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EXPERIMENTAL ARTICLES

Microbiological Studies in the Deepwater Area of the Southern Caspian Sea

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Abstract—For the first time, microbiological monitoring was performed in the deepwater area of the Southern Caspian Sea. It revealed seasonal and interannual variations in total microbial content and concentrations of saprophytes and other physiological groups of bacteria in water and bottom sediments. The biomass, generation time, and bacterial production were determined. The most profound variations in microbial content, biomass, and production were found to occur in the water column and in bottom sediments at depths to 200–250 m.

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Key words: microbiological processes, degradation, biomass, production, anthropogenic eutrophication.

Earlier data on the microflora of the Caspian Sea, in particular, its southern part, were obtained in the 1930s, and they were rather fragmentary. Most of these studies involved isolation of live cells from benthic and core samples [1–4]. Later, in 1952, Kriss investigated a single cross section, from the mouth of the Kura River to Ogurchinsk [5].

These poor, obsolete, and fragmentary data, taken together with dramatic changes in the ecosystem of the Caspian Sea, which have occurred for more than half a century since then, do not allow proper characterization of the current state of the Southern Caspian microflora [6]. Therefore, microbiological study of this water body is of great importance. The goals of the present work were to (1) determine the occurrence of the microflora, (2) elucidate the relation of its parameters to environmental conditions and seasonal changes, and (3) understand the effect of anthropogenic eutrophication on quantitative and qualitative parameters of microflora in the water column and bottom sediments of the deepwater part of the Southern Caspian Sea.

MATERIALS AND METHODS

Our microbiological monitoring of the Southern Caspian Sea has been performed since 1960 [7, 8]. Seasonal observations of its deepwater area were performed four times, in 1971, 1973, 1986, and 1998. For the first time, we examined bottom sediments in the deepwater central area of the Southern Caspian Sea in 1966 and repeated the examination in 1988 and 1995 [7]. In addition to this integrated research, water and bottom sediment samples were taken in four sections from west to east, each including six to eight sites, in the winters of 1995 and 1999 (Fig. 1). Water samples for microbiological examination were collected in special-purpose sterile glass vials according to the Sorokin method [9] and treated on board the ship no later than 1.5–2 h after the sampling. Bottom grounds were taken with an Okean dredge. Samples for microbiological studies were taken from the upper layer with a sterile spatula. For inoculation, the samples were suspended in sterile water.

Total water and ground microorganisms were counted by the Razumov [10] and Vinogradskii [11] methods on membrane filters no. 2 or 3 produced in the former Soviet Union. Filters with adhering microorganisms were stained with 3% erythrosine and additionally stained with 2% fuchsine (which was further diluted prior to use).

Saprophytic bacteria were isolated by submerged inoculation to dry nutrient agar and beef-extract agar. Bacteria of certain physiological groups were isolated on corresponding selective media [12]. Biomass, generation time, and production were determined by the Romanenko radiocarbon method [13]. Water samples for microbiological studies were taken from the following depths (m): 0.3; 25, 50, 75, 100, 150, 200, 250, and then down to 980 m at 100 m intervals and also at the thermocline, occurring at depths from 28 to 30 m at various sites.

RESULTS AND DISCUSSION

Total content of microorganisms in water. Table 1 shows the history of the microbial content in the water over the seasons of 1998 in various sea horizons at the

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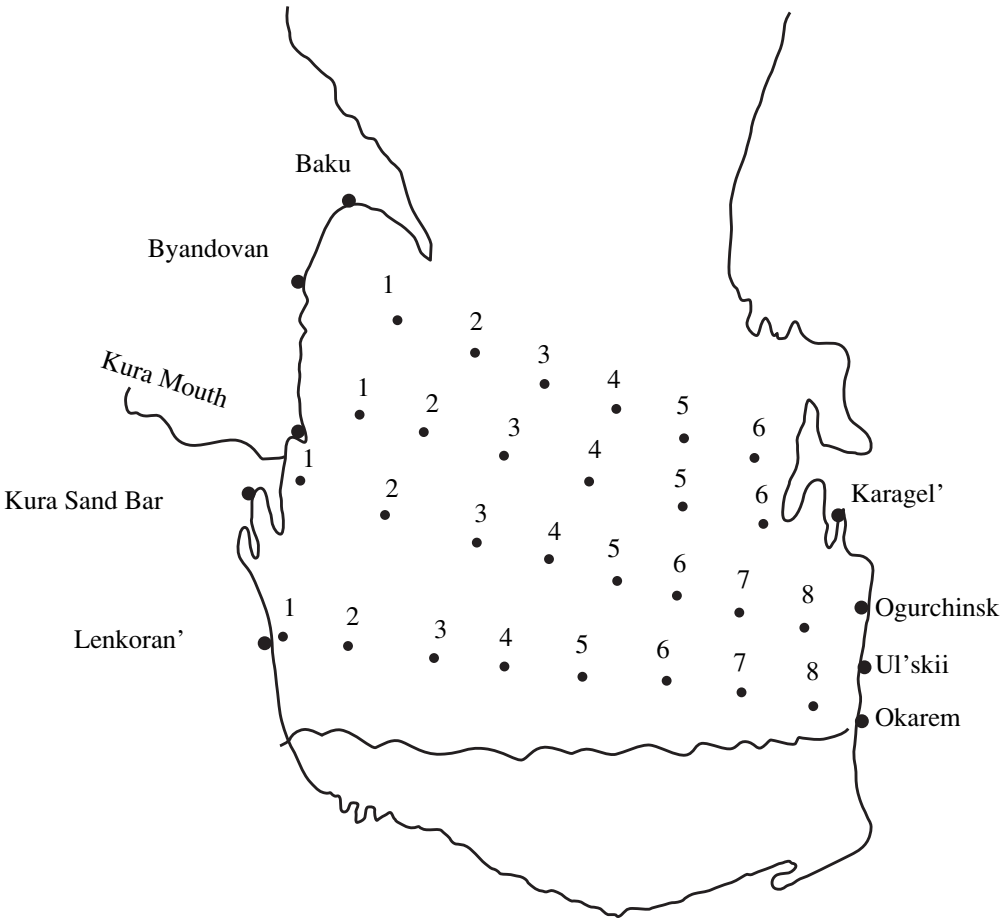

Fig. 1. Map of the Southern Caspian Sea indicating sections and sites. Numerals indicate sites.

deepest sites of the Kura–Ogurchinsk (site 4) and Kura Sand Bar–Ul'skii (site 4) sections. It is apparent that the total content of microorganisms in the upper 100-m layer varies from season to season. The minimum microbial content was recorded in winter. Our earlier studies showed that the mean annual content in the western part of the sea, particularly in water areas of the Kura mouth, was nearly twice as large as that determined for southern and southeastern areas. Probably, this is related to the effect of river water, rich in organic matter and biogenic elements [6].

Studies of variation in microbial content revealed a tendency toward an increase in its density (Table 2). Apparently, the microbial content had increased by a factor of 2–2.5 for 30 years throughout the water area. The densest microbial population was noted in the area adjacent to the Kura River, although an increase in the mean annual microflora density was also noted in the water columns of deepwater areas in the Southern Caspian Sea (Table 2, sites 5 and 6 in all sections).

The contrastive data obtained in the water area of the Kura-Ogurchinsk section are presented in Table 3. It shows that the total microbial content at great depths also increased more than twofold for 20 years (1973–1993).

Biomass, generation time, and microbial production in the water of the central Southern Caspian Sea. Seasonal variations in the biomass, generation time, and microbial production of the Southern Caspian Sea, including its central area, were first determined in 1998. The results are shown in Table 4. They indicate that these parameters vary noticeably according to the season only in upper horizons of the central Southern Caspian Sea, at depths to 100 m, showing smaller seasonal variability in deeper water layers.

The highest levels of biomass and the shortest generation times of bacteria and, correspondingly, the maximum values of biomass production were also recorded in upper horizons of the water column, at depths to 100 m (Table 4).

According to data from the literature, the qualitative and quantitative composition of these bacteria makes it possible to characterize the trophic state of the water body, the features of the organic matter present in the water, and the degree of its biodegradation [13, 14]. It was shown previously that variations in the content and morphological composition of saprophytic bacteria in

Table 1. Total content of microorganisms (10^3 cells/ml) in the water column of the Southern Caspian Sea over seasons of 1998

Section	Depth, m	Winter (II)	Spring (IV)	Summer (VIII)	Autumn (XI)
Kura Mouth–Ogurchinsk	0.5	440	690	1220	890
	30	340	580	2410	610
	50	200	340	1410	710
	75	140	220	910	390
	100	120	160	870	320
	200	86	94	510	180
	300	56	49	64	47
	400	46	51	61	39
	500	30	41	51	30
	600	20	24	27	19
	700	19	18	21	16
	800	20	16	19	17
	950	40	44	37	30
Kura Sand Bar–Ul'skii	0.5	240	360	940	720
	30	160	270	1840	620
	75	120	210	860	410
	100	116	211	810	396
	200	80	83	110	96
	350	60	58	66	30
	750	20	19	26	19
	960	40	29	34	24

different areas of the central Southern Caspian Sea were seasonally related. This variation was most pronounced in summer [6]. Our studies demonstrated a tendency toward an increasing content of saprophytic bacteria in the water over the years (Table 5).

The content of saprophytic bacteria varies over a wider range than the total microbial content, both with time and from site to site. A dramatic increase in saprophytic population is observed in the upper 50 m of the water column in the region under study. Data on the seasonal variation in phytoplankton production, total microbial content, and saprophyte content in the central Southern Caspian are presented in Table 6.

Bacteria of particular physiological groups. We studied the occurrence of free-living aerobic and anaerobic microbial nitrogen fixators, denitrifiers, sulfate reducers, and cellulose degraders in the central Southern Caspian Sea. Aerobic nitrogen fixators were found in samples from surface water horizons at 12 (out of 30) sites at concentrations of 15–25 cells/ml. Anaerobic nitrogen fixators (*Clostridium pasteurianum*) and denitrifiers were found in all water samples starting from a depth of 250–300 m at concentrations of 10–100 cells/ml.

Large-scale phytoplankton growth and a dramatic increase in daily organic matter degradation in the water of the region under study [9] prompted us to focus on the quantitative distribution of sulfate-reducing bacteria. In most benthic samples (88–90%), sulfate-reducing bacteria were present at concentrations of 80–100 cells/ml. It is worth noting that analysis of benthic samples collected at 46 sites of the central Southern Caspian Sea in the 1970s revealed sulfate-reducing bacteria at concentrations of 11–14 cells/ml only in 12 samples (12%) [16].

Aerobic cellulose-degrading bacteria occur mainly in layers of intense photosynthesis, and their maximum

Table 2. Mean annual concentrations of microorganisms (10^3 cells/ml) in the near-surface water horizon of the Central Southern Caspian Sea (over all sites of the section)

Section	Sites									
	1		3		4		5		6	
	1973	2003	1973	2003	1973	2003	1973	2003	1973	2003
Byandovan–Karasengir	260	410	140	280	170	380	140	290	110	210
Kura Mouth–Ogurchinsk	980	2670	840	1370	610	900	280	340	800	1810
Kura Sand Bar–Ul'skii	480	990	180	340	110	190	100	140	200	410
Lankaran–Okarem	940	1980	600	870	340	610	130	280	400	1300
Mean	665	1513	440	715	307	520	162	263	377	932

Table 3. Microflora content in the water column of the Kura–Ogurchinsk section over seasons of 1973, 1976, and 1993 (10^3 cells/ml)

Season	Horizon, m					
	100–200			over 200		
	1973	1976	1993	1973	1976	1993
Spring	130	350	510	50	65	160
Summer	360	700	870	200	430	640
Winter	96	186	210	33	41	89

concentrations (100–150 cells/ml) were recorded in layers of temperature discontinuity. Anaerobic cellulose-degrading bacteria were found in benthic water layers at concentrations no higher than 10–12 cells/ml.

It is of special importance that, in the winter of 1999, at two sites in the western part of the Kura Sand Bar–Ul'skii (depths in the range 360–470 m) water samples taken from horizons below 200 m contained anaerobic cellulose-degrading bacteria at concentrations of 35–40 cells/l, values which were higher than the corresponding values determined in 1988. The same horizons showed a decrease in oxygen saturation by 25–30% in comparison with the results of the 1980s (Fig. 2).

Thus, the data presented in Tables 5 and 6 and in Fig. 2 indicate that the central Southern Caspian Sea experiences anthropogenic eutrophication. Bacterial concentrations and biochemical activity, including oxygen consumption, have increased in the water column of the region, thereby inducing the growth of anaerobic bacteria.

Total content of microorganisms in bottom grounds. Long-term studies indicate that the total content of microorganisms in bottom sediments of the deepwater Southern Caspian (from 150 to 950 m) varies with both the season and the sea depth (Table 7). The content of microorganisms is season-dependent to depths of 100–150 m. Starting from depths of 200–250 m, the microbial content gradually decreases and becomes virtually invariable the year round.

The table also indicates that the content of bacteria in bottom sediments increases from north to south in all seasons. The decrease in the average content of microflora from west to east follows nearly the same regularity (Fig. 3). Figure 3 shows that the content of microorganisms at site 1 of the Kura Sand Bar–Ul'skii in the west (depth of 100–120 m) is 1.12×10^9 cells/g, and in bottom sediments of site 9 in the east, at the same depth, it is no more than 3.4×10^8 cells/g.

One of the notable features of the Caspian Sea is that its western shelf is richer than the eastern one according to many parameters [6, 7].

Saprophytic bacteria. The qualitative and quantitative composition of saprophytic bacteria in bottom grounds of the deepwater Southern Caspian varies from

Table 4. Biomass (mg C/m^3), biomass doubling time (h), and bacterial production (mg C/m^3) in the central Southern

Depth, m	Biomass				Generation time				Production			
	II	IV	VII	X	II	IV	VII	X	II	IV	VII	X
0.5	11	21	39	28	49	37	23	19	17	34	68	46
30	11	17	58	20	51	39	27	23	14	28	138	40
50	10	15	42	18	54	44	26	27	12	18	33	27
100	8	10	32	17	59	51	47	38	10	14	20	17
200	6	7	11	8	106	86	71	63	5	6	7	8
300	5	6	9	6	138	129	117	109	3	4	5	6
400	4	5	6	4	143	138	139	138	2	2	3	4
500	3	4	4	3	157	144	139	141	2	2	3	4
600	2	3	3	3	166	159	153	160	2	2	2	3
700	2	3	3	3	171	166	157	149	2	2	2	3
800	3	3	3	3	183	178	183	128	2	2	2	2
950	3	4	6	4	–	–	–	–	3	4	4	4
Mean	5.7	8.2	18	9.8	116	106	98	90	6.2	9.8	23.9	13.6

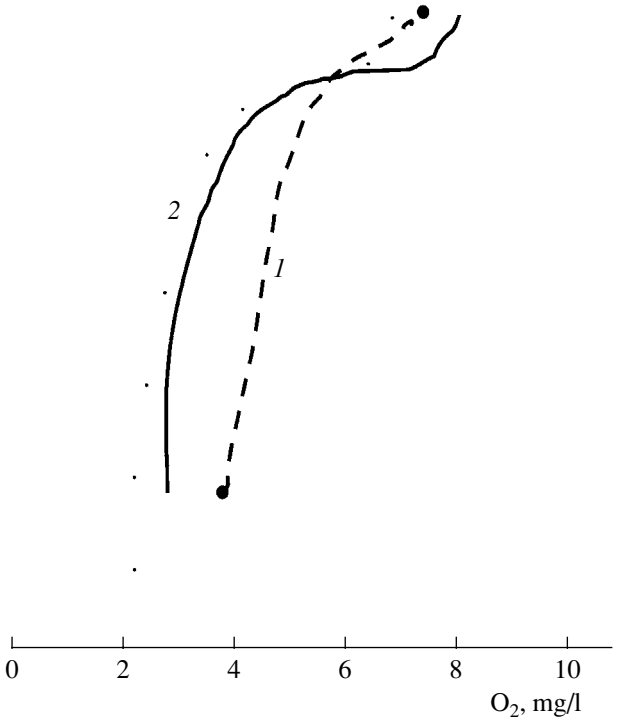

Fig. 2. Oxygen distribution in the water column of the Central Southern Caspian in summer: (*1*) 1988, (*2*) 1995.

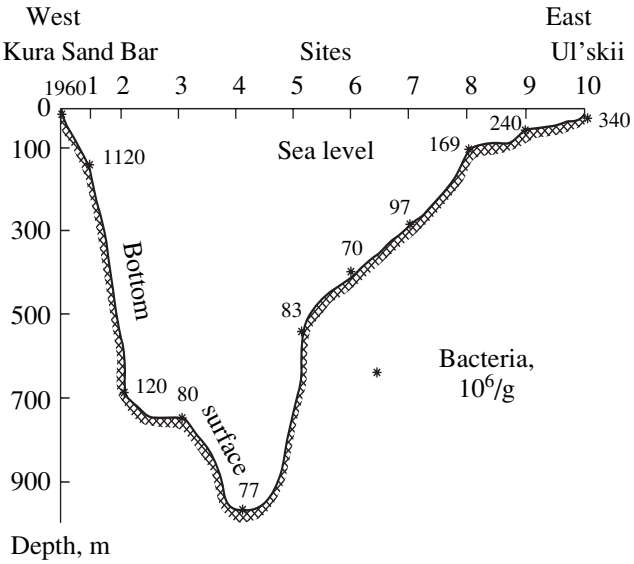

Fig. 3. Distribuion of microorganism concentrations in bottom sediments over the Kura Sand Bar–Ul'skii section.

site to site mainly according to its depth, the spatial dependence being less variable (Table 8).

The qualitative composition of the saprophytes isolated varies with depth. In grounds from depths of no more than 100 m, spore-forming species constitute 60–68%, whereas they reach 90% among saprophytes isolated from grounds at depths of 700–900 m. The

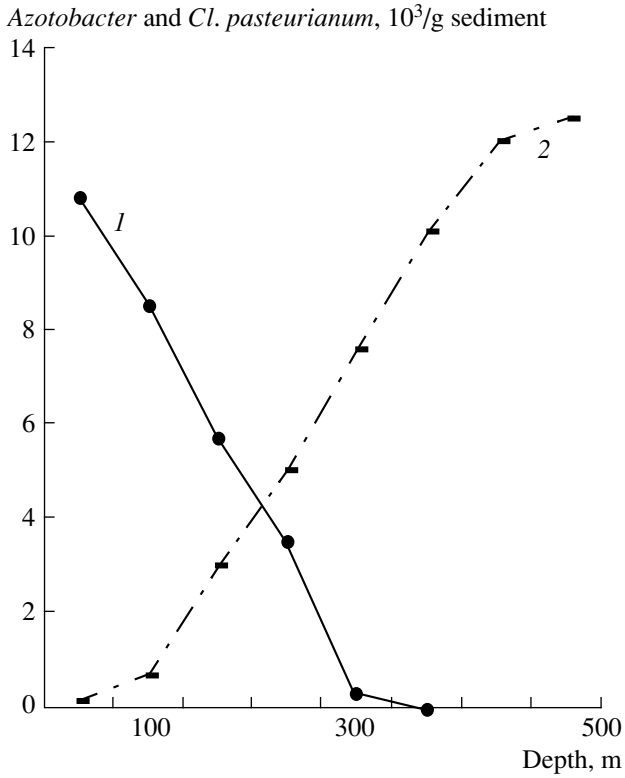

Fig. 4. Distribution of free-living nitrogen-fixing bacteria in bottom grounds of the Central Southern Caspian Sea: (*1*) *Azotobacter*, (*2*) *Cl. pasteurianum*.

increase in saprophyte content from year to year follows the same pattern as the total microbial content. Therefore, it is reasonable to suggest that the water column and bottom sediments of the deepwater area of the Caspian Sea are also being enriched in organic matter, largely of autochthonous origin [8, 9].

Bacteria of various physiological groups in bottom sediments. In bottom grounds of the deepwater Southern Caspian Sea, the content of aerobic and anaerobic bacteria changes dramatically with depth (Fig. 4). Cellulose-degrading bacteria follow the same pattern: aerobic species are predominant in grounds at depths to 150–200 m, and anaerobic species, at greater depths. It is worth noting that the contents of denitrifying and anaerobic nitrogen-fixing bacteria are nearly equal, varying within the range $3–5 \times 10^3$ cells/g.

Sulfate-reducing bacteria constitute a significant proportion of anaerobic bacterial species, reaching 100%. Their content varies within the range $3.9–12 \times 10^3$ cells/g.

Note that studies of bottom grounds of the Caspian Sea performed in recent years revealed increasing abundance of sulfate-reducing, denitrifying, methane-producing, and other anaerobic bacteria. In particular, analysis of samples taken in December 1995 from depths of 270, 340, 460, and 540 m showed that the

Table 5. Variation in the content of saprophytic bacteria (cells/ml) in the water column of the central Southern Caspian Sea in the summers of 1968, 1973, 1978, 1986, and 1998 (site 4 of the Kura Mouth–Ogurchinsk section)

Depth, m	Content of saprophytic bacteria					Multiplied by
	1968	1973	1978	1986	1998	
0.5	600	860	1027	1340	2300	4
10	617	680	932	1200	1840	3
30	967	1240	1826	2410	3600	4
50	721	824	1030	1440	1880	3
100	411	570	760	820	1200	3
200	84	114	440	511	570	7
300	27	36	61	83	90	3
400	21	29	43	56	66	3
500	16	23	37	48	59	4
600	11	18	29	35	41	4
700	9	14	21	31	39	4
800	7	11	16	24	30	4
950	21	32	33	53	66	3

Table 6. Comparison of total contents of microorganisms (10^3 cells/ml), contents of saprophytic bacteria (cells/ml), and daily photosynthetic production of plankton (g C/m^2) in surface water layers of the central Southern Caspian Sea in seasons of 1969 and 1988 (site 4 of the Kura Mouth–Ogurchinsk section)

Season	Primary production		Total content of microorganisms		Saprophyte content	
	1969	1988	1969	1988	1969	1988
Winter	46.2	59.4	210	463	136	246
Spring	70.8	116.3	440	980	290	687
Summer	50.3	129.0	1027	2410	680	1126
Autumn	63.2	119.0	680	1840	440	721

mean content of bacteria of these groups had increased almost threefold in comparison with 1969 [9].

It is of special importance that the increase in the content of anaerobic bacteria correlates with the rate of organic matter degradation in grounds of the water region under study (Table 9). Therefore, we suggest that the intensification of mineralization of organic matter in the water column and the uppermost layers of bottom sediments, involving aerobic microorganisms, favors

Table 7. Total contents of microorganisms (10^6 cells/g) in bottom sediments of the central Southern Caspian Sea in seasons of 1993 (from west to east)

Section	Site depth, m	Winter	Spring	Summer	Autumn
Byandovan–Karagel'	100	180	300	440	370
	150	100	168	176	184
	250	100	120	133	143
	600	90	96	98	94
	100	75	81	79	86
Kura Mouth–Ogurchinsk	100	250	420	510	380
	150	110	210	360	156
	250	120	130	120	110
	800	70	90	98	93
	950	73	81	84	84
Kura Sand Bar–Ul'skii	100	200	378	460	320
	150	110	170	163	120
	300	76	83	92	88
	600	68	74	81	76
	951	67	70	74	70
Lankaran–Okarem	100	210	400	900	610
	130	100	210	290	140
	300	96	98	110	96
	500	84	81	92	87
	600	76	76	86	80
	700	70	70	77	78
	950	81	73	74	76

Table 8. Contents of saprophytic bacteria in bottom sediments of the central Southern Caspian Sea in seasons of 1995 (10^3 cells/g)

Section	Depth, m	II	V	VIII	X
Byandovan–Karagel	65	120	160	230	220
	150	56	81	120	117
	230	45	56	98	110
	600	43	63	88	98
	700	44	60	76	81
Kara Mouth–Ogurchinsk	36	180	210	468	330
	400	67	98	110	119
	800	71	73	79	71
	951	70	66	73	68
Kura Sand Bar–Ul'skii	30	120	360	400	340
	300	60	88	120	97
	600	61	60	79	–
	900	68	73	72	–
Lankaran–Okarem	70	330	440	780	640
	500	70	67	81	89
	700	65	87	82	73
	946	92	98	91	88

Table 9. Mean annual primary production (10^3 tons C), OM degradation in water (mg C/l) and bottom grounds (mg C/m^2 per day), and contents of saprophytic and sulfate-reducing bacteria in the near-bottom water layer (cells/ml) and in bottom sediments (10^3 cells/g) at site 4 of the Kura Mouth–Ogurchinsk section

Year	Primary production	Degradation		Saprophytes		Sulfate-reducing bacteria in grounds
		Water	Ground	Water	Ground	
1969	20176	0.48	20	21	46	2.1
1973	26340	0.96	32	33	68	4.3
1995	30700	1.48	41	60	73	6.8

consumption of oxygen, present at minute concentrations, which promotes growth of anaerobic bacteria.

The results of our study, taken together with the comparison of microbial contents, bacterial production, and organic matter degradation, suggest that the microbial content in the deepwater part of the Southern Caspian Sea has increased two- to threefold over the last thirty years. The main cause is the enrichment of the water column in organic matter of allochthonous (in the western area near the Kura mouth) or autochthonous (in the central area) origin. Seasonal and perennial changes of biomass amounts, generation times, and microbial production are more pronounced in upper water layers (100–150 m). Intense production and degradation in deep water layers and bottom sediments favor biological oxygen consumption. This enhances the growth of anaerobic bacterial species, which can give rise to anaerobiosis in bottom layers of deepwater depressions in the Southern Caspian Sea.

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