 prove the ability of fucus algae to digest oil hydrocarbons. Consequently, this result opens up a possibility to use fucus algae for biodegradation of oil films.

The measurements of OHC levels in fucus plants provide the basis for preliminary assessment of biofiltering properties of a sanitary algae plantation. After seven-day storage in a refrigerator, the fucus plants metabolized 3466 mg/kg of OHC at the average daily rate of 495 mg/kg/day. During the second week of storage, the daily rate dropped considerably, and the plants metabolized 252 mg/kg of OHC, at the average daily rate of 36 mg/kg/day.

III.3.2.2.2 ***Laminaria saccharina***

Morphological and functional characteristics

During construction of the experimental plantation, we used the described above sowing method. Laminaria spores were sewn on the vertical slings. These spores sprouted and developed young sporophytes, which grew to the length of several centimeters during the period of the experiment. The laminaria thalluses, germinated from spores in November 2007, had the following morphological parameters by the end of August: weight 26±9.2 grams, leaf length 32 cm, leaf width 9 cm and leaf area 288 cm^2. Each vertical sling supported about 150 laminaria plants on average.

Along with spore sowing, we entwined young laminaria sporophytes in the vertical slings in April. The divers collected these young plants from the depths of 5-7 meters at the mouth of Olenya Bay. Young sporophytes successfully adapted at the plantation. Between April and June, their weight increased fourfold, and their length increased twofold. Photosynthetic production of laminaria increased twofold during the period of the experiment and reached 0.48 mg of carbon per gram of wet weight per hour. In August and September, the entwined laminaria sporophytes measured 290±36.2 of wet weight, 98±35 cm in leaf length, 25 cm in leaf width, and 2450 cm^2 in leaf area.

Levels of oil products

The young naturally growing laminaria sporophytes, which were collected for entwining, initially contained 0.08 mg/kg of OHC, which was just above MAC for seawater. After several major oil spills in June and August, laminaria contained 725 mg/kg of OHC, which was still lower than the corresponding value for fucus algae. Unfortunately, we could not differentiate between the levels of OHC in laminaria tissue and thalline surface because of technical difficulties.

Fig. 14 Visual examination of laminaria thalluses

Biochemical analysis

We measured carbohydrates in laminaria thalluses in June and late August. The levels of alginate and mannite in June were 16% and 12%, respectively. In late August, they increased to 26% and 22%, respectively. General life function theory predicts that accumulation of carbohydrates in the fall season is regulated endogenously; the plant gets ready for spore-bearing and long polar winter.

III.4 Discussion and Appraisal of Project Results

III.4.1.1 Harvesting and utilization of algae

Harvesting, transportation and utilization of algae took place between September 15 and September 30.

Fucus

By the end of the project, there were 12600 fucus plants of 3-4 years of age at the plantation. Total weight of fucus plants was 201.6 kg.

The horizontal cable ropes supported a large quantity of fucus sprouts, but we did not include these sprouts in our estimates.

The pilot project demonstrated that fucus algae, which come in contact with oil film on the water surface, contain oil propagation and absorb oil pollution. The cable ropes themselves were highly polluted, and there was little sense in leaving them on the project site for reuse after completion of the project.

We cut fucus plants off the ropes, and packed them in polypropylene bags with 100-200 liter volume. Our experience showed that it was convenient to cut the ropes and pack them together with fucus plants in polypropylene bags.

We detected high levels of oil products on the plant surface and in plant tissue. The contaminated fucus plants were incinerated, while a small portion of the plants (20%) was dried up for further investigation of their utilization options. After drying, these plants contained 5-8% of moisture. They were tested for alternative disposal technologies.

This stage of the experiments showed that:

1) The staff harvesting the polluted algae should work in rubber gloves, resistant to organic compounds. We recommend to collect the plants in 200-liter polypropylene bags.
2) The plants could be simply harvested in a cargo vessel without any polypropylene bags. In this case, vessel bottom and boards should be covered with a fishing net and a layer of polyethylene to prevent vessel contamination.
3) The plants are transported ashore and utilized by incineration in furnaces. An alternative solution (drying and fabrication of fuel brickets) proved inefficient from economic

standpoint. We also tried to clean fucus plants from the accumulated oil products for further utilization of clean plants. This option was not economically viable either. Fucus plants were incinerated at Murmansk incinerator *Monogar Ltd*.

Laminaria

We collected laminaria plants from the slings mounted on the nearest (coastal) and the farthest (open-sea) cable ropes. These plants were then analyzed for chemical and morphological parameters. We also analyzed bacteria, which lived on the surface of these plants.

An estimated 9200 laminaria plants aged one year and older grew on the plantation by the end of the experiment. Their total weight was 2668 kg and their total surface (double-faced) was 9016 m^2.

An estimated 30000 laminaria plants under one year of age grew at the plantation by the end of the experiment. Their weight was 780 kg, and their total surface (double-faced) was 1060 m^2.

These estimates provided the basis for calculation of potential harvest. Laminaria plants with total wet weight 2660 kg and humidity 90% were transported ashore for drying and chemical analyses. We measured concentrations of mono- and polysaccharides; the levels of environmental pollutants, and tested alternative utilization options.

We left some laminaria plants intact on the plantation. To be specific, we left the young plants and one or two grown-up sporophytes on every tenth sling for natural spore-seeding of laminaria.

Harvesting of laminaria is a labor-consuming process, because it cannot be mechanized. We harvested laminaria from a motor boat “Dori” using a cable hoist and a cargo boat. It is best to cover the bottom and the boards of a cargo boat with a cargo net. We lifted the vertical slings out of the water, cut off laminaria thalluses and put them in the cargo net. A cable hoist was the best option for unloading of laminaria from the cargo boat.

Figure 15. Drying of fucus and laminaria plants in a ventilated camera

We measured the dimensions and wet weight of harvested algae plants ashore. After these measurements we dried the plants for chemical measurements of content of polysaccharides, heavy metals, oil products.

After the experiment, plantation was partly liquidated because it could obstruct navigation. The remaining part of the plantation (0.25 hectares) was handed over to "*Biofriz Ltd*.", a subsidiary of SRY *Nerpa*, under the agreement on further development of the pilot project. This part was to become a test site for new experiments in marine oil pollution cleanup technologies.

III.4.1.2 Recommendations for processing of clean algae for extraction of commercial products

Presently there are no applicable sanitary standards for maximum allowable concentrations of oil products in marine macrophytes in Russia and abroad. Tutelian and co-authors (1999) proposed to use 0.1 mg/kg standard as a MAC of OP in marine life which is used for production of bioactive additives (BAA).

Processing of fucus algae

The main purpose of biofiltering plantation is to clean up marine environment from moderate oil pollution (i.e., when the environmental concentrations of OP are just above MAC levels). It is expected that, under normal operating conditions at the plantation, the concentrations of oil products in seawater would stay below MAC for extended periods of time. In this situation, fucus algae may be subsequently utilized for production of BAA, because the bioaccumulated levels of OP would be quite modest. The following recommendations should be observed in this case:

1) Only the plants aged over 5-6 years are harvested, because such old plants have lower metabolic activity and lower levels of bioaccumulated OP.
2) The plants are dried in two steps: preliminary airing and main phase.
3) Harvested algae plants are placed on a stone floor or a grass lawn and aired off in open air.
4) The plants are overturned every 8-12 hours and dried until the moisture content falls down to 23-25%.
5) The main phase of drying takes place in a ventilated camera, where the plants are dried until the moisture content falls down to 12-14%. These dried plants are suitable for long-term storage. The recommended temperature in a ventilated camera is 60-70ºC. It is important to prevent gluing or blocking of algae plants. Long-term storage of minced algae with moisture content above 14% is not recommended, because a variety of microorganisms develop on the plant surface and eventually destroy the product.
6) Dried algae are minced in a grinder in 0.2-0.5 cm pieces, packed in 16-kg kraft packs and stored in a dry place.

Processing of laminaria algae

1) Laminaria thalluses are transported ashore, where their leaves are separated from stipitates. The leaves are cleaned from biofouling and encrusting organisms.
2) The leaves are hanged on hargers or left on stone or grass floor for preliminary airing, which takes 1-3 days. The leaves have to be regularly overturned during airing.
3) The climate at Barents Sea is rather humid, which precludes complete drying of leaves in open air. Final drying takes place in a ventilated camera until the moisture content drops to 12-14%. We used common heater fans for drying.
4) Laminaria stipitates are dried in the same way.

5) Several alternative dryers are available for final drying, including infrared dryers. Infrared dryers can be efficiently used only after preliminary airing of laminaria.
6) Laminaria leaves are either minced in grinders until coarse-grained texture, or grinded until fine powder texture.
7) Fine powder fraction is packed into inner polyethylene bags and then in outer kraft bags, while coarse-grained plants and laminaria stipitates may be simply packed in kraft bags.
8) High water-absorbing (hygroscopic) capacity of grinded laminaria should be considered during drying and storage.
9) Laminaria leaves, coarse-grained or finely powdered products can be sold to final consumers - companies which produce bioactive additives and pharmaceutics. All commercial goods should meet sanitary standards, including the applicable maximum allowable level/concentration standards. Russian Federal standard "SanPin 2.3.2.560-96" sets the following maximum allowable concentrations/limits for such products:
   - Colonies of mesophilic anaerobic facultative microorganisms $5 \cdot 10^4$ CFC/g
   - Coliforms in 1 g sample – not allowed
   - Pathogenic microflora including salmonella in a 25-gram sample– not allowed
   - Fungus and mildew, 100 CFC/g
   - Lead 0.5 mg/kg
   - Cadmium 2.0 mg/kg
   - Arsenic 5.0 mg/kg
   - Copper 30 mg/kg
   - Zinc 200 mg/kg
   - Mercury 0.1 mg/kg
   - Cesium-137 200 Becquerel/kg
   - Strontium-90 100 Becquerel/kg

We measured these parameters in dried fucus and laminaria and certified that our products complied with all applicable standards. Below we summarize the results of quality control measurements:

Sanitary standard	*Laminaria saccharina*	*Fucus vesiculosis*
Colonies of mesophilic anaerobic facultative microorganisms, CFC/g	0	0
Coliforms in one-gram sample	Not found	Not found
Pathogenic microflora including salmonella in a 25-gram sample	Not found	Not found
Fungus and mildew, 100 CFC/g,	Not found	Not found
Lead 0.5 mg/kg	0.36 mg/kg	0.24 mg/kg
Cadmium 2.0 mg/kg	1.8 mg/kg	1.0 mg/kg
Arsenic 5.0 mg/kg	4.1 mg/kg	2.4 mg/kg
Copper 30 mg/kg	18 mg/kg	24 mg/kg
Zinc 200 mg/kg	180 mg/kg	120 mg/kg
Mercury 0.1 mg/kg	Less than 0.1 mg/kg	Less than 0.1 mg/kg
Cesium-137 200 Becquerel/kg	46.2 Becquerel/kg	20.4 Becquerel/kg
Strontium-90 100 Becquerel/kg	32.4 Becquerel/kg	26.2 Becquerel/kg

The most profitable solution in utilization of kelp, fucus and brown algae is combined extraction of several biologically active substances. These substances are used as raw material for production of drugs and BAA.

Table 12 summarizes the results of chemical analyzes of dried algae after plantation cultivation.

Table 12. Chemical composition of clean laminaria and fucus algae, which were cultivated at the experimental plantation

Algae	Percentage content in dry weight								
	Alginic acid	Laminarane	Fucoidane	Mannitol	Organic matter	Minerals	Nitrogen	Oidine	Fibers
L. saccharina	18-26	12-14	6-8	20-22	55-68	27-35	8-14	0.12-0.24	15-16
F. vesiculosus	17-21	0.5-1.0	12-16	6-9	56-72	18-22	4-13	0.10-0.12	8-10

Chemical composition of plantation algae is similar to that of thalluses of the same age, which grow in natural substrates in Olenya Bay.

III.4.2 Additional experiments to assess effectiveness of cleanup of oil pollution with symbiotic plant association: brown algae and hydrocarbon oxidizing bacteria

In situ studies performed during implementation of this pilot project were quite time-consuming, especially taking in account environmental conditions at the Barents Sea coast. Rapid changes of air temperature and wind, wind currents and waves, tidal dynamics – all these factors can be regarded as extreme working conditions. Relatively calm days provided rare opportunity for sampling of algae plants and water, for hydrodynamic measurements. Additional measurements were conducted in chemical labs in Murmansk Institute of Marine Biology and Dalnie Zelentsy biological station, in Moscow State University and Saint-Petersburgh Marine Institute.

These measurements confirmed efficiency of sanitary algae plantation in marine oil pollution cleanup. Table 13 lists the selected microbiological indicators, which characterize the populations of HOM on the surface of fucus and laminaria algae, and their capacity to purify seawater from oil products. Table 13 provides the basis for assessment of relative roles of fucus and laminaria algae in oxidization of hydrocarbons, or, rather, the roles of supported populations of symbiotic microorganisms.

Table 13. Populations of HOM on the surface of fucus and laminaria algae

Sample	Hydrocarbon oxidizing bacteria in marine mineral media			Saprotrophic bacteria in Zobell solution		
	growth	Mean value, bc/ml*	Mean value, bc/cm^2	growth	Mean value, bc/ml	Mean value, bc/cm^2
1	*2*	*3*	*4*	*5*	*6*	*7*
Polluted water near the pier	10^1 10^1	50		10^2 10^3	1200	
Clean water taken from relatively clean coastal area	10^2 10^1	120		10^2 10^3	1200	
Fucus sample taken from relatively clean coastal area	10^1 10^1		125	103 10^2		2987
Fucus algae taken from polluted area near the pier	10^3 10^2		1728	10^4 10^4		71984
Laminaria taken from relatively clean coastal area	10^2 10^1		150	10^3 10^3		313
Laminaria taken from polluted area near the pier	10^1 10^2		150	10^3 10^2		1500

*) bc=bacterial clumps

All calculations below are based on the values reported in Column 4 of Table 13. An estimated 6250 bacterial clumps may live on each square centimeter of laminaria surface. This estimate includes 150 bc/cm^2 of hydrocarbon oxidizing bacteria. Given the mean area of two-year-old laminaria leaf (3600 cm^2), we estimated that each two-sided leaf supports 22500000 bacterial clumps, including 540000 clumps of HOB. Average fucus thallus supports eleven times more HOB clumps than average laminaria thallus.

Comparative studies of laminaria algae from polluted and clean areas showed that the former supported four times more HOB than the latter. In real life, a significant fraction of HOB live in the boundary layer of the leaf. This layer may reach up to 2 cm, depending upon the leaf size and the current flow velocity.

The results reported in Tables 13 and 14 provided the basis for calculation of hydrocarbon oxidization capacity of HOB living on fucus and laminaria algae. Our estimate is conservative, in a sense that real hydrocarbon oxidization capacity could be even greater.

The next step in our experiments was to determine oxidizing capacity of epiphyte HOM, which use algae thallus as a substrate. In our experiments, we used ultrasonic waves for desorbtion of bacteria. We took the thalluses with the weight of 1.78-1.90 grams, washed them in 3% sterile NaCl solution, and placed them in a sterile 100-ml glass with 50 ml of sterile marine mineral media. After ultrasonic treatment of this sample, fucus was cleaned and removed from the glass, and the remaining mixture was poured in a half-liter shaking bath, which contained 150 ml of marine mineral media. Then we added 1 ml (0.76 g) of sterile diesel fuel, which modeled oil pollution.

The shaking-bath was incubated at 20ºC for three weeks, and then the remaining DF was extracted with carbon tetrachloride, and settled for a day. The lower layer of the extractant was analyzed at the spectrophotometer. The results of these analyses are reported in Table 14.

Table 14. Laboratory measurements of oxidizing capacity of native HOM.

Sample number	Sample description	Weight of fucus sample	Remaining concentration of diesel fuel in shaking bath, mg/l	Diesel fuel consumption, %
1	*2*	*3*	*4*	*5*
1	Clean fucus	1.80	40.0	40.0
2	Clean fucus	1.78	38.0	42.0
3	Polluted fucus	1.76	33.0	49.0
4	Polluted fucus	1.94	33.0	49.0
5	Polluted fucus	35.0	0.174	63.0

Notes to Table 14:

1) Sterile diesel fuel (DF) with initial concentration of 65.0 mg/l was used to simulate oil pollution.
2) Epiphyte HOM were collected by desorbtion from the surface of *Fucus vesiculosis* algae.
3) Sample 5 was prepared in a different way, than samples 1-4. Fucus algae were collected in a polluted area of the sea and put in 100 ml of DF solution with initial concentration 0.316 mg/l.

Table 14 illustrates the ability of epiphyte microorganisms to destruct oil products in laboratory conditions. Maximum consumption of DF during three-week exposition was 49% of the control. Microorganisms desorbed from polluted fucus had higher oxidizing capacity than microorganisms desorbed from clean fucus. The difference reached 7-9% of DF consumption. Incubation of fucus algae in the polluted seawater increases its OHC-oxidizing property. Therefore, preliminary treatment of fucus with small quantity of DF intensifies the process of biological oxidization of oil products.

Based on the results of this laboratory experiment, we recommend preliminary treatment of cable ropes with fucus algae and HOM in pools with polluted seawater, before SAP construction.

Clean fucus was able to absorb DF at varying concentrations of the latter in the pools and in the glass vessels in a controlled thermostatic environment (Table 15).

Table 15. Results of laboratory tests of capacity of fucus plants to clean up seawater

Sample description and incubation period	Concentrations of oil products in seawater (mg/l) and algae (mg/kg)					
	Pure water	Water + fucus	Water+DF	Water +fucus+DF	Water +fucus+DF	Water +fucus+DF
Test No.	1	2	3	4	5	6
Control Water/fucus	0.05	0.05	1.54	1.53	6.42	12.24 / 0.165
3 days	-	-	-	0.41	1.9	-
7 days	0.05	0.034	1.54	0.26 (0.32)	0.72	2.84 / 5.62
14 days	0.05	0.03	1.52	0.06 (0.09)		0.09 / 8.68
21 days	0.05	0.03	1.54	0.032(0.04)	0.04 (0.8)	-

Notes to Table 15:

1) MAC for OP concentration in seawater is 0.05 mg/l.
2) We used three-year-old thalluses with 6-7 dichotomous branchings.
3) We used summer grade diesel fuel.
4) The tests were performed in three-liter battery glasses in magnetic mixers at 8-10ºC.
5) We added DF added to the glass, intensely mixed it and let it settle for 24 hours, then we removed the surface film.
6) The numbers in brackets in columns 4 and 5 are the results of two repetitions of the same test.
7) Column 6 reports the ratios of DF in water and DF in fucus thalluses.

Table 15 illustrates that the adsorption rate in the system ‘water-DF-fusus’ is significantly higher than that in the system ‘water-OP-fucus’. This is probably related to better conditions of interaction between fucus and DF. We observed reduction of adsorption capacity in the system ‘water-DF-fucus’ on the last week of the experiment, just like with the system ‘water-OP-fucus’. If average rate of DF adsorption during the first 10 days of the experiment was 0.38

mg_{DF}/kg_{FP}/day, then the same rate during the next seven days was only 0.31 mg_{DF}/kg_{DF}/day, or 18% less. This trend was more pronounced in the system 'water-DF-fucus'.

The latest experiments showed that younger sections of fucus thallus have greater adsorption capacity. This is likely explained by higherer metabolic rate of younger sections of fucus thallus. We observed the reduction of metabolic rate of fucus thalluses older than 5-6 years. Therefore, we recommend to use fucus thalluses aged 3-4 years for the most efficient neutralization of oil products.

When marine vessel Rebecca sank in Dalnezelenetskaya Bay, diesel fuel started to penetrate in the bay and formed continuous film around the vessel. This accident provided an opportunity to test oil film containment properties of sanitary algae plantation. The staff of Sirena Ltd. and Murmansk Institute of Marine Biology constructed SAP and isolated the accident site (Figure 16). They entwined fucus plants in the cable ropes anchored around the sunk ship.

The effect of oil film containment was observed in the experiments with in-line modules (Figure 17). In these experiments, we observed a rapid decrease of oil film containment capacity of fucus plants at the wind speed above 5 points. Probably, this drawback can be overcome by denser entwining of fucus algae in the artificial substrate.

Fig. 16 Biofiltering algae strip around MV *Rebecca* which sunk in Dalnezelenetskaya Bay provided an example of successful containment of oil film

Peretrukhina at al (2006) observed a 20% increase of hydrocarbon oxidizing capacity of microorganisms in the boundary layer of fucus leaves adjacent to thalline surface, relative to the hydrocarbon oxidizing capacity of microorganisms, which live in the seawater. We confirmed this observation in our experiments, and regarded it as an important factor of assessment of SAP effectiveness. The reason for increase of hydrocarbon oxidizing capacity is photosynthetic activity of fucus. HOM living in fucus substrate receive more oxygen than HOM living in water column. In the result, symbiotic HOM have higher rate of metabolic rates.

Fig. 17 Experimental plantation in Dalnezelenetskaya Bay

CONCLUSIONS OF PILOT PROJECT "CLEANUP OF ARCTIC MARINE ENVIRONMENT USING BIOLOGICAL FILTRATION POTENTIAL OF BROWN ALGAE"

1) SAP has been correctly designed and set up to serve its main purpose (regarding the number and positions of anchors, ropes and floats).
2) Cultivation of algae requires meticulous implementation of melioration measures, especially in the spring and summer season.
3) SAP was an efficient instrument of containment and adsorption of oil film on the water surface.
4) Fucus algae not only adsorb oil products but also metabolize and neutralize them.
5) Hydrocarbon oxidizing capacity of HOM – epiphytes of fucus algae – increases in a marine environment, polluted by oil products, which should be taken in account during construction of SAP.
6) It is very likely that the hydrocarbon oxidizing capacity of microorganisms in the boundary layer of fucus leaves adjacent to the thallus surface increases by 20% relative to the hydrocarbon oxidizing capacity of microorganisms, which live in the water column.
7) Individual modules of SAP can be effectively used for containment of oil films and sustainable development of aquaculture in Barents Sea.

8) Our estimates of SAP effectiveness, based on both *in-situ* measurements and laboratory experiments, showed that one hectare of biofiltering plantation may neutralize about 100 kg of oil products per week.

III.4.3 Economic analysis of the proposed method. Dissemination of project results in littoral areas of Russian North, Far East and South

The valuation of economic benefits of avoided environmental damage from water pollution was based on regional unit costs of pollution. Such unit costs are the regional estimates of economic damage per one ton of effective mix of pollutants.

According to the Guidelines of State Committee on Environmental Protection (1999), the economic benefit, or avoided damage, AD_{nr} of a certain environmental protection activity *n*, implemented at an enterprise-polluter *k* in a region *r*, is estimated by Equation 1:

$$AD_{nr} = \Sigma (U_{rj} \times \Sigma M_{nk}) \times K_e \qquad (1)$$

Where:

U_{rj} is unit damage to water resources of region *r*, defined as economic damage associated with the discharge of one ton of pollutant mix in watershed *j*. The Guidelines (1999) recommend using the value U_{rj} = 5609.6 Rubles per ton of pollutant mix discharged in the watershed of Barents Sea;

M_{nk} is the adjusted (for toxicity) volume of avoided discharge of pollutants in the water basin *j* from enterprise *k* in the result of environmental protection activity *n,* measured in tons;

K_e is the weighing factor which is proportional to the overall environmental burden and environmental importance of the watershed. These values are specified in Table 1 of the cited Guidelines (State Committee on Environmental Protection 1999); K_e=1 for the watershed of Barents Sea.

The toxicity-adjusted volume of avoided discharge of pollutants is calculated by Equation 2:

$$M_{nk} = \Sigma m_i K_{ei} \qquad (2)$$

where:

m_i is the discharge of pollutant *i* (or a group of pollutants with the same coefficient of relative environmental toxicity), which was avoided in the result of implementation of environmental protection activity *n* at enterprise *k*, tons;

K_{ei} is the coefficient of relative environmental toxicity for pollutant (group of pollutants) *i,* specified in Table 2 of the cited Guidelines (State Committee on Environmental Protection 1999).

The coefficients of relative environmental toxicity for pollutant (group of pollutants) *i* are inversely proportional to maximum allowable concentrations of these pollutants in water reservoirs used for commercial fishing activities and specified in (Russian Institute for Fishing Industry and Oceanography 1999); K_{ei} =1/MAC.

The discharge of pollutant *i* is reported by an enterprise-polluter *k* in the annual environmental report “2TP-Water”. Alternatively, the volume (rate) of pollutant discharge can be directly measured by regional environmental surveillance authorities or their certified laboratories, hydrometeorology departments, domestic or international experts.

Table 16 summarizes the parameters used for estimation of avoided environmental damage in the framework of our pilot project.

Table 16. Raw data for calculation of avoided environmental damage

	Water pollutant						Total
	Cu	Ni	Pb	Cd	Zn	OHC	
1.MAC, mg/l	0.001	0.01	0.006	0.005	0.01	0.05	
2. Relative environmental toxicity coefficient (K_{ei})	1000	100	167	200	100	20	
3. Sorption coefficient (K_s), g/kg							
- maximum value	64	59	207	112	52	1920 g/m^2	
- value used in calculations	16	15	52	28	13	960 g/m^2	
4. Volume of avoided pollution (m_i), tons	0.48	0.45	1.56	0.84	0.39	9.6	13.32
5. Adjusted for toxicity volume of avoided discharge (M_{nk}), tons	480	45	260.5	168	39	192	1184.5

Sorption coefficient (K_s) for metals is calculated per 1 kg of algae, and K_s for oil hydrocarbons is calculated per 1 m^2 of plantation per year. In our calculations, we used the following values: total area of algae plantation is 5000 m^2, and total mass of cultivated algae plants is 30 tons.

The volume of avoided discharge of pollutant *i* is given by the following equation:

$$m_i = K_s S$$

where K_s is sorption coefficient, and S is the area of algae plantation. Monetary valuation of avoided environmental damage to water resources during the period of project implementation was calculated as the product of U_{uj} (5609.6 Rubles per ton of pollutant mix for the watershed of Barents Sea) and M_{nk} (total toxicity-adjusted volume of avoided discharge from Table 16):

$$AD_{nr} = 5609.6 \cdot 1184.5 = 6{,}644{,}571 \text{ Rubles.}$$

The unit costs of pollution were set by the cited Guidelines for year 1999. Total deflator of consumer price index in the Russian Federation between 2000 and 2006 was 2.48, according to Russian Ministry of Economic Development and Russian Committee on Statistics (Rosstat). Using the annual inflation rates for the same period, which gradually diminished from 19% to 8% p.a, we estimated this deflator for the same period as 2.32. Therefore, the unit cost of water pollution should be multiplied by CPI deflator, and the final estimate of avoided damage AD_{nr} is 13,289,000 Rubles, or about USD 415,000.

Commercial sales of laminaria algae, harvested at the experimental plantation, may generate ancillary economic benefit. We estimated this benefit as 450,000 Rubles, or USD 16,000-18,000.

The results of monetary valuation of economic effectiveness of the experimental algae plantation in the conditions of Russian Arctic seas are summarized in Table 17. These are the preliminary results, which have to be considered with caution. Interested organizations exploring feasibility of construction of biofiltering algae plantations will have to make more precise and specific calculations in the conditions of Barents Sea or elsewhere.

Table 17. Comparison of effectiveness of monoculture and biculture sanitary algae plantations

Scenario of plantation design	Main parameters							
	Depth of algae cultivation, m	Density of algae, plants per m^2	Mass of algae, kg/ m^2	Substrate area for epiphyte HOM[1], m^2	Density of epiphyte HOM, bacterial clumps per m^2	Decomposition of oil products by fucus[2], g/ m^2/day	Oxidization of oil products[3], g/ m^2/day	Total neutralization of oil products, g/ m^2/day
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Monoculture fucus plantation	0-0.3	90	27	9	$1727 \cdot 9 \cdot 10^4 = 1.55 \cdot 10^8$	13.5	220.0	233.5
Monoculture laminaria plantation	0.8-6.0	243	120	87	$150 \cdot 87 \cdot 10^4 = 1.3 \cdot 10^8$	-	180.0	180.0
Biculture fucus+ laminaria plantation	0-03 0.8-6.0	90 243	27 120	9 87	$1.55 \cdot 10^8$ $1.3 \cdot 10^8$	13.5	220.0 180.0	413.5

Notes to Table 17:

[1]) HOM = Hydrocarbon oxidizing microorganisms; the area is measured one year after construction of the experimental plantation; accuracy of this estimate is ±15%.

[2]) This parameter refers to direct inclusion of oil products in metabolism of algae.

[3]) This parameter is measured under the following conditions: surface current velocity is 0.5 m/s; concentration of oil products is 1 g/m^2.

The following conclusions follow from Table 17:

1. The main effect of SAP is attributed to utilization of natural symbiotic association "macrophyte algae + HOM".
2. The effectiveness of biculture SAP is two times greater than the effectiveness of monoculture SAP.
3. Direct metabolism of oil products by fucus algae at the temperatures 2-12ºC contributes only 6% to the total effect of monoculture fucus plantation.
4. The main advantage of monoculture fucus plantation is its ability to contain oil spill on the water surface and create OP-enriched environment for rapid development of HOM. Monoculture laminaria plantation does not have this property, because laminaria plants develop underneath the surface and do not have oil film on their leaves.
5. The proposed design of SAP can be implemented in south seas. The effectiveness of SAP in warm waters, at the temperatures about 25ºC, should be fivefold-sevenfold greater due to increase in oxidization rate.

The proposed design of SAP uses natural ability of fucus plants to thrive on any suitable substrate on seawater surface. Fucus plants are resistant to ultraviolet solar radiation, fresh water, negative air temperatures in winter, waves, storms and currents. Table 18 compares these vital parameters and habitats of several widespread species of fucus algae.

The scenarios in Table 18 do not consider positive effect of a 20% increase of hydrocarbon oxidizing capacity of HOM in the boundary layer, adjacent to the surface of fucus thalluses, compared to hydrocarbon oxidizing capacity of freely flowing HOM.

Table 18. Species of fucus algae suitable for marine oil pollution cleanup

Sea	Species	Habitat	Reference
1	*2*	*3*	*4*
Barents Sea, White Sea	*Fucus vesiculosus*	Littoral	Kuznetsov and Shoshina 2003; Voskoboinikov 2006
Ochotsk Sea, Sea of Japan	*Fucus evanescens*	Littoral Well-lit shallow waters	Klochkova 1998
Azov Sea Black Sea	*Cystoseira crinita* *Cystoseira barbata*	Well-lit shallow waters Up to 20-m depth	Blinova 2007; Kalugina-Gutnik 1975

Table 19 lists laminaria species suitable for cultivation under the water surface. Azov and Black Sea Basin is an exceptional case, because laminaria does not live there. We suggest to use fucus algae *Cystoseira barbata* instead of laminaria algae in this basin.

Table 19. Species of laminaria algae suitable for marine oil pollution cleanup

Sea	Species	Habitat	Reference
1	*2*	*3*	*4*
Barents Sea, White Sea	*Laminaria saccharina* *Laminaria digitata*	Depths between 1 and 25 m	Kuznetsov and Shoshina 2003
Ochotsk Sea, Sea of Japan	*Laminaria japonica* *Laminaria ochotnsis* *Laminaria longipes*	Depths between 2 and 25 m	Klochkova 1998
Pacific coast of Kamchatka, Avachinskaya Bay[1]	*Laminaria bongardiana*	Depths between 1 and 12 m	Klochkova and Berezovskaya 1997; 2001
Azov Sea Black Sea[2]	-	-	Blinova 2007; Kalugina-Gutnik 1975

Notes: to Table 19:

1) Avachinsksya Bay is the only polluted region in Kamchatka, and the proposed species is suitable for artificial propagation.

2) *Cystoseira barbata* lives at the depths up to 20 meters and may replace laminaria in this basin. Dry weight of *Cystoseira barbata* contains up to 35% of alginic acid, which chemically fixes heavy metals.

Cultivation of laminaria in water column under the surface provides substrate for microorganisms which destruct oil products. At the same time, laminaria purifies seawater from a variety of common pollutants which are typical for industrial wastewater effluents. The capacity of laminaria to adsorb heavy metals depends upon the content of alginic acid. Dried laminaria contains 15-33% of this acid by weight. For reference, dried fucus contains 9-28% of alginic acid. Growing algae absorb large quantities of ions of heavy metals, and this biological property is widely used for purification of coastal waters from industrial effluents (Kamnev 1989; Christophorova 1989).

The proposed design of sanitary algae plantation utilizes two different types of algae: fucus and laminaria. Fucus algae grows on the sea surface, where the concentrations of oil products are maximal. Laminaria grows underneath the surface and enriches seawater column with oxygen. Laminaria neutralizes the dissolved and emulsified fraction of oil products, and absorbs heavy metals. The system "laminaria+fucus" possesses natural self-recovering property and effectively cleans up oil pollution.

We constructed SAP in several stages, as described in this report. Implementation of the same design in other regions will require alterations and modification of the described method, depending upon local aquatic life, microflora and climate.

III.5 List of intellectual property generated by this research project

Several publications have discussed the results of this project:

Voskoboinikov GM, Stepanian OV (2007) Morphological and functional changes of macroalgae and forecast of development of coastal marine plant associations at Murmansk coast under the influence of oil pollution. In: Dynamics of marine ecosystems and current problems of preservation of biological potential of Russian seas. Dalnauka, Vladivostok, pp 392-417

Voskoboinikov GM, Ilyinsky VV, Lopushanskaya EM, Pugovkin DV (2008) Possible role of macrophytes in purification of seawater from oil pollution. Proceedings from International Conference “Oil and Natural Gas of Arctic Continental Shelf”. Murmansk Institute of Marine Biology, Murmansk, pp 63-66

Voskoboinikov GM, Makarov MV, Ryzhik IV, Korobkov VA (2009) Theoretical basis and perspectives of utilization of biofiltering plantation for cleanup of seawater from oil pollution. Murmansk, in print

Obluchinskaya ED (2008) Comparative chemical composition of the Barents Sea brown algae. Appl. Biochem and Microbiol 44:305-309

Obluchinskaya ED, Klindukh MP (2008) Rheological properties of natural polysaccharide solutions. Proceedings from International Conference “Current problems of algology”. South Scientific Center of Russian Academy of Sciences. Rostov-on-Don, pp 256-259

Patent application No. 2007106573/13(007130). Authors: Voskoboinikov GM, Korobkov VA and Makarov MV. Patent holder: Sirena Ltd. “A method of purification of coastal seawater from oil films and oil products, dispersed in the surface layer”. Approved patent priority date: 21.02.2007

III.6 Project information dissemination for implementation of biotechnology options for marine oil pollution cleanup

The results of this pilot project have been published in mass media and presented at several regional and federal radio programs and TV shows.

Formal presentations have been delivered at several scientific forums:

- International conference “Oil and Natural Gas of Arctic Continental Shelf”, Murmansk, 2008;
- International conference “Current problems of algology”, Rostov-on-Don, 2008;
- Academic council of Murmansk Institute of Marine Biology;
- Session of Environmental Protection Department of Administration of Murmansk Region.

Project implementing agency organized a field workshop for students of Natural Sciences Department of Murmansk State Pedagogical University at the project workstation in Olenya Bay.

IV. CONCLUSIONS

The importance of this project has been demonstrated during the three major oil spills, which occurred in Olenya Bay during 2007 and 2008. Ecosystems of Barents Sea experience increasing stress due to intense transportation, and construction of oil terminals and oil refineries on its coast. Construction of sanitary algae plantation facilitates protection of marine life from oil pollution. Successful implementation of this pilot project demonstrated effectiveness of the proposed solution. Biofiltering properties of algae have been confirmed during lab tests. The experimental algae plantation contained oil film, neutralized oil products and prevented spreading of oil pollution during oil spills.

We explored the ability of SAP to withstand extremely high loads of oil products. We proved that fucus algae retain vitality and effectively contain oil pollution even after major oil spills, when OP concentrations in Olenya Bay exceeded 8.0 mg/l (200 MAC). It is worth mentioning that fucus algae can live for several years in the littoral areas constantly covered with oil film. We also observed spills of oil film over the fucus “slick bars” during storms, when the rip exceeded 4-5 points on Beaufort scale.

The experimental SAP plantation worked for 18 months in very harsh environmental conditions and withstood several storms. Functional properties of SAP proved its ability to localize, contain and neutralize hydrocarbon pollution. The experimental plantation could have worked even longer.

A possible application of the proposed biofiltering method can be relevant for aquaculture economics, e.g. fish and crab farming, which becomes an important activity at Murmansk seacoast. The proposed method can be implemented at other seas, taking in account regional features.

This project showed the importance of geographical, hydrodynamic and hydrobiological features of the project site. A typical systemic approach to construction of sanitary algae plantations in different regions is described in the Appendix.

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APPENDIX. A typical systemic approach to construction of sanitary algae plantations in different regions

Introduction

A typical systemic approach to construction of sanitary algae plantations for cleanup of marine oil pollution in different regions should take in account geographical, hydrodynamic and hydrobiological features of the project site. Chemical characteristics of industrial effluents will differ from one site to another. Regional emergency environmental response systems also will react differently in case of emergency oil spills. These considerations limit the scope of application of standard approaches and make each project truly individual in a sense that it will inevitably differ from the original prototype. Even the species of algae will be different in each location. For example, since laminaria does not grow in Black Sea, it makes little sense to reproduce there the exact type of the plantation implemented in Barents Sea.

A typical approach should present a systemic approach to solution of the problems, which arise during selection of design of a prospective sanitary algae plantation, assessment of its functional capabilities and future scenarios of utilization of waste produced by such sanitary plantation. For example, important considerations should include the amounts of oil products neutralized per day, periods of regular replanting of new algae for most effective containment and oxidization of oil pollution. In the shallow waters, it will be important to maintain clean water space in case of pollution of land or sea bottom. Taking all the above considerations in account, the purpose of this Appendix is to propose a typical systemic approach to construction of SAP in various regions, rather than to propose a standard SAP design.

Exploratory design stage

2. Monitoring of seawater pollution is essential to clearly identify the goals of water purification efforts at the project site. The main function of biofiltering plantation is everyday preventive purification of seawater.
3. Assessment of geographical and hydrological properties of the project site is essential for correct design of anchoring system and stable orientation of SAP. Typical wind directions and marine current velocities should be considered at this stage to provide additional margin of stability for the anchored plantation. Alternatively, SAP orientation can be manually adjusted to withstand wind and

hydraulic forces during storms. SAP orientation and location will depend upon navigation routes, location of main sources of pollution, directions of dispersion of marine pollution. All these factors present certain limitations for design and construction of biofiltering plantations.

4. Implementation of the pilot project, described in this report, provided the basis for realistic estimates of total area of sanitary algae plantation, its uptime period, and waste generation.
5. Project feasibility study should include assessment of waste utilization options, available infrastructure and waste utilization capacities. Local waste utilization infrastructure may include incinerators, methane tanks for utilization of agricultural waste, thermal power plants fueled by coal and peat. Installation of own incinerators similar to those installed on ships for household waste combustion will most likely be unprofitable, because such incinerators usually have low combustion temperatures suitable for incineration of paper and cardboard.

The above considerations provide the rationale for identification of the basic module of SAP.

SAP design documentation

SAP design documents typically include:

1.2.1 Technical drawings for parts manufacturing and assembling;

1.2.2 Technical drawings of individual parts, similar to state standard GOST 21.501;

1.2.3 Specification of equipment of assembling works under GOST 21.110-95;

1.2.4 Specification of materials under GOST 21.110;

1.2.5 Other documents under the applicable System of project documentation for construction projects (SPDS);

1.2.6 Economic estimates and cost sheets in established formats.

Approvals

Our experience of project implementation in Barents Sea showed the need to obtain approvals for construction of engineering structures. The following authorities will have to approve the planned construction project:

1.3 The owner of the project site;

1.4 Department of Natural Resources and Environmental Protection;

1.5 Engineering and Environmental Surveillance Authority;

1.6 Marine Inspectorate.

Examples of SAP design

The examples below differ in marine flora, characteristic microorganisms and climate

Variant 1, implemented in Barents Sea

Preparations for construction. Preliminary studies of the project site include assessment of geographical, geophysical and hydrochemical properties, type of sea bottom, prevailing directions and velocities of marine currents, potential sources of pollution, directions of spreading of oil pollution on the sea surface, character and intensity of seawater pollution. The project site may need cleaning from litter and floating objects. Diving survey may be needed to discover and remove any sunk objects which interfere with SAP.

Water samples are analyzed for background concentrations of oil products, heavy metals, and radioactive nuclides. Hydrodynamic calculations provide the basis for assessment of main construction elements: SAP configuration, mass and positions of gravitation anchors, length of ropes and fasteners, load-carrying frame and any elements which provide substrate for algae.

The cable ropes and slings are cut into required lengths. The fasteners are mounted on these ropes. Cultivation of fucus requires nylon ropes with 10-20 mm diameter and at least 20 m length. Vertical slings with 10 mm diameter and 3-6 m length provide the substrate for cultivation of laminaria. Preliminary treatment of these slings may involve sterilization in NaClO solution and soaking in fresh water for 10-12 hours.

The type of laminaria seeding depends upon the season. There are two options. *Spore seeding:* the slings are soaked in the barrels with seawater and specially prepared suspension of laminaria spores for 24 hours. The best way to monitor sedimentation and seeding of laminaria spores is to use special object-plates. Spore seeding option is less labor-intensive than *entwining* of young sporophytes into the nylon slings. If the spore seeding is performed in August or September in Barents Sea, then laminaria plants may begin functioning in July-August of the following year. It takes almost a year for young sporophytes to develop.

Young *L. saccharina* plants are collected for entwining in the lower littoral horizon. The length of these plants should be 10-20 cm. These plants are entwined into the nylon slings so that each 10-15 cm of the sling length carries 3 plants.

Fufus plants for planting are collected on the littoral and entwined so that each 5 cm of the rope length carries the knot of 3-4 plants.

To speed up colonization of algae thalluses by microorganisms, we recommend soaking algae in the seawater enriched with nutrients and natural mix of colonies of hydrocarbon oxidizing bacteria and colonies of other microorganisms which destruct oil products. These microorganisms should be specially cultivated. For Kola Bay of Barents Sea, we suggest using the mix of yeast-like fungus *Candida* sp. and bacterial microflora of *Pseudomonas* sp., *Corynebacterium* sp., *Brevibacterium* sp., *Nocardia* sp., *Arthrobacter* sp., *Streptomyces* sp., *Acremonium* sp. This natural mix of aquatic life may be found elsewhere in Barents Sea irrespective to the levels of oil pollution.

After entwining or seeding, the prepared substrates with algae are transported to the workstation.

Assembling – 1st stage

Gravitational anchors are placed in the selected points of the project site. The floats are attached to the anchors with wire cables or nylon ropes. These buoys are needed to keep the plantation load-carrying frame afloat. The frame consists of polymer ropes with 30-40 mm diameter. The substrate cable ropes are attached to the frame ropes at the second stage.

Assembling – 2nd stage

Horizontal cable ropes with entwined fucus plants are fastened to the load-carrying frame. The floats (floats) are mounted on the cable ropes.

Assembling – 3rd stage

Vertical slings with laminaria sporophytes are fastened to the floats. The free-flowing sling ends should have metal weights to keep them in vertical position. After this, the load-carrying cables are tightened to maintain the required configuration and orientation of SAP in relation to the expected direction of propagation of oil pollution.

SAP servicing

Early stages of plantation operation require regular monitoring of algae development and overall integrity of plantation structure. Any damaged modules may have to be replaced. Plantation thinning or additional planting may be required.

Subsequent stages require periodical checks of integrity of the construction and plant cover. Addition of new modules or changes in plantation configuration may be required. Water samples are taken periodically for lab tests. The purpose of such tests is monitoring of biofiltering properties of the plantation.

Variant 2, proposed for Black Sea

This variant differs from Variant 1 in several aspects:

1. Fucus algae *Cystoseira crinita* are collected in advance and entwined in the horizontal cable ropes. Relatively more heliophobic fucus plants *C. barbata* are collected and entwined in the vertical slings.
2. There is no need for preliminary seeding of algae with microorganisms. High water temperatures facilitate rapid natural colonization of algae thalluses immediately after their immersion in the sea.

The succession of assembling stages is the same as in Variant 1.

Variant 3, proposed for the Sea of Japan

This variant uses different algae species. There is no need for artificial seeding of algae with microorganisms either. In case of ice formation on the sea surface, the plantation should be submerged under the surface. This will prevent rope abrasion by ice blocks.

Variant 4, proposed for Avachinsky Bay

Same as Variant 3.

The analysis of project implementation highlighted the following limitations of the proposed method:

1. SAP cannot be constructed if the underlying depths exceed 25-30 m. Large sunk objects, piers or other man-made constructions may create risks for plantation assembling, mounting of anchors, or diving operations.
2. The passing ships may destroy the plantation. The plantation itself may obstruct navigation. In the areas with intense navigation, SAP must be clearly marked up by signal buoys, radiolocation devices or lights.
3. SAP cannot operate in high seas or open-sea parts of bays because high rip entangles the ropes and destroys the plantation carcass.
4. Fresh water currents near river estuaries, in fiords of harbors may change hydrochemical properties of seawater and suppress algae development. Fresh waters also increase the risk of ice formation and plantation failure.

There are several considerations which favor construction of SAP in a particular location. Below we summarize these considerations in the form of checklist:

1. The prospective project site is adequately protected from strong storms and high rip.
2. Hydrochemical parameters of seawater are relatively stable.
3. Neighboring settlements provide workforce for the plantation.
4. Nearby transportation routes facilitate logistics and delivery of plantation parts (modules).
5. Nearby companies help utilize plantation waste.
6. Nearby clean littoral areas provide algae plants for seeding or planting.

Results of analysis of operations of the experimental plantation

The proposed method of water purification was originally designed for fine-cleaning of surface waters. This is the final stage of water purification, which takes place after mechanical removal of bulk of oil products after oil spills. We also anticipated that SAP would be used as a preventive stand-by cleanup strategy. In real life, SAP appeared to be more viable than we expected. The experience of major oil spills in Olenya Bay in 2007 and 2008 showed that SAP withstood very high levels of oil pollution. Fucus algae retained vitality even in the conditions of continuous oil film, at OP concentrations above 100 mg/l after accidental oil spills. Even after 15 days of exposure to extremely high concentrations of oil products, fucus algae contained and accumulated oil pollution. The content of oil products in fucus plants after this exposure approached 6 g/kg of dry mass. Their functional capability decreased only by 30-40% after such stress. *L. digitata* was the preferred species for using in the areas of Barents Sea with high water mobility, because this species better withstood strong turbulent currents.

The pilot SAP worked for 18 months in very harsh environmental conditions; survived several storms and successfully neutralized oil pollution. It could have worked even longer.

The parallel experiment was conducted in Dalnezelenetskaya Bay of Barents Sea. This experiment confirmed oil pollution containment properties of SAP. The plantation in Dalnezelenetskaya Bay adequately protected mariculture from oil pollution. Sanitary algae plantations may be used in intensely developing crab farming and pisciculture along the coast of Barents Sea.