

<p align="center"><b>Bioindication of the Black Sea Environment Quality by Microphytobenthos</b> <i>(Oleksandra Rachynska, Liudmyla Krasota)</i></p>	<p align="center">“Cercetări Marine“  Issue no. 50  Pages 84 - 94</p>	<p align="center">2020</p>
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## BIOINDICATION OF THE BLACK SEA ENVIRONMENT QUALITY BY MICROPHYTOBENTHOS

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### ABSTRACT

In summer and autumn of 2019, the quality assessment of the Black Sea environment was carried out by the method of bioindication by indicators of microphytobenthos development. It was found that on the ecological status of marine environment of researched water areas was influenced by anthropogenic and natural factors, which reflected on indexes of development of indicator organisms. It was established that the state of environment of the sea water areas which are under the influence of river and waste waters inflows was significantly worse compared to the open waters of the shelf of Ukraine and other Black Sea countries.

**Key-words:** Black Sea, environment, quality, bioindication, microphytobenthos.

### AIMS AND BACKGROUND

The aim of the work was to evaluate the quality of the Black Sea environment according to the bioindication method with the use of systematic, quantitative, halobiont and saprobiont indicators of microphytobenthos development.

The bioindication is one of the important methods for assessing the quality of marine environment. Benthic microalgae, which are intensively developing on different substrates and cannot leave its inhabited place, take the important place among the communities of monitoring organisms. The microphytobenthos reacts sensitively to changes in environmental factors: therefore, its development indices (species composition, abundance, biomass, halobiont and saprobiont compositions) clearly reflect the ecological status of the marine

environment and are used in bioindication (Guslyakov et al., 1992; Oksiyuk et al, 1994; Oksiyuk et al, 2006; Barinova et al., 2006).

## **EXPERIMENTAL**

In the summer and autumn of 2019, the assessment of the environmental status of the Black Sea was performed by the bioindicator method according to the microphytobenthos development. Samples of microalgae from soft substrates (silt, sand) were taken in the areas of influence of the Danube, Dniester, Dnieper and Southern Bug rivers, in places of domestic wastewater discharge of the port cities of Odessa and Chornomorsk (in Fig. 1 soil sampling stations are marked in yellow and red), as well as in the shelf waters of Ukraine, Romania, Bulgaria and Turkey (blue and purple markings).

Sampling of microphytobenthos was performed by immersing in 1 cm of cylindrical plastic containers with a plane of 7 cm<sup>2</sup>, in the surface layer of bottom sediments raised by a grab sample and then fixed with 4% formalin. The microscopic and statistical processing of the samples was carried out mainly in accordance with the generally accepted methods (Wasser *et al.*, 1989; Mordukhai-Boltovskoi, 1975).

The trophic characteristics of the studied water areas were determined by the parameters of microphytobenthos biomass (Oksiyuk *et al.*, 1994), which were calculated by "volume" method, measuring the linear dimensions of the microalgae cells and equating their shapes with certain geometric shapes (Bryantseva *et al.*, 2005).

## **RESULTS AND DISCUSSION**

During 2019, the impact of anthropogenic factors (municipal sewage discharges, port operations, etc.) and natural factors (especially low salinity of water bodies in places of river runoff) on the quality of the Black Sea environment was investigated by the parameters of the indicator organisms (of benthic microalgae).

### **Bioindication of the Black Sea environment in the zones influenced by river and wastewaters**

In summer and autumn, samples of microphytobenthos were taken in regions expose to constant influence of river waters: of Danube (opposite the exits from the mouth of the Bystryi and Kiliya arm), Dniester (at the outlet of the Dniester estuary near Zatoka), of Dnipro (in the Dnipro River, in estuary of the Southern Bug River, at the exit of the Dnipro-Bug estuary near Ochakiv).



In autumn, samples were also taken in the anthropogenized waters of the Dniester region of northwestern part of the Black Sea (NWBS) (near the place of discharge from Chornomorsk waste waters treatment plant (WWTP) and near the place of discharge from WWTP "South" of Odessa).

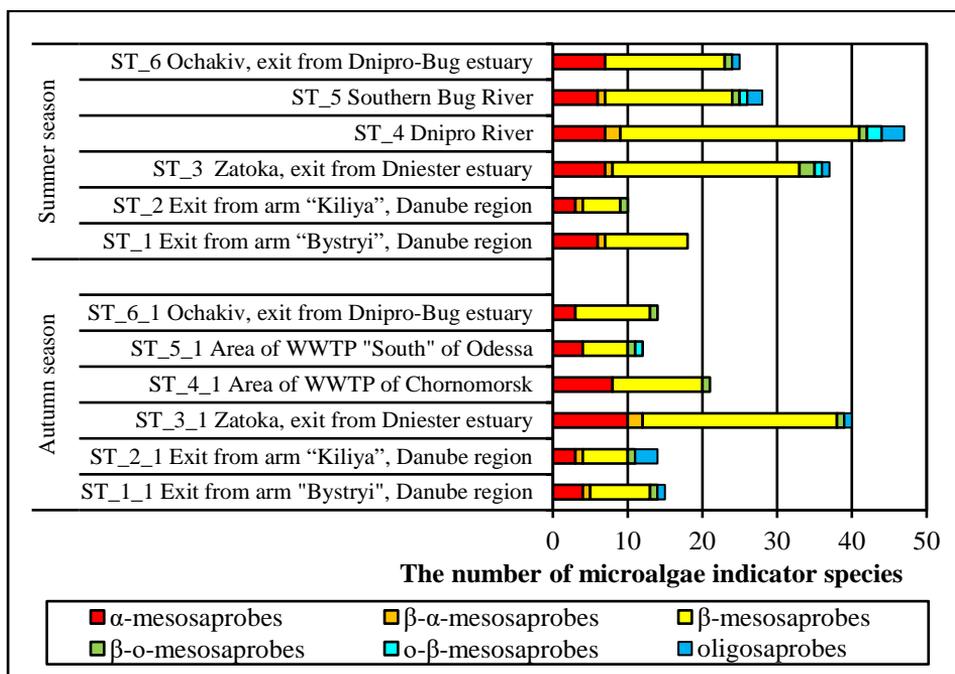
In the zones of influence of river waters, diatoms predominated everywhere (11-48 species in summer and 16-57 in autumn). The genera *Nitzschia* and *Navicula* were most widely represented. Species of the genera *Cymbella* and *Cyclotella* were common in summer, and *Gyrosigma* and *Cocconeis* – in autumn. The total abundance of microalgae was  $527\text{-}20\,274 \times 10^6$  cells/m<sup>2</sup> in summer and  $2\,852\text{-}10\,723 \times 10^6$  cells/m<sup>2</sup> in autumn. Their biomass was  $77.31\text{-}17\,025.90$  mg/m<sup>2</sup> and  $241.82\text{-}22\,694.25$  mg/m<sup>2</sup>, respectively. The basis of the abundance of microphytes everywhere was cyanoprokaryotes, and of the biomass – diatoms. The brackish and freshwater algae species dominated: *Detonula confervacea*, *Aulacoseira granulata*, *Melosira varians*, *Fragilaria construens*, and species of the genus *Cyclotella*. Microalgae developed most intensively in the estuarine waters of the Dnipro River and in the area of Zatoka.

According to biomass indicators (by the trophic characteristics of water bodies of Ukraine (Oksiyuk et al, 1994)), the environment of the sea near Zatoka, the Dnipro River and of the Southern Bug estuary belonged to the class "eutrophic", and of the Dnipro-Bug estuary near Ochakiv to the class "mesotrophic". The environment of the Danube region at the exit from the arm "Bystryi" changed during the year from "mesotrophic" to "eutrophic", and opposite the exit from the arm "Kiliya" – from "oligotrophic" to "mesotrophic".

Regarding the connection with salinity of water, the species of benthic microphytes were represented, first of all, by meso- and oligohalobes (halophiles and indifferentes). They were most widely represented in the Dnipro River and opposite Zatoka. Polyhalobes predominated only in autumn near Ochakiv, and in the Dnipro they were absent.

During the year, the total number of saprobiont species of microalgae near the arm "Bystryi" and Zatoka decreased by 1.2, and near Ochakiv – in 1.8 times (Fig. 2). Species  $\beta$ -mesosaprobies (indicators of moderate organic pollution (Barinova et al., 2006)) predominated everywhere. Among them the most abundant and most mass were diatoms *A. granulata*, *M. varians* and *F. construens*. The  $\beta$ - $\alpha$ -mesosaprobic diatom *Cyclotella meneghiniana* was also numerous.

Among  $\alpha$ -mesosaprobites (indicators of significant organic pollution) *Cyclotella choctawhatcheeana*, *Stephanodiscus hantzschii*, *Paralia sulcata*, *Navicula salinarum* were most common. The highest quantity of saprobionts species was observed in the area of Zatoka. During the year, the content of  $\alpha$ -mesosaprobites here increased 1.5 times.



**Fig. 2.** The saprobiont composition of microphytobenthos of the Black Sea's Ukrainian part in zones of influence by river and wastewaters in 2019

A significant contribution to the formation of systematic and quantitative indicators of microphytobenthos development in the zones of influence of rivers also belongs to the indicators of low organic pollution – to the o- $\beta$ -mesosaprobites and pure water indicators – oligosaprobic microalgae.

In the NWBS anthropogenically loaded areas microphytobenthos was represented mainly by diatoms: 18 species were found near WWTP "South" (the most widely were genera *Diploneis* and *Amphora*) and 37 – near the WWTP of Chornomorsk (due to species of the genera *Navicula* and *Cocconeis*). In the area of the sea adjacent to Chornomorsk, abnormal cells of the diatom *Synedra crystallina* were observed.

The total abundance of microalgae in these waters was 3 140-5 641 x 10<sup>6</sup> cells/m<sup>2</sup>, and biomass was 587.51-1 456.62 mg/m<sup>2</sup>,

respectively. Therefore, the number of microphytobenthos in NWBS near Chornomorsk was by 1.8, and biomass – by 2.5 times higher than near the WWTP "South". Diatoms *Halamphora coffeiformis*, *P. sulcata*, *Tabularia fasciculata* and species of the genus *Pleurosigma* dominated. By biomass indicators (according to the trophic classes of water bodies of Ukraine (Oksiyuk et al, 1994), the aquatic environment of NWBS near treatment plant of Chornomorsk belonged to the class "eutrophic", and near the WWTP "South" – to the class "mesotrophic".

In contrast to the waters which are under the influence of rivers, in anthropogenized marine areas polyhalobes dominated. There were almost twice as many of them near the Chornomorsk treatment plant (compared to the water area near the WWTP "South").

In relation to organic contamination, most of the species found were  $\beta$ -mesosaprobies (Fig. 2). These are mainly diatoms of *Achnanthes longipes*, *Cocconeis scutellum*, species of the genera *Navicula* and *Nitzschia*. The  $\alpha$ -mesosaprobies of *P. sulcata* and *T. fasciculata*, *Melosira moniliformis* were also abundant. The content of saprobionts in the water area, located near the treatment plant of Chornomorsk, was by 1.8 times, and of  $\alpha$ -mesosaprobies – twice as more than near the WWTP "South" of Odessa city. However, in contrast to the areas of the sea under the influence of rivers, in the waters located opposite the treatment plant of Chornomorsk and Odessa, no species-indicators of clean water (oligosaprobies) were found. Only 1 species-indicator of low organic pollution ( $\alpha$ - $\beta$ -mesosaprobic diatom *Synedra pulchella*) was found near WWTP "South".

Thus, the bioindication the quality of the NWBS marine environment showed that the highest systematic, quantitative, halobiont and saprobiont indicators of microphytes development in river impacted zones were in the marine waters of regions Dniester (opposite Zatoka) and Dnipro (near Ochakiv). In the anthropogenized areas of NWBS, the most organic polluted (eutrophicated), was the water area located in the zone of influence of waste waters from the treatment plant of Chornomorsk.

### **Bioindication of environmental quality of the Black Sea shelf's open zone**

In autumn, benthic microalgae samples were taken on the shelves of Ukraine (in the Danube region ShW\_UA\_1 (ST NMS UA-15, depth 25.0 m), in the Mixing zone 2 ShW\_UA\_5 (ST NMS UA-16, 24.8 m), in the Central region ShW\_UA\_7 (ST NMS UA-17, 40.4 m), and in the

regions of Romania ShW\_RO\_1 (ST ANE-RO-5, 65.0 m), Bulgaria ShW\_BG\_2 (ST ANE-BG-1, 41.0 m) and Turkey ShW\_TR\_1 ANE-TR-1, 69.0 m) (Fig. 1).

On the Ukrainian shelf in the microphytobenthos composition the following numbers of species were found: 31 – in the Central region, 54 – in the Danube region, and 77 – in the Mixing zone 2. Diatoms (22-70 species) predominated everywhere. The total abundance of microphytes in these water areas was  $4336-12\,276 \times 10^6$  cells/m<sup>2</sup>, and the biomass 606.27-51 991.67 mg/m<sup>2</sup>. The highest quantitative indicators of microphytes development were in the Mixing zone 2, the lowest biomass – in the Central, and the lowest number – in the Danube region. The basis of the abundance was formed everywhere by cyanoprokaryotes, of biomass – by diatoms. In the Mixing zone 2, the total abundance of microphytes was by 2.5 times and the biomass was almost by 86 times higher than in the Central region, due to the development of diatoms *Toxarium undulatum*, *Thalassionema nitzschioides*, species of the genus *Pleurosigma*. In the Danube region predominated *T.fasciculata* and *Amphora proschkiniana*. Solitary abnormal cells of *Diatoma tenue* were observed in this area. In the Central region the most abundant was *Navicula ramosissima*.

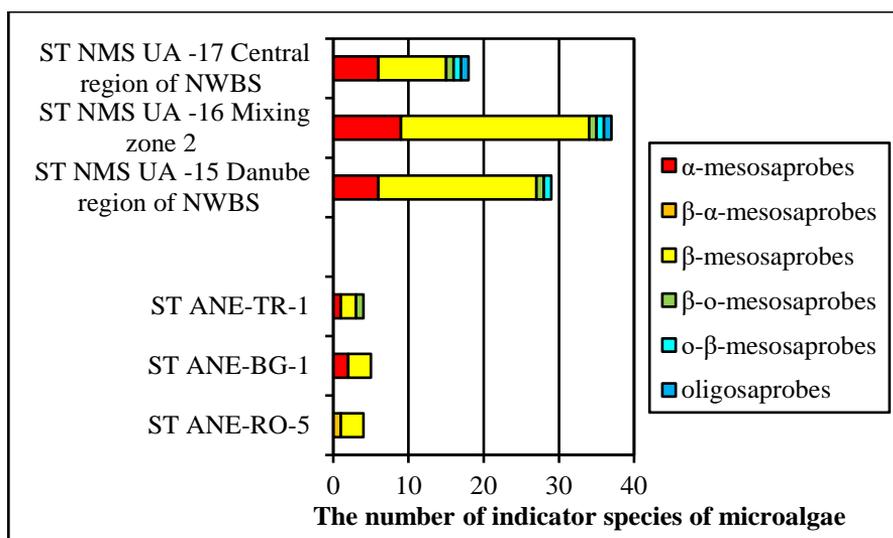
According to the microphytobenthos biomass indexes (by the trophic characteristics of Ukrainian water bodies (Oksiyuk et al, 1994)), the environment of the Mixing zone 2 belonged to the class "hypertrophic", Danube region – to the "eutrophic", and Central – to the "mesotrophic".

Polyhalobes predominated in all water areas (in relation to the water salinity), their highest number was in the Mixing zone 2, where mesohalobes, halophiles and indifferentes are also widely represented. In the Central region there were the least polyhalobes.

Everywhere most of the algae found were  $\beta$ -mesosaprobies – indicators of moderate organic pollution (Barinova et al., 2006). There were 4.0-7.4 times more saprobionts on the Ukrainian shelf compared to Romania, Bulgaria and Turkey shelves (Fig.3). Most saprobionts, in particular  $\alpha$ -mesosaprobies, were observed in the Mixing zone 2. But here and in the Danube region microphytes were found, which is indicators of low organic pollution ( $\alpha$ - $\beta$ -mesosaprobies) and clean water (oligosaprobies), indicating a good ecological status.

In the Romanian shelf waters 12 species of microphytobenthos algae were found. Diatoms predominated – 6 species (mostly of the genera *Diatoma* and *Cyclotella*). Golden algae, inherent in pure waters,

were represented by 3 species. The number of microphytes here was  $997 \times 10^6$  cells/m<sup>2</sup> (due to blue-green algae of the genus *Microcystis*), and biomass – 110.27 mg/m<sup>2</sup> (inresult of the development of diatoms *Grammatophora serpentina*, *C. meneghiniana* and species of the genus *Diatoma*). In the halobiont composition of microphytobenthos, polyhalobes predominated. Halophiles were common, which clearly reflects the influence of freshwaters input. The found algae were mostly  $\beta$ -mesosaprobies, which indicates moderate organic pollution of this area.



**Fig. 3.** Saprobiont composition of microphytobenthos of the Black Sea shelf waters in autumn 2019.

These are mainly diatoms *Ceratoneis closterium*, *D. tenue*, *D. vulgaris* var. *breve*. The  $\beta$ - $\alpha$ -mesosaprobic *C. meneghiniana* was also numerous. Importantly, there were no  $\alpha$ -mesosaprobies.

Near the coast of Bulgaria benthic microphytes were represented by 16 species. Most were diatoms – 11 species. The total number of microphytobenthos was  $481 \times 10^6$  cells/m<sup>2</sup>, and biomass was 172.06 mg/m<sup>2</sup>. Cyanoprokaryotes predominated by abundance and diatoms – by biomass. Large-celled *Coscinodiscopsis jonesiana*, *S. crystallina* dominated. Regarding the salinity of water, almost half of the found species of microalgae were polyhalobes. The  $\beta$ -mesosaprobies dominated in the saprobiont composition of benthic microphytes such as: *C. closterium*, *D. tenue*, etc. The  $\alpha$ -mesosaprobies were also numerous, among these *P. sulcata* predominated. A slightly higher number of  $\alpha$ -

mesosaprobies was found off the Bulgarian coast compared to the shelf water areas of Romania and Turkey.

In the species composition of microphytobenthos on the Turkish shelf, 12 species of algae were found, among which there were only 5 diatoms. The microphytobenthos abundance here was  $856 \times 10^6$  cells/m<sup>2</sup>, and biomass – 126.45 mg/m<sup>2</sup>. *P. sulcata* dominated. Diatoms *N. ramosissima* and *Thalassiosira baltica* were also numerous. According to the halobiont composition, the algae species found were mostly poly- and mesohalobes. Among saprobionts, the majority were  $\beta$ -mesosaprobies. These are diatom *C. closterium* and green algae *Monoraphidium arcuatum*.

By the biomass of microphytobenthos, the environment of the shelf waters of Romania, Bulgaria and Turkey belonged to the class "mesotrophic" (Oksiyuk et al, 1994).

In contrast to the Ukrainian shelf, neither  $\alpha$ - $\beta$ -mesosaprobies nor oligosaprobies were found in the studied waters of the other Black Sea countries.

It should be emphasized that the quality of the marine environment of the Romanian shelf had positive saprobiological characteristics – no  $\alpha$ -mesosaprobies (indicators of significant organic pollution) were registered, but several species of golden algae, inherent to pure waters, were found.

Thus, the indicators of the highest systematic, quantitative, halobiont and saprobiont of microphytes development in the places of river influence were characteristic of the marine waters of Dniester regions (opposite Zatoka) and Dnipro (near Ochakiv). Among the areas studied anthropogenized by NWBS, the most polluted organically was the water space in the water area near the discharges of wastewater from Chornomorsk. The environment state of the sea water areas affected by the river and wastewater inflow was significantly worse compared to the environment quality of open shelf waters of Ukraine and other Black Sea coast countries.

## CONCLUSIONS

In the summer and autumn seasons 2019, the influence of environment on marine areas indicators of organisms-monitors (algae of microphytobenthos) development was studied during the bioindication of the Black Sea environment quality.

It was found that the ecological status of the researched marine water areas was influenced by both anthropogenic factors (urban

wastewater discharges, port operations, etc.) and natural ones (in particular, low salinity water masses with at river runoff sites), which affected on indexes of the state algae-indicators.

The bioindication of the quality of marine environment showed that the highest systematic, quantitative, halobiont and saprobiont indexes of benthic microphytes status in places influenced by river runoff were recorded in water areas Dniester region (opposite Zatoka) and Dnipro region (near Ochakiv). In the NWBS anthropogenic areas, the most eutrophicated was the water affected by effluents from the Chornomorsk treatment plants. The state of the environment of the marine areas that are located under the influence of river and waste waters was much worse than the open waters of the shelf of the countries on the Black Sea coast. The Romanian shelf bottom environment was of better quality than in Turkey (at depths of 65.0 m and 69.0 m, respectively), as evidenced by the absence of  $\alpha$ -mesosaprobies in the Romanian microphytobenthos community. The Bulgarian marine environment (at a depth of 41.0 m) was less eutrophicated than the Ukrainian at a depth of 40.4 m, where the  $\alpha$ -mesosaprobic bottom microphytes were 3 times more.

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