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Original article

The Content of Hydrocarbons and Indicator Groups of Bacteria in the Marine Environment of Laspi Bay (Southern Coast of Crimea)

E. A. Tikhonova, O. V. Soloveva, Yu. S. Tkachenko *,
N. V. Burdiyan, Yu. V. Doroshenko, E. V. Guseva, S. V. Alyomov

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Sevastopol, Russia
* *e-mail: yulechkatkachenko.90@mail.ru*

Abstract
The paper assesses the quality of the marine environment of Laspi Bay near the Batiliman Stow according to main chemical and microbiological parameters under various recreational impacts on the water area. The material for the study was water and fouling samples taken in May, July and October 2023. The qualitative and quantitative composition of hydrocarbons was determined by gas chromatography on a Crystal 5000.2 chromatograph with a flame ionization detector in the Scientific and Educational Center for Collective Use «Spectrometry and Chromatography» of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS. Diagnostic markers of the origin of hydrocarbons were used to identify possible sources of organic substances. The abundance of bacteria groups (saprophytic heterotrophic, hydrocarbon-oxidizing, lipolytic and phenol-oxidizing) was determined by the method of tenfold dilutions using elective nutrient media. From May to October 2023, the concentration of hydrocarbons in the coastal waters of the Batiliman Stow was 0.013–0.304 $mg \cdot L^{-1}$. The composition of n-alkanes indicated the absence of oil pollution in the studied water area. The exceedance of the maximum permissible concentration for hydrocarbons, noted in July at one of the stations, is of natural origin and is associated with an active intake of allochthonous compounds. The quantitative assessment of the mentioned bacteria groups in the water and microperiphyton of macrofouling indicates an increase in the abundance of indicator groups of bacteria in all samples taken in July. Nevertheless, the results of the hydrocarbon content study and the quantitative assessment of the main microbiological indicators in the water and microperiphyton of macrofouling suggest that there are active bacterial self-purification processes in the water area of the Batiliman Stow. According to microbiological indicators, the studied area can be classified as conditionally clean.

Keywords: coastal water area, recreational load, seawater, markers, periphyton, heterotrophic bacteria, hydrocarbon-oxidizing bacteria, lipolytic bacteria, phenol-oxidizing bacteria, macrophytes, Laspi Bay, anthropogenic pollution, petroleum hydrocarbons

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Содержание углеводородов и индикаторных групп бактерий в морской среде бухты Ласпи (Южный берег Крыма)

Е. А. Тихонова, О. В. Соловьёва, Ю. С. Ткаченко *,
Н. В. Бурдиян, Ю. В. Дорошенко, Е. В. Гусева, С. В. Алёмов

ФГБУН ФИЦ Институт биологии южных морей имени А. О. Ковалевского РАН,
Севастополь, Россия
* *e-mail: yulechkatkachenko.90@mail.ru*

Аннотация

Оценено качество морской среды бухты Ласпи в районе урочища Батилиман по основным химико-микробиологическим параметрам в периоды различной рекреационной нагрузки на акваторию. Материалом для исследования послужили пробы воды и обрастаний, отобранные в мае, июле и октябре 2023 г. Качественный и количественный состав углеводородов определялся на базе НОЦКП «Спектрометрия и хроматография» ФИЦ ИнБЮМ методом газовой хроматографии на хроматографе «Кристалл 5000.2» с пламенно-ионизационным детектором. Для идентификации вероятных источников поступления органических веществ использовали диагностические маркеры происхождения углеводородов. Численность групп бактерий (сапрофитных гетеротрофных, углеводородокисляющих, липолитических и фенолокисляющих) определяли методом предельных десятикратных разведений с использованием элективных питательных сред. Концентрация углеводородов в прибрежных водах урочища Батилиман с мая по октябрь 2023 г. составляла 0.013–0.304 мг·л$^{-1}$. Состав н-алканов указывал на отсутствие нефтяного загрязнения в исследуемой акватории. Превышение ПДК для углеводородов, отмеченное в июле на одной из станций, носит природный характер и связано с активным поступлением аллохтонных соединений. Количественная оценка обозначенных групп бактерий в воде и микроперифитоне макрообрастаний указывает на возрастание численности индикаторных групп бактерий во всех пробах, отобранных в июле. Тем не менее результаты исследования углеводородного фона и количественной оценки основных микробиологических показателей в воде и микроперифитоне макрообрастаний указывают на то, в акватории урочища Батилиман активно происходят процессы бактериального самоочищения. По микробиологическим показателям исследуемый участок можно отнести к условно-чистым акваториям.

Ключевые слова: прибрежная зона, рекреационная нагрузка, морская вода, маркеры, перифитон, гетеротрофные бактерии, углеводородокисляющие бактерии, липолитические бактерии, фенолокисляющие бактерии, макрофиты, бухта Ласпи, антропогенное загрязнение, нефтяные углеводороды

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Introduction

The coastline of the Batiliman Stow (a coastal-aquatic complex between Cape Sarych and Laspi Bay), from where the mountains of the Southern coast of Crimea begin, stretches from the base of Mount Kush-Kaya to Laspi Bay. The area is characterised by intense water exchange with the open sea and by high aeration. Runup and surge phenomena typical of the Southern coast of Crimea result in water salinity fluctuating from 17.70 to 18.47 [1].

From the water's edge to shallow depths (10 m) the bottom is represented by boulders (boulder bench) (Fig. 1) with rare areas of sandy bottom (Fig. 2). The granulometric composition of bottom sediments and peculiarities of morphodynamic conditions of the environment (drift of fine fractions to shallow zones) determine the absence of organic carbon accumulation within this coastal area [2].

This is correlated with the reports [3] on the low number of microbial population in the loose bottom sediments of nearby Laspi Bay where the number of saprophytic heterotrophic bacteria averages 2500 cells$\cdot$g^{-1} and the number of hydrocarbon-oxidising bacteria does not exceed 2 cells$\cdot$g^{-1}. Of the macrophytes, *Cystoseira crinita* (Dyby, 1830) and *Ceramium diaphanum* (Roth, 1806) dominate year-round in terms of occurrence, while *C. crinita* and *C. barbata* (C. Agardh, 1820) dominate in terms of phytomass [4]. *Cystoseira spp.* is the main component of coastal phytocenosis and one of the main sources of organic matter [5]. Besides, *Cystoseira spp.* is considered to be the most suitable object of algomonitoring in assessing the environmental quality of marine coastal water areas, including the water area of Batiliman Stow [6].

The Batiliman Stow bordering the sea from the west of Cape Aya is a state natural landscape reserve of regional significance[1] and includes 208 ha of the Black Sea water area. The territory up to the next protected object, hydrological natural monument Coastal Aquatic Complex at Cape Sarych, has no nature protection status, although works in this regard are being done. The authors of work [7] proposed

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[1] Government of the City of Sevastopol, 2016. *On Amendments to the Resolution of the Government of the City of Sevastopol no. 409-ПП “On Approval of the Regulations on the State Natural Landscape Reserve of Regional Significance Cape Aya” as of 29 April 2016.* Resolution of the Government of the City of Sevastopol no. 178-ПП as of 25 April 2022. Sevastopol: Government of the City of Sevastopol (in Russian).

to create a national park Yuzhnoberezhny from Balaklava Bay to Cape Sarych including the adjacent water area and protected areas of regional significance.

This area is highly appealing for tourists, but due to its small recreational capacity there is a danger of negative impact of mass unregulated visitation on the state of the water area ecosystem. Over the last decades, the ecological state of the concerned water area has deteriorated. This is due to the increase in the flow of visitors and development of the coastal zone, as well as the placement of mussel farms nearby (with an output of up to 83 t (per dry weight) of biosediments per year, including 3 t of protein and 1 t each of carbohydrates and lipids [8]). Recent studies have shown that the number of polychaete species has decreased from 64 (1983) to 45 (2019) [9], the macrophytobenthos stock of the Black Sea environment-forming species have decreased by about 1.5 times, whereas some bottom areas have lost vegetation at all [10]. According to the authors of work [11], in 2017–2018, the content of petroleum hydrocarbons (PHC) in the water of Laspi Bay was close to the maximum permissible levels. At the same time, in the summer of 2018, the maximum permissible concentration (MPC) was exceeded 3–4 times. The PHC content in Laspi Bay was higher than their average content in Sevastopol bays, and in 2016 the frequency of recorded cases of exceeding MPC in the bottom horizon of the water area of Laspi Bay was 25% [12, 13]. Thus, the authors indicate that the study area, previously classified as reference clean, is under a significant anthropogenic impact. Probably, the obtained results are related to the recent active development of the coastline of Laspi Bay [11].

Of note, this part of the coast is often affected by landslides, mudflows and coastal abrasion [14]. Development of this area only worsens the situation.

Preservation of the Batiliman Stow coastal-aquatic complex requires an integrated approach to the study of the coastal water area to calculate the current level of anthropogenic pressure and the stability of the complex against it. This approach will also allow proposing measures to minimise the negative effects of increasing recreational load without affecting the established cycle of matter and energy as well as aesthetics of this unique place.

Within an integrated approach to the study of the ecological state of the Batiliman beach water area, there has been little research of the hydrocarbon (HC) content in seawater and characteristics of its bacterial population, which is the first link in the process of biological self-purification of the marine environment.

The work aims to assess the content of HCs and indicator groups of bacteria in the marine environment of Laspi Bay.

The objectives of the study include determination of:

– qualitative and quantitative composition of HCs in the coastal water area of Laspi Bay;

– the number of saprophytic heterotrophic bacteria – the main destructors of readily available organic compounds in water and in microperiphyton of macrofouling;

– abundance of indicator groups of bacteria – oil, phenol and fat destructors in water and in microperiphyton of macrofouling.

Material and methods

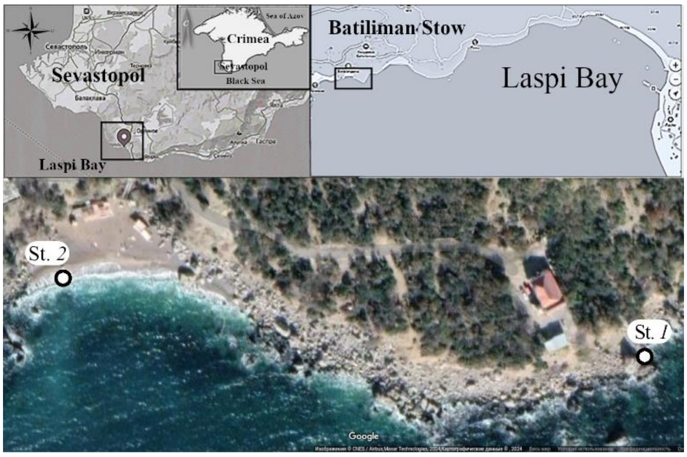
Water samples for HC analysis were taken in May, July and October 2023 at two stations. Station *1* – biostation, low-exploited area with a low anthropogenic load. Station *2* – Tavrida Beach, an area with a high anthropogenic load in summer. Both at Station *1* and Station *2*, water was sampled near the water edge (Fig. 1).

Water samples were taken in glass-stoppered glass bottles with a capacity of 1 dm^3, pre-washed with chromium mixture, tap and distilled water and rinsed with hexane. Before sampling, the bottles were pre-washed with n-hexane and rinsed with the sampled water.

Sample preparation was carried out according to the procedure 2). A water sample (250 mL) acidified with sulfuric acid (1:1) (1.5 mL) was extracted twice with n-hexane (25 mL each). The hexane extract was passed through a glass column filled with aluminium oxide and concentrated to a volume of 1 mL at room temperature in a fume hood.

The qualitative and quantitative composition of HCs was determined at the Scientific and Educational Center for Collective Use “Spectrometry and Chromatography” of IBSS using a Crystal 5000.2 gas chromatograph with a flame ionization detector (FID).

An aliquot of the concentrated extract was injected with a microsyringe into the gas chromatograph evaporator heated to 250 °C. HCs were separated



F i g . 1 . The map of seawater and macrofouling sampling in the water area of the Batiliman Stow, 2023. Google Maps image (available at https://www.google.ru/maps)

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2) Drugov, Yu.S. and Rodin, A.A., 2020. [*Ecological Analyses in Oil and Petroleum Product Spills. A Practical Guide*]. Moscow: Laboratoriya Znaniy, 270 p. (in Russian).

on a TR-1MS capillary column 30 m long, 0.32 mm in diameter and with the stationary phase film thickness of 0.25 μm (Termo Scientific). The column temperature was programmed from 70 to 280 °C (rate of temperature rise: 8 $°C \cdot min^{-1}$). The carrier gas (nitrogen) flow in the column was 2.5 $mL \cdot min^{-1}$ without flow splitting. The detector temperature was 320 °C.

Quantitation of the total HC content was performed by absolute calibration of the FID with a standard mixture of HCs (C_{10}–C_{40}) ranging 0.01–0.5 $mg \cdot L^{-1}$. A standard sample of ASTMD2887 Reference Gas Oil standard (SUPELCO, USA) was used as a HC mixture. The total HC content was determined by the sum of the areas of eluted n-alkanes peaks and the unresolved complex mixture (UCM). The results were processed with the Chromatec Analytical 3.0 software (the absolute calibration and percentage normalization method).

The following diagnostic indices were used to identify HC genesis: terrigenous/aquatic ratio (TAR) [15], average chain length (ACL) [16], and low-molecular weight to high-molecular weight homologues ratio (LWH/HWH) [17]. The P_{aq} index [18] (aquatic to terrestrial plant index) determines the type of vegetation prevailing in the organic matter formation. Carbon Preference Indices CPI_1 [19], calculated for lighter n-alkanes, and CPI_2 [16], calculated for the high-molecular weight part of the spectrum, are used to identify the petroleum and biogenic origin of HCs. The HC genesis markers were determined according to the ratios presented in Table 1.

Seawater samples for microbiological analysis were collected in sterile 50 cm^3 tubes and fouling samples were collected in sterile jars. In this study, the macrofouling community was assessed from which microperiphyton was then washed out

Table 1. Main diagnostic indices for identification of the hydrocarbon genesis

Index	Formula
TAR	$\sum(C_{27} + C_{29} + C_{31})/\sum(C_{15} + C_{17} + C_{19})$
LWH/HWH	$\sum(C_{13}–C_{21})/\sum(C_{22}–C_{37})$
ACL	$(27C_{27} + 29C_{29} + 31C_{31} + 33C_{33} + 35C_{35} + 37C_{37})/$ $(C_{27} + C_{29} + C_{31} + C_{33} + C_{35} + C_{37})$
CPI_1	$\frac{1}{2}\{(C_{15} + C_{17} + C_{19} + C_{21})/(C_{14} + C_{16} + C_{18} + C_{20}) +$ $+ (C_{15} + C_{17} + C_{19} + C_{21})/(C_{16} + C_{18} + C_{20} + C_{22})\}$
CPI_2	$\frac{1}{2}\{(C_{25} + C_{27} + C_{29} + C_{31} + C_{33} + C_{35})/(C_{24} + C_{26} + C_{28} + C_{30} + C_{32} + C_{34}) +$ $+ (C_{25} + C_{27} + C_{29} + C_{31} + C_{33} + C_{35})/(C_{26} + C_{28} + C_{30} + C_{32} + C_{34} + C_{36})\}$
P_{aq}	$(C_{23} + C_{25})/(C_{23} + C_{25} + C_{29} + C_{31})$

to determine bacterial abundance. In all sampling periods, macrofouling was represented exclusively by Cystosira. Its abundance decreased naturally from May to October, and its biomass was slightly higher in all sampling periods at St. *1*. The abundance of saprophytic heterotrophic (HB), hydrocarbon-oxidising (HOB), lipolytic (LB) and phenol-oxidising (POB) bacterial groups was determined in each sample. The abundance of these bacterial groups was determined by the method of tenfold dilutions using selective nutrient media. For HBs, a peptone medium was used [20]. HOBs and LBs were cultured on a Voroshilova–Dianova medium [21], to which sterile oil or vegetable fat (1% of the volume) was added as the only source of carbon and energy. For phenol-oxidising bacteria, a modified Kalabina–Rogovskaya medium was used [22]. When preparing the media, the salinity of seawater was taken into account. The most probable number of microorganisms per unit volume was calculated using McCready's table (in triplicate) based on the method of variation statistics 3).

Results and discussion

The total HC content in water at the studied stations from May to October 2023 ranged from 0.013 to 0.304 mg·L^{-1} (Fig. 2). In July, at St. *1*, the exceedance of MPC for fishery water bodies (0.05 mg/L) 4) by 6 times was recorded (Fig. 2), at St. *2* during the study period, the value of HC concentration was rather low and did not exceed the MPC.

Comparing the indicators of the low-exploited coastal area and the beach, it is difficult to speak about the increase of HC content on the beach in summer, when the anthropogenic load on the coast increases significantly. Probably, other factors play the leading role in formation of hydrocarbon content in the coastal waters of this area.

The study of the individual composition of n-alkanes, as well as calculation of markers characterising the sources of organic substances origin in water, allow more reliable identification of HC input sources.

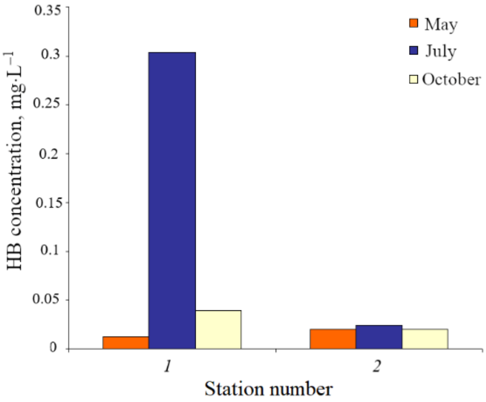

Fig. 2. Hydrocarbon concentrations in the coastal waters of the Batiliman Stow, May–October 2023

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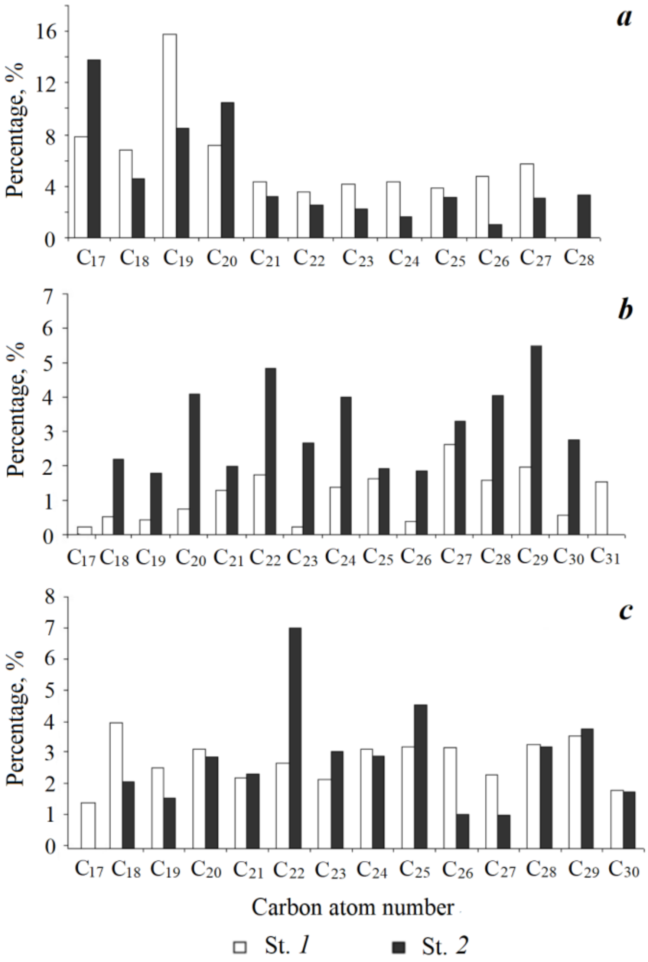
3) Netrusov, A.I., ed., 2005. [*Practical Course on Microbiology*]. Moscow: Akademiya, 608 p. (in Russian).

4) Ministry of Agriculture of Russia, 2016. *On the Approval of Water Quality Standards for Water Bodies of Commercial Fishing Importance, Including Standards for Maximum Permissible Concentrations of Harmful Substances in the Waters of Water Bodies of Commercial Fishing Importance*: Order of the Ministry of Agriculture of Russia dated December 13, 2016, No. 552. Moscow: Ministry of Agriculture of Russia (in Russian).

In water samples collected from May to October 2023, n-alkanes in the range n-C_{17}–C_{31} were identified (Fig. 3), the n-alkane C_{31} was detected only once at St. *1* in July (Fig. 3, *b*). The homologues of C_{29} and C_{30} were not detected in May at both stations (Fig. 3, *a*), the other n-alkanes were represented everywhere.

The distribution of n-alkanes obtained in May at both stations was unimodal. Surface water samples collected in May 2023 were dominated by low-molecular weight homologues (Fig. 3, *a*), in particular heptadecane (n-C_{17}), which is the main alkane produced by phyto- and zooplankton [23, 24], and alkane n-C_{19}, also of phytoplanktonic genesis. The C_{18} and C_{20} peaks were well pronounced. They are associated with the development of bacterial community [25]. Markers *TAR* and CPI_1 (Table 2) show the predominance of autochthonous matter in water, formed as a result of microbiological degradation of organic matter [26].



F i g . 3 . Distribution of n-alkanes in the coastal waters of the Batiliman Stow: May (*a*), July (*b*), October (*c*), 2023

Table 2. Content and composition of n-alkanes (markers) in the water of the coastal water area of the Batiliman Stow, May–October 2023

Station number	D	C	LWH/ HWH	P_{aq}	TAR	ACL	CPI_1	CPI_2
				May				
1	C_{17}–C_{27}	0.013	1.59	1.00	0.23	26.17	1.78	1.50
2	C_{17}–C_{28}	0.020	2.66	1.00	0.12	26.00	1.79	1.23
				July				
1	C_{17}–C_{31}	0.304	0.21	0.34	13.57	28.00	1.08	2.50
2	C_{18}–C_{30}	0.024	0.34	0.52	4.50	27.40	0.48	0.92
				October				
1	C_{17}–C_{30}	0.040	0.65	0.65	1.27	27.00	0.73	0.93
2	C_{18}–C_{30}	0.020	0.34	0.65	3.33	26.90	0.60	1.32

Note: D – the range of identified n-alkanes; C – total concentration of identified n-alkanes, $mg \cdot L^{-1}$.

Thus, the n-alkanes in the water samples collected in May 2023 are predominantly autochthonous and are associated with phytoplankton and bacterial production. The above season is characterised by active phytoplankton development [27].

In July, a relatively uniform distribution of n-alkanes was recorded at St. *1*, where the MPC exceedance was observed. At St. *2*, the distribution differed, showing signs of bimodality (Fig. 3, *b*): the first peak (even-numbered n-alkanes in the range C_{18}–C_{24}) may be associated with the work of the bacterial community, whereas the second peak (C_{27}–C_{30}) is usually associated with higher plants of both aquatic and terrestrial origin [25].

At St. *1*, the n-C_{17} homologue was identified in small amounts, while at St. *2* it was not detected at all (Fig. 3, *b*). The *TAR* index at both stations was significantly greater than unity (Table 2), indicating the predominance of allochthonous matter coming from land.

Though at St. *1* in July 2023, the exceedance of MPC by 6 times was recorded, the CPI_2 index value was 2.5 (Table 2), indicating the biogenic origin of organic matter. Moreover, the C_{17}/C_{25} ratio was 0.08 (Table 2), indicating the predominance of allochthonous homologues [28]. The *TAR* index value significantly exceeded unity and was 13.57 (Table 2), which also shows the predominance of allochthonous matter in the water area.

Thus, despite the relatively uniform distribution of n-alkanes, which may signify fresh oil pollution, markers of the latter were absent. Diagnostic indices clearly indicate the predominance of biogenic allochthonous matter. It can be concluded that no oil pollution was detected in the studied samples, and the increased values of HC concentrations were due to natural processes.

In surface water samples collected in October 2023, the distribution of n-alkanes was relatively monotonous (Fig. 3, *c*). The n-C_{22} homologue was dominant at St. *2*; together with the CPI value (Table 2), this indicates the presence of HC microbial degradation products in open surface water [25, 29]. In the high-molecular weight part of the spectrum, the peak associated with the n-alkane C_{25}, which is of allochthonous origin, was pronounced for St. *2*. The homologue C_{17}, which is a marker of phyto- and zooplankton, was absent at St. *2* (Fig. 3, *c*). The values of TAR and LWH/HWH indices (Table 2) at the studied stations indicate the dominance of allochthonous matter coming from land. No signs of oil pollution were detected, as indicated by the CPI_2 marker value (Table 2).

The ACL marker is used to reveal changes in the ecosystem. The marker remains stable for a long time and decreases abruptly in case of oil pollution [30]. High values of the ACL marker indicate the predominant contribution of herbaceous vegetation to HC formation, while low ACL values are characteristic of HCs of wood origin. This index ranged from 26 to 28 (mean 26.9 ± 0.7) (Table 2) at both stations, which indicates the absence of fresh oil inputs and reflects approximately the same contribution of woody and herbaceous plants to the formation of organic matter of surface open waters in the water area. The P_{aq} indicator [18] (Table 2) allows determining the type of vegetation prevailing in the process of organic matter formation: terrigenous or aquatic [18]. The indicator shows that in May, HCs of aquatic origin prevailed, whereas in July, terrigenous matter dominated at St. *1*. The proportions of autochthonous and allochthonous matters were approximately equal at St. *2*. In October, a slight predominance of autochthonous compounds was noted at both stations.

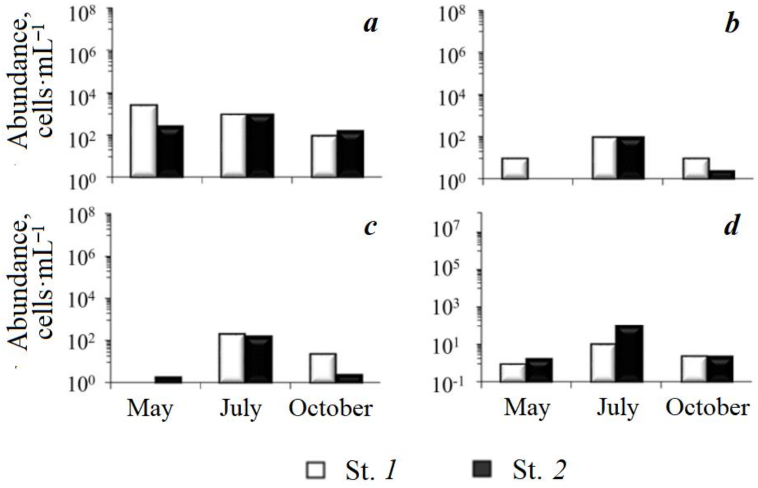
From the analysis results of the water samples taken in the water area of the Batiliman Stow from May to October 2023, including during the high recreational season, it was not possible to establish oil pollution of the bay waters. The main sources of formation of hydrocarbon background of the water area in May were autochthonous processes associated with the production of phytoplankton and bacterial destruction of organic matter. In subsequent periods, the importance of phytoplankton production decreased, bacterial processes and the input of allochthonous compounds came to the foreground. The exceedance of sanitary norm values (MPC = 0.05 $mg \cdot L^{-1}$), observed in July (0.304 $mg \cdot L^{-1}$) at one of the stations, is of natural character and associated with active input of allochthonous compounds.

An important indicator of the marine environment quality is the state of the bacterial community, for which organic matter, including HCs, entering the water area is a nutritious substrate. Our results on the origin of HCs indicate active participation of bacteria in the synthesis and transformation of HCs.

The results of the performed microbiological studies show that the maximum HB abundance ($2.5 \cdot 10^3$ cells·mL^{-1}) in water was observed once in the May sample of St. *1* (Fig. 4, *a*), while in the other samples of St. *1*, the number of HBs varied from 95 to 950 cells·mL^{-1}. In the beach water area (St. *2*), HB abundance ranged from 150 to 950 cells·mL^{-1} (Fig. 4, *a*). In May and July, the HB abundance at both stations exceeded the HB values in October samples, which is probably related to the phytoplankton blooms, typical of spring [31], and the increase in water temperature in summer. The HB abundance in water of the studied sites is similar to the data [32] obtained earlier in the conditionally clean water area.

At St. *1*, HOBs were detected in all samples. The maximum (95 cells·mL^{-1}) was observed in the July sample, while in the remaining samples, the abundance of HOBs did not exceed 10 cells·mL^{-1} (Fig. 4, *b*). In the May sample of St. *2*, no HOBs were detected, and in October they were represented by single cells in a millilitre of seawater (Fig. 4, *b*). The HOB maximum at St. *2* was identified in July (95 cells·mL^{-1}). The HOB share of the HB abundance in water samples at St. *1* did not exceed one per cent in May, and in July and October it was 10%. At St. *2* , the HOB share of the HB abundance was 10% in July and it decreased to 1.6% in the October sample. In clean waters, hydrocarbon-oxidising microorganisms are considered to account for up to 7% of saprophytic heterotrophic microflora [33].

No lipolytic bacteria were cultured in the May sample of St. *1* (Fig. 4, *c*). The maximum LB abundance was observed in July (200 cells·mL^{-1}), and in October, the LB value was an order of magnitude less. The LB abundance in the water of St. *2* ranged from 2 to 150 cells·mL^{-1} (Fig. 4, *c*). The LB maximum was determined in July, as at St. *1*.



F i g . 4 . Dynamics of the abundance (cells·mL^{-1}) of heterotrophic (*a*), hydrocarbon-oxidizing (*b*), lipolytic (*c*), phenol-oxidizing (*d*) bacteria in the water

POBs at St. *1* were detected in all samples (Fig. 4, *d*). The maximum (10 cells·mL^{-1}) was detected at station *1* in July, while in the other samples of St. *1*, this group of bacteria was represented by single cells per millilitre of seawater. At St. *2*, POBs were also detected in all samples (Fig. 4, *d*). The maximum (95 cells·mL^{-1}) was detected at St. *2* in July. In other samples, the POB abundance was under 10 cells·mL^{-1}.

The obtained results (Fig. 5, *a*) for the assessment of HB abundance in the macrofouling microperiphyton showed that at St. *1* the maximum HB abundance (2.5·10^6 cells·g^{-1}), indicating a sufficient amount of highly digestible organic matter, was observed in the May sample. In subsequent determinations, the HB abundance was 9.5·10^3 cells·g^{-1}. At St. *2*, the HB abundance in the May and October samples ranged from 2.5·10^4 – 4.5·10^4 cells·g^{-1}, with the abundance value of HBs decreasing by an order of magnitude in July. The highest HB abundance at St. *2*, as at St. *1*, was determined in the May sample (4.5·10^4 cells·g^{-1}).

HOBs were cultured from all fouling samples from the mentioned stations (Fig. 5, *b*). At both stations, the HOB abundance varied from 95 to 2.5·10^2 cells·g^{-1}. However, at St. *1*, the lowest (95 cells·g^{-1}) HOB value was recorded in the May sample, while in the other samples of St. *1*, the HOB abundance ranged from 1.5·10^2 to 2.5·10^2 cells·g^{-1}. In the beach fouling (St. *2*), the maximum (2.5·10^2 cells·g^{-1}) was obtained in July and the minimum (95 cells·g^{-1}) was recorded in October. The HOB share of HB in July samples of St. *1* was 1.6%, and in July samples of St. *1* and *2* it was 2.6 %. In other months the HOB share at both stations was under 1%. The quantitative indicators of HOB obtained in the water area of the Batiliman Stow are much lower than those obtained in the microperiphyton of breakwaters of the Sevastopol water area, which are under a significant anthropogenic load [34].

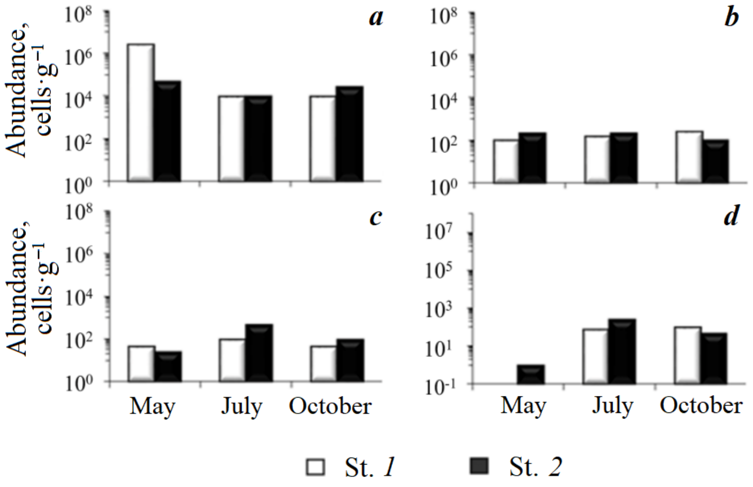


Fig. 5. Dynamics of the abundance (cells·g^{-1}) of heterotrophic (*a*), hydrocarbon-oxidizing (*b*), lipolytic (*c*), phenol-oxidizing (*d*) bacteria in the fouling

LBs were detected in 100% of fouling samples from St. *1* and *2* (Fig. 5, *c*). At St. *1*, the abundance of LBs ranged from 45 to 95 cells·g^{-1}, while at St. *2*, the range of LB abundance was 25–450 cells·g^{-1}. The highest LB abundance values at both stations were obtained at the height of the holiday season. The minimum LB abundance (25 cells·g^{-1}) was determined in the May sample at St. *2*. In the other samples at St. *1* and *2*, the abundance of LBs varied from 45 to 95 cells·g^{-1}.

POBs at St. *1* were not cultured in the May sample, the results of follow-up observations at St. *1* showed an increase in the POB abundance in July and October samples, 75 and 95 cells·g^{-1}, respectively (Fig. 5, *d*). At St. *2*, the POB abundance ranged from 1 to 250 cells·g^{-1} (Fig. 5, *d*). The maximum (250 cells·g^{-1}) at St. *2* was recorded in the July sample, the minimum in May, and in October the POB abundance in beach fouling decreased to 45 cells·g^{-1}.

The data analysis showed that the absence of a pronounced abundance peak of saprophytic heterotrophic bacteria (Fig. 4, *a*) and the observed increase in the hydrocarbon content in July at St. *1* (Fig. 2), given the composition of n-alkanes, can be related to the entry of high-molecular weight allochthonous compounds, which are less susceptible to bacterial degradation [35]. The input of allochthonous material can be related to precipitation occurred the day before (21–23 July 2023) (available at: https://goodmeteo.ru/pogoda-batiliman-orlinoe-sevastopol/23-7/) and the peculiarities of the station location (possibility of mudflows). The HOB share of the HB abundance in fouling at both stations was rather low and did not exceed 2.6%, which corresponds to the values for clean water areas [5)]. The obtained quantitative characteristics of POBs and LBs in the fouling of the Batiliman Stow water area at the investigated sites were much lower than similar indicators of Golubaya Bay Beach (Sevastopol water area), at the same time the POB content was much higher than that in the periphyton of Golubaya Bay Beach [32]. The increase in the abundance of indicator groups of bacteria (HOBs, LBs and POBs) at St. *1* and *2* in July both in the water samples and in the microperiphyton is a response of the microbial community to seasonal changes in the ecosystem, including an increase in the anthropogenic load on the water area of the Southern Coast of Crimea.

Conclusions

From May to October 2023, the HC concentration in the coastal waters of Batiliman Stow was 0.013–0.304 mg/L. The composition and content of HCs in the coastal waters of the Batiliman Stow are caused by natural processes. Oil pollution was not recorded.

Quantitative assessment of the indicated groups of bacteria in water and microperiphyton of macrofouling and the obtained results of hydrocarbon content study indicate that, despite a significant anthropogenic load in summer, there are active bacterial self-purification processes in the water area of the Batiliman Stow. Based on the microbiological indicators, the studied area can be classified as conditionally clean.

[5)] Mishustina, I.E., Shcheglova, I.K. and Mitskevich, I.N., 1985. [*Marine Microbiology*]. Vladivostok: DVGU, 184 p. (in Russian).

Taking into account the increasing anthropogenic load on this part of the coast, associated with the construction of tourist facilities, the results of baseline studies can be used further for comparative analysis of the state of the waters of the Batiliman Stow during environmental monitoring or environmental assessment in emergencies.

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About the authors:

Elena A. Tikhonova, Leading Research Associate, A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave, Sevastopol, 299011, Russian Federation), PhD (Biol.), **ORCID ID: 0000-0002-9137-087X, Scopus Author ID: 57208495804, ResearcherID: X-8524-2019**, *tihonoval@mail.ru*

Olga V. Soloveva, Leading Research Associate, A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave, Sevastopol, 299011, Russian Federation), PhD (Biol.), **ORCID ID: 0000-0002-1283-4593, Scopus Author ID: 57208499211, ResearcherID: X-4793-2019**, *kozl_ya_oly@mail.ru*

Nataliya V. Burdiyan, Senior Research Associate, A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave., Sevastopol, 299011, Russian Federation), PhD (Biol.), **ORCID ID: 0000-0001-8030-1556, Scopus Author ID: 57208497483, ResearcherID: AAD-1704-2022,** *burdiyan@mail.ru*

Yulia S. Tkachenko, Junior Research Associate, A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave, Sevastopol, 299011, Russian Federation), **ORCID ID: 0009-0001-1752-1043**, **Scopus Author ID: 1220495;** *yulechkatkachenko.90@mail.ru*

Yulia V. Doroshenko, Research Associate, A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave., Sevastopol, 299011, Russian Federation), PhD (Biol.), **ORCID ID: 0000-0003-0498-3369; Scopus Author ID: 57211643141, ResearcherID: AAD-1706-2022,** *julia_doroshenko@mail.ru*

Elena V. Guseva, Junior Research Associate, A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave., Sevastopol, 299011, Russian Federation), **Scopus Author ID: 57208488324;** *guseva_ev@ibss-ras.ru*

Sergey V. Alyomov, Leading Research Associate, A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (2 Nakhimova Ave., Sevastopol, 299011, Russian Federation), PhD (Biol.), **ORCID ID: 0000-0002-3374-0027, Scopus Author ID: 24070027300,** *alyomov_sv@ibss-ras.ru*

Contribution of the authors:

Elena A. Tikhonova – statement of aims and objectives of the complex study, water and fouling sampling, manuscript writing

Olga V. Soloveva – analysis of the results obtained on the hydrocarbon composition of water, results discussion, manuscript writing

Yulia S. Tkachenko – preparation of water samples, determination of qualitative and quantitative composition of hydrocarbons in the water, writing and presentation of the article

Nataliya V. Burdiyan – statement of the objectives of the microbiological study, analysis of the results obtained in the microbiological study, results discussion, manuscript writing, microbiological works to determine the abundance of indicator groups of bacteria in the periphyton

Yulia V. Doroshenko – analysis of the obtained results on microbiological research, results discussion, manuscript writing, microbiological works to determine the abundance of indicator groups of bacteria in the water

Elena V. Guseva – study rationale, preparation of the literature review, fouling sampling, construction of graphical materials

Sergey V. Alyomov – sampling, description of the macrofouling community and its changes during different sampling periods

All the authors have read and approved the final manuscript.