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Species Diversity of Epilithon Diatoms and the Quality of the Waters of the Donuzlav Gulf Ecosystem (Crimea, the Black Sea)

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Abstract: In this work, the species diversity of epilithon diatoms of Donuzlav Gulf (Crimean coastal waters of the Black Sea) is presented for the first time. A total of 60 taxa of Bacillariophyta belonging to 3 classes, 18 families and 34 genera were found. The largest number of species (45) of diatoms was observed in summer, and the smallest number of species (12) in winter. A total of 25 saprobity indicator species of diatoms was found on the epilithon, 12 taxa of which belong to a group of betamesosaprobionts—indicators of moderate organic pollution of water. Marine species comprise 55% and brackish-marine ones make up 37% of them, but no freshwater species were identified. The diatom community was dominated by cosmopolites (32%) and arctic–boreal–tropical species (27%). The maximal abundance (186.2×10^3 cells cm^{-2}) and biomass (0.083 mg cm^{-2}) of diatoms was noted in June 2018 at salinity 16.58‰ and T = 24.4 °C with the dominant cosmopolites species of *Licmophora abbreviata* and *Cocconeis scutellum*. The minimum values, 14.8×10^3 cells cm^{-2} and 0.005 mg cm^{-2}, were observed in January 2019 at salinity 16.86‰ and T = 9.0 °C with the dominant *Berkeleya rutilans* and *Nitzschia hybrida* f. *hyalina*. The high values of BOD_5, oxidizability, silicon, N_{org} and P_{org} were noted, indicating a certain degree of water pollution by domestic sewage. It has been shown that the pollution in the southwestern part of the Donuzlav Gulf is greatest since this part of the coast was populated most densely in comparison with the northern coast of the Gulf where there are no coastal settlements except for two mariculture farms.

Keywords: species diversity; diatoms; stones; bioindication; indicators; organic pollution; Crimean coastal waters; Donuzlav Gulf; the Black Sea

1. Introduction

Donuzlav Gulf of the Black Sea is a unique semi-enclosed marine with a surface area of 47.5 km^2, located off the West coast of the Crimea 150 km from Sevastopol. The length of the sand embankment separating it from the sea is 9 km and its width is about 200–600 m. Most of the depth is less than 4–5 m; in the area of the central basin along the lake, the depth reaches 12–20 m. The first complex studies of biota, including phytoplankton, and monitoring data of hydrochemical and chemical–toxicological characteristics of Donuzlav marine ecosystems were conducted in different years [1–3]. The authors showed that in the ecosystem of Donuzlav marked high trophic waters, productivity of meio- and macrobenthos, as well as the presence of natural populations of mollusks (mussels) and fish make the reservoir very promising for the development of mariculture. The period 2015–2017 showed that the average content of dissolved oxygen in the water changed in the range of 8.77–9.40 mg L^{-1} [3]. These values are satisfactory for the life of hydrobionts and the degree of pollution the water environment with heavy metals and a petroleum hydrocarbon contaminated site is quite low. The ecosystem of

Donuzlav Gulf is of particular interest. On the one hand, it has twice undergone its formation. At first, it was a sea, then it was separated from the sea by a mound, then again, it became a Gulf. Currently, the ecosystem of Donuzlav is affected by such anthropogenic environmental factors as industrial sand mining and the location of the mussel–oyster farms. Therefore, the primary producers of the microphytobenthos as an important food source for all species of aquatic organisms have never been studied. Diatom indices used in biomonitoring of water quality of the mostly of the rivers [4–10] but very poorly marked on the marine benthic ecosystems [11–18].

Despite the fact that diversity species of the microphytobenthos of the Black Sea is well studied in different ecotopes [18–22]. However, diatoms of microphytobenthos in different seasons of the year have never been studied in the Donuzlav Gulf. In this regard, it became necessary to conduct a study of benthic diatoms as indicators of water quality using the known environmental saprobity indicators. These data are poorly understood in the seas of the World Ocean in general and in the Black Sea in particular.

The aim of this work was the study of species diversity of benthic diatoms and their indicator role in the Donuzlav Gulf of Crimean coastal waters of the Black Sea.

2. Materials and Methods

Sampling of stones was carried out from April 2018 to April 2019 at 9 stations at a depth of 0.5 to 1.5 m in the Donuzlav Gulf (45°20′00″ N, 33°00′00″ E) of the Crimean coastal waters of the Black Sea (Figures 1 and 2). Among them, Stations 1, 2, 3, 5, 7 are located near areas for the cultivation of mussels and oysters. In this work, different methods of studying diatoms were used [16,18,19]. Samples of stones were taken manually, by three stones from each sampling station. In the laboratory, each stone was scraped for suspension for qualitative and quantitative processing. The collected stones were carefully cleaned by a scraper to collect suspensions. Samples of the suspension from live diatoms were used for the taxonomic study and the samples were fixed by 2% formaldehyde for the quantitative assessment. The sizes of the stones were of the following range: length (x):4–12 cm; width (y):2–7 cm; height (z):1.5–3.5 cm. Determination of the surface area (S, cm^2) of the stones was carried out by the method of Graham et al. [23]:$S = \pi/3\ (xy + yz + xz)$, where x, y, z are the linear of the substrate sizes. The error in determining the surface area of the stone was ± 5%. Goryaev's camera with a volume of 0.9 mm^3 was used for counting of diatom cells.

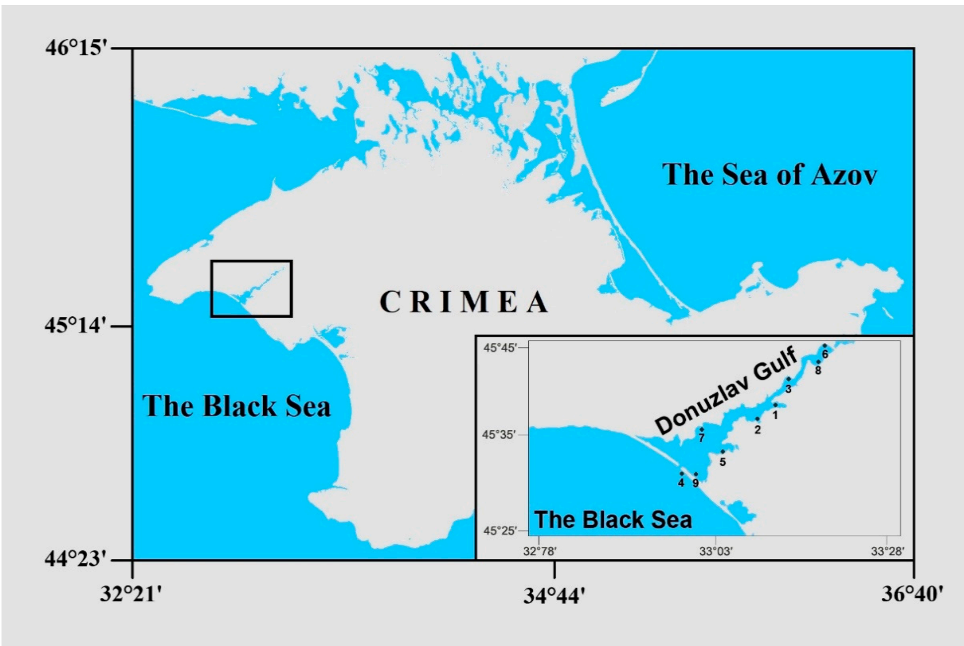

Figure 1. Crimean area of the studied diatoms—Donuzlav Gulf of the Black Sea.

Figure 2. Northern (**A**) and southern (**B**) coasts of the Donuzlav Gulf of the Black Sea.

The study of diatoms was carried out on a light microscope of C. Zeiss "Axioskop 40" with software AxioVision Rel. 4.6 at magnifications of 10 × 40, 10 × 100 under oil immersion in living material and in permanent slides prepared according [23] and mounted in the El'yashev media (n = 1.67). A total of 64 samples was collected and processed. Analysis of number of species (*n*), species richness (*R*), abundance (*N*, cells cm^{-2}) and biomass (*B*, mg cm^{-2}) of community diatoms in each sample was carried out according to the methods described earlier [16,18,20]. The abundance and biomass of diatoms were calculated according to the formulae: $N = n\ V/S\ V$, where n is the number of diatom cells in the Goryaev counting chamber with volume equal to 0.9 mm^3; V is the sample volume, ml; $B = h\ V\ b/SV_{chamber}$, where b is total $V_{chamber}$, mm^3, h, the unit weight of benthic diatoms, is equal to 1.2×10^{-9} mg μm^{-3}, and others are 1×10^{-9} mg μm^{-3} [24].

The hydrological characteristic of Donuzlav Gulf waters had the following range: the temperature varied from 7.0 °C (March) to 27.0 °C (July) and salinity from 16.58‰ (June) to 18.62‰ (May). The hydrochemical parameters of Donuzlav Gulf waters (pH, O_2, alkaline permanganate oxidizability, N_{org}, NO_2, NO_3, NH_4, P_{org}, PO_4, Si) at the stations are determined according to conventional methods [25,26].

In our work we used the method of monitoring the quality water of the environment on saprobity indicators developed for diatoms and adopted species names to modern system [4–15,18,27–29]. The species composition of diatoms was determined using the following sources [18,29–32]. The maps of distribution of environmental variables values and indicator taxa number were generated in the Statistica 12.0 program [33].

3. Results

Species diversity of microphytobenthos of the stone substrates in the Donuzlav Gulf are 60 taxa of Bacillariophyta (Table 1), belonging to 3 classes, 18 families and 34 genera. In the diatom flora, marine and cosmopolites dominated, which is typical for the phytobenthos of the Crimean coastal waters of the Black Sea [18,21].

Table 1. Checklist of the epilithon diatoms in the Donuzlav Gulf of the Black Sea, 2018–2019 and their ecological and phytogeographical characteristics.

Taxa	ECD		PhG
	S	RS	
Achnanthes brevipes C.A. Agardh 1824	*β*	BM	K
Achnanthes longipes C.A. Agardh 1824	*β*	M	ABT
Amphora angusta W. Gregory 1857	–	BM	K
Amphora arcus W. Gregory 1857	–	M	AB
Amphora ovalis (Kützing) Kützing 1844	*o-β*	B	K
Amphora proteus W. Gregory 1857	*α-β*	M	K
Ardissonea crystallina (C.A. Agardh) Grunow 1880	*β*	BM	BT
Bacillaria paxillifera (O.F. Müller) T. Marsson 1901	*o-α*	BM	K
Berkeleya micans (Lyngbye) Grunow 1880	*o*	BM	B not

Table 1. *Cont.*

Taxa	ECD		PhG
	S	RS	
Berkeleya rutilans (Trentepohl ex Roth) Grunow 1880	–	BM	AB not
Caloneis liber (W. Smith) P. Cleve 1894	–	M	K
Carinasigma rectum (Donkin) G. Reid 2012	–	M	BT not
Cocconeis costata W. Gregory1855	–	M	K
Cocconeis scutellum Ehrenberg 1838	*β*	BM	K
Cylindrotheca closterium (Ehrenberg) Reimann et Lewin 1964	*β*	M	K
Diploneis bombus (Ehrenberg) Ehrenberg 1894	–	M	ABT
Diploneis chersonensis (Grunow) P. Cleve 1894	–	M	ABT
Diploneis smithii (Brébisson) P. Cleve 1894	–	BM	K
Entomonei spaludosa (W. Smith) Reimer 1975	–	BM	AB not
Falcula media var. *subsalina* Proschkina-Lavrenko 1963	*o*	M	B
Grammatophora marina (Lyngbye) Kützing 1844	*β*	M	K
Gyrosigma prolongatum (W. Smith) Griffith et Henfrey 1856	–	M	ABT
Halamphora coffeiformis (C.A. Agardh) Levkov 2009	–	BM	ABT
Halamphora costata (W. Smith) Levkov 2009	–	M	BT
Halamphora hyalina (Kützing) Rimet et R. Jahn 2018	*β*	M	ABT not
Haslea ostrearia (Gaillon) Simonsen 1974	–	M	B
Licmophora abbreviata C.A. Agardh 1831	*β*	M	K
Licmophora dalmatica (Kützing) Grunow 1867	–	M	B
Licmophora flabellata (Greville) C.A. Agardh 1831	*β*	M	BTnot
Licmophora gracilis (Ehrenberg) Grunow 1867	–	M	ABT
Licmophora hastata Mereschkowsky 1901	–	M	B
Lyrella clavata (W. Gregory) 1990	–	M	BT
Melosira lineata (Dillwyn) C.A. Agardh 1824	*α*	BM	ABT
Melosira moniliformis (O.F. Müller) C.A. Agardh 1824	*o-β*	BM	K
Navicula cancellata Donkin 1873	–	M	K
Navicula directa (W. Smith) Ralfs ex Pritchard 1861	–	M	K
Navicula distans (W. Smith) Ralfs ex Pritchard 1861	–	M	B
Navicula pennata var. *pontica* Mereschkowsky 1902	–	BM	BT
Navicula perrhombus Hustedt ex Simonsen 1962	–	M	BT
Navicula salinarum Grunow 1880	*β-o*	B	AB not
Nitzschia hybrida f. *hyalina* Proschkina-Lavrenko 1963	*β*	BM	B
Nitzschia lanceolata W. Smith 1853	–	B	BT not
Nitzschia sigma (Kützing) W. Smith 1853	–	B	ABT
Nitzschia tenuirostris Mereschkowsky 1902	–	B	B
Parlibellus delognei (Van Heurck) E.J. Cox 1988	–	M	ABT
Petroneis monilifer (Cleve) A.J. Stickle et D.G. Mann 1990*	–	M	AB
Plagiotropis lepidoptera (W. Gregory) Kuntze 1898	*o*	M	ABT
Pleurosigma elongatum (W. Smith) 1852	–	BM	K
Psammodictyon panduriforme (W. Gregory) D.G. Mann 1990	–	M	BT not
Rhaphoneis amphiceros (Ehrenberg) Ehrenberg 1844	–	BM	ABT
Seminavis ventricosa (W. Gregory) M. Garsia-Baptista 1993	*β*	M	K
Striatella delicatula (Kützing) Grunow ex Van Heurck 1885	–	BM	ABT
Striatella unipunctata (Lyngbye) C.A. Agardh 1832	–	M	BT
Tabularia fasciculata (C.A. Agardh) D.M. Williams et Round 1986	*x-o*	BM	K
Tabularia parva (Kützing) D.M. Williams et Round 1990	*α*	BM	ABT
Tabularia tabulata (C.A. Agardh) Snoeijs 1992	*β-α*	BM	K
Thalassiosira eccentrica (Ehrenberg) P. Cleve 1904	–	M	K
Trachyneis aspera (Ehrenberg) P. Cleve 1894	*β*	M	ABT not
Tryblionella coarctata (Grunow) D.G. Mann 1990	–	BM	B
Undatella lineolata (Ehrenberg) L.I. Ryabushko 2006	–	BM	ABT

Note: (–)—the absence of the diatoms species, (*)—the species was first recorded in the benthos of the Black Sea; ecological characteristic of diatoms (ECD): *S*—species-specific index of saprobity according Sládeček [4]: *α*—alphamesosaprobiont, *α-β*—alpha-betamesosaprobiont, *β*—betamesosaprobiont, *β-α*—beta-alphamesosaprobiont, *β-o*—beta-oligosaprobiont, o—oligosaprobiont, *o-α*—oligo-alpha-mesosaprobiont, *o-β*—oligo-beta-mesosaprobiont, *x-o*—xeno-oligosaprobiont; RS—the ratio of species to the water salinity: M—marine species, BM—brackish-marine, B—brackish; phytogeographycal elements (PhG): B—boreal species, AB—arcto–boreal, BT—boreal–tropical, ABT—arcto–boreal–tropical, K—cosmopolite, not—notal species, found in the southern hemisphere.

From the total species of diatoms on stone substrates in the Donuzlav Gulf the Bacillariophyceae class dominated. Mass species of pennate diatoms presented of genera *Licmophora, Grammatophora, Cylindrotheca, Trachyneis, Cocconeis,* and *Striatella*, etc.

The geographical characteristic of the diatoms of Gulf was carried out. It is shown that in the flora diatom, predominant were cosmopolites (32%) and arctic–boreal–tropical species (27%), which is common species for the microphytobenthos of the Crimean coastal waters of the Black Sea (Table 1). The number of species (40; 12; 29; 30; 21; 37; 38) was distributed by stations according to the values of water salinity (16.58; 16.86; 17.06; 17.81; 18.52; 18.54; 18.62‰). From all 24 species found throughout, the range of investigated water salinity values was from 16.58 to 18.62‰ (June). Only two species of *Amphora arcus* and *Berkeleya rutilans* were found at all stations in the Donuzlav Gulf.

The 18 species found once or twice in the range of the whole water salinity spectrum were as follows: *Berkeleya micans, Carinasigma rectum, Gyrosigma prolongatum, Halamphora coffeiformis, Haslea ostrearia, Licmophora dalmatica, L. flabellata, L. gracilis, Lyrella clavata, Melosira moniliformis, Navicula perrhombus, N. salinarum, Nitzschia sigma, N. tenuirostris, Parlibellus delognei, Pleurosigma elongatum, Tabularia tabulata, Tryblionella coarctata* (Table 1).

The largest number of species (45) was observed in July at salinity of 16.58‰ and temperature 24.4 °C. The smallest number of species (12) was in January at salinity of 16.86‰ and T = 9.0 °C. Despite the low salinity of the Gulf waters, marine species dominated (55% of all species) and brackish-marine species were at 37% (Table 1), which are generally characteristic of the microphytobenthos of the Black Sea [16,18,19]. No freshwater species have been found, although in the past the Gulf was separated from the sea and was called a lake or liman. However, according to our data, the studied area can be considered a marine Gulf.

Other data on the species diversity is the dynamic of their quantitative values (species richness, abundance and biomass of the community), which together with the species composition, and environmental and phytogeographical characteristics can be used to estimate the productivity and water quality of the studied reservoir. Therefore, the following values were obtained. The richness of species varied from 11 to 24 (Figure 3).

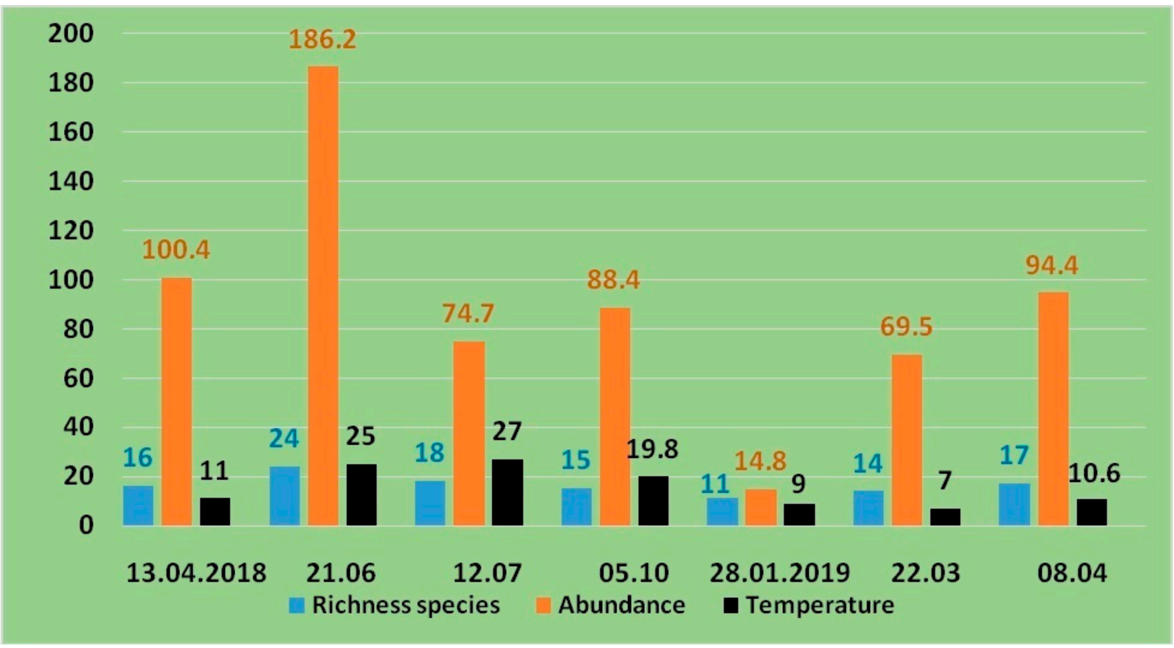

Figure 3. Species richness, abundance of diatoms and water temperature in Donuzlav Gulf of the Black Sea (2018–2019).

From these, 10 species dominated in abundance and biomass: *Berkeleya rutilans*, *L. abbreviata* in April, maximum values of species cosmopolites *L. abbreviata* and *C. scutellum* in June at T = 24.4 °C, *A. hyalina*, *Th. eccentrica* in July, *N. perrhombus* and *A. longipes* in October, and minimum values of *B. rutilans*, *N. hybrida* var. *hyalina* in January, *G. marina* and *C. closterium* in March.

The values of abundance (*N*) diatoms changed (14.8–186.2) × 10^3 cells cm^{-2} with average *N* = 100.5 × 10^3 cells cm^{-2} (Figure 3). The minimum abundance was *N* = 14.8 × 10^3 cells cm^{-2} with *B* = 0.005 mg·cm^{-2} were in January at salinity 16.86‰ and T = 9.0 °C with dominant of *B. rutilans* and *N. hybrida* f. *hyalina*. The maximum abundance 186.2 × 10^3 cells cm^{-2} with a biomass of 0.083 mg·cm^{-2} were found in June 2018 with dominant *L. abbreviata* and *C. scutellum*. The biomass (*B*) varied from 0.006 to 0.083 mg·cm^{-2} with an average *B* = 0.044 mg·cm^{-2} (Figure 4).

Figure 4. Species biomass diatoms in the Donuzlav Gulf of the Black Sea (2018–2019).

Another environmental characteristic is the ratio of species to organic water pollution. Diatoms are indicators of water quality; this is known in particular for freshwater bodies. The study of marine waters on this issue is still lagging behind.

Bioindication of organic pollution in the coastal waters of the Black Sea microphytobenthos communities was gathered for the first time; therefore, the materials for comparison are not yet available. With the help of ecological characteristics of these species, the proportion of saprobiont species was studied. A total of 25 species-indicators of saprobity were found in the epilithon of the Donuzlav Gulf from which 12 taxa belong to a group of betamesosaprobiontes species of moderate organic pollution of water (Table 1). Analysis of the distribution of species-indicators of organic pollution in the epilithon showed that class 2, 4, and 5 indicators of water quality have been also presented. We choose indicators of class 2 (low organic pollution) and class 5 (high organic pollution) of water quality to demonstrate the contrast of its distribution in Donuzlav stations (Figure 5A,B).

For the development of bottom-diatoms important are not only hydrological values, but also the hydrochemical parameters of the environment (Table 2).

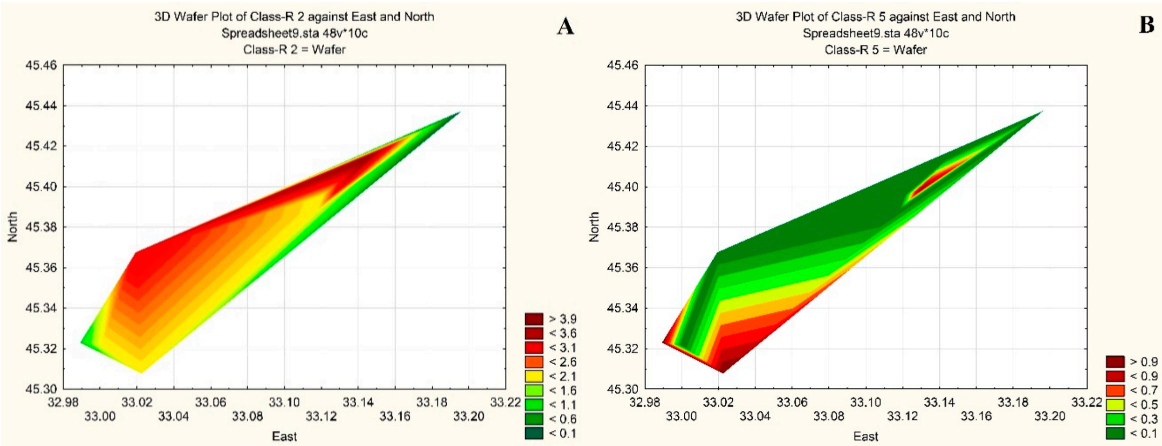

Figure 5. Maps of saprobity indicators of class 2 (**A**) and 5 (**B**) of water quality in the Donuzlav Gulf of the Black Sea (2018–2019).

Table 2. The hydrochemical parameters of Donuzlav Gulf waters of the Black Sea.

№ st.	O_2		BOD_5,	NO_2	NO_3	NH_4	Norg	PO_4	Porg	Si	Oxid.,
	mg L^{-1}	%	mg O_2 L^{-1}	µg L^{-1}							mg O_2 L^{-1}
1	10.22	103.3	0.55	0.3	12.4	10.6	875	0.6	7.6	66.6	2.98
2	10.42	105.1	0.56	0.6	17.6	8.3	955	1.4	6.1	52.3	3.37
3	10.32	103.8	0.61	0.8	13.7	14.2	1072	0.9	6.9	49.7	2.68
5	10.30	103.6	0.34	0.6	17.8	2.7	1104	1.0	7.8	75.1	2.69
6	10.35	104.1	0.57	0.8	11.6	0.6	972	1.7	6.7	124.4	3.40
7	10.32	103.8	0.71	0.9	15.4	7.7	1093	1.1	8.1	51.7	3.57
8	10.14	106.7	0.82	0.5	9.2	7.7	1094	1.1	9.5	137.4	3.67

The analysis showed that the dissolved oxygen content in the range from 10.14 to 10.42 mg L^{-1} with an average value of 10.29 mg L^{-1} in 2018–2019 (Table 2, Figure 6A). The minimum of these values was observed in the winter at station 8, the maximum was in June in the middle part of the Donuzlav Gulf (station 2).

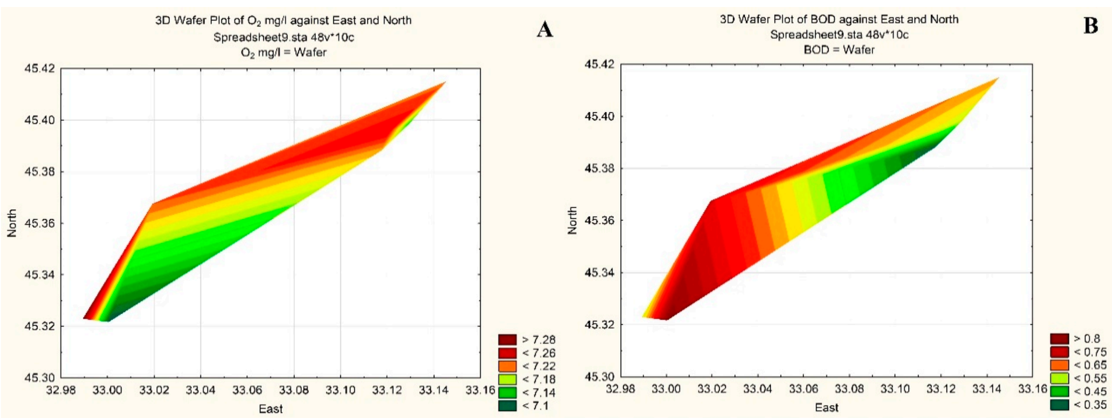

Figure 6. The average annual concentrations of dissolved oxygen (**A**) and BOD_5 (**B**) in the water column at the sampling stations in the Donuzlav Gulf of the Black Sea.

The values of BOD_5 were low, ranging from 0.34 to 0.82 mg O_2 L^{-1} (Figure 6B). They were 2.5–6 times lower than the minimum permissible concentration (2.0 mg O_2 L^{-1}), which indicates the relative cleanness of the waters. The magnitude of the oxidizability changed in the range of 2.68 to 3.67 mg O_2 L^{-1} and were below the minimum permissible level (4.0 mg O_2 L^{-1}) confirming no water pollution in this area.

The values of organic nitrogen varied from 875 to 1104 μg L^{-1} and was highest at station 7 (Figure 7A); inorganic forms of nitrogen varied within the following limits: nitrites (NO_2) ranged from 0.3 to 0.9 μg L^{-1}, nitrates (NO_3) from 9.2 to 17.8 μg L^{-1} and ammonium nitrogen (NH_4) from 0.6 to 14.2 μg L^{-1} (Table 2, Figures 7B and 8A,B). The maximum concentration of nitrite nitrogen was below the maximum permissible concentration (20 μg L^{-1}).

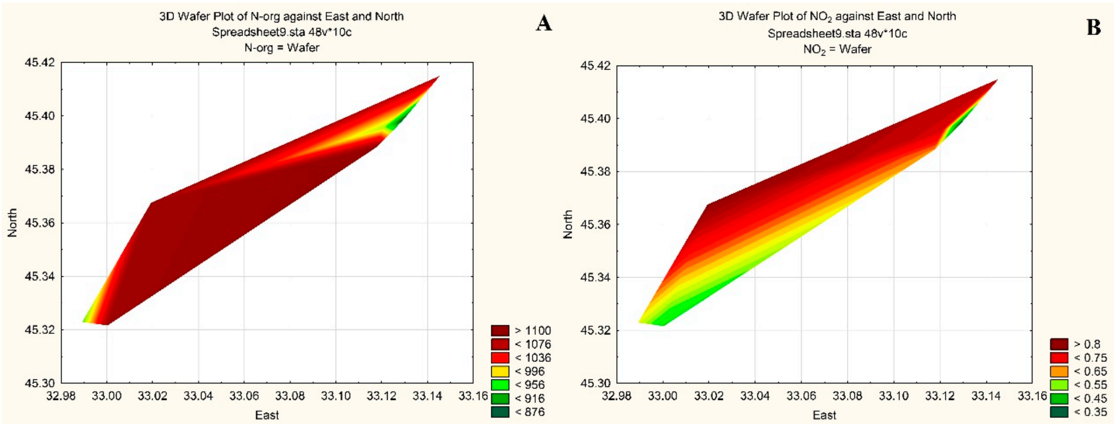

Figure 7. Average annual concentrations of organic nitrogen (**A**) and dissolved nitrites (**B**) in the water column at the sampling stations in the Donuzlav Gulf of the Black Sea.

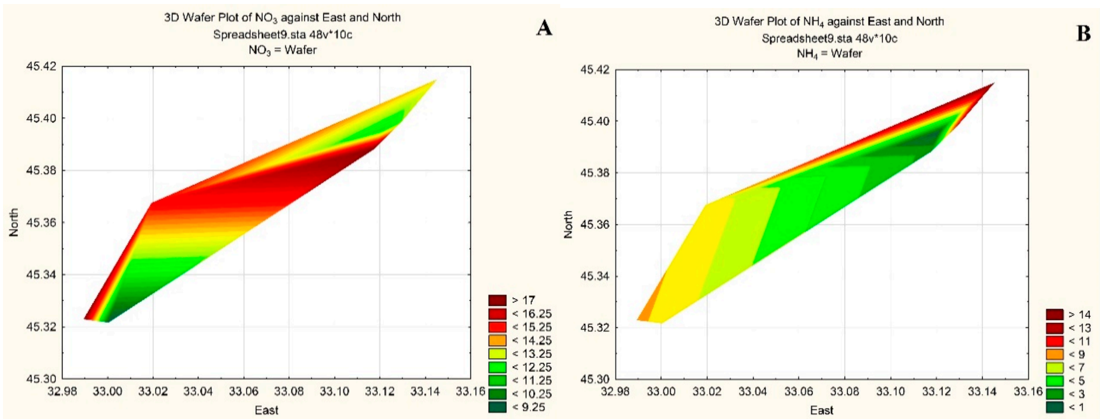

Figure 8. Concentrations of dissolved nitrates (**A**) and ammonium (**B**) in the water column at the sampling stations in the Donuzlav Gulf of the Black Sea.

The values of organic phosphorus (P_{org}) varied from 6.1 to 9.5 μg L^{-1} and the concentrations of mineral phosphorus (PO_4) varied in a narrow range (from 0.9 to 1.7 μg L^{-1}) (Figure 9). In general, the content of nutrients in the Donuzlav Gulf increased from April to June and decreased from October to January.

The concentrations of dissolved silicon varied from 49.7 to 137.4 μg L^{-1} in the waters of the Donuzlav Gulf in January 2019 (Figure 10). The maximum was marked at station 8 in autumn and winter. The minimum was near the farm at station 3, most likely due to the development of diatoms.

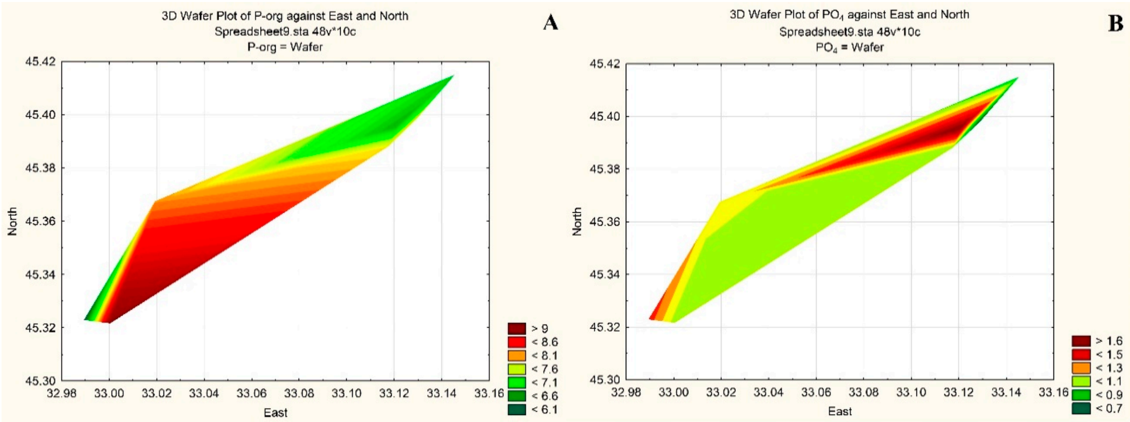

Figure 9. Dissolved organic phosphorus (**A**) and orthophosphate (**B**) concentrations in the water column at the sampling stations in the Donuzlav Gulf of the Black Sea.

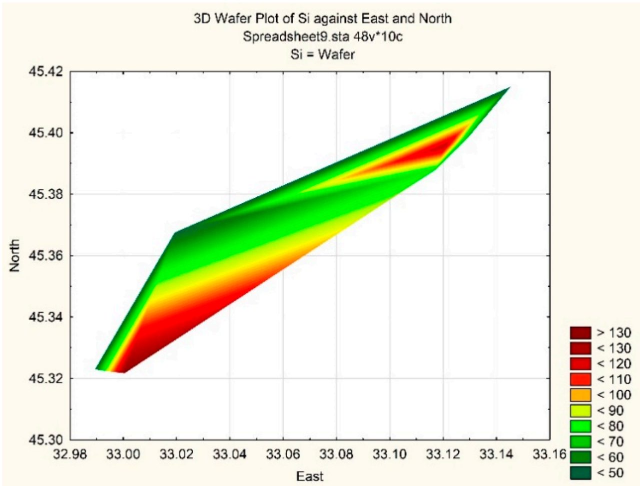

Figure 10. Concentrations of dissolved silicon in the water column at the sampling stations in the Donuzlav Gulf of the Black Sea.

4. Discussion

A comparison of the quantitative data obtained for benthic diatoms from other areas of the Black Sea are recorded for diatom communities as follows. In the epilithon of stones from Karantinnaya Bay (Sevastopol) the species richness (R = 16) and abundance diatoms (average N= 338.6 ± 160.0 × 10^3 cells cm^{-2}) at maximum N = 641.4 × 10^3 cells cm^{-2} with dominant of diatom *Nitzschia tenuirostris* (N = 477.2 × 10^3 cells cm^{-2}) was in April 1988 at T = 13.0 °C [18], which was also marked in the Donuzlav Gulf. In the epiphyton of the brown alga *Cystoseira crinita* Duby 1830 in the Donuzlav Gulf 35 species of diatoms were found with an average abundance of 104 × 10^3 cells cm^{-2} in June 2018 at T = 25 °C [22]. The maximum values of the species richness (26) and abundance (74.78 × 10^3 cells cm^{-2}) of algae were observed in epizoon of Karantinnaya Bay in February 2016 at T = 9.7 °C and in April 2015 (62.0 × 10^3 cells cm^{-2}, T = 10.3 °C), as well as in January 2016 (65.1 × 10^3 cells cm^{-2}, T = 9.5 °C) [21]. The largest biomass was registered in August of 2016 (0.272 mg cm^{-2}, T = 25.5 °C). Near the area for the cultivation of mussels, 24 species-indicators have been detected, among which 16 are beta-mesosaprobionts [21]. It should be noted that in the microphytobenthos of the Azov, Black and Japanese seas this group of saprobity also dominated [11,13,16,18,19].

For comparison, we present data on the structure and functioning of the plankton and benthic communities of Donuzlav in the conditions of anthropogenic pollution of 2000 [2]. From a total number of 82 taxa in phytoplankton of Donuzlav, including 45 species of diatoms, 12 species of benthic diatoms were found that we observed in the microphytobenthos of this area. The largest abundance of 5823 million cells m^{-3} with biomass of 1932.4 mg m^{-3} of phytoplankton was found in May [2]. However, the authors noted that these values were lower in the Black Sea as a whole. Currently phytoplankton is developing worse than before. The abundance and biomass of diatoms in Donuzlav benthos were also lower than in the Sevastopol region.

Over the study period, microphytobenthos in the value of pH = 8.41 in the environment was constant and was close to that found in Kazach'ya Bay (Sevastopol) = 8.14 [18]. The range of oxygen saturation variability (103–105%) was small. The maximum value of saturation corresponds to the maximum value of the absolute oxygen content, and the minimum value of oxygen saturation was marked at Station 1 in Donuzlav. In general, there was a slight super saturating of oxygen from 3–5%. Here the average content of dissolved oxygen in the water changed in the range of 8.77–9.40 mg L^{-1} in the period 2015–2017 [3]. There was a greater range of variation and seasonal variation than in the open waterbody [1].

In Karantinnaya Bay, as a comparison, the corresponding range was 93–125% [21]. The seasonal variability of concentrations of organic nitrogen and phosphorus was not observed. The maximum values of BOD_5, oxidizability, silicon and a high content of N_{org} were noted, which indicates a certain degree of contamination by domestic sewage.

5. Conclusions

The species diversity of benthic diatoms on stony substrates in the Donuzlav Gulf was presented for the first time. A total of 60 taxa of Bacillariophyta, belonging to 3 classes, 18 families, and 34 genera were found. The largest number of diatom species (45) was observed in summer and the smallest (12) was detected in winter. Marine species comprise 55% and brackish-marine ones make up 37% of them, but no freshwater species was identified. The diatom community is dominated by cosmopolites (32%) and arctic–boreal–tropical species (27%).

The maximal abundance (186.2 10^3 cells cm^{-2}) and biomass (0.083 mgcm^{-2}) of diatoms were noted in June 2018 at salinity 16.58‰ and T = 24.4 °C with the dominant species of *L. abbreviata* and *C. scutellum*. The minimum values, $14.8{\cdot}10^3$ cells cm^{-2} and 0.005 mg cm^{-2}, were observed in January 2019 at salinity 16.86‰ and T = 9.0 °C with the dominant species of *B. rutilans* and *N. hybrida* f. *hyalina*.

A total number of 25 saprobity indicator species of diatoms were found on the epilithon, 12 taxa of which belong to a group of betamesosaprobionts—indicators of moderate organic pollution of water. The high values of BOD_5, oxidizability, silicon, N_{org} and P_{org} were noted, indicating a certain degree of water pollution by domestic sewage. It has been shown that the pollution in the southwestern part of the Donuzlav Gulf was greatest since this part of the coast is populated most densely in comparison with the northern coast of Donuzlav where there are no coastal settlements except for two mariculture farms.

Thus, the species diversity, environmental and quantitative characteristics of diatomic benthos and hydrochemical parameters of the Donuzlav Gulf indicate a high supply of oxygen-enriched waters and a sufficient supply of nutrients for the development of biota. The nutrient levels in the water of the gulf are typical for semi-enclosed waters of the Crimean coastal shelf.

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References

1. Kovrigina, N.P.; Nemirovsky, M.S. Hydrochemical characteristics of waters near Lake Donuzlav according to 1990–1997. *Ecol. Morya* **1999**, *48*, 10–15. (In Russian)
2. Samyshev, E.Z.; Senichkina, L.G.; Sergeeva, N.G.; Mikhailova, T.V.; Pankratova, T.M. Structure and functioning of the plankton and benthic communities of oz. Donuzlav in the conditions of anthropogenic pollution and assessment of prospects of its fishery use. In *Systems Control Environment: Proceeding Sci./NAS of Ukraine*; IGI Publisher: Sevastopol, Russia, 2001; pp. 301–325.
3. Zhugaylo, S.S.; Avdeeva, T.M.; Pugach, M.N.; Adzhiumerov, E.N. Current state of water quality and bottom sediments in Lake Donuzlav in modern times. *Aquat. Bioresour. Environ.* **2018**, *1*, 32–38.
4. Barinova, S.S.; Bilous, O.P.; Tsarenko, P.M. *Algal Indication of Water Bodies in Ukraine: Methods and Prospects*; Publishing House of Haifa University: Haifa, Israel; Kyiv, Ukraine, 2019; 367p. (In Russian)
5. Barinova, S.S.; Medvedeva, L.A.; Anisimova, O.V. *Diversity of Algal Indicators in the Environmental Assessment*; Pilies Studio Publisher: Tel'Aviv, Israel, 2006; 498p. (In Russian)
6. Harding, W.R.; Taylor, J.C. *The South African Diatom Index (SADI)—A Preliminary Index for Indicating Water Quality in Rivers and Streams in Southern Africa*; WRC Report; Water Research Commission: Pretoria, South Africa, 2011; pp. 1–11.
7. Harding, W.R.; Taylor, J.C. *Diatoms as Indicators of Historical Water Quality: A Comparison of Samples Taken in the Wemmershoek Catchment (Western Province, South Africa) in 1960 and 2008*; Water Research Commission: Pretoria, South Africa, 2014; pp. 601–606.
8. Potapova, M.; Charles, D.F. Diatom metrics for monitoring eutrophication in river of the United States. *Ecol. Indic.* **2007**, *7*, 48–70. [CrossRef]
9. Prygiel, J. Management of the diatom monitoring network in France. *J. App. Phycol.* **2002**, *14*, 19–26. [CrossRef]
10. Szczepocka, E.; Żelazna-Wieczorek, J. Diatom biomonitoring—Scientific foundations, commonly discussed issues and frequently made errors. *Oceanol. Hydrobiol. Stud.* **2018**, *47*, 314–325. [CrossRef]
11. Barinova, S.S.; Bondarenko, A.V.; Ryabushko, L.I.; Kapranov, S.V. Microphytobenthos as indicator of water quality and organic pollution in the Western coastal zone of the Sea of Azov. *Oceanol. Hydrobiol. Stud.* **2019**, *48*, 21–35. [CrossRef]
12. Begun, A.A. Of the state of the marine environment on diatoms of epiphytonmacrophytes (Peter the Great Bay, Japan Sea). *Izv. Tinro* **2012**, *169*, 1–17.
13. Ryabushko, L.I.; Begun, A.A. *Diatoms of Microphytobenthos of the Sea of Japan*; Book 1; N. Orianda Publishers: Sevastopol-Simferopol, Russia, 2015; 288p. (In Russian)
14. Zalat, A.A. Distribution and origin of diatoms in the bottom sediments of the Suez canal lakes and adjacent areas, Egypt. *Diat. Res.* **2002**, *17*, 243–266. [CrossRef]
15. Desrosiers, C.; Leflaivea, J.; Eulinb, A.; Ten-Hagea, L. Bioindicators in marine waters: Benthic diatoms as a tool to assess water quality from eutrophic to oligotrophic coastal ecosystems. *Ecol. Indic.* **2013**, *32*, 25–34. [CrossRef]
16. Ryabushko, L.I.; Bondarenko, A.V. The Qualitative and Quantitative Characteristics of the Benthic Diatoms near Kazantip Cape of the Sea of Azov. *J. Black Sea Mediterr. Environ.* **2016**, *22*, 237–249. (In Russian)
17. Bondarenko, A.V.; Ryabushko, L.I.; Sadogurskaya, S.A. Microalgae of Benthos and Plankton in the Coastal Waters of the Nature Reserve "Kazantipskiy" (the Sea of Azov, Crimea). *In Biodiversity and Environment of Protected Areas* **2018**, *4*, 25–48. (In Russian)
18. Ryabushko, L.I. *Microphytobenthos of the Black Sea*; Gaevskay, A.V., Ed.; ECOSI-Gidrofizica: Sevastopol, Russia, 2013; 416p. (In Russian)
19. Ryabushko, L.I.; Balycheva, D.S.; Ryabushko, V.I. Microphytobenthos Diatoms of the Black Sea: Biodiversity and Ecology. *Ecol. Montenegrina* **2017**, *10*, 1–15.
20. Ryabushko, L.I.; Balycheva, D.S.; Strizhak, A.V. Diatoms of epiphyton some species of green algae-macrophytes and periphyton of anthropogenic substrates of the Crimean coastal waters of the Black Sea. *Algologia* **2013**, *23*, 419–437. (In Russian) [CrossRef]
21. Ryabushko, L.I.; Pospelova, N.V.; Balycheva, D.S.; Kovrigina, N.P.; Troshchenko, O.A.; Kapranov, S.V. Epizoon microalgae of the cultivated mollusk *Mytilus galloprovincialis* Lam. 1819, phytoplankton, hydrological and hydrochemical characteristics in the mussel-and-oyster farm area (Sevastopol, the Black Sea). *Mar. Biol. J.* **2017**, *2*, 67–83. (In Russian) [CrossRef]

22. Ryabushko, L.I.; Lishaev, D.N.; Shiroyan, A.G. The species, ecological and geographical diversity of microphytobenthos microalgae in the Crimean coast of the Black Sea. In Proceedings of the 2nd International UNIDOCAP the BlacкSea Symposium on Biodiversity, Samsun, Turkey, 28–30 November 2018; Progr. & Abstract. Book: Samsun, Turkey, 2018; p. 33. (In Russian).
23. Graham, A.A.; McCaughan, D.J.; McKee, F.S. Measurement of surface area of stones. *Hydrobiologia* **1988**, *157*, 85–87. [CrossRef]
24. Oksiyuk, O.P.; Yurchenko, V.V. About the weight diatoms. *Hydrobiol. J.* **1971**, *7*, 116. (In Russian)
25. Grasshoff, K. (Ed.) *Methods of Seawater Analysis*; Verlag Chemie: Weinheim, Germany; New York, NY, USA, 1976; 317p.
26. *Methods of Hydrochemical Studies of Basic Hydrochemical Elements*; VNIRO: Moscow, Russia, 1988; 120p. (In Russian)
27. Sladeček, V. Diatoms as indicators of organic pollution. *Acta Hydrochem. Hydrobiol.* **1986**, *14*, 555–566. [CrossRef]
28. Guiry, M.D.; Guiry, G.M. AlgaeBase. World-Wide Electronic Publication, National University of Ireland, Galway. Available online: http://www.algaebase.org (accessed on 20 December 2018).
29. Guslyakov, N.E.; Zakordonets, O.A.; Gerasimyuk, V.P. *Atlas of Diatoms of the Benthos in the North-Western Part of the Black Sea and Adjacent Reservoirs*; Nauk. Dumka: Kyiv, Ukraine, 1992; 112p. (In Russian)
30. Proshkina-Lavrenko, A.I. *Diatoms of the Black Sea Benthos*; Nauka Publishers: Leningrad, Moscow, 1963; 244p. (In Russian)
31. Ryabushko, L.I.; Begun, A.A. *Diatoms of the Microphytobenthos of the Sea of Japan (Synopsis and Atlas)*; Book 2; PK «KIA» Publishers: Sevastopol, Russia, 2016; 324p. (In Russian)
32. Smith, W.F. *A Synopsis of the British Diatomaceae*; John Van Voorst: London, UK, 1853; Volume 1, 89p.
33. Barinova, S. Ecological Mapping in Application to Aquatic Ecosystems BioIndication: Problems and Methods. *Int. J. Environ. Sci. Nat. Resour.* **2017**, *3*, 1–7. [CrossRef]