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Ecological state assessment of the Novik Bay (Peter the great Bay, Sea of Japan) by biomonitoring methods During Summer 2024

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Abstract: The content and distribution of indicator groups of microorganisms were studied. Biotesting of water and aqueous extracts from bottom sediments of Novik Bay (Russky Island, Peter the Great Bay, Sea of Japan) was conducted. It was established that at the time of observation, the waters of the bay belonged to the category of α-β-mesosaprobic, and the obtained values of the oligotrophic index indicated the accumulation of easily accessible organic matter. The biological pollution of water was indicated by a high number of bacteria of the sanitary indicator group, exceeding permissible standards, and the activity of plant communities was indicated by the presence of large quantities of indicators of phenolic contamination of the environment. Nickel-resistant bacteria stood out in terms of numbers and distribution among the groups of metal-resistant microorganisms, which is due to the combustion of hydrocarbon fuels, confirming the widespread distribution of oil-oxidizing microorganisms. The bioassay showed that water and aqueous extracts from bottom sediments had a depressing effect on the Ph. tricornutum culture in the acute experiment, indicating the presence of pollutants in the environment that are toxic to microalgae. None of the water samples showed any toxic effects on the microalgae culture in the chronic experiment. However, bioassay of aqueous extracts from the soil revealed a stimulating effect, most likely due to the accumulation of organic matter in the bay's soils.

Keywords: *Bioassay, Biomonitoring, sanitary and bacteriological assessment, Environmental monitoring, Peter the great Bay, The Novik Bay.*

1. Introduction

Novik Bay is the largest of the island bays by Peter the Great Bay. It is long and narrow and stretches across Russky Island from northwest to southeast for 13 km. The Novik Bay is closed off from the waves of the open sea; waves that are coming from the northwest of Amur Bay reach it only. Circulation in the Novik Bay is limited due to its geographical location. The natural monsoon self-cleaning mechanism is weak within the bay. The circulation is "locked" due to the surge effect of the drift current on the apex part during northern winds here, and the circulation is weakened by the orography of Russky Island during southern winds [1]. Limited circulation complicates the ecological state of this water area, especially in the apex. Wastewater from the FEFU campus on Russky Island is pumped to the sewage treatment plants located in Lesnoye village, from where it flows into the head of Novik Bay (10,000 m^3 daily, or 4 million m^3 annually) [2]. The bay's waters are actively used for year-round commercial fishing, and in the summer, they attract many tourists. It is surrounded by small villages, boat stations, and recreation centers that do not have a centralized sewer system. Thus, the waters of Novik Bay are subject to significant domestic and recreational impacts, which affect the composition of the bay's microbial communities [3]. This is especially noticeable in the corner of the bay and at its entrance. This is why it is necessary to conduct regular monitoring studies of the bay's environmental quality, including the use of biological methods such as microbial indication and bioassay.

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2. Materials and Methods

The work was carried out in the water area of Novik Bay in summer 2024 (Figure 1). Water samples for microbiological analysis and biotesting were taken from the surface (0.5 m) horizon with a bathometer from aboard a small boat. Bottom sediments were collected with a dredger from the surface horizon (0.1 m) at a depth of 5 m. A total of 19 samples of surface waters (for microbiological analysis and bioassay of toxicity) and 9 samples of bottom sediments (for bioassay of toxicity) were selected and analyzed.

Figure 1.
A map showing the location of sampling stations in the water area of the Novik Bay.

2.1. Sanitary and Bacteriological Analysis

Sanitary and bacteriological analysis was carried out on water samples. The number of heterotrophic saprophytic bacteria (Heterotrophic Plate Count, HPC) was determined on SMM medium (medium for marine microorganisms) with the addition of 1.5% agar using the Koch plate method [4]. The most probable number of bacteria of individual physiological groups (oil-oxidizing, phenol-oxidizing) was estimated based on the tenfold dilution method using selective media. Yeast extract (0.005%) with mineral salts was used as a basis for the preparation of selective media, to which oil or phenol was added at a final concentration of 0.1% as the only carbon source for the development of bacteria [5]. The number of oligotrophs (O) in 1 ml of water was determined by tenfold dilutions and subsequent seeding of an aliquot in two replicates on solid Mills medium modified for marine microorganisms [6]. The number of metal-resistant forms in a community of heterotrophic cultured microorganisms was determined by the plate method using SMM supplemented with metal salts at concentrations inhibiting the growth of sensitive forms of bacteria. The metal chlorides used were Zn, Cu, Cd, Ni, and Pb [7]. Resistance testing was performed for each toxic additive (element by element) for all collected samples. Coliform bacteria (CB) were detected using Endo's selective medium. Catalase-positive, oxidase-negative gram-negative bacteria were identified [5]. Fecal streptococci (enterococci) (E) were identified on azide medium using membrane filtration. Pathogenic enterobacteria (PE) were cultured on Ploskirev medium and XLD agar. The results were processed using Microsoft Excel: mean values and standard deviations were calculated.

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2.2. Biotesting

Water extracts from the bottom sediments were prepared for biotesting. The toxicity of water and bottom sediments was assessed by changes in the number of cells of the cultured microalgae *Phaeodactylum tricornutum* in the prepared suspension and filtered water samples. Biotesting was performed under standard conditions, under illumination by fluorescent lamps with a light-dark period of 16 h light: 8 h dark at a temperature of 20 ± 2°C. Acute toxicity of microalgae extracts was assessed after 72 and 96 hours from the start of the experiment, and chronic toxicity was evaluated after 7 days [8]. The calculation of the number of cells and the degree of inhibition of microalgae growth in the experiment was performed in relation to the control. Bottom sediment extraction was considered non-toxic if, at the end of the study, the number of algae cells in it was ≥ 90%, slightly toxic – 65-89%, medium toxic 50-64%, highly toxic 0-49% of the control [9]. The chlorophyll a content was determined by the standard method of extraction from cells with acetone, followed by measurement on a Shimadzu-UV 1800 spectrophotometer. The results were processed using Microsoft Excel and Statistica. Mean values and standard deviations were calculated, and the significance of differences from the control was assessed using the Mann-Whitney U test.

3. Results and Discussion

3.1. Microbiological Parameters of Surface Waters

The results of microbial analysis of the bay waters are presented in Tables 1-2. Heterotrophic organisms are consumers of easily oxidizable organic matter, and their numbers are used to assess the saprobity of waters. According to the saprobity scale, waters with heterotroph counts up to 10^3 cells/ml are considered oligosaprobic, up to 10^5 - mesosaprobic, and 10^6 cells/ml and above polysaprobic [10].

The total number of heterotrophs ranged from 10^4 to 10^5 CFU/ml during the summer period (Table 1). The waters of Novik Bay were characterized as α-β-mesosaprobic, indicating enrichment in organic compounds.

Table 1.
The number of ecological-trophic groups of microorganisms, sanitary indicators of surface waters of Novik Bay and the value of the oligotrophic index (CFU/ml of water).

Station	HPC	O	I (oligotrophic index)	CB/E.coli	E	PE
1	(6.5±0.2)*10^4	(1.3±0.2)*10^3	0.02	(3.6±0.1)*10^3/0	(1.4±0.2)*10	0
2	(1.8±1.4)*10^5	(1.8±0.7)*10^4	0.1	(4.0±1.2)*10^3/0	0	0
3	(5.7±0.5)*10^4	(5.5±0.2)*10^3	0.09	0/0	0	0
4	(1.3±0.3)*10^5	(2.0±0.3)*10^2	0.001	0/0	(6.4±0.1)*10	0
5	(1.8±0.3)*10^5	(4.8±0.7)*10^4	0.26	(1.3±0.5)*10^3/0	(9.2±0.3)*10	0
6	(9.9±3.2)*10^4	(4.5±0.2)*10^4	0.45	0/0	0	0
7	(4.6±1.3)*10^4	(1.0±1.2)*10^4	0.21	(5.5±3.1)*10^3/0	0	0
8	(5.7±3.3)*10^5	(1.7±0.1)*10^4	0.02	0/0	0	0
9	(7.7±3.1)*10^4	(2.3±1.4)*10^3	0.02	(5.0±0.2)*10^3/0	0	0
10	(1.5±0.2)*10^5	(1.5±1.3)*10^3	0.01	0/0	0	0

Oligotrophs are an important indicator of microbial assimilation of small amounts of organic matter, with their numbers ranging from 10^3 to 10^4 CFU/ml. The simultaneous detection of saprophytes and oligotrophs, along with the comparison of their numbers, reveals the degree of mineralization of organic matter and provides a quantitative indicator of the oligotrophic index that reflects the enrichment of a habitat with nitrogen-containing organic matter and the intensity of its mineralization.

An oligotrophic index greater than one indicates a balanced bacterial mineralization of organic matter. Index values less than one indicate that the aquatic environment is polluted with organic matter, and that organic matter accumulation processes in the biocenosis predominate over its destruction. During the summer, the index was minimal, indicating an imbalance between the groups of organisms consuming organic matter.

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Furthermore, coliform bacteria (CB) stand out among this heterotrophic pool. They were detected at most stations, exceeding the standard level of 5 CFU/ml and indicating biological contamination of the water area. However, *E. coli*, a direct marker of fecal contamination, was not detected. Enterococcus, a marker of fresh fecal contamination, was also detected at three stations in July, in quantities exceeding the standard level. Pathogenic enterobacteria were not detected at any station.

Oil pollution indicators were detected everywhere, although their numbers were low and ranged from 10^2–10^3 CFU/ml. However, zero quantitative values were not detected (Table 2). Nevertheless, of the entire pool of abundance indicators, the highest value was determined throughout the summer period and amounted to 10^3 CFU/ml. According to the well-known classification [11], waters with the number of microorganisms indicators of oil pollution, not exceeding 10^4 CFU/ml are classified as "slightly polluted." Our data indicate that the number of microorganisms growing on oil suggests minor pollution of the waters with oil products, characterizing the waters of the bay according to this indicator as waters with a slight excess of the background.

Table 2.
Number of oil and phenol pollution indicators and metal-resistant microorganisms in surface waters of Novik Bay (CFU/ml of water).

Station	Oil-oxidizing bacteria	Phenol-oxidizing bacteria	Cu	Zn	Ni	Cd
1	(5.1±0.2)*10³	(1.4±2.2)*10⁴	(1.3±0.4)*10²	0	(6.5±1.7)*10²	(3.0±0.2)*10³
2	(2.7±0.2)*10³	(2.6±0.4)*10⁴	(3.0±0.1)*10²	(2.1±0.2)*10²	(1.2±0.3)*10³	0
3	(9.5±2.2)*10³	(1.3±2.2)*10⁴	(5.1±0.4)*10²	(5.9±1.4)*10²	(3.2±0.9)*10³	0
4	(2.6±0.7)*10³	(7.0±0.7)*10³	0	0	(4.7±1.1)*10³	0
5	(2.4±0.5)*10³	(3.0±0.7)*10⁴	(1.8±0.2)*10	0	(9.5±0.4)*10²	0
6	(1.3±0.4)*10³	(1.9±0.2)*10⁴	(9.2±0.8)*10	0	(1.3±1.2)*10³	0
7	(1.2±7.1)*10³	(1.2±1.1)*10⁴	0	0	(1.5±0.2)*10³	0
8	(3.0±0.3)*10³	(6.6±0.4)*10⁴	0	0	(8.0±3.7)*10²	0
9	(1.1±3.1)*10³	(3.4±2.1)*10⁴	0	0	(1.5±0.4)*10²	(2.7±0.5)*10⁴
10	(2.3±0.6)*10³	(1.3±0.2)*10⁴	(4.2±0.4)*10	0	(6.5±2.7)*10²	0

Indicators of phenolic pollution were detected at all stations and in all seasons of observation. Bacterial counts of this ecotrophic group ranged from 10^2 to 10^4 CFU/ml, reaching 10^4 CFU/ml at 9 of the 10 stations studied (Table 2).

Phenols are highly toxic substances, and even at counts reaching 102 cells/ml, they are dangerous pollutants. Water pollution by phenols is divided into several categories: noticeable $>10^2$; significant $>10^3$; severe $>10^4$ and higher [7].

The obtained data indicate significant and measurable environmental pollution by phenols. The presence of significant numbers of phenol-resistant microorganisms in the environment may have several causes, the main ones being the activities of pulp and paper, wood processing, and oil refining industries. Furthermore, phenols may reflect environmental pollution by organochlorine pesticides and may also be of faecal origin. There are no large industrial enterprises on the island that produce phenolic pollution, and a significant portion is of natural origin. The main sources of this pollutant are indigenous phenols, formed primarily as a result of the decomposition of animal and plant remains. Significant quantities of phenols also enter the environment through household wastewater, including sewage effluents, leading to environmental contamination with phenols of faecal origin and also reflecting environmental pollution by petroleum products.

Metal-resistant microorganisms, being part of heterotrophs, allow us to assess the level of specialized pressure on surface watercourses. While the content of Cd, Pb, and Ni ions and complexes in water primarily indicates man-made impacts on the environment, Cu and Zn indicate anthropogenic impacts.

Overall, the microbiological study results revealed a mosaic distribution of metal-resistant microorganisms (Table 2). The most common ecotrophic groups recorded at most stations were Cu-,

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Cd-, and Zn-resistant bacteria. Ni-resistant heterotrophs were the dominant group, recorded at all stations in higher quantities (by 1-2 orders of magnitude) than Cu- and Cd-resistant microorganisms. The high abundance of this metal-resistant group is undoubtedly related to the combustion of hydrocarbon fuels (boilers, marine engines).

3.2. Biotesting of Environmental Toxicity

The results of the water and bottom sediments bioassay from Novik Bay using a culture of *Ph. tricornutum* microalgae are shown in Figures 2-5. As observed in figure 2, none of the stations recorded a deviation in microalgae abundance greater than 50% of the control; the bay waters did not exert a highly toxic effect on culture growth throughout the experiment. Most stations demonstrated a decrease in the toxic effect of water on *Ph. tricornutum* by day 7 from the start of the experiment, compared to days 3 and 4. Water samples from stations 5, 8, 9, and 10 showed inhibition of population growth, indicating moderate water toxicity in the acute experiment (72 hours); the remaining stations showed no toxic effect on microalgae. During a 96-hour exposure, samples collected at 6 of the 9 stations (except stations 3, 4, and 9) also showed a moderate toxic effect on *Ph. tricornutum.* A weak toxic effect was observed for the remaining water samples. Samples containing stage 10 continued to exert a weak inhibitory effect on the culture after 168 hours of testing.

The change in chlorophyll a content in the samples relative to the control generally follows the pattern of changes in the number of microalgae (figure 2): in the acute experiment, a deviation from the control of over 20% was recorded for water samples from stations 2, 3, 9, and 10; in the chronic experiment, a decrease in chlorophyll concentration relative to the control was not observed at any of the stations, indicating a toxic effect on *Ph. tricornutum.*

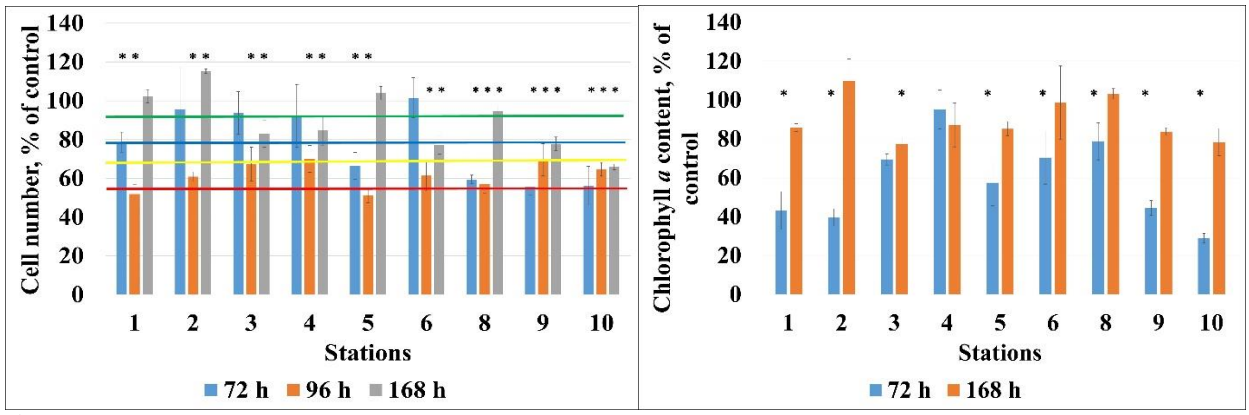

Figure 2.
The abundance dynamics and chlorophyll *a* content in the cells of *Ph. tricornutum* microalgae in water samples (*– the difference from the control is significant at $p < 0.05$).

In water samples collected at stations 1, 5, 8, 9, and 10 during the acute experiment, the inhibition rate exceeded 20% (Figure 3), confirming the toxic effect of the test samples on microalgae. This toxicity level was maintained during the 96-hour exposure. By the end of the experiment, no station recorded a deviation from control of more than 20%. Overall, it can be assumed that a number of stations are characterized by a constant influx of pollutants via surface runoff that can have a toxic effect on microalgae cultures; however, these substances degrade over time in the aquatic environment.

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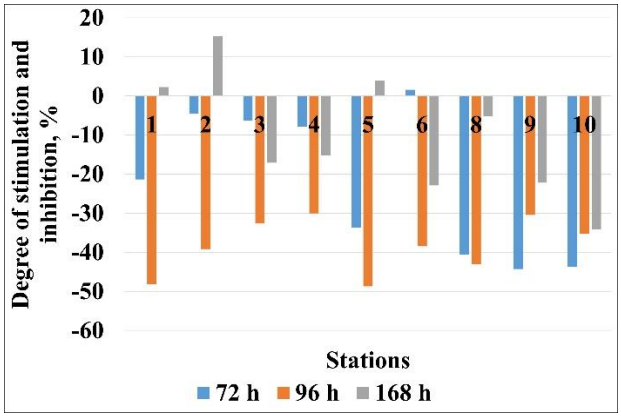

Figure 3.
Deviation of the abundance of *Ph. tricornutum* from the control (degree of stimulation and inhibition).

The dynamics of *Ph. tricornutum* abundance in aqueous extracts from bottom sediments of Novik Bay differ from the dynamics described above in water samples of the bay (figure 4): all stations were characterized by suppression of the growth of the culture population relative to the control, and for soil samples from a number of stations (1, 2, 4, 5, 8, 9, and 10) in the acute experiment. Moreover, high toxicity (inhibition over 50%) was recorded for soil samples from several stations, medium toxicity for station 6, and low toxicity for station 3. A similar pattern persists after 96 hours of exposure: only station 2 is characterized by moderate toxicity of aqueous extracts from bottom sediments, while the remaining samples exhibit high toxicity. Bottom sediments typically accumulate pollutants from the water column and can serve as a depot for toxicants. Furthermore, they can also be a source of secondary pollution of water bodies.

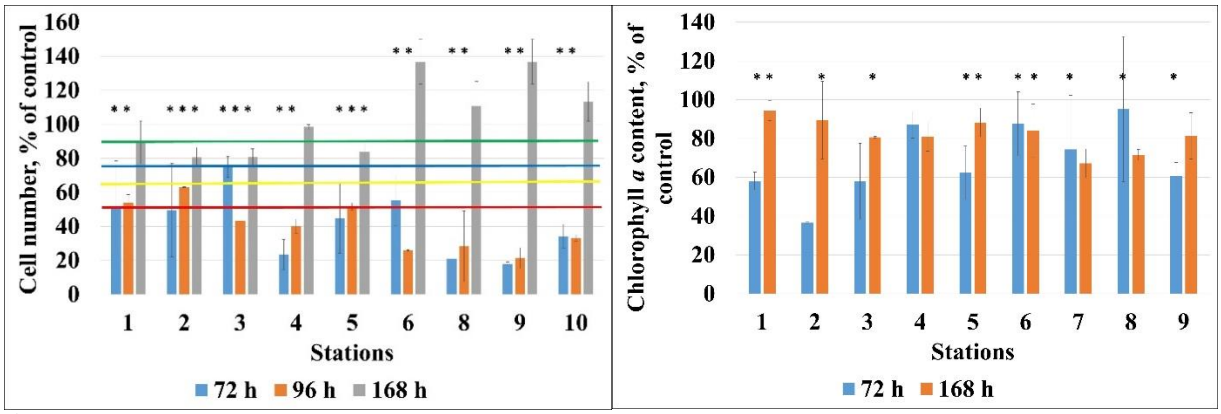

Figure 4.
The dynamics of the abundance and content of chlorophyll *a* in the cells of *P. tricornutum* microalgae in water extracts of bottom sediments (*- the difference from the control is significant at $p < 0.05$).

Stimulation of the growth of microalgae populations relative to the control was noted in water extracts from stations 4, 6, 8, 9, 10 by the end of the experiment, and for stations 6 and 9 by more than 20% relative to the control, which also allows us to characterize the samples as toxic in accordance with [12]. However, this fact does not indicate the true toxicity of the bay soils but rather the significant content of biogenic and organic substances in them, which can have a stimulating effect on the growth of microalgae [13]. A similar effect can also be caused by, for example, copper ions [14], which are constantly present in environments exposed to municipal and industrial wastewater. Weak toxicity is

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characteristic of aqueous extracts from bottom sediments from stations 4, 1, 2, 3, and 5 in the chronic experiment.

The change in chlorophyll a content relative to the control at nearly all stations (except station 9 in the acute experiment) throughout the experiment did not exceed 20%, indicating the absence of a toxic effect of aqueous extracts of bottom sediments from Novik Bay on microalgae cultures. The presence of both stimulating and inhibitory effects is clearly demonstrated in Figure 5: during 72-hour and 96-hour exposures, inhibition of microalgae growth was observed across all samples. However, by the end of the experiment, samples from stages 6 and 9 showed a reversal, with inhibition replaced by stimulation of population growth exceeding 20%. Station 9, located near the village of Podnozh'e, and station 6, near the village of Ekipazhny, are influenced by municipal wastewater and recreational activities during summer, which can contribute to organic matter pollution in bay waters and its accumulation in bottom sediments. For the remaining samples, no toxic effects were detected after 168 hours of exposure.

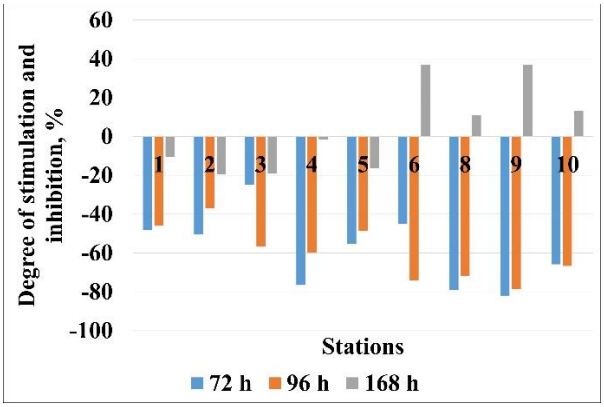

Figure 5.
Deviation of the abundance of *P. tricornutum* from the control (degree of stimulation and inhibition).

4. Conclusion

Thus, according to the results of microbiological monitoring, the waters of the Novik Bay in the summer period belonged to the category of α-β-mesosaprobic, enriched with organic compounds, and the obtained values of the oligotrophic index indicated the accumulation of easily accessible organic matter, where accumulation processes prevailed over destruction. At most stations, bacteria of the sanitary indicator group, indicating biological pollution of the waters, were found in sufficient quantities, but a direct marker of fecal pollution of the environment - *E. coli*, was not recorded. Bacteria growing in media containing crude oil were recorded at all studied stations, characterizing the environment as slightly polluted, and phenol-resistant microorganisms were also recorded in fairly high quantities, which signaled noticeable and significant pollution of the environment by this pollutant, caused by both natural and anthropogenic processes.

Metal-resistant microorganisms have shown that the level of specialized (technogenic) pressure on the bay's surface waters is insignificant. The dominant group of metal-resistant bacteria is Ni-resistant heterotrophs.

A bioassay of water and aqueous extracts from bottom sediments in Novik Bay revealed that, in an acute experiment (72 hours), water from Novik Bay at stations 5, 8, 9, and 10, as well as aqueous extracts from soils at all sampling stations, had a depressing effect on *Ph. tricornutum* culture, indicating the presence of pollutants toxic to microalgae in the Novik Bay environment. In a chronic experiment (168 hours), none of the water samples exhibited toxic effects on the microalgae culture. Furthermore, a bioassay of aqueous extracts from Novik Bay soils over a 168-hour exposure revealed a stimulating

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effect (bottom sediments collected near villages), which is most likely due to the accumulation of organic matter in the bay soils.

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Transparency:
The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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