

Original paper

## The Abundance and Distribution of Individual Bacterial Groups in Coastal Waters of the Kamchatka Peninsula

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### Abstract

The paper presents the results of microbiological study in the coastal waters of the northwestern Pacific Ocean and the Sea of Okhotsk, obtained during the expedition of cruise 23/4 of PV *Professor Multanovsky* (August–September 2023) within the Floating University Program. Quantitative characteristics and spatial distribution of bacteria transforming major classes of organic compounds, including petroleum hydrocarbons (diesel fuel), lipids, and phenols, in the surface and bottom layers of the water column were determined. The abundance of heterotrophic bacteria in the surface and bottom layers ranged from  $10^3$  to  $10^5$  cells/mL. The abundance of hydrocarbon-oxidizing bacteria ranged from 1 to  $10^2$  cells/mL in the surface layer and from 1 to 10 cells/mL in the bottom layer. The abundance of lipolytic bacteria varied from 10 to  $10^3$  cells/mL in both layers. The phenol-oxidizing bacterial group ranged in abundance values from 1 to  $10^2$  cells/mL in both the surface and bottom layers. The investigated bacterial groups exhibited non-uniform distribution in the coastal waters of the Kamchatka Peninsula. Maximum values are associated with the anthropogenically loaded Avacha Bay, exploited bays, and sites of active river runoff. During the study period, the water temperature of the surface layer ranged from 11.0 to 16.1°C, and the bottom layer ranged from 1.3 to 11.3°C. The pH values in surface water varied from 8.38 to 8.49. The depth at the bottom water sampling stations was from 13.5 to 780 m in the Pacific Ocean and from 26 to 62 m in the Sea of Okhotsk. No significant correlation was found between the abundance of identified microbial groups and the recorded physical and chemical parameters.

**Keywords:** heterotrophic bacteria, coastal waters, Kamchatka Peninsula, Avacha Bay, Sea of Okhotsk

**Acknowledgements:** The work was carried out during cruise 23/4 of PV *Professor Multanovsky* as part of the Floating University Program (Agreement no. 075-01593-23-06)

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with the support of the Ministry of Science and Higher Education of the Russian Federation. The nutrient media for determining the abundance of indicator groups of bacteria were prepared as part of the IBSS state research assignment “Study of biogeochemical patterns of radioecological and chemoecological processes in the ecosystems of water bodies of the Sea of Azov–Black Sea Basin in comparison with other areas of the World Ocean and individual aquatic ecosystems of their drainage basins to ensure sustainable development in the southern seas of Russia” (No. 124030100127-7).

**For citation:** Zaripova, K.M., Demidova, E.A., Tikhonova, E.A., Burdiyan, N.V., Doroshenko, Yu.V. and Basova, E.D., 2025. The Abundance and Distribution of Individual Bacterial Groups in Coastal Waters of the Kamchatka Peninsula. *Ecological Safety of Coastal and Shelf Zones of Sea*, (2), pp. 135–148.

## **Численность и распределение отдельных групп бактерий в воде прибрежной акватории полуострова Камчатка**

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### **Аннотация**

Представлены результаты микробиологических исследований прибрежной акватории северо-западной части Тихого океана и Охотского моря, полученные в ходе экспедиции рейса 23/4 ПС «Профессор Мультиановский» (август – сентябрь 2023 г.) в рамках программы «Плавучий университет». Определены количественные характеристики и изучено пространственное распределение бактерий, трансформирующих основные классы органических соединений, включая углеводороды нефти (дизельное топливо), липиды и фенолы в поверхностном и придонном слоях водной толщи. Численность гетеротрофных бактерий в этих слоях варьировала от  $10^3$  до  $10^5$  кл/мл. Численность углеводородокисляющих бактерий составляла от 1 до  $10^2$  кл/мл в поверхностном слое и от 1 до 10 кл/мл – в придонном. Численность липолитических бактерий изменялась от 10 до  $10^3$  кл/мл в обоих слоях, а фенолоксилирующей группы бактерий колебалась от 1 до  $10^2$  кл/мл. Исследованные группы бактерий распределены в прибрежной акватории п-ова Камчатка неравномерно. Максимальные показатели были зафиксированы в антропогенно нагруженной акватории Авачинской губы, эксплуатируемых бухтах и местах активного речного стока. В рассматриваемый период температура воды поверхностного слоя изменялась от 11.0 до 16.1 °С, придонного слоя – от 1.6 до 11.3 °С. Значения водородного показателя в воде поверхностного горизонта колебались в диапазоне от 8.38 до 8.49. Глубина на станциях отбора придонной воды варьировала от 13.5 до 780 м в Тихом океане и от 26 до 62 м в Охотском море. Значимых корреляционных связей между численностью определяемых групп микроорганизмов и указанными физико-химическими параметрами выявлено не было.

**Ключевые слова:** гетеротрофные бактерии, прибрежные воды, полуостров Камчатка, Авачинский залив, Охотское море

**Благодарности:** работы выполнены в рейсе 23/4 ПС «Профессор Мультановский» в рамках научно-образовательной программы «Плавучий университет» (соглашение № 075-01593-23-06) при поддержке Министерства науки и высшего образования РФ, питательные среды для определения численности индикаторных групп бактерий подготовлены в рамках темы госзадания ФГБУН ФИЦ «Институт биологии южных морей имени А. О. Ковалевского РАН (ФИЦ ИнБИОМ) «Изучение биогеохимических закономерностей радиоэкологических и хемотропических процессов в экосистемах водоемов Азово-Черноморского бассейна в сравнении с другими акваториями Мирового океана и отдельными водными экосистемами их водосборных бассейнов для обеспечения устойчивого развития на южных морях России» (№ гос. регистрации 124030100127-7).

**Для цитирования:** Численность и распределение отдельных групп бактерий в воде прибрежной акватории полуострова Камчатка / К. М. Зарипова [и др.] // Экологическая безопасность прибрежной и шельфовой зон моря. 2025. № 2. С. 135–148. EDN VUFBCX.

## Introduction

Coastal marine waters represent a contact zone where land and sea pollutant fluxes are mixed. Furthermore, these bodies of water are frequently subject to intensive economic activities. A variety of organic substances are introduced into marine ecosystems, thereby becoming a significant and permanent environmental factor. These substances are then degraded by microorganisms [1]. Hydrocarbons are the most prevalent organic pollutants in ecosystems [2, 3]. Petroleum hydrocarbons and phenols play a substantial role in the contamination of the marine waters surrounding Kamchatka <sup>1)</sup>.

When water bodies are polluted, increases in populations of individual bacterial groups are often observed before changes in water chemistry can be detected. Therefore, microbiological methods can be significantly more sensitive than sanitary-chemical ones [4]. Thus, we consider the number of hydrocarbon-oxidizing bacteria (HOB) as indicators of water pollution by hydrocarbons, the number of phenol-oxidizing bacteria (POB) – by phenol, the number of lipolytic bacteria (LLB) – by lipid substances [5]. In consideration of the fact that diesel-fuelled ships are moored in the study area <sup>2)</sup> and that fuel transportation and bunkering of ships are in constant operation [6, 7], one of the common types of hydrocarbon raw materials, namely diesel fuel, was selected as the sole source of carbon and energy for HOB.

Studies aimed at monitoring pollution levels and assessing the quality of natural waters pay special attention to the heterotrophic component of microbial communities. This component plays a key role in the energy balance of aquatic ecosystems and in the self-purification of water bodies.

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<sup>1)</sup> Ministry of Natural Resources and Environment of the Kamchatka Krai, 2023. [*A Report on the State of the Environment in the Kamchatka Krai in 2022*]. 418 p. (in Russian).

<sup>2)</sup> Kasperovich, E.V., 2011. [*Technogenic Influence of Marine Vessels on the State of Near-Kamchatka Ecosystems*]. Extended Abstract of PhD Thesis. Petropavlovsk-Kamchatsky, 25 p. (in Russian).

In the coastal waters of East Kamchatka and the Sea of Okhotsk, the microbial indication method was previously used for operational characterisation of the water pollution degree (the number of heterotrophic microorganisms, including some physiological groups such as HOB and POB) in the surface water layer in the area of Avacha Bay, in the coastal water areas of Sakhalin and in the water area of the port of Magadan in 2001 [8]. Furthermore, in 1997–1999, microbiological studies were conducted in Avacha Bay and in the vicinity of the north-eastern coast of Sakhalin Island (as well as in the northern Primorye region and in the Peter the Great Gulf). Heterotrophic microorganisms from the surface and bottom water layers were studied, though within the context of evaluating the ecological status of water bodies subject to elevated levels of heavy metal pollution [9, 10]. In 2004–2006, the abundance of heterotrophic bacteria (HB), *E. coli* bacteria, HOB and LLB was investigated in the coastal waters of southern Sakhalin Island<sup>3)</sup>. In 2015, a study was conducted in Avacha Bay to ascertain the number of saprophytic microorganisms in marine water and other sanitary and microbiological indicators by determining the relevant measurements at six stations in different seasons [11]. In 2021, HOB were studied as indicators of watercourse pollution in the city of Petropavlovsk-Kamchatsky [12]. It can be concluded that studies of the abundance of heterotrophic microorganisms of different ecological and trophic groups were mainly episodic. Furthermore, the studies were localized in Avacha Bay, within the area of coastal waters of the Kamchatka Peninsula, while other areas were insufficiently investigated. The 2022 report on the state of the environment in Kamchatka Krai contains information on marine pollution in only two areas: Avacha Bay and Khalaktyrsky Beach<sup>1)</sup>.

The work aims to reveal the peculiarities of the distribution of different bacterial indicator groups in the southeastern and southwestern coastal waters of the Kamchatka Peninsula in summer and autumn period.

### Materials and methods

The studies were carried out in the summer and autumn period of 2023 during the cruise of PV *Professor Multanovsky* in the northwestern Pacific Ocean and the Sea of Okhotsk (Fig. 1, Table 1).

Sampling stations are linked to areas of potentially high anthropogenic impact: they are located in bays, in the marine mouths of large rivers and in pre-estuary areas with settlements, agricultural and industrial facilities in their catchment areas (Fig. 1). Thus, stations 1–3 are located in Avacha Bay, including directly at the mouth of the Avacha River, station 4 – in the strait connecting Avacha Bay to the ocean, station 15 – a few kilometres from the bay's outlet. Stations 6–8 are located near Khalaktyrsky Beach – next to the mouth of Nalycheva River where “red tides” were recorded in 2020 [13, 14]; station 12 – near the mouths of the Vakhil and Ostrovnyaya rivers; station 24 – in the water area of Vilyuchinskaya Bay

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<sup>3)</sup> Repina (Smirnova), M.A., 2009. [*Petroleum- and Hydrocarbon-Oxidizing Microorganisms of Coastal Waters of the Southern Sakhalin Island*]. Extended Abstract of PhD Thesis. Vladivostok: FEFU, 22 p. (in Russian).

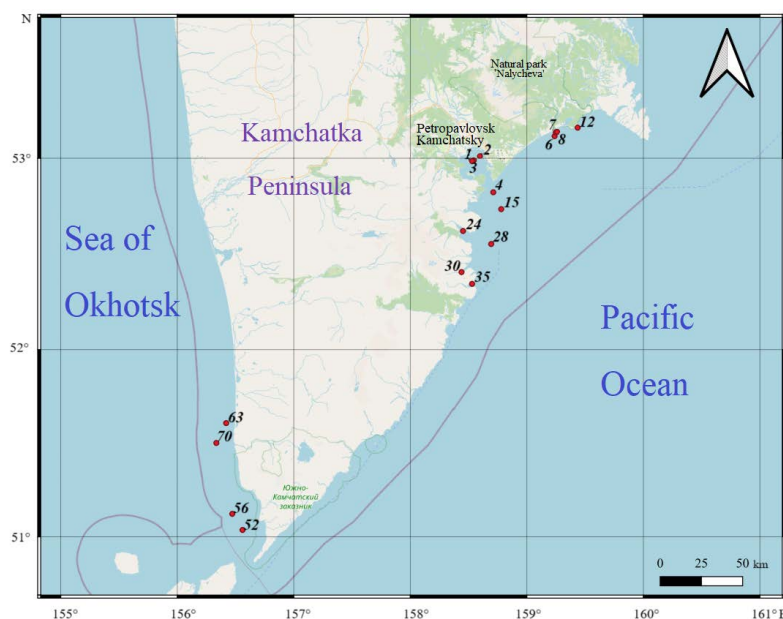


Fig. 1. Map of sampling points in the coastal zone of the Kamchatka Peninsula, 2023

where the Vilyucha and Zhirovaya rivers flow into; station 30 was carried out in Russkaya Bay; station 35 – in Listvennichnaya Bay where the river of the same name flows into; station 56 – near the mouths of the Lysaya and Krivaya rivers; station 70 – around the mouth of the Ozernaya River near the settlement of Ozernovskiy.

Water samples were taken from the surface layer using a bathometer, while bottom water was taken directly from a box corer.

HB, HOB, LLB, POB abundance was determined in surface and bottom water layers. No inoculation was carried out for samples from the surface layer at station 28, from the bottom layer at station 7 (and at station 15 for LLB and POB). Bacterial abundance was determined by the method of limiting dilutions using liquid nutrient media. Peptone medium was used for HB <sup>4)</sup>, Voroshilova–Dianova medium – for HOB and LLB [15], modified Kalabina medium – for POB [16]. As the sole carbon and energy source, 1% sterile diesel fuel was added to each tube after inoculation for HOB and 1% sterile vegetable oil for LLB. The salinity of seawater was taken into account when preparing the media. The most probable number of microorganisms per unit volume was calculated using the McCredie table based on the method of variation statistics. Samples were processed no later than two hours from the time of collection <sup>5)</sup>.

<sup>4)</sup> Mironov, O.G., ed., 1988. [*Biological Aspects of Oil Pollution of the Marine Environment*]. Kiev: Naukova Dumka, 248 p. (in Russian).

<sup>5)</sup> Netrusov, A.I., ed., 2005. [*Practical Course of Microbiology*]. Moscow: Akademiya, 608 p. (in Russian).

Table 1. Numbers, coordinates and depths of sampling stations on cruise 23/4 of PV *Professor Multanovsky*

Station number	Latitude, N	Longitude, E	Depth, m
1	52° 59.53'	158° 32.42'	25.0
2	53° 00.79'	158° 35.61'	22.0
3	52° 59.30'	158° 31.62'	26.0
4	52° 49.63'	158° 42.48'	59.0
6	53° 08.08'	159° 14.30'	15.0
7	53° 08.16'	159° 14.76'	15.0
8	53° 08.34'	159° 15.43'	13.6
12	53° 09.64'	159° 26.02'	24.0
15	52° 44.53'	158° 46.80'	780.0
24	52° 37.52'	158° 26.95'	14.0
28	52° 33.41'	158° 41.42'	119.0
30	52° 24.65'	158° 26.16'	26.0
35	52° 20.94'	158° 31.61'	31.0
52	51° 02.34'	156° 33.73'	33.0
56	51° 07.49'	156° 28.44'	62.0
63	51° 36.75'	156° 25.30'	26.0
70	51° 30.38'	156° 20.24'	57.0

To profile coastal waters as microbial habitat, temperature and salinity in the surface and bottom layers were recorded using a CTD probe (CTD Sea-bird SBE 911plus) and pH values in the surface water layer were determined using a pH meter (FireSting-PRO).

The map of sampling points was constructed in QGIS 3.34.11 software using an OpenStreetMap basemap.

### Results and discussion

During the study period, surface water temperature at stations 1–35 in the north-western Pacific Ocean varied from 11.0 to 15.7°C, bottom water temperature ranged from 1.6 to 11.3°C. Water salinity ranged from 26.8 to 30.8 PSU in the surface layer and from 31.2 to 34.1 PSU in the bottom layer. Bottom water sampling depths ranged from 13.6 to 780 m (Tables 1, 2).

In the Sea of Okhotsk, at sampling stations 52–70, surface water temperature varied from 7.8 to 12.1°C and bottom water temperature ranged from 3.6 to 6.7°C. Recorded water salinity in the surface layer varied between 31.9 and 32.5 PSU and in the bottom layer between 32.5 and 32.9 PSU. Bottom water sampling depths ranged from 26 to 62 m (Tables 1, 2).

Table 2. Temperature and salinity in the surface and bottom water layers at sampling stations

Station number	Surface layer		Bottom layer	
	<i>t</i> , °C	<i>S</i> , PSU	<i>t</i> , °C	<i>S</i> , PSU
1	14.40	27.48	4.05	31.70
2	14.53	27.97	3.57	31.95
3	14.30	26.83	4.04	31.95
4	11.04	30.43	2.13	32.93
6	15.30	29.66	9.80	31.49
7	14.70	30.03	9.07	31.50
8	15.00	29.54	11.32	31.15
12	15.57	29.66	5.40	32.28
15	15.73	29.86	3.58	34.11
24	13.31	30.50	8.70	31.60
28	14.40	30.80	1.60	33.05
30	12.77	30.18	3.99	32.67
35	13.90	30.16	3.61	32.71
52	7.80	32.55	6.70	32.50
56	10.20	32.46	4.23	32.87
63	12.05	32.01	6.56	32.63
70	11.47	31.87	3.57	32.94

Organic matter-degrading bacteria were detected in all water samples from both layers (Fig. 2). HB abundance in both surface and bottom layers varies from  $10^3$  to  $10^5$  cells/mL. No statistically significant differences between the bacterial abundance values in these two layers were observed. Based on the HB abundance we determined, according to state standard GOST 17.1.2.04-77, 75% of water samples from the northwestern Pacific Ocean are classified as oligo- and beta-mesosaprobic waters. Exceptions are samples from the surface layer at stations 8, 24 and 30 and from the bottom layer at stations 30 and 35: water from these samples belongs to polysaprobic, i. e. to “dirty” water according to the organic pollution degree. In the Sea of Okhotsk, water samples taken from the surface layer (from the bottom layer at station 52) are classified as polysaprobic (state standard GOST 17.1.2.04-77) based on the number of heterotrophic microorganisms. High HB abundance indicates water pollution with organic substances and high degree of adaptation of microorganisms to destruction of these substances.

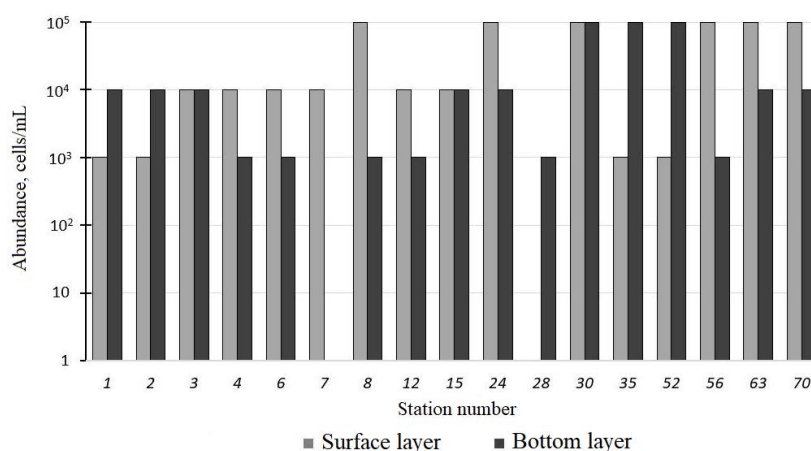


Fig. 2. Abundance of heterotrophic bacteria in the surface and bottom layers of the water column at the studied stations

Station 8 is located near the mouth of the Nalycheva River (the largest catchment area among the rivers of the Avacha group of volcanoes) and probably falls within the high turbidity contour of the river turbidity plume [17]. Higher HB abundance in the surface layer compared to the bottom layer at station 8 can indicate the input of organic matter with river runoff.

Stations 24, 30 and 35 are located in bays with high recreational value, which are often visited by tourists travelling by water transport, resulting in a certain anthropogenic load. Furthermore, these bays contain the mouths of minor rivers, which can serve as a source of organic matter input to the water areas under study.

HOB were isolated in 100% of samples. The abundance of hydrocarbon-oxidizing bacteria in seawater at most stations is 10 cells/mL in samples from the surface layer. The exceptions are station 3 (in Avacha Bay) and station 35 (in Listvennichnaya Bay) on

the Pacific coast of southeastern Kamchatka: at these stations, their abundance reaches 100 cells/mL in the surface layer and 1 cell/mL at stations 15 and 24. The highest concentrations of dissolved hydrocarbons in Avacha Bay are regularly recorded in areas where ships are moored, ship repair plants and transport enterprises discharge wastewater. At the same time, tidal and surge currents contribute to the spread of petroleum hydrocarbons over the entire water area of the bay [18]. It should be noted that in May 2022, a discharge of oil products was detected near the pier of *Okeanrybflot* JSC (15 tons of oil products fell into the bay). During the spill, high concentrations of petroleum hydrocarbons were observed in the surface layer of the bay (at the level of 22–38 MPC, up to 1.7 mg/L), but six months after the spill clean-up, the content of hydrocarbons in the water in the accident area decreased 2.5 times [18].



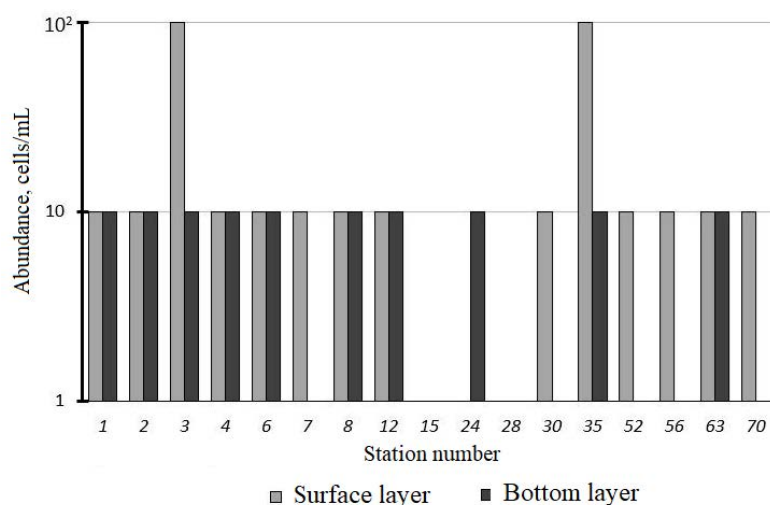


Fig. 3. Abundance of hydrocarbon-oxidizing bacteria in the surface and bottom layers of the water column at the studied stations

The abundance of HOB in the bottom layer at most stations is also 10 cells/mL but minimum single values were recorded at stations 28, 30, 52, 56 and 70 (Fig. 3).

No statistically significant differences between the bacterial abundance values in the surface and bottom layers of the marine water were recorded.

Oil hydrocarbons and phenols make the greatest contribution to the pollution of near-Kamchatka waters. It is noteworthy that, according to Kamchatka Hydrometeorological Service, the mean content of petroleum products in the water of 22 rivers of the peninsula from 2019 to 2022 decreased more than eight times and in 2022, the exceedance of MPC was approximately two times. In Kamchatka marine coastal waters – in Avacha Bay and in the coastal part of the gulf (Khalaktyrsky Beach area) – the mean content of dissolved petroleum hydrocarbons decreased from about 2 MPC in 2019 to 0.3 MPC in 2022 but in isolated cases, elevated values were observed in Avacha Bay<sup>1)</sup>.

POB were isolated in 87.5% of samples from the surface layer. At most stations, their abundance was 10 cells/mL, at stations 7 and 8 (Khalaktyrsky Beach area, at different distances from the mouth of the Nalycheva River) and at station 30 in Russkaya Bay (Fig. 4) it reached 100 cells/mL and at stations 1 and 24 it was 1 cell/mL.

POB were isolated in 93.3% of samples from the bottom layer. At most stations, their abundance was 10 cells/mL, at station 6 (Khalaktyrsky Beach area) and at station 30 in Russkaya Bay (Fig. 4) it reached 100 cells/mL and at stations 3, 52 and 70 it was 1 cell/mL.

No statistically significant differences between the POB abundance in the surface and bottom layers were recorded.

Over the five-year (2018–2022) observation period of Kamchatka Hydrometeorological Service, the mean annual amount of phenols in coastal marine waters

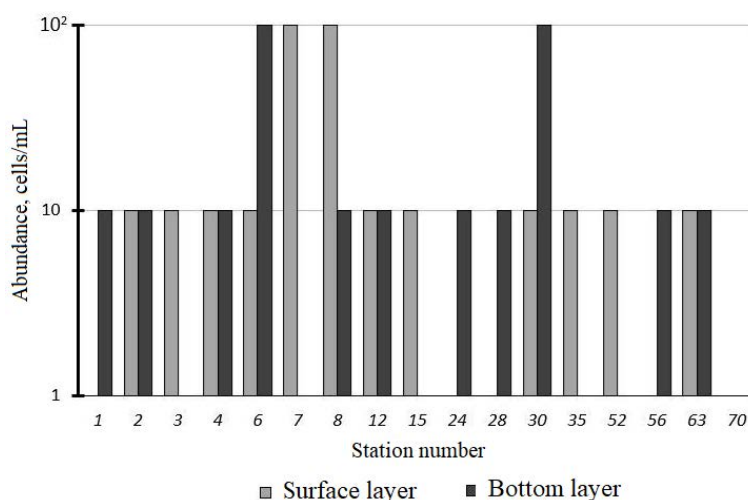


Fig. 4. Abundance of phenol-oxidizing bacteria in the surface and bottom layers of the water column at the studied stations

decreased from 2 to 0.3 MPC. Concurrently, phenol is a principal pollutant contaminating the river network of the peninsula. Its mean content in the river water remained at a level of 5–6 MPC from 2019 to 2022<sup>1)</sup>.

LLB were isolated in 100% of samples. The abundance of bacteria capable of lipid oxidation varied greatly from 1 to 1000 cells/mL in water samples from both surface and bottom layers (Fig. 5).

In the surface layer, the maximum abundance value (1000 cells/mL) was observed at stations 3 и 70, the minimum one (1 cell/mL) was at station 63. In the bottom layer, the maximum value (1000 cells/mL) was recorded at station 63, the minimum one (1 cell/mL) was at station 70.

No statistically significant differences between the LLB abundance in the surface and bottom layers were recorded. The correlation coefficient between the abundance of HOB and LLB is 0.4 ( $P = 0.05$ ), which corresponds to a weak positive correlation. It is important to note that lipids can be formed during the process of oil biodegradation.

The abundance of microorganisms in seawater is determined by a number of factors, including temperature, salinity, depth, etc. The data on water temperature obtained during the study correspond to optimal conditions for the development of psychrophilic and psychrotrophic microorganisms. The findings of the study indicate that the values of the water hydrogen index in the designated area are conducive to the existence of HOB [19].

No significant correlations were found between the abundance of the studied microorganism groups and values of such indicators as surface and bottom water temperature, salinity and depth.

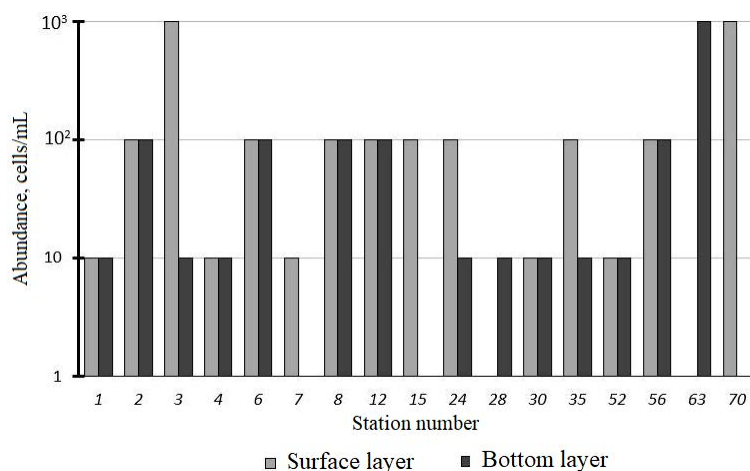


Fig. 5. Abundance of lipolytic bacteria in the surface and bottom layers of the water column at the studied stations

The obtained HB abundance is comparable to the data for the coastal waters of Sakhalin Island in 2004–2006: the summer range of fluctuations in the average abundance of heterotrophic microorganisms was from  $6 \cdot 10^3$  cells/mL (the settlement of Prigorodnoye) to  $45 \cdot 10^7$  cells/mL (the port of Korsakov). In summer, the waters of the ports of Kholmsk and Korsakov as well as Lososey Bay corresponded to the category “very dirty” and the waters of the other stations – “dirty”. Sakhalin coastal waters represent an area with multiple sources of oil pollution of both anthropogenic and natural origin: in summer, the share of HOB in the total number of heterotrophs here ranged from 60% (the village of Okhotskoye) to 80% (the village of Zolotorybnoye)<sup>3)</sup>, which is several times higher than the HOB quantitative characteristics we obtained (see Fig. 3). It is also noted that the values of the absolute abundance of planktonic heterotrophic colony-forming microorganisms were previously studied in water samples collected from Avacha Bay in July 1999: their abundance was from  $1.63 \cdot 10^4 \pm 0$  cells/mL in Turpanka Bay to  $7.98 \cdot 10^5 \pm 0.83 \cdot 10^5$  cells/mL in Rakovaya Bay [9].

### Conclusions

The study characterised the spatial distribution and quantitative characteristics of various organic-oxidising bacteria in the surface and bottom water layers near the southeastern and southwestern coasts of the Kamchatka Peninsula in August–September 2023. The abundance of all investigated groups of bacteria fluctuated within wide limits and was unevenly distributed in the peninsula coastal waters. The maximum values were recorded in the anthropogenically loaded water area of Avacha Bay, in occupied bays and places of active river runoff. Local high abundance of HB indicates both pollution of waters with organic substances and high potential of waters for self-purification.

The studied physiological groups of bacteria exhibited no significant differences in abundance in the surface and bottom layers. No significant correlations were identified between the abundance of bacteria of different groups and such parameters as salinity, temperature and depth.

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Submitted 22.05.2024; accepted after review 23.07.2024;  
revised 25.03.2025; published 30.06.2025

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**Ekaterina A. Demidova** – construction of graphical material, preparation of the article, sampling, microbiological work to determine the number of indicator bacteria groups

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*All the authors have read and approved the final manuscript.*