

Conference Paper

Current State of Heterotrophic Bacterioplankton and Bacteriobenthos in the Northern and Middle Parts of the Caspian Sea

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Abstract

Data on the abundance of heterotrophic bacterioplankton and bacteriobenthos in the northern and middle parts of the Caspian during the period from 2013 to 2017 are presented. In the waters of the northern part of the Caspian, a peak in the number of saprotrophs and crude oil degraders was recorded in 2013. In the middle part of the Caspian and in the region of the Mangyshlak regression, high numbers of heterotrophic bacteria were recorded in 2013 and 2016. The biodiversity of bacterioplankton in the surveyed areas is almost the same; the species composition of saprotrophs and crude oil degraders is identical. In the bottom sediments of the northern and middle parts of the Caspian Sea, two peaks of saprotrophic bacteriobenthos development (in 2013 and 2016) were recorded; in the region of the Mangyshlak threshold, the concentration of saprotrophs remained stable. The number of crude oil degraders was inferior to those of saprotrophs, while the proportion of crude oil degraders to saprotrophs changed annually. The species composition of saprotrophs is relatively stable, the biodiversity of oil-oxidizing bacteriobenthos has changed in the direction of increasing the proportion of non-fermenting bacteria. Constant changes in the ratio of the number of saprotrophs and crude oil degraders, together with the transformations of the dominant groups of the species composition of bacteria, indicated the instability of bacteriobenthos, and, as a result, the vulnerability of the microecosystem of bottom sediments under changing environmental conditions.

Keywords: Caspian Sea, bacterioplankton, bacteriobenthos, saprotrophs, crude oil degraders

1. Introduction

The Caspian Sea is the largest brackish water body without any connection with the World Ocean. Considering the morphological structure and physical and geographical features, the sea is divided into three parts: the northern, middle and southern Caspian [1]. The ecosystem of each part of the sea is unique and features differences in geographical location, climate, as well as hydrological and hydrochemical properties [2, 3].

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The economic importance of the Caspian Sea is difficult to overestimate. In its water area, ideal conditions are created for feeding young and adult individuals of semi-migratory and migratory fish species; active hydrocarbon production is ongoing on the shelf; developed shipping and port areas also complement the anthropogenic impact on the Caspian basin [4]. Based on these areas of economic use of the Caspian Sea, and taking into account the presence of protected areas in the sea [5], annual environmental studies are necessary, including monitoring of both hydrochemical indicators and various biotope groups, including the number and ratio groups of heterotrophic bacteria, in particular, saprotrophs and crude oil degraders [6], since they play a decisive role in the mineralization of organic matter and the circulation of biogens and are actively involved in the processes of self-purification of the sea [7], which makes the determination of the number and species composition of oil-oxidizing and saprotrophic bacterioplankton and bacteriobenthos high on the agenda.

2. Methods and Equipment

The studies were carried out in the summer from 2013 to 2017 in the northern part of the Caspian Sea (sectors 324, 402, 405), near the Mangyshlak regression---the conditional border line between the northern and middle parts of the Caspian Sea (sectors 354, 464, 467). In the middle part of the Caspian Sea, samples were taken in the western part of the sea to a depth of 200 m (sectors 568, 587, 639) and in the deep-water zone (sectors 597, 700, 722, 743). To determine the number of saprotrophic and oil-oxidizing bacteria, samples of bottom sediments (at a depth of up to 90 m) and water from the surface and bottom horizons were taken. Bottom sediment samples were taken using a bottom sampler (Okean 50), and water samples were taken using the bathometers of a SBE 32 Carousel Water Sampler. A total of 440 water and bottom soil samples were collected and processed for microbiological research. To determine the number of different groups of heterotrophic bacteria, the limiting dilutions method was used, followed by seeding on solid nutrient media [8]. Nutrient agar was used to determine for saprotrophic bacteria, and Tepper medium with the addition of 1.0% sterile oil as the sole carbon source was used to determine oil-oxidizing bacteria. Pure bacterial cultures were isolated according to generally accepted methods [9]. Species identification of the isolated bacteria was carried out using the Bergey's Manual of Determinative Bacteriology [10].

3. Results

During the monitoring period from 2013 to 2017, the maximum abundance of saprotrophic bacteria in the northern part of the Caspian Sea (75.72 ± 33.09 thousand CFU/ml) was recorded in 2013 (Table 1).

TABLE 1: The number of saprotrophic and hydrocarbon-oxidizing bacteria in the water of the Caspian Sea in 2013--2017 (thousand CFU/ml).

Research area	Year				
	2013	2014	2015	2016	2017
Saprotrophs					
North Caspian	75.72 ± 33.09	1.29 ± 0.36	1.53 ± 0.44	3.72 ± 0.82	1.78 ± 1.01
Mangyshlak regression	26.15 ± 10.59	2.03 ± 0.71	1.78 ± 0.70	11.16 ± 9.30	2.24 ± 0.91
Middle Caspian (depth down to 200 m)	32.69 ± 12.45	1.24 ± 0.68	2.30 ± 1.45	15.00 ± 6.15	1.20 ± 0.32
Middle Caspian (depth of 250-700 m)	17.98 ± 3.11	4.81 ± 1.55	0.83 ± 0.27	11.33 ± 4.66	0.56 ± 0.18
Oil oxidizing bacteria					
North Caspian	7.96 ± 4.60	0.20 ± 0.03	0.63 ± 0.17	0.95 ± 0.17	0.30 ± 0.09
Mangyshlak regression	8.60 ± 5.22	0.23 ± 0.09	0.63 ± 0.22	1.60 ± 0.78	0.76 ± 0.24
Middle Caspian (depth down to 200 m)	1.87 ± 0.47	0.23 ± 0.07	0.33 ± 0.09	2.13 ± 0.55	0.19 ± 0.05
Middle Caspian (depth of 250-700 m)	1.72 ± 0.59	0.87 ± 0.16	0.27 ± 0.11	2.18 ± 0.84	0.16 ± 0.03

In 2014, a decrease in the concentration of saprotrophs by an order of magnitude was noted; in subsequent years, the number of these bacteria did not change significantly. Oil-oxidizing bacteria were 2-10 times inferior in number to saprotrophs. The number of crude oil degraders, as well as saprotrophs, was maximum in 2013 (7.96 ± 4.60 thousand CFU/ml). In general, the dynamics of the number of oil-oxidizing bacteria in the period from 2013 to 2017 corresponded to saprotrophs (Table 1).

The Mangyshlak regression is a conditional boundary between the northern and middle parts of the Caspian. The combination of various hydrochemical and hydrophysical factors provides water exchange between the northern and middle parts of the Caspian Sea. Saprotrophic bacterioplankton in this region ranged from 1.78 ± 0.70 to 32.57 ± 10.59 thousand CFU/ml (Table 1). The maximum number of bacteria was noted in 2013 (26.15 ± 10.59 thousand CFU/ml). In 2014 and 2015, the number of saprotrophs decreased by an order of magnitude and stabilized at the level of 2--3 thousand CFU/ml, with the exception of 2016, when a 5-fold jump in the number of saprotrophic bacteria was noted (11.16 ± 9.30 thousand CFU/ml).

As in the north of the Caspian, in the Mangyshlak region, the number of crude oil degraders was 3--9 times lower than that of saprotrophs. The maximum concentration of these bacteria also occurred in 2013 (8.60 ± 5.22 thousand CFU/ml), the dynamics of the number of oil destructors in the period from 2013 to 2017 was similar to saprotrophs, with the exception of 2016, when a 2.5-fold increase was noted for crude oil degraders, while a 10-fold increase was noted in the saprotrophic bacteriocenosis abundance.

In the middle part of the Caspian, the dynamics of the number of saprotrophs corresponded to that in the region of the Mangyshlak regression. The maximum concentration of bacteria was recorded in 2013, while the number of saprotrophic bacterioplankton in the west of the middle part of the Caspian was slightly higher than in deep water (32.69 ± 12.45 and 17.98 ± 3.11 thousand CFU/ml, respectively). In 2014, a 25-fold decrease in the number of saprotrophs was noted in the west of the middle part of the Caspian Sea, while at deepwater stations, the number decreased by 4 times. In 2015, the concentration of bacteria in the west did not change significantly, and in the deep-water zone, a sharp decrease in the number of saprotrophs was noted. In 2016, an increase in the concentration of bacteria was noted in the Middle Caspian. In 2017, the number of saprotrophs decreased to the level of 2015.

The dynamics of the number of crude oil degraders in the middle part of the Caspian during the period from 2013 to 2017 corresponded to that of saprotrophs (Table 1). High concentrations of these bacteria were noted in 2013 and 2016. There were no significant differences in the number of crude oil degraders in the west of the middle part of the Caspian Sea and in its deepwater part.

Bottom sediments were taken in the waters of the northern part of the Caspian Sea, the Mangyshlak regression and in the west of the middle part of the Caspian at a depth of up to 200 m. The number of heterotrophic bacteria in the bottom ground was naturally higher than in water, due to the nature of the soil as a habitat (adhesive properties, abundance of nutrients and other).

During the study period, the number of North Caspian saprotrophic bacteriobenthos varied widely, with a maximum (299.66 ± 113.07 thousand CFU/g) in 2013 and a minimum (11.76 ± 6.53 thousand CFU/g) in 2014 (Table 2).

High concentrations of oil-oxidizing bacteria in the soil of the northern part of the Caspian were recorded in 2013, 2015 and 2016. (up to 25.50 ± 13.32 thousand CFU/g), the minimum was recorded in 2014 (1.91 ± 1.23 thousand CFU/g).

In the region of the Mangyshlak regression, the abundance of saprotrophic bacteriobenthos in 2013--2017 changed slightly. In 2013--2014 the concentration of bacteria in the soil reached 70--80 thousand CFU/g. In 2015--2017, the number of saprotrophs

TABLE 2: Number of saprotrophic and hydrocarbon-oxidizing bacteria in the bottom soil of the Caspian Sea in 2013--2017 (thousand CFU/g).

Research area	Year				
	2013	2014	2015	2016	2017
Saprotrophs					
North Caspian	299.66±113.07	11.76±6.53	91.62±33.75	168.5±95.66	21.62±5.32
Mangyshlak regression	69.96±41.28	79.95±41	41.77±20.04	48.75±18.16	44.46±17.18
Middle Caspian (depth down to 200 m)	271.84±133.54	3.40±1.25	129.71±93.58	401.50±248.75	29.88±8.29
Oil oxidizing bacteria					
North Caspian	14.47±7.6	1.91±1.23	26.5±13.32	25.12±11.32	7.93±4.28
Mangyshlak regression	25.97±17.89	6.30±3.85	4.63±1.49	35.58±21.46	12.51±6.00
Middle Caspian (depth down to 200 m)	4.11±1.41	0.74±0.22	9.73±3.86	72.51±41.83	10.13±2.98

slightly decreased and stabilized at the level of 41–48 thousand CFU/g. The dynamics of the number of oxidizing bacteriobenthos in the period from 2013 to 2017 slightly differs from that of saprotrophs. High concentrations of these bacteria were noted in 2013 and 2016, the minimum abundance was recorded in 2015 (Table 2).

The change in the number of saprotrophic bacteriobenthos in the west of the Middle Caspian during the study period varied within two orders of magnitude, the maximum concentration of saprotrophs was noted in 2016 (401.50±248.75 thousand CFU/g), the minimum was in 2014 (3.40±1.25 thousand CFU/g). The dynamics of the number of oil-oxidizing bacteriobenthos during the study period corresponded to that of saprotrophs (Table 2).

During the study period (2013--2017), the species composition of saprotrophic and oil-oxidizing bacteria was also studied in the northern and middle parts of the Caspian. To determine the bacterial biodiversity, enrichment cultures of saprotrophs and crude oil degraders were cultivated, followed by isolation of pure isolates of microorganisms. In total, more than 1,500 cultures were analyzed. Significant differences in the species composition of bacterial communities in the studied areas were not noted, therefore, generalized data are presented in the work.

Isolated saprotrophic and oil-oxidizing bacteria are divided into the following groups: gram-positive bacteria (*Arthrobacter*, *Bacillus*, *Corynebacterium*, *Enterococcus*, *Marinococcus*, *Micrococcus*, *Nocardia*, *Planococcus*, *Rhodococcus*, *Salinococcus*, *Staphylococcus*) Enterobacteriaceae family (*Citrobacter*, *Edwardsiella*, *Enterobacter*, *Esherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*), Neisseriaceae family (*Acinetobacter*, *Moraxella*), Pseudomonadaceae family

TABLE 3: Biodiversity of heterotrophic bacterioplankton in the northern and middle parts of the Caspian in 2013–2017.

Bacteria group	Number of isolates [%]				
	2013	2014	2015	2016	2017
Saprotrophic bacteria					
Gram-positive bacteria	3.60	25.20	44.50	31.40	29.60
Enterobacteriaceae	30.40	39.20	16.50	19.60	18.40
Neisseriaceae family	17.60	4.90	17.30	7.80	8.20
Pseudomonadaceae family	19.70	11.70	6.20	11.80	25.40
Vibrionaceae family	17.90	13.40	8.20	9.80	11.20
Alcaligenaceae family	-	2.80	5.20	7.80	3.10
Flavobacteriaceae family	10.80	2.80	2.10	11.80	4.10
Oil oxidizing bacteria					
Gram-positive bacteria	22.10	5.20	33.30	23.50	20.60
Enterobacteriaceae family	24.90	18.40	20.80	11.80	11.40
Neisseriaceae family	11.80	10.50	16.70	17.70	23.50
Pseudomonadaceae family	19.10	47.60	12.50	11.80	22.60
Vibrionaceae family	8.90	13.10	8.30	23.40	11.80
Alcaligenaceae family	4.40	2.60	4.20	-	2.90
Flavobacteriaceae family	8.80	2.60	4.20	11.80	7.20

(*Pseudomonas*), Vibrionaceae family (*Aeromonas*, *Photobacterium*, *Vibrio*) Alcaligenaceae family (*Alcaligenes*), Flavobacteriaceae family (*Flavobacterium*).

In saprotrophic bacterioplankton, the proportion of isolated microorganisms of the families Flavobacteriaceae, Alcaligenaceae, Vibrionaceae and Neisseriaceae did not change significantly during the study period and did not exceed 19% (Table 3).

The bacteria of Enterobacteriaceae family were met annually. In 2013 and 2014, the share of these microorganisms was maximum. Although since 2015, their decrease has been noted. The proportion of gram-positive bacteria was increasing until 2015. In 2016 and 2017, a decrease in their occurrence was recorded. In 2017, an increase in the number of bacteria of the Pseudomonadaceae family was also noted (up to 25.40%).

The species diversity of oil-oxidizing bacteria corresponded to that of saprotrophs. All isolated bacterial genes, with the exception of *Escherichia*, *Planococcus*, and *Micrococcus*, are capable of oil destruction; however, the arrangement of the dominant groups did not correspond to saprotrophs. In the enrichment cultures of oil-oxidizing bacteria, gram-positive bacteria were most often found annually. The number of representatives of the Enterobacteriaceae family gradually decreased during the study period, while the bacteria of the Neisseriaceae family, in contrast, were more common, peaking in 2017 (23.50%). In 2014, a peak in the development of bacteria of the Pseudomonadaceae

family was noted (47.60%); in 2016, an increase in the number of representatives of the Vibrionaceae family (23.40%) was recorded. The bacteria of the Alcaligenaceae and Flavobacteriaceae families were isolated singly annually.

In the saprotrophic bacteriobenthos of the northern and middle parts of the Caspian, as well as in bacterioplankton, microorganisms of the families Flavobacteriaceae, Alcaligenaceae, Vibrionaceae and Neisseriaceae were found less often than others (Table 4).

TABLE 4: Heterotrophic bacteriobenthos biodiversity in the northern and middle parts of the Caspian in 2013–2017.

Bacteria group	Number of isolates 5				
	2013	2014	2015	2016	2017
Saprotrophic bacteria					
Gram-positive bacteria	10.80	39.50	48.30	59.10	40.20
Enterobacteriaceae family	24.30	20.70	12.90	6.80	2.00
Neisseriaceae family	13.50	2.90	9.70	6.80	16.60
Pseudomonadaceae family	24.30	16.40	6.50	9.10	9.80
Vibrionaceae family	10.90	14.70	12.90	9.10	9.80
Alcaligenaceae family	8.10	2.90	9.70	-	8.80
Flavobacteriaceae family	8.10	2.90	-	9.10	12.80
Oil oxidizing bacteria					
Gram-positive bacteria	31.70	24.30	27.90	19.20	6.70
Enterobacteriaceae family	27.10	16.20	11.20	7.70	-
Neisseriaceae family	9.00	13.50	16.70	19.20	33.30
Pseudomonadaceae family	18.50	32.50	27.50	23.10	13.30
Vibrionaceae family	9.00	10.80	5.60	19.20	13.30
Alcaligenaceae family	4.50	2.70	11.10	-	6.70
Flavobacteriaceae family	-	-	-	11.50	26.70

Gram-positive bacteria were the dominant group, with the exception of 2013, when the Enterobacteriaceae and Pseudomonadaceae families were most often observed in enrichment cultures (24.30% each). The share of bacteria of the Enterobacteriaceae family decreased annually; in 2017, these microorganisms were observed singly (2.00%).

Other tendencies were revealed in the composition of the oil-oxidizing bacteriobenthos. The share of gram-positive bacteria and representatives of the Enterobacteriaceae family in enrichment cultures decreased annually; in 2017, the bacteria of the Enterobacteriaceae family were completely absent. Peak incidence of the Pseudomonadaceae family was in 2014, starting in 2015, their share also decreased. During the research period, an annual increase in the number of oil-oxidizing bacteria of the Neisseriaceae family up to 33.30% was noted in 2017. The representatives of the Flavobacteriaceae

families from 2013 to 2015 were not recorded among bacteriobenthos, but in 2017, their number reached 26.70% of the number of all selected cultures. Representatives of the Vibrionaceae and Alcaligenaceae families were noted almost annually; their share did not exceed 19.20%.

4. Discussion

In the northern and middle parts of the Caspian, the numbers of different groups of heterotrophic bacterioplankton differed, while the dynamics of the numbers of saprotrophs and oil destructors in the temporal aspect was similar. In the northern part of the Caspian, high concentrations of saprotrophs and oil destructors were noted only in 2013. Starting from 2014 and until the end of the research period, the number of heterotrophic bacterioplankton significantly decreased, and there were no sharp fluctuations in the numbers. The number of heterotrophic bacteria largely depended not only on the hydrochemical and biotic factors of the water area, but also on the flow of the Volga River. For instance, year 2013 was the most high-water [11], which influenced the concentration of heterotrophic bacterioplankton in the northern part of the Caspian Sea. Moreover, the decrease in the number of heterotrophic bacterioplankton by an order of magnitude in 2014 and its stabilization at the level of 1-3 thousand CFU/ml indicated not only the active processes of self-purification of the marine environment, but also the stability of the microecosystem as a whole.

In the middle part of the Caspian and near the Mangyshlak regression, the peak of bacterioplankton abundance also occurred in 2013, while in 2016, an increase in the concentration of bacteria was observed. The amount of bacterioplankton in the west of the middle part of the Caspian compared to its deep-sea part was 2-3 times higher, with the exception of 2014, when the opposite trend was noted. Despite the differences in hydrochemical conditions, in particular, the content of nutrients and organic carbon, changes in water temperature and depth [12], differences in the number of bacterial communities were small. It is likely that the growth of the hydrogen sulfide layer could affect the number of facultative anaerobic bacteria in the deep-water area of the surveyed water area [12, 13]. In the region of the Mangyshlak regression, the dynamics of bacterioplankton abundance corresponded to the average Caspian indicators, which indicated the predominant influence of the waters of the middle part of the Caspian Sea with their hydrological and hydrochemical parameters on bacterioplankton in this water area.

The species composition of the bacterioplankton of the Caspian Sea was quite stable throughout the entire period of research. Each year, the majority of the isolates belonged to the group of opportunistic bacteria, while true saprotrophs, unable to colonize living objects, were much less common in enrichment cultures. The prevalence of opportunistic bacteria is caused not only by their high enzymatic activity and antagonistic properties [14], but also by the elective conditions of the accumulating media, which sharply differed from the natural living conditions of the marine bacteriocenosis. Therefore, the predominance of opportunistic bacteria in selective conditions, perhaps, did not provide this group of bacteria with a dominant position in the saprotrophic microcenosis, but confirmed their persistence in the water in active or static form, which poses a potential danger for both aquatic organisms and humans [15]. Interestingly, there was almost complete species identity of saprotrophic and oil-oxidizing bacterioplankton, which indicated the high adaptive ability of the isolated bacteria to changing living conditions against the background of active anthropogenic activity.

The number of heterotrophic bacteria in the soil in different parts of the Caspian Sea varied significantly. In the northern and middle parts of the Caspian Sea, two peaks of development of saprotrophic bacteriobenthos (in 2013 and 2016) were recorded, while in the region of the Mangyshlak regression, the concentration of saprotrophs remained stable throughout the entire study period. Crude oil degraders were always inferior in number to saprotrophs; however, the share ratio of crude oil degraders to saprotrophs changed annually. Constant changes in the proportional composition of heterotrophic bacteria indicated instability of the microbial biocenosis in each surveyed area of the sea, which is also confirmed by a gradual change in the species composition of oil destructors and saprotrophs. Gram-positive bacteria, annually widely represented among saprotrophs, gradually lost the ability to utilize petroleum hydrocarbons, so their share among the distinguished crude oil degraders decreased annually. Increasing the proportion of non-fermenting oil-oxidizing bacteria of the Neisseriaceae and Flavobacteriaceae families against the background of their insignificant share in the saprotrophic community, together with a decrease in the occurrence of gram-positive bacteria, indicated a transformation of the heterotrophic bacterial community of bottom sediments in the northern and middle parts of the Caspian.

5. Conclusion

Studies conducted in 2013--2017 in the waters of the northern and middle parts of the Caspian Sea showed that in all the studied areas the peak abundance of saprotrophic

and oil-oxidizing bacterioplactone was in 2013. The average concentration of bacteria in the Caspian also showed a jump in bacteria concentration in 2016, while in the northern part of the sea the number of heterotrophic bacterioplactone in the period from 2014 to 2017 remained stable. The dynamics of the number of bacteria in the region of the Mangyshlak regression corresponded to the average Caspian figures, which indicated the predominant influence of the hydrochemical conditions of the middle part of the Caspian. The species composition of saprotrophic and oil-oxidizing microorganisms was almost identical; conditionally pathogenic bacteria predominated in both groups. In bottom sediments, the number of heterotrophic bacteria varied significantly both temporally and depending on the location of the sampling site. Constant changes in the ratio of the number of saprotrophs and crude oil degraders, together with the transformations of the dominant groups of the species composition of bacteria, indicated the instability of bacteriobenthos, and, as a result, the vulnerability of the microecosystem of bottom sediments under changing environmental conditions.

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Conflict of Interest

The authors have no conflict of interest to declare.

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