

Microphytobenthos as an indicator of water quality and organic pollution in the western coastal zone of the Sea of Azov

by

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Abstract

Bioindication of organic pollution and seawater quality was carried out for the first time in the western part of the Sea of Azov on the basis of species composition and quantitative characteristics of microphytobenthos. A total of 229 algal samples were collected at 17 sites over the period of 2005–2014 on three different substrates in three areas under study: Sivash Gulf, the Kerch Strait and Cape Kazantip. In total, 200 taxa of algae were found, which belong to six taxonomic divisions with a predominance of diatoms. Among those, 108 taxa are indicators of substrate, water temperature, salinity, water pH, trophic state, the type of nutrition and organic pollution of water. It has been shown that the most active self-purification of water takes place in communities on stony substrates. The largest number of algae species (50%) occurs in the cleanest waters of the Kazantip Nature Reserve (Water Quality Classes I–II). The species composition of organic pollution indicators in Sivash Gulf corresponds to waters of Classes III–IV, which are more polluted than those of the Kerch Strait and Cape Kazantip. All the studied areas of the Sea of Azov are cleaner compared to some waters of the Eastern Mediterranean and the Sea of Japan.

Key words: bioindication, water quality, organic pollution, microphytobenthos, ecological mapping, the Sea of Azov

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Introduction

The Sea of Azov is a unique reservoir considered to be the most continental and shallow sea in the world. It is characterized by strong variations in salinity in its different regions and considerable fluctuations in water temperature. Environmental changes affect the species composition and quantitative characteristics of microalgae communities, especially benthic ones, which reside on the substrate. Benthic microalgae communities of the western coastal zone of the Sea of Azov have been unevenly and insufficiently explored (Sadogurskaya et al. 2006; Ryabushko & Bondarenko 2011; 2016; Bondarenko 2012; 2017). Currently, the most studied groups of the benthic area of the Sea of Azov are diatoms, Dinophyceae, Haptophyceae, Chrysophyceae, and cyanobacteria (Ryabushko & Bondarenko 2011).

In the coastal zone of many water bodies, heterotrophic processes prevail over the autotrophic synthesis of organic matter (Smith & MacKenzie 1987; Smith & Hollibaugh 1993; Kepkay et al. 1997). It is known that in addition to photosynthesis, algae actively use the heterotrophic type of nutrition, especially in the photolimitation conditions (Rivkin & Putt 1987). The tendency of algae, e.g. diatoms, to heterotrophy is important for explaining the mechanisms of excessive organic matter use by algae

in their environment (Lewin 1953; Admiraal & Peletier 1979; Saks 1983).

Microalgae are known to be bioindicators of the ecological state of freshwater bodies (Makrushin 1974; Watanabe et al. 1984; 1986; Barinova & Medvedeva 1996; Barinova et al. 2006; Barinova 2011). Bioindication of seawater quality based on the species composition and quantitative characteristics of microalgae has just started being developed. It is particularly important to consider changes in microphytobenthos, as its morphological, floristic, quantitative and production characteristics as well as saprobity can be used for assessing the quality of any water body and for predicting such negative phenomena as water blooms and red tides (Ryabushko 2003; 2013). This methodological approach is relatively inefficient for microphytobenthos of the Sea of Azov, the Black Sea, the Sea of Japan and the Egyptian coasts of the Mediterranean Sea, and only limited data have been provided (Guslyakov et al. 1992; Lyalyuk & Lipnitskaya 1997; Zalat 2002; Begun & Ryabushko 2010; Begun 2012; Bondarenko 2012; 2017; Ryabushko 2013; Ryabushko & Begun 2015). In addition, the ecological characteristics of water bodies include salinity as an indicator of water quality for Bacillariophyta (Proshkina-Lavrenko 1953; Barinova et al. 2006; Ryabushko 2013; Bondarenko 2017).

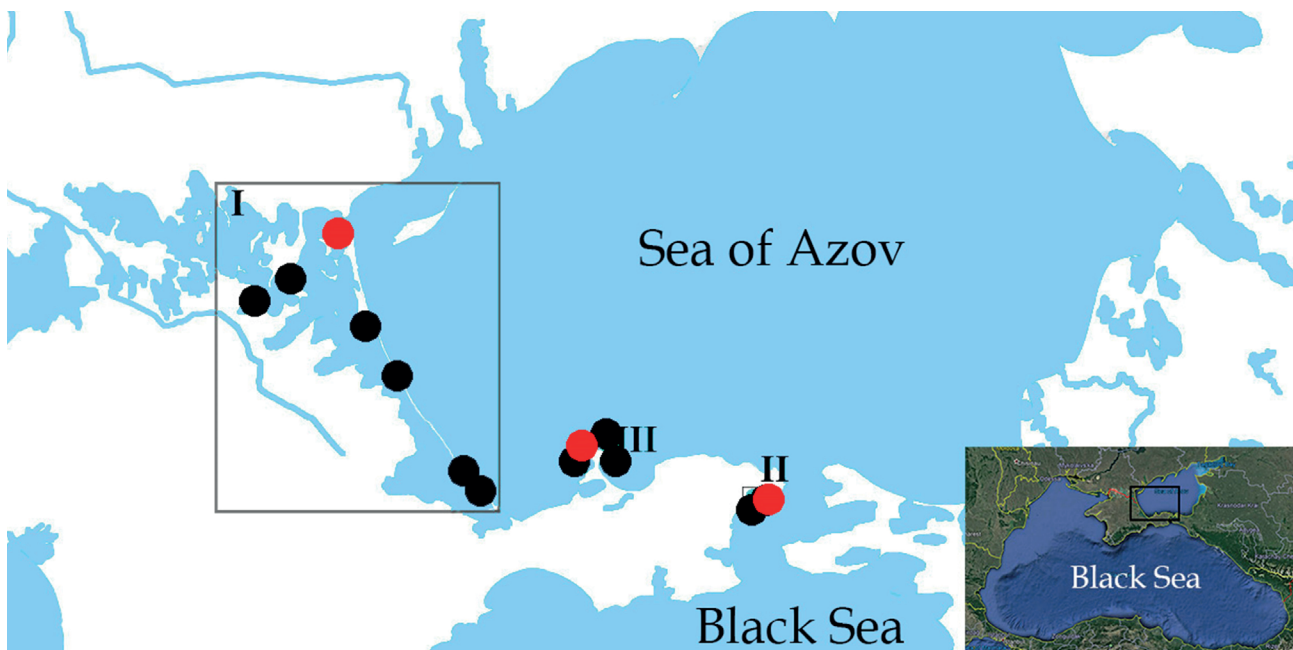


Figure 1

Sampling areas in the western coastal zone of the Sea of Azov. Red dots denote the sampling sites where information for mapping was accumulated, and black dots denote other sampling sites. (I) Sivash Gulf, (II) the Kerch Strait and (III) Kazantip Cape

**Figure 2**

Coastal zone of Sivash Gulf (a) and sandy coast of Cape Kazantip (b). Microphytobenthos habitats in the Kazantip Natural Reserve: submerged stones (c) and shell fouling (d)

Bioindication and assessment of the environmental situation in local marine areas, as well as in nature reserves, require a combination of different approaches and research methods. It is therefore particularly important and promising to study the microphytobenthos of the marine environment with varying levels of organic pollution.

The presented work aims at bioindication of properties of the western coastal waters of the Sea of Azov based on the distribution of microphytobenthos.

Materials and methods

A total of 229 microphytobenthos samples were collected for the study. Sampling of microalgae was performed at 17 sites in 2005–2006, 2008–2011 and 2014 in different seasons and different ecotopes of the western coastal zone of the Sea of Azov (Fig. 1). The samples were collected at a depth of 0.1–1.5 m in three shallow water areas: (I) Sivash Gulf (45°93'N and 34°46'E), (II) the Kerch Strait (45°29'N and 36°42'E), and (III) Cape Kazantip, including waters of the Kazantip Nature Reserve (45°28'N and 35°52'E; Fig. 2).

Water salinity was 34–46 PSU, 13–15 PSU and 11.5 PSU in areas I, II, and III, respectively. The water temperature varied during the study from –0.5 in February to +29.0°C in August (Bondarenko 2017).

The number of samples collected in the study areas varied as follows: 55 (I), 50 (II) and 124 (III), including epilithon, epiphyton, silt and sand ecotopes (Bondarenko 2017). Microalgae were examined under an optical microscope Axioskop 40 (C. Zeiss, Germany) with ocular lens 10, objective lens 40 and immersion objective lens 100. The AxioVision Rel. 4.6 software was used to determine the size and morphological structure of the cells. The taxonomic composition has been adapted to algaebase.org (Guiry & Guiry 2018). Taxonomic identification was performed according to Cleve-Euler (1953a,b), Gollerbach et al. (1953), Proshkina-Lavrenko (1963), Guslyakov et al. (1992), Witkowski et al. (2000), Al-Yamani & Saburova (2011) and Ryabushko & Begun (2016).

Environmental preferences of algal species were used in the bioindication analysis (Barinova & Medvedeva 1996; Barinova et al. 2006). Ecological mapping was performed using Statistica 12.0.

Results

Species diversity

The first research stage of the research consisted in creating a checklist of microphytobenthos algae from different ecotopes of the western coastal zone of the Sea of Azov. A total of 200 taxa of microalgae from six taxonomic divisions, with a predominance of the Bacillariophyta group (79%), were found (Bondarenko, 2017). Among them, 108 taxa from four taxonomic divisions with known bioindicator properties were bioindicators of ten environmental variables (Table 1, Fig. 3).

Bioindication

Bioindicator taxa account for about half of the algae occurring near the western coast of the Sea of Azov. The large number of the indicator species found is very important for marine bioindication as this method was primarily developed for the diversity of continental algae.

Taxonomic distribution of species richness and ecological characteristics of flora are presented for each region and ecotope (Table 1). The following distribution of indicator species was observed in different ecotopes: 65 species in epiphyton, 35 species in epilithon and 46 species in silt and sand.

Table 1

Distribution of microalgae species indicators on different types of substrate and in different study areas in the western coastal zone of the Sea of Azov

| Taxa | EP | EL | SE | Siv | Ker | Kaz | Hab | T | Oxy | pH | Hal | D | Sap | S | Tro | Aut-Het |
|--|----|----|----|-----|-----|-----|--------|------|--------|-----|-----|----|-----|------|-----|---------|
| Cyanobacteria | | | | | | | | | | | | | | | | |
| <i>Aphanocapsa incerta</i> (Lemmermann) G.Cronberg & Komárek | 1 | 0 | 0 | 0 | 0 | 1 | B-P | - | - | - | i | - | b | 2.2 | me | - |
| <i>Aphanothece stagnina</i> (Sprengel) A.Braun | 1 | 1 | 1 | 1 | 0 | 1 | B-P | - | - | ind | hl | - | b-a | 2.4 | me | - |
| <i>Chamaecalyx swirenkoi</i> (Sirsov) Komárek & Anagnostidis | 0 | 1 | 1 | 1 | 0 | 1 | Ep | - | - | - | - | - | - | - | - | - |
| <i>Chroococcus limneticus</i> Lemmermann | 1 | 0 | 0 | 0 | 1 | 1 | P | - | - | - | - | - | - | - | o-m | - |
| <i>Leptolyngbya foveolara</i> (Gomont) Anagnostidis & Komárek | 1 | 0 | 0 | 0 | 0 | 1 | B, S | - | aer | - | - | - | b-a | 2.4 | - | - |
| <i>Leptolyngbya fragilis</i> (Gomont) Anagnostidis & Komárek | 1 | 0 | 1 | 1 | 0 | 0 | B, S | warm | - | - | ph | - | b-o | 1.7 | - | - |
| <i>Lyngbya aestuarii</i> Liebman ex Gomont | 1 | 0 | 0 | 1 | 0 | 0 | B-P, S | - | - | - | mh | - | o | 1.3 | - | - |
| <i>Lyngbya lutea</i> Gomont ex Gomont | 0 | 0 | 0 | 0 | 1 | 0 | B-P | - | - | - | mh | - | - | - | - | - |
| <i>Lyngbya semiplena</i> J.Agardh ex Gomont | 1 | 0 | 0 | 1 | 0 | 0 | B, S | - | - | - | ph | - | - | - | - | - |
| <i>Merismopedia glauca</i> (Ehrenberg) Kützing | 0 | 0 | 1 | 1 | 0 | 0 | B-P | - | - | ind | i | - | b-o | 1.75 | o-m | - |
| <i>Microcystis aeruginosa</i> (Kützing) Kützing | 1 | 1 | 1 | 0 | 0 | 1 | P | - | - | - | hl | - | b | 2.1 | e | - |
| <i>Microcystis pulverea</i> (H.C.Wood) Forti | 1 | 0 | 1 | 0 | 0 | 1 | B-P, S | - | - | - | i | - | o-b | 1.5 | - | - |
| <i>Microcystis wesenbergii</i> (Komárek) Komárek ex Komárek | 1 | 0 | 1 | 0 | 1 | 1 | P | - | - | - | - | - | o-a | 1.9 | e | - |
| <i>Nodularia harveyana</i> Thuret ex Bornet & Flahault | 1 | 0 | 0 | 0 | 0 | 1 | B, S | - | - | - | mh | - | o | 1.2 | - | - |
| <i>Oscillatoria nitida</i> Schkorbatov | 1 | 0 | 0 | 0 | 0 | 1 | P | - | st-str | - | i | - | - | - | - | - |
| <i>Phormidium breve</i> (Kützing ex Gomont) Anagnostidis & Komárek | 1 | 0 | 0 | 1 | 0 | 0 | B-P, S | - | st,aer | - | - | - | a | 3.1 | - | - |
| <i>Phormidium puteale</i> (Montagne ex Gomont) Anagnostidis & Komárek | 0 | 1 | 0 | 0 | 0 | 1 | B, S | - | st-str | - | - | - | - | - | - | - |
| <i>Pleurocapsa minor</i> Hansgirg | 0 | 1 | 0 | 0 | 1 | 0 | B | - | st-str | - | - | - | x-b | 0.9 | o | - |
| <i>Spirulina tenuissima</i> Kützing | 1 | 0 | 1 | 1 | 0 | 1 | B | - | st-str | - | - | - | o-b | 1.4 | - | - |
| Bacillariophyta | | | | | | | | | | | | | | | | |
| <i>Achnanthes brevipes</i> C.Agardh var. <i>brevipes</i> | 1 | 1 | 1 | 1 | 1 | 1 | B | - | - | alf | hl | - | b | 2.0 | me | - |
| <i>Achnanthes brevipes</i> var. <i>intermedia</i> (Kützing) Cleve | 0 | 1 | 1 | 0 | 1 | 0 | B | - | st | - | mh | - | - | - | - | - |
| <i>Achnanthes longipes</i> C.Agardh | 1 | 1 | 1 | 1 | 1 | 1 | B | - | - | - | hl | - | - | - | - | - |
| <i>Amphora commutata</i> Grunow in Van Heurck | 1 | 0 | 0 | 1 | 0 | 0 | B | - | - | - | mh | - | - | - | e | - |
| <i>Amphora delicatissima</i> Krasske | 1 | 1 | 0 | 0 | 0 | 1 | B | - | - | - | mh | - | - | - | - | - |
| <i>Amphora ovalis</i> (Kützing) Kützing | 0 | 0 | 1 | 1 | 0 | 1 | B | temp | st-str | alf | i | sx | o-b | 1.5 | me | ate |
| <i>Bacillaria paxillifera</i> (O.F.Müller) T.Marsson | 1 | 1 | 1 | 0 | 1 | 1 | B-P | - | - | ind | hl | es | b | 2.3 | me | ate |
| <i>Berkeleya rutilans</i> (Trentepohl ex Roth) Grunow | 1 | 1 | 1 | 0 | 1 | 1 | B | - | - | alf | hl | - | - | - | - | - |
| <i>Cocconeis costata</i> Gregory | 1 | 1 | 1 | 0 | 1 | 1 | B | - | - | alf | hl | - | b | 2.0 | me | - |
| <i>Cocconeis disculus</i> (Schumann) Cleve | 1 | 1 | 1 | 1 | 1 | 1 | B | - | st | alf | i | es | o-x | 0.7 | me | - |
| <i>Cocconeis pediculus</i> Ehrenberg | 1 | 0 | 0 | 1 | 0 | 0 | B | - | st-str | alf | i | sx | o-a | 1.8 | me | ate |
| <i>Cocconeis placentula</i> var. <i>intermedia</i> (Héribaud-Joseph & M.Peragallo) Cleve | 1 | 1 | 1 | 1 | 1 | 1 | B | - | st-str | alf | i | - | o-b | 1.4 | ot | ate |
| <i>Cocconeis scutellum</i> Ehrenberg | 1 | 1 | 1 | 1 | 1 | 1 | B | - | - | alf | hl | - | b | 2.0 | me | - |
| <i>Ctenophora pulchella</i> (Ralfs ex Kützing) D.M.Williams & Round | 0 | 0 | 1 | 0 | 1 | 0 | B-P | - | st-str | alf | i | - | b | 2.3 | o-m | ate |
| <i>Cylindrotheca closterium</i> (Ehrenberg) Reimann & J.C.Lewin | 1 | 1 | 0 | 0 | 1 | 1 | B | - | - | alf | l | - | b | 2.0 | - | - |
| <i>Diatoma tenuis</i> C.Agardh | 1 | 1 | 0 | 0 | 0 | 1 | B-P | - | st-str | ind | hl | sx | o | 1.3 | e | ate |
| <i>Diatoma vulgare</i> Bory | 1 | 0 | 0 | 0 | 0 | 1 | B-P | - | st-str | ind | i | sx | b | 2.2 | me | ate |
| <i>Diploneis didyma</i> (Ehrenberg) Ehrenberg | 0 | 0 | 1 | 0 | 0 | 1 | B | - | - | - | mh | - | b | 2.0 | - | - |
| <i>Entomoneis alata</i> (Ehrenberg) Ehrenberg | 0 | 0 | 1 | 1 | 0 | 0 | B-P | - | st | alf | mh | - | b | 2.0 | - | - |
| <i>Entomoneis paludosa</i> (W.Smith) Reimer | 1 | 1 | 1 | 0 | 1 | 1 | B-P | - | - | alf | hl | - | b-a | 2.5 | m | - |

Microphytobenthos as an indicator of water quality in the Sea of Azov

| Taxa | EP | EL | SE | Siv | Ker | Kaz | Hab | T | Oxy | pH | Hal | D | Sap | S | Tro | Aut-Het |
|--|----|----|----|-----|-----|-----|--------|------|--------|-----|-----|----|-----|-----|-----|---------|
| <i>Fallacia pygmaea</i> (Kützing) A.J.Stickle & D.G.Mann | 0 | 1 | 0 | 0 | 1 | 0 | B-P | – | st-str | alf | mh | es | a-o | 2.7 | e | hne |
| <i>Fragilaria capucina</i> Desmazières | 1 | 1 | 1 | 0 | 1 | 1 | B-P | – | – | ind | i | es | b-o | 1.6 | m | – |
| <i>Fragilaria crotonensis</i> Kitton | 1 | 0 | 0 | 0 | 1 | 1 | P | – | st-str | alf | i | es | o-b | 1.5 | m | ate |
| <i>Fragilariforma virescens</i> (Ralfs) D.M.Williams & Round | 1 | 1 | 1 | 0 | 1 | 1 | B-P | – | st | ind | i | es | x-o | 0.4 | o-m | ats |
| <i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst | 1 | 0 | 1 | 1 | 0 | 1 | B | – | – | – | – | es | – | – | – | – |
| <i>Gyrosigma balticum</i> (Ehrenberg) Rabenhorst | 1 | 0 | 1 | 1 | 0 | 0 | B | – | – | – | hl | – | – | – | e | – |
| <i>Gyrosigma fasciola</i> (Ehrenberg) J.W.Griffith & Henfrey | 0 | 0 | 1 | 1 | 0 | 0 | B | – | – | alf | mh | – | o | 1.0 | – | – |
| <i>Gyrosigma scalproides</i> (Rabenhorst) Cleve | 1 | 0 | 1 | 1 | 0 | 1 | B | – | – | alf | i | es | b | 2.2 | – | – |
| <i>Gyrosigma wansbeckii</i> (Donkin) Cleve | 0 | 0 | 1 | 1 | 0 | 0 | B | – | st-str | alf | mh | es | b | 2.0 | e | – |
| <i>Halamphora acutiuscula</i> (Kützing) Levkov | 1 | 1 | 1 | 0 | 1 | 1 | B-P | warm | – | alf | mh | sp | b | 2.0 | – | – |
| <i>Halamphora coffeiformis</i> (C.Agardh) Levkov | 1 | 1 | 1 | 1 | 1 | 1 | B | – | st-str | alf | mh | – | a | 3.0 | e | ate |
| <i>Haslea spicula</i> (Hickie) L.Bukhtiyarova | 0 | 1 | 0 | 0 | 0 | 1 | B-P | – | – | – | mh | – | – | – | – | – |
| <i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski | 0 | 1 | 1 | 0 | 0 | 1 | B | temp | st-str | alf | hl | es | b | 2.1 | me | ate |
| <i>Hyalodiscus scoticus</i> (Kützing) Grunow | 1 | 0 | 0 | 0 | 1 | 0 | – | – | – | alf | i | – | b | 2.0 | – | – |
| <i>Mastogloia smithii</i> Thwaites ex W.Smith | 1 | 0 | 1 | 1 | 0 | 1 | B | – | – | alf | mh | sx | o | 1.3 | me | – |
| <i>Melosira lineata</i> (Dillwyn) C.Agardh | 1 | 0 | 0 | 0 | 1 | 0 | B-P | cool | – | alf | mh | es | o-a | 1.8 | e | ats |
| <i>Melosira moniliformis</i> (O.F.Müller) C.Agardh var. <i>moniliformis</i> | 1 | 1 | 1 | 1 | 1 | 1 | B-P | – | str | alf | hl | – | b | 2.0 | – | – |
| <i>Melosira moniliformis</i> var. <i>octogona</i> (Grunow) Hustedt | 1 | 0 | 1 | 0 | 1 | 0 | B-P | – | – | – | hl | – | – | – | – | – |
| <i>Melosira moniliformis</i> var. <i>subglobosa</i> (Grunow) Hustedt | 1 | 0 | 0 | 0 | 1 | 0 | B-P | – | str | alf | hl | – | b | 2.0 | – | – |
| <i>Navicula ammophila</i> Grunow | 1 | 1 | 1 | 1 | 1 | 1 | B | – | – | – | hl | – | – | – | – | – |
| <i>Navicula cancellata</i> Donkin | 1 | 1 | 1 | 0 | 1 | 1 | B | – | – | – | hl | – | – | – | – | – |
| <i>Navicula cryptocephala</i> Kützing | 1 | 0 | 0 | 1 | 0 | 0 | B-P | temp | st-str | ind | i | es | b | 2.1 | o-e | ate |
| <i>Navicula digito-radiata</i> (Gregory) Ralfs | 1 | 1 | 0 | 1 | 1 | 1 | B | – | – | alf | i | es | b | 2.0 | me | – |
| <i>Navicula lanceolata</i> var. <i>tenuirostris</i> Skvortzov | 1 | 0 | 0 | 0 | 0 | 1 | B | – | – | alf | i | – | – | – | – | – |
| <i>Navicula peregrina</i> (Ehrenberg) Kützing | 1 | 0 | 1 | 1 | 1 | 1 | B-P | – | – | alf | mh | es | o-b | 1.5 | o-m | – |
| <i>Navicula ramosissima</i> (C.Agardh) Cleve | 1 | 1 | 1 | 1 | 1 | 1 | B | – | – | – | ph | – | – | – | – | – |
| <i>Navicula radiosa</i> Kützing | 1 | 0 | 0 | 1 | 0 | 0 | B | temp | st-str | ind | i | es | o | 1.3 | me | ate |
| <i>Navicula salinarum</i> Grunow | 1 | 1 | 0 | 1 | 0 | 1 | B-P | – | st-str | ind | mh | – | b | 2.1 | me | ate |
| <i>Navicula veneta</i> Kützing | 1 | 1 | 0 | 0 | 0 | 1 | B | – | – | alf | hl | es | a-o | 2.7 | – | – |
| <i>Nitzschia amphibia</i> Grunow | 1 | 0 | 0 | 0 | 1 | 0 | B-P, S | temp | st-str | alf | i | sp | b | 2.1 | e | hne |
| <i>Nitzschia dissipata</i> (Kützing) Rabenhorst | 1 | 0 | 1 | 1 | 1 | 1 | B | – | st-str | alf | i | sx | b-o | 1.7 | me | ate |
| <i>Nitzschia gracilis</i> Hantzsch | 1 | 0 | 1 | 1 | 0 | 0 | B-P | temp | st-str | ind | i | sp | o-a | 1.8 | m | – |
| <i>Nitzschia holsatica</i> Hustedt | 1 | 0 | 1 | 1 | 0 | 0 | B-P | – | – | ind | i | es | b | 2.3 | – | – |
| <i>Nitzschia lanceolata</i> W.Smith | 1 | 1 | 1 | 0 | 1 | 1 | B | – | – | alf | hl | – | b | 2.0 | e | – |
| <i>Nitzschia linearis</i> W.Smith | 1 | 0 | 0 | 0 | 1 | 0 | B | temp | st-str | alf | i | es | b-o | 1.7 | me | ate |
| <i>Nitzschia macilenta</i> Gregory | 0 | 0 | 1 | 1 | 0 | 0 | – | – | – | – | hl | – | – | – | – | – |
| <i>Nitzschia obtusa</i> W.Smith | 1 | 1 | 0 | 1 | 1 | 1 | B | – | – | – | hl | es | b-a | 2.4 | m | – |
| <i>Nitzschia recta</i> Hantzsch ex Rabenhorst | 1 | 0 | 0 | 1 | 0 | 0 | B | – | st | ind | i | es | o-b | 1.5 | o-m | ate |
| <i>Nitzschia scalpelliformis</i> Grunow | 1 | 0 | 0 | 0 | 0 | 1 | B | – | – | alf | hl | sp | b | 2.0 | me | – |
| <i>Nitzschia sigma</i> (Kützing) W.Smith | 1 | 1 | 1 | 1 | 1 | 1 | B | temp | st-str | alf | mh | es | a | 3.0 | e | ate |
| <i>Nitzschia sigmoidea</i> (Nitzsch) W.Smith | 1 | 1 | 1 | 1 | 1 | 1 | B-P | – | st-str | alf | i | – | b-a | 2.5 | e | ate |
| <i>Nitzschia vermicularis</i> (Kützing) Hantzsch | 1 | 1 | 0 | 1 | 0 | 1 | B-P | – | str | alf | i | – | b | 2.2 | m | – |
| <i>Odontella aurita</i> (Lyngbye) Agardh | 0 | 1 | 1 | 1 | 1 | 0 | – | – | – | alf | mh | – | b | 2.0 | – | – |
| <i>Petronis humerosa</i> (Brébisson ex W.Smith) A.J.Stickle & D.G.Mann | 0 | 0 | 1 | 0 | 0 | 1 | B | – | – | – | mh | – | – | – | – | – |
| <i>Petronis marina</i> (Ralfs) D.G.Mann | 0 | 0 | 1 | 1 | 0 | 0 | B | – | – | – | mh | – | – | – | – | – |
| <i>Plagiotropis lepidoptera</i> (Gregory) Kuntze | 1 | 1 | 1 | 1 | 1 | 0 | B | – | – | – | i | – | – | – | – | – |
| <i>Planothidium delicatulum</i> (Kützing) Round & Bukhtiyarova | 1 | 0 | 0 | 1 | 0 | 0 | B-P | – | – | alb | hl | es | b | 2.0 | o-m | – |
| <i>Planothidium haukianum</i> (Grunow) Round & Bukhtiyarova | 1 | 0 | 0 | 0 | 0 | 1 | B-P | – | – | alf | hl | – | o | 1.0 | o-m | – |
| <i>Pleurosigma angulatum</i> (J.T. Queckett) W.Smith | 1 | 0 | 1 | 1 | 1 | 1 | B | – | – | alf | hl | – | b | 2.0 | – | – |
| <i>Pleurosigma elongatum</i> W.Smith | 1 | 1 | 1 | 1 | 1 | 1 | B | – | – | alf | mh | – | b | 2.0 | – | – |
| <i>Pseudostaurosira brevistriata</i> (Grunow) D.M.Williams & Round | 1 | 1 | 0 | 0 | 0 | 1 | B-P | – | st-str | alf | i | – | o | 1.2 | ot | – |
| <i>Rhoicosphenia abbreviata</i> (C.Agardh) Lange-Bertalot | 1 | 1 | 1 | 0 | 0 | 1 | B | – | st-str | alf | i | es | o-a | 1.9 | me | ate |
| <i>Rhoicosphenia marina</i> (Kützing) M.Schmidt | 1 | 1 | 1 | 1 | 1 | 1 | B | – | – | – | hl | – | – | – | – | – |
| <i>Rhopalodia gibberula</i> (Ehrenberg) Otto Müller | 0 | 0 | 1 | 1 | 0 | 0 | B | temp | str | alf | mh | es | b | 2.0 | me | – |
| <i>Rhopalodia musculus</i> (Kützing) Otto Müller | 0 | 0 | 1 | 1 | 0 | 0 | B-P, S | – | str | alb | mh | – | o | 1.0 | – | – |
| <i>Skeletonema subsalsum</i> (A.Cleve) Bethge | 1 | 0 | 0 | 0 | 0 | 1 | P | – | – | ind | i | – | o | 1.0 | me | – |
| <i>Surirella ovalis</i> Brébisson | 1 | 0 | 1 | 1 | 0 | 1 | B-P | – | st-str | alf | i | es | a | 3.0 | me | ate |
| <i>Tabularia fasciculata</i> (C.Agardh) D.M.Williams & Round | 1 | 0 | 0 | 0 | 0 | 1 | B-P | – | st | ind | mh | es | b-a | 2.5 | e | ate |
| <i>Tabularia parva</i> (Kützing) Williams et Round | 1 | 1 | 1 | 1 | 1 | 1 | – | – | – | alf | mh | – | a | 3.0 | m | – |
| <i>Thalassionema nitzschioides</i> (Grunow) Mereschkowsky | 1 | 1 | 1 | 0 | 1 | 1 | P | – | – | – | i | – | – | – | – | – |
| <i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve | 1 | 0 | 1 | 1 | 1 | 1 | P | – | – | ind | i | – | – | – | – | – |
| <i>Tryblionella acuminata</i> W.Smith | 1 | 1 | 0 | 1 | 1 | 0 | B | – | st | alf | hl | sx | a-o | 2.9 | me | – |

| Taxa | EP | EL | SE | Siv | Ker | Kaz | Hab | T | Oxy | pH | Hal | D | Sap | S | Tro | Aut-Het |
|---|----|----|----|-----|-----|-----|------------|-------|--------|-----|-----|----|-----|-----|-----|---------|
| <i>Tryblionella apiculata</i> Gregory | 0 | 0 | 1 | 1 | 0 | 0 | B | – | – | alf | hl | es | a-o | 2.7 | e | – |
| <i>Tryblionella granulata</i> (Grunow) D.G.Mann | 0 | 0 | 1 | 1 | 0 | 0 | B | – | – | alf | hl | – | – | – | – | – |
| <i>Tryblionella hungarica</i> (Grunow) Frenguelli | 1 | 1 | 1 | 1 | 1 | 1 | B-P | – | – | alf | mh | sp | a-o | 2.9 | e | ate |
| <i>Tryblionella levidensis</i> W.Smith | 0 | 1 | 1 | 0 | 1 | 1 | B-P | – | st-str | ind | mh | sp | a-o | 2.6 | e | ate |
| <i>Tryblionella punctata</i> W.Smith | 1 | 0 | 1 | 1 | 0 | 0 | B | eterm | – | – | mh | – | – | – | – | – |
| Chrysophyta | | | | | | | | | | | | | | | | |
| <i>Chrysococcus</i> sp. | 1 | 0 | 0 | 0 | 0 | 1 | P | – | – | – | – | – | o-b | 1.4 | – | – |
| Chlorophyta | | | | | | | | | | | | | | | | |
| <i>Chlorella vulgaris</i> Beyerinck | 1 | 0 | 0 | 0 | 0 | 1 | B-P, pb, S | – | – | – | hl | – | a | 3.1 | – | – |
| Total number of taxa | 84 | 48 | 65 | 58 | 50 | 70 | | | | | | | | | | |

Note: EP – epiphyton, EL – epilithon, SE – sediments, Siv – Sivash, Ker – Kerch, Kaz – Kazantip. Indicators of habitat preferences: P – planktic, B-P – benthic-planktic, B – benthic, wide range, need some substrate; S – soil; pb – phycobiont. Indicators of water temperature preferences: warm – warm-water inhabitants; cool – cool-water inhabitants; temp – temperate water temperature inhabitants or indifferent. Oxygenation indicators: st – standing water, str – streaming water, st-str – low streaming water; aer – aerophile. Salinity: i – oligohalobes-indifferents, mh – mesohalobes, hl – halophiles; ph – polyhalobes. Watanabe indicators of organic pollution: sx – saproxene; es – eury saprobe; sp – saprophile. Sap – self-purification zone, x-b – xeno-beta-mesosaprobies; o – oligosaprobies; o-a – oligo-alpha-mesosaprobies; x-o – xeno-oligosaprobies; o-x – oligo-xenosaprobies; o-b – oligo-beta-mesosaprobies; b-o – beta-oligosaprobies; b-a – beta-alpha-mesosaprobies; b – beta-mesosaprobies; a-o – alpha-oligosaprobies; a – alpha-mesosaprobies. Index S, S – species-specific index of saprobity according to Sládeček. Nitrogen uptake metabolism indicators: ats – nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate – nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne – facultatively nitrogen-heterotrophic taxa, periodically requiring elevated concentrations of organically bound nitrogen. Trophic state indicators: ot – oligotraphentic; o-m – oligo-mesotraphentic; m – mesotraphentic; me – meso-eutraphentic; e – eutraphentic; o-e – oligo- to eutraphentic (hypereutraphentic)

The distribution of species in the study areas was as follows: 43 species in Sivash Gulf, 37 species in the Kerch Strait and 53 species in bays of Cape Kazantip.

The distribution of 77 identified indicators of substrate preferences is presented in Figure 4.

Benthic-planktic and benthic species occur in all ecotopes, with the latter dominating. The occurrence

of planktic species in benthic communities in all the regions under study can be explained by the shallowness of the coastal waters as they precipitate from the water column onto substrates. Temperature indicators (12 taxa) show an increase in warm-water species from north to south as well as from epiphyton to sediments (Fig. 4).

The distribution of water flow and oxygen enrichment indicators (39 taxa) shows medium water enrichment with oxygen from north to south, with aerophilic indicators being present in the Kazantip waters (Fig. 5). This allows us to assess the increase in water oxygenation toward the protected area of Kazantip, where the environment of epiphyton is most oxygenated. Among the water pH indicators (66 taxa and 20 taxa with a known pH range; Table 1), we recognize the prevalence of alkaliphilic species (Fig. 5), which indicates a decrease in pH from north to south and its increase from epiphyton to sediments.

Classification of microalgae species composition in relation to water salinity (75 taxa) shows that marine species predominate in the areas under study (despite low water salinity); however, freshwater and freshwater-brackish species are also significantly represented (Fig. 6). Among the species found, we have identified 79 saprobionts known as indicators of organic pollution (Table 1). These are species belonging to Cyanobacteria (13), Bacillariophyta (65) and Chlorophyta (1). Bacillariophyta are represented by 39 taxa in the Watanabe system (Fig. 6) and they show an increase in organic pollution from Sivash Gulf to the Kerch Strait with a maximum on stony substrates and in sediments.

The analysis of bioindication properties of microalgae was carried out also in the Sládeček system (Sládeček 1973). We have identified four Water Quality

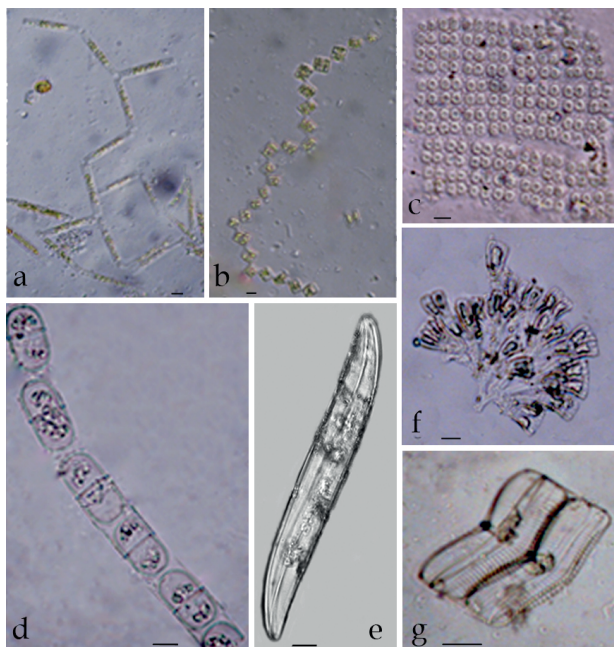


Figure 3

Some indicator species from the western coast of the Sea of Azov: *Diatoma tenuis* (a), *D. vulgaris* (b), *Merismopedia glauca* (c), *Melosira lineata* (d), *Gyrosigma wansbeckii* (e), *Rhoicosphenia abbreviata* (f), *Achnanthes brevipes* (g). Scale bar: 10 μ m

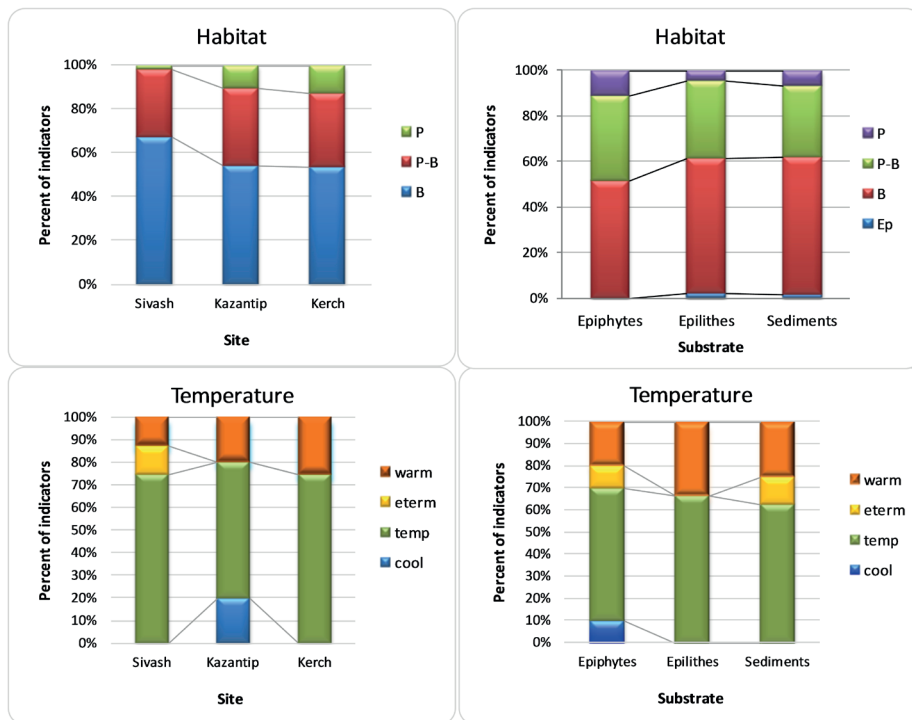


Figure 4

Percentage distribution of species indicators of natural habitat preferences and water temperature over different substrates and regions under study in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

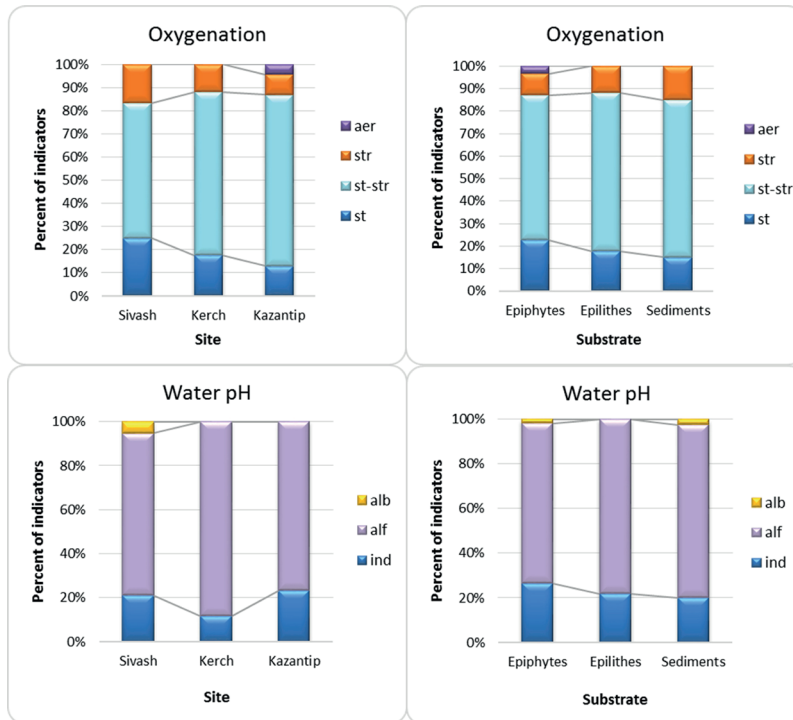
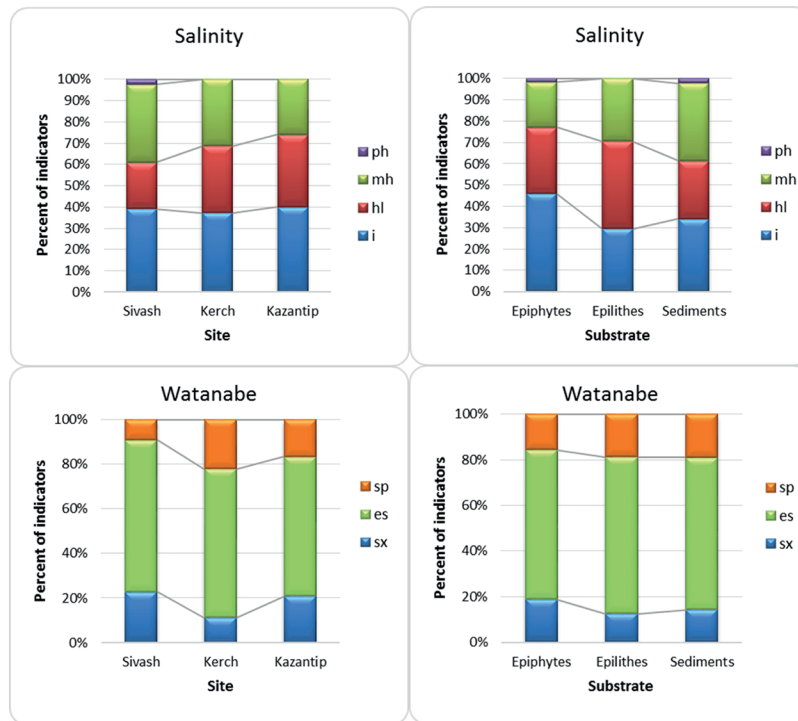


Figure 5

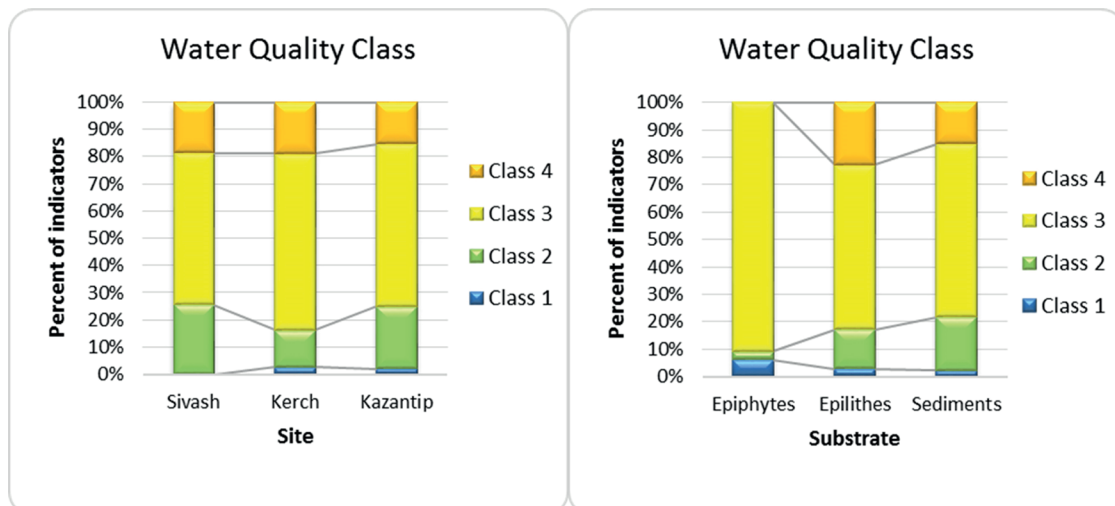
Percentage distribution of water oxygenation and pH indicators over different substrates and regions under study in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

**Figure 6**

Percentage distribution of salinity and Watanabe organic pollution indicators over different substrates and regions under study in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

Classes with a predominance of beta-mesosaprobionts (18 species) typical for slightly and moderately polluted waters (Class III; Table 1) and the maximum value of the water saprobity group index of $s = 2.0$. The second largest group of species (10) corresponds to the group

of oligosaprobionts with a minimum group index of 1.0–1.3. The minimum value of 0.4 was recorded for xeno-oligosaprobionts. Each group of saprobity indicators corresponds to a certain water quality class defined by the EU color code (Fig. 7).

**Figure 7**

Percentage distribution of saprobity indicators related to the water quality classes over different substrates and regions under study in the western coastal zone of the Sea of Azov with EU color codes

Using the ecological characteristics of these microalgae, the proportion (%) of saprobiontic species was studied separately for each ecotope and each surveyed area, and the water quality class was identified according to this proportion (Fig. 7).

The analysis of the distribution of organic pollution indicators over the ecotopes shows almost no difference between the substrates, since indicators of Water Quality Class III, characteristic of moderate pollution, dominate the communities (Fig. 7). However, a significant number of Class IV indicators (polluted water) were recorded in epilithon as compared to other substrates.

The comparison of the species composition of indicators for the studied areas shows that indicators of polluted waters (Classes III–IV) are more numerous in Sivash Gulf. Up to 50% of the species typical of Classes I–II (clean water) occur in the communities of microalgae of Kazantip bays. Consequently, the waters of Cape Kazantip can be assessed as cleaner than the rest of the studied areas.

Trophic state indicators (54 taxa of microalgae) show that water eutrophication increases from the Sivash to Kerch areas. While epiphyton species are mainly typical for mesotrophic waters, microalgae of epilithon and sediments are common in eutrophic

waters (Fig. 8). Nutrition type indicators (27 taxa) are mainly represented by autotrophic species. Facultative heterotrophs are found in the Kerch Strait, where they reside mainly on stony substrates (Fig. 8).

Ecological mapping

A database was compiled for statistical surface mapping of the western coastal waters of the Sea of Azov (Figs 9–12). Ecological and taxonomic data on microalgae in this region were generalized for the three studied areas, which are represented by red dots (Fig. 1). The results demonstrate major trends in different ecological properties of the obtained taxa distribution in this sea. The distribution of water flow and oxygen saturation indicators is presented in Figures 9a, b. It appears that standing waters with low oxygen concentration are found near Sivash Gulf, whereas well-oxygenated waters are near the Kazantip Nature Reserve. Benthic communities occur in all the study areas and planktic species are found in Sivash and Kazantip waters (Figs 9c, d).

Temperature indicator species show warmer waters in the Sivash area, whereas the Kazantip waters are favorable for cool-water indicators (Figs 10a, b). Among the 108 indicator species, truly marine

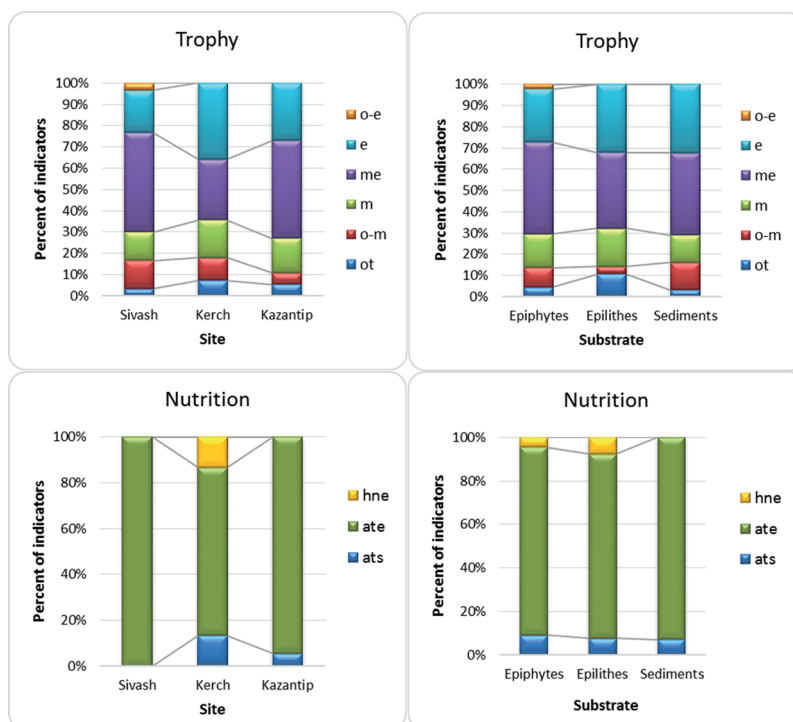


Figure 8

Percentage distribution of species indicators of the trophic state and the type of algae nutrition over different substrates and regions under study in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

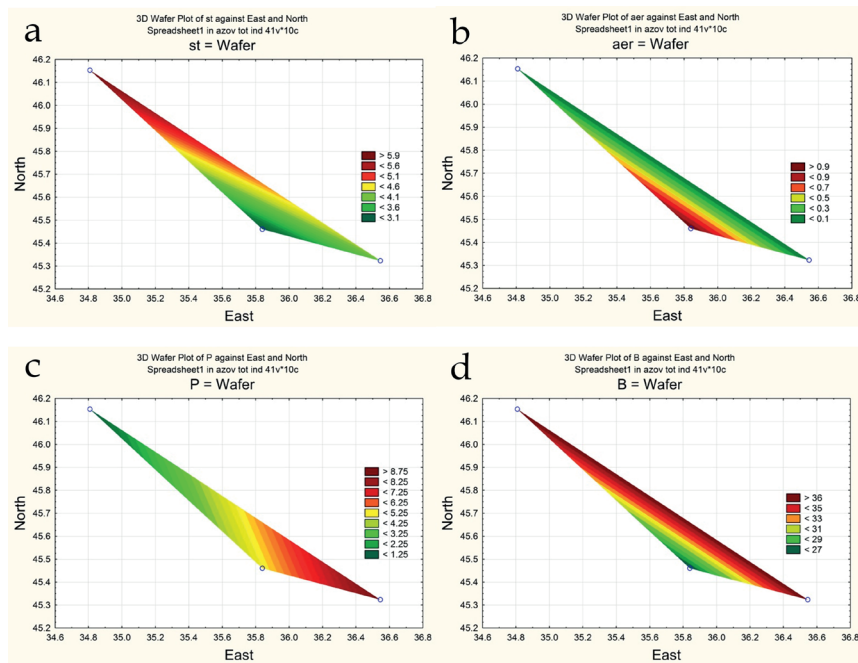


Figure 9

Statistically generated maps of water flow and oxygen saturation indicators (a – standing water inhabitants, b – aerophilic species) and substrate preferences (c – planktic, d – benthic species) in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

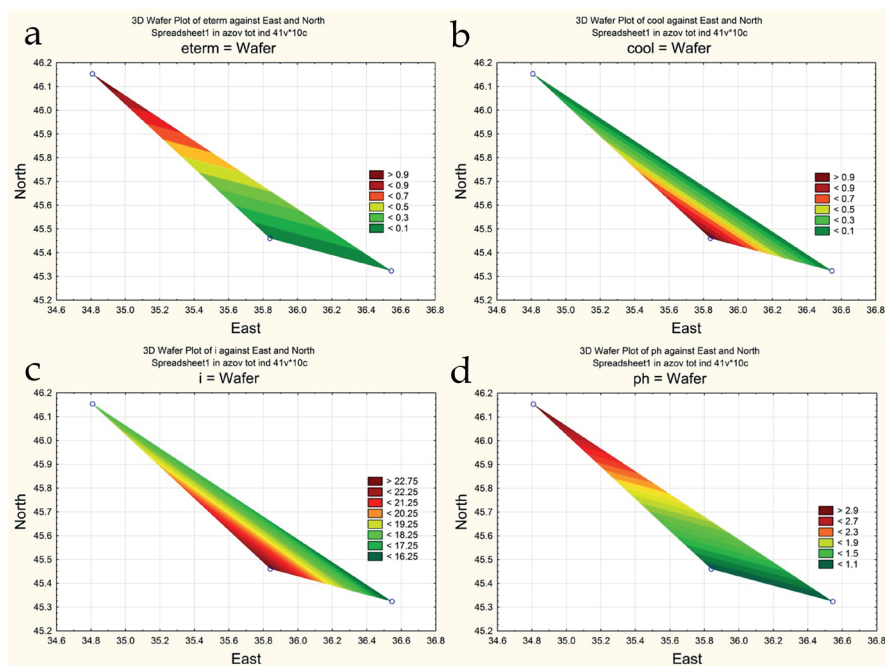


Figure 10

Statistically generated maps of water temperature indicators (a – eurythermic, b – cold water) and water pH indicators (c – indifferents, d – polyhalobes) in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

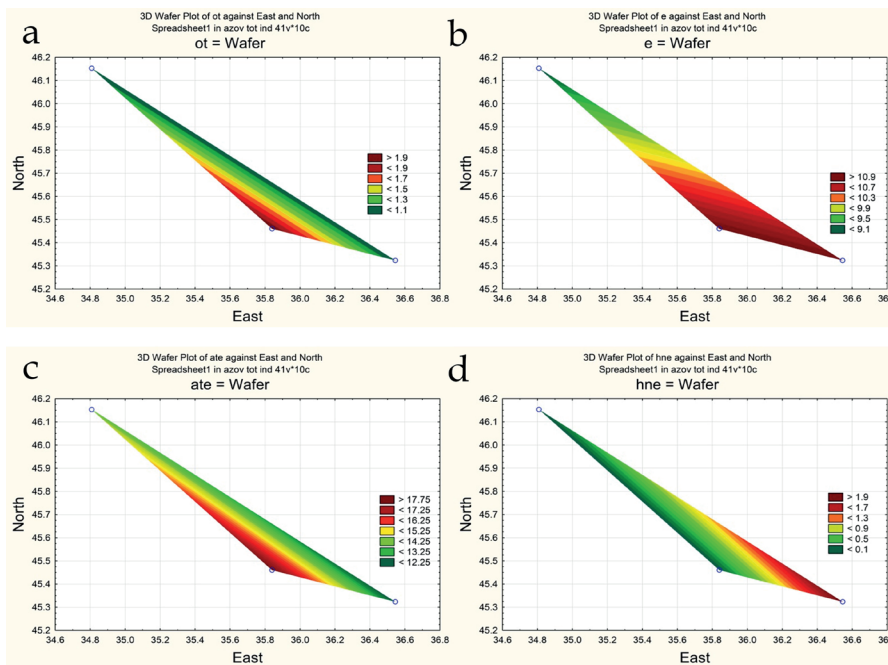


Figure 11

Statistically generated maps of organic trophic state indicators (a – oligotraphents, b – eutrathents) and indicators of algae nutrition types (c – autotrophs, d – heterotrophs) in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

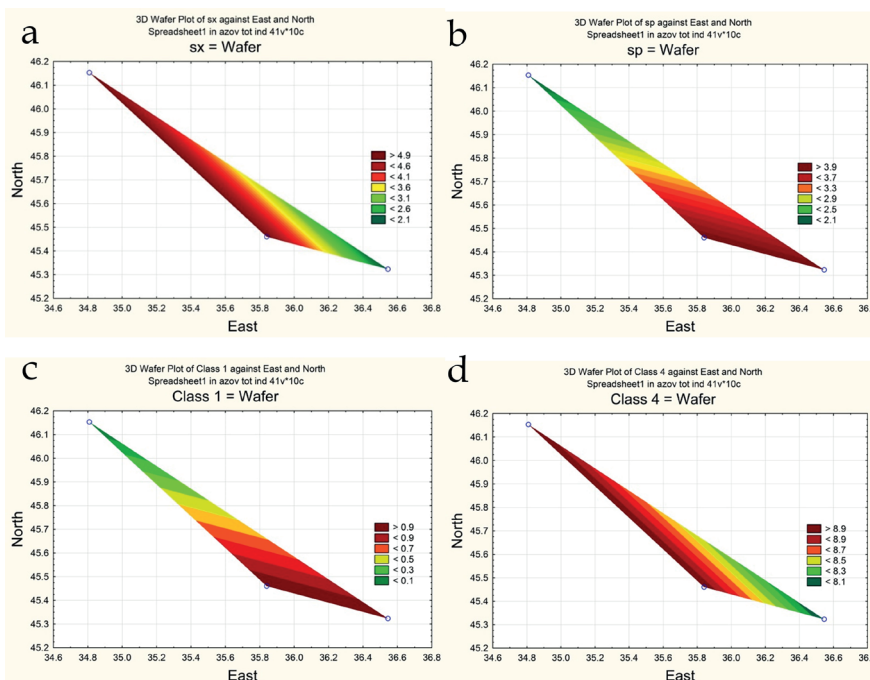


Figure 12

Statistically generated maps of organic pollution indicators: according to Watanabe (a – saproxenes, b – saprophiles), and according to Sládeček (c – Class 1, d – Class 4) in the western coastal zone of the Sea of Azov. Abbreviations of the ecological groups are as in Table 1.

species dominate the three areas. Among the halobity indicators, algal species (indifferents) are concentrated near the Kazantip Nature Reserve, while true polyhalobes occur in Sivash Gulf (Figs 10c, d).

The presented distribution of trophic indicators shows that oligotrophic species are dominant in the Kazantip Nature Reserve and the abundance of eutrophic species increases starting from Cape Kazantip, with the maximum number in the Kerch Strait (Figs 11a, b).

The distribution of nutrition type indicators related to eutrophication (Figs 11c, d) demonstrates the dominance of true autotrophic species in Kazantip bays, whereas facultative heterotrophic algae occur in the Kerch Strait.

The organic pollution indicators are mapped using two approaches: with diatom species only (Figs 12a, b) and with all identified indicator species (Figs 12c, d). The occurrence of diatoms according to the first approach indicates that waters of Sivash Gulf are clean, whereas waters dominated by saprophytic species in the two other areas are considered polluted.

Indication on the basis of species that represent not only diatoms, but also other groups of algae is at variance with the results based on diatoms only. Thus, indicators of Water Quality Class I (clean waters) indicate Cape Kazantip and Kerch areas as low-polluted areas, whereas indicators of Class IV (polluted waters) are mostly found in Cape Kazantip and Sivash Gulf.

Discussion

Both our research and other authors' reports have demonstrated that the β -mesosaprobic group (about 50% of all species), in terms of the scale of the Pantle-Buck saprobic system modified by Sládeček (Sládeček 1973; 1986), is dominant among the species indicators of organic water pollution in the coastal zone of the Black Sea, the Sea of Azov and the Sea of Japan (Guslyakov et al. 1992; Ryabushko 2013; Ryabushko & Begun 2015; Bondarenko 2017).

Bioindication of organic pollution in the coastal waters of the Sea of Azov based on microphyto-benthos communities has been carried out for the first time, and hence reference data are not available. However, the algal bioindication has been implemented in recent years in neighboring areas such as the Black Sea (Barinova et al. 2016) and the Dnieper River (Klochenko et al. 2014). In the Eastern Mediterranean, in the area polluted by the Qishon River and one of the largest ports, Haifa (Barinova et al. 2004), the microalgae community is mainly

represented by alpha-mesosaprobionts and the saprobity index ranges from 2.31 to 2.65 (Barinova et al. 2008). It should be noted that the microphyto-benthos of the Black Sea and the Sea of Japan was also dominated by the beta-mesosaprobiont group (Ryabushko 2013; Ryabushko & Begun 2015).

An example of organic pollution bioindication in coastal waters is also known from the opposite side of Eurasia, i.e. the Sea of Japan, polluted by the Rudnaya River, along which several plants of heavy metal and borate industry are located (Barinova et al. 2004). The species composition here is an indicator with a wide amplitude of saprobity group values and dominance of beta- and beta-alpha-mesosaprobiont communities, whose indices are around 2.3–2.5 (Barinova et al. 2008). Therefore, organic pollution of the coastal waters of the Sea of Azov can be assessed as significantly lower than in some areas of the Mediterranean Sea and the Sea of Japan, as indicators of Water Quality Classes II and III (oligo- and beta-mesosaprobionts) are predominant in the Sea of Azov. Ecological mapping in pollution analysis helps us to identify coastal areas as a zone where organic pollution indicators show the value of dissolved organic matter as well as demonstrate high self-purification capacity of coastal ecosystem (Sládeček 1973). In this case, the Kerch Strait is defined as an area with smaller organic pollution, where indicators of Class I occur, and the Kazantip site appears to be an area with a high activity of using organic matter by algae.

Most of the identified species of benthic diatoms are alkaliphilic and, to a lesser degree, pH-indifferent forms occurring locally in these seas. It should be taken into account that pH in seawater varies within a narrow range as compared to freshwater bodies; thus, pH characteristics of seawater may not be so important. The technique has recently undergone a number of modifications (Barinova et al. 2006; Barinova 2011; Barinova & Krassilov 2012) and is now widely used in the interpretation of a variety of data.

In addition to bioindication and pH analysis, we have implemented a statistical map construction method in Statistica 12.0 based on the environmental data presented in Table 1. Statistical maps visualize the distribution of taxonomic and ecological data over the waterbody surface (Barinova 2017). Previously, we conducted a statistical analysis of algal bioindication data for rivers of Pakistan (Khuram et al. 2017), Kazakhstan lakes (Krupa et al. 2018) and the Black Sea (Barinova et al. 2016) and concluded that these methods can provide important ecological data for different water bodies. We have combined the ecological assessment methods for ecosystems of

large continental water bodies such as Lake Balkhash (Krupa al. 2018), Shardara reservoir (Barinova & Krupa 2017; Krupa et al. 2017) and estuarine waters of Sasyk reservoir (Barinova et al. 2016). However, this analysis has never been applied to coastal seawaters.

Conclusions

Ecological analysis of indication properties of the identified species for ten environmental factors in the three areas of the western coastal waters of the Sea of Azov has been carried out for different substrates using two methods, bioindication and statistically generated mapping of the distribution of indicator groups. The analysis of the marine environment using these two methods has been performed for the first time. Considering 108 indicator species from four taxonomic divisions with the dominance of diatoms, the richest algal species composition was determined in the Kazantip Nature Reserve. Benthic organisms are dominant in Sivash Gulf. Benthic communities are most developed in the Sivash and Kerch areas and decline southward. It has been found that planktic species prefer the Kazantip waters. Temperature indicators have demonstrated an increase in the number of warm-water species from north to south, as well as from epiphyton to sediments. Indicators of good oxygenation are more numerous in the water of the Kazantip protected area, with epiphyton living in the most oxygenated environment. Water salinity indicator species, previously found in continental waters with a wide salinity gradient, have been observed mostly near the Kazantip Nature Reserve, while true polyhalobes have been found in Sivash Gulf. Alkaliphilic species are predominant among water pH indicators, and their number is consistent with the pH increase from south to north and from epiphyton to sediments.

The water trophic state index increases from Sivash Gulf to the Kerch Strait, which are mainly mesotrophic. True autotrophic species occur in the Kazantip area, whereas facultative heterotrophic algal species are found in the Kerch Strait, mostly on stony substrates. Bioindication showed an increase in the number of beta-mesosaprobionts with increasing organic pollution from Sivash Gulf to the maximum in the Kerch area (Water Quality Classes III–IV), as well as in epilithon and sediments.

The greatest diversity of algae (about 50% of the identified species) has been found in the clean waters of the Kazantip Nature Reserve (Water Quality Class I–II). The most active self-cleaning occurs in epilithon communities, and the cleanest water occurs around

Cape Kazantip. Thus, Cape Kazantip is an area with a high rate of organic matter use by algae. All three surveyed areas of the Sea of Azov have been found cleaner than some areas of the Eastern Mediterranean and the Sea of Japan, where the distribution of saprobity indicators was previously analyzed.

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