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ANALYSIS OF THE ECOLOGICAL CONDITION OF WATER AND BOTTOM SUBSTRATES BIOTESTING METHOD ON MICROALGAE

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ABSTRACT

In the summer and autumn of 2019, biotesting of water and bottom sediments of Ukrainian, Romanian and Bulgarian reservoirs with different anthropogenic load and the influence of various anthropogenic factors was carried out. Our studies show that the presence of toxic substances for the reproduction of the laboratory culture of the planktonic alga *Desmodesmus communis* in the vast majority of the studied territories has not been established. The results of biotesting showed that the state of the aquatic environment in shelf waters is better than in coastal water bodies of Northwestern Black Sea (NWBS). Installed that the water areas of the NWBS, which include: coastal reservoirs of Ukraine, areas affected by river runoff (Danube, Dniester, Dnieper-Bug); areas of NWBS ports; urban waste water and their influence areas; the zone of mixing of river and sea waters; the central part of the Ukrainian; part of the shelf zone of the Black Sea; as well as water bodies in Romania and Bulgaria - constant environmental monitoring is required.

Key-Words: biotesting, reproductive, laboratory culture, planktonic algae

AIMS AND BACKGROUND

The aim of the study was to establish the quality of seawater and bottom sediments of Ukrainian, Romanian and Bulgarian with different nature of anthropogenic factors and anthropogenic load using the ecological method of biotesting, depending on the indicator reproductive changes in the laboratory culture of microalgae.

EXPERIMENTAL

A study of the effect of pollutants contained in seawater and extracted from bottom sediments on the marine environment was carried out on a population of microalgae in the laboratory. Biotesting was performed according to the generally accepted method of biotesting on laboratory cultures of unicellular algae at certain constant values of temperature (20 ± 2^0 C) and illumination (2200 lux). pH of the artificial

environment equal to 7.8 without changing solutions, under artificial illumination in a place protected from direct sunlight (luminostat). We used fluorescent lamps mounted on top at a distance of 60 cm from the flasks. The criterion of toxicity was the changes in algal cells affecting their reproductive ability and changes in the number of cells (Tsyban A., 1980; Gypsy A., 1988; Semenova O., Bazeljan V., 2006; Lanskaya L.,1971).

Research conducted with water samples, mainly from the surface layer, and bottom sediments taken in the summer and autumn of 2019 (Fig.1).

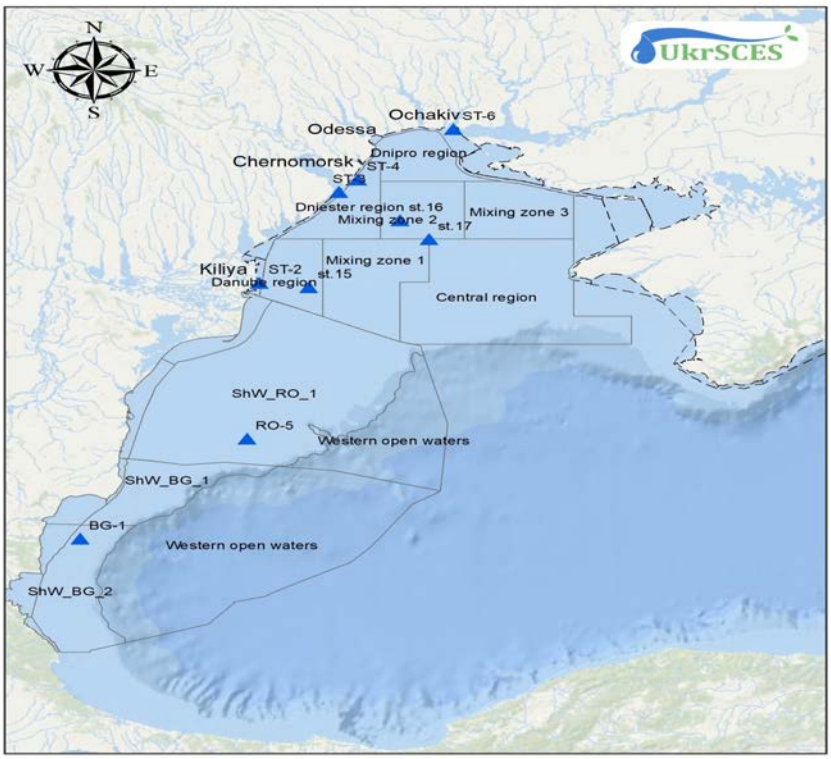


Fig. 1. Map-scheme of sampling UkrSCES by conducting environmental monitoring in 2019

The laboratory culture of planktonic algae *Desmodesmus communis* (E. Hegewald) E. Hegewald was used as a test object. The test function was the reproducibility of the test object. The research results are subjected to variational statistical processing (Semenova O., Bazeljan V., 2006; Plokhinsky N. 1970). The studied concentrations were 1.0 ml • l^{-1};10.0 ml • l^{-1} and 1.0 g • $l^{-1;}$ 10.0 g • l^{-1} (Lanskaya L., 1971, Semenova O., Bazeljan V., 2006). The sample water was filtered through a membrane filter No. 6, sterilized three times by heating in a water bath to 75° C and cooled to room temperature. The chronic experiment lasted 10 days. We have developed an express tool for assessing the quality of the

aquatic environment (water and sediments) (Semenova O., Bazeljan V., 2006; Plokhinsky N., 1970; Ukrainian V. *et al*., 2018). The result of the assessment of environmental quality of water and sediments is defined and included in accordance with its own weight (selected by the expert) in a qualitative response: stimulating -> 100%; invalid - 75% - 100%; toxic - 50% - 75%; acute-toxic - 25% -50%; lethal - <25%. The final assessment of the quality of the aquatic environment ranges from - <25% to >100% of the control values and corresponds to 5 classes (Table 1).

Table 1. Scale of environmental quality of water and bottom sediments by biotesting on microalgae

Survival of algae cells during a laboratory experiment, % of control	**Color code**	**Ecological status class**	**Characteristics of aquatic environment**
> 100 %	Blue	High	Stimulating
75 % - 100 %	Green	Good	Inactive
50 % - 75 %	Yellow	Moderate	Toxic
25 % - 50 %	Orange	Poor	Acutely toxic
- < 25 %	Red	Bad	Lethal

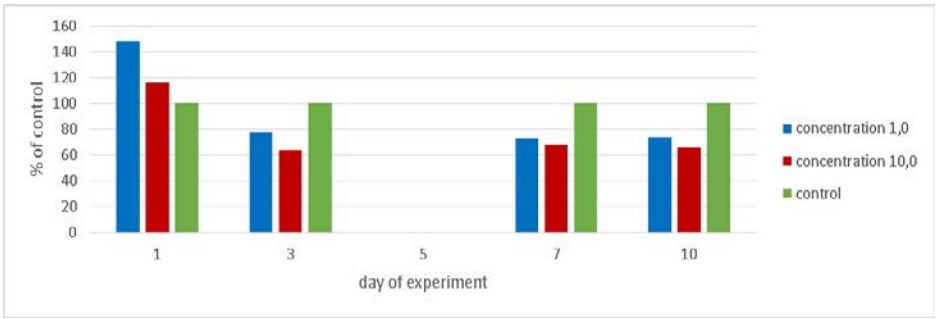
RESULTS AND DISCUSSION

Biotesting of water and bottom sediments of Ukrainian coastal water bodies

The ecological state of the aquatic environment in all the studied Ukrainian marine water bodies located near the mouths of the Dnieper, Southern Bug, Dniester and Danube rivers, is influenced by both natural abiotic and anthropogenic factors. The results of our research are presented in Annex 1, Table 1.

In the waters of Dniester region (the city of Chernomorsk, St.4), which are under the influence of domestic discharges from sewage treatment plants, the presence of substances that affect the reproduction of algal test object was not detected.

In the surface waters of the Danube region (exit of the city of Kiliya arm, St.2), the presence of substances that affect the reproduction of test object was not detected also. On the contrary, the bottom sediments contained substances toxic to the reproductive processes of test object (for concentration 1.0 $g \cdot l^{-1}$ the number of cells was 74.11% and for 10,0 $g \cdot l^{-1}$ 66.22%, of the control values, respectively) (Fig. 2).



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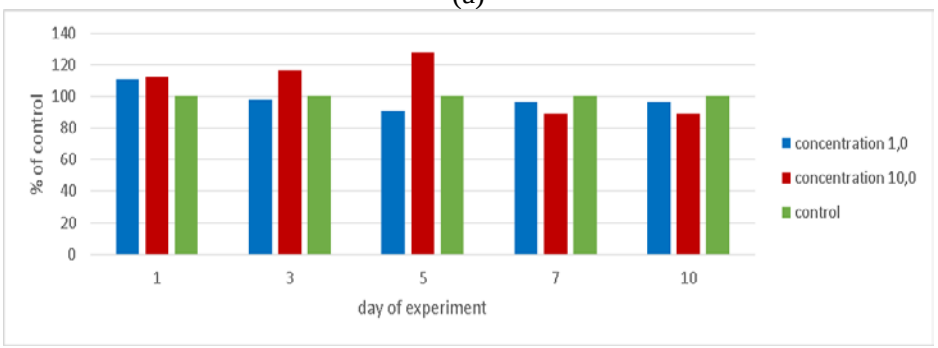
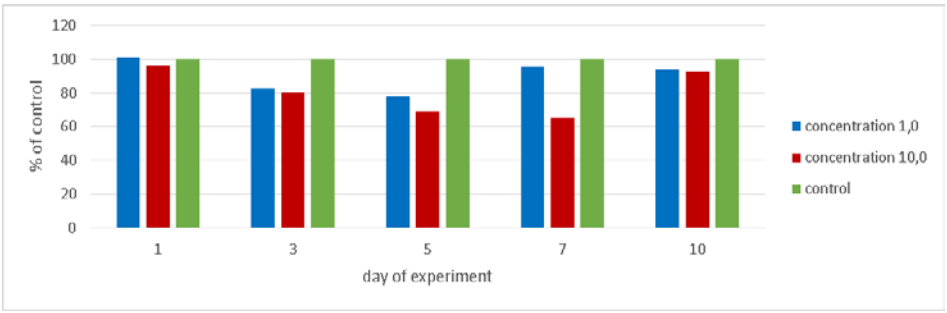


(b)

Fig. 2. Change in the number of *D. communis* cells (in % of control) during the biotestin of the surface waters (a) and bottom sediments (b) taken in the Danube region (exit of the city of Kiliya arm, St.2, 09.11.2019)

The surface layers of mesogalin open in shallow water at the outlets of the Dniester and the Dnieper-Bug estuary, and contain active compounds, which caused sharp changes in the number of algae cells during the chronic experiment. However, the experimental object successfully adapted to a mixture of these substances in samples of experimental solutions - the number of algae cells in the experiment and control was approximately the same (Fig. 3,4). Bottom sediments at the outlet of the Dnieper-Bug estuary (5.1 m depth), at a concentration of 10.0 had a stimulating effect on cell proliferation. And bottom sediments at the exit of the Dniester (1m depth) contained toxic substances that negatively affected the test function of the studied object (the number of studied objects is 29.31% lower than the control) (Fig. 3, 4).

Thus, the surface waters of the Ukrainian coastal water bodies of the Dniester and Dnieper-Bug areas can be characterized as those belonging to the “good” and “high” ecological status classes. The bottom sediments of the Dniester area are "moderate - good", the Dnieper-Bug districts are "good - high".



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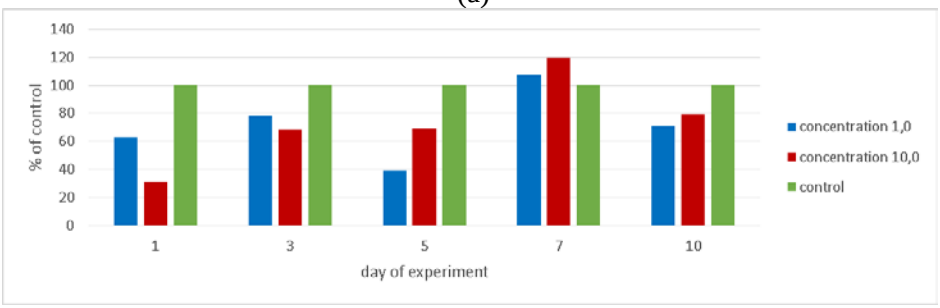
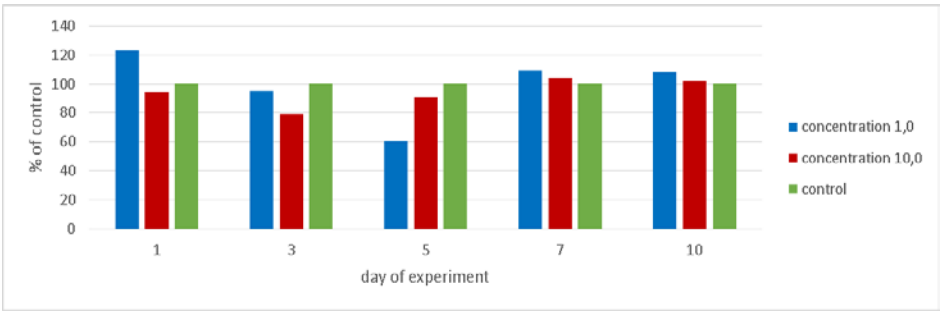


(b)

Fig. 3. Change in the number of *D. communis* cells (in % of control) during the biotesting of the surface waters (a) and bottom sediments (b) taken in the Dniester region (exit from Dniester estuary Zatoka, St.3, 14.06.2019)



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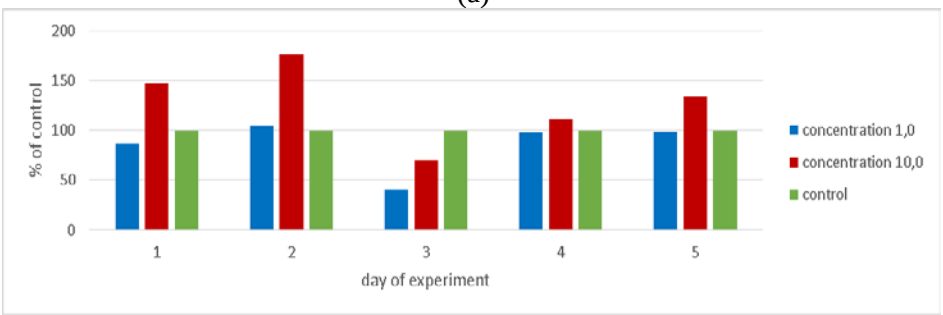
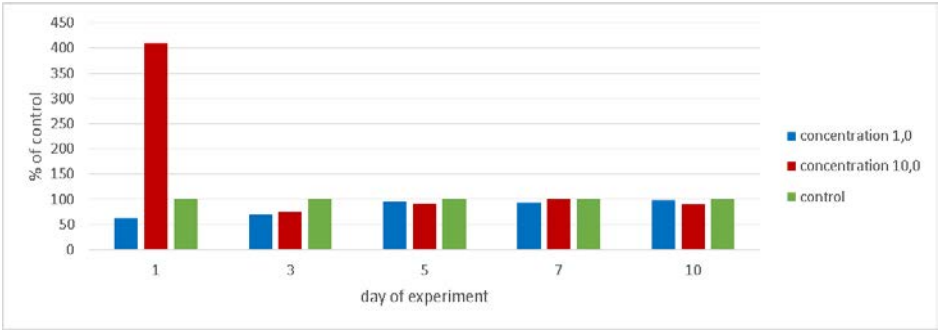


(b)

Fig. 4. Change in the number of *D. communis* cells (in % of control) during the biotesting of the surface waters (a) and bottom sediments
(b) taken in the Dnipro-Bug region
(city of Ochakov, exit from Dnipro-Bug estuary, St.6, 18.06.2019)

Biotesting of water and bottom sediments of the Black Sea shelf zone

In autumn 2019, the surface water layer and bottom sediments of the Danube region (Snake Island - Zmeinyj Island) taken from a depth of 26 m contained substances that actively affected the reproductive function of the test-object (Fig. 5, Annex 1, Table 1).



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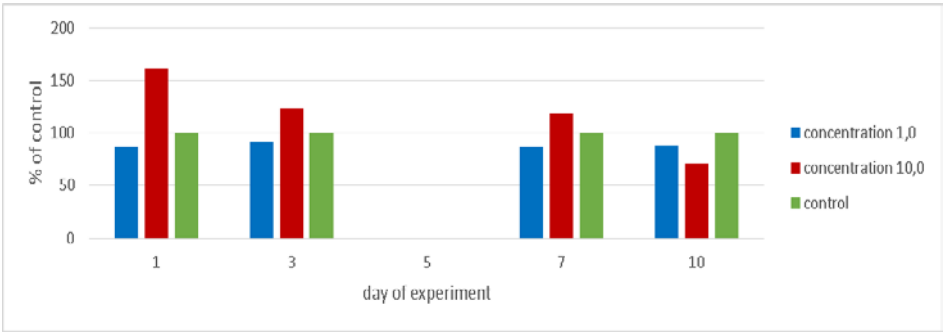


(b)

Fig. 5 Change in the number of *D. communis* cells (in % of control) during the biotesting of the surface waters (a) and bottom sediments (b) in the Danube region (Snake Island- Zmeinyj Island, St.15, 30.09.2019)

However, at the end of the experiment, the number of algal cells in both experimental concentrations of surface water samples were close to control, in the experiment with bottom sediments 29.20% below control.

Thus, the surface layer of the shelf waters of the Danube region can be described as belonging to the class of ecological status of waters "good", while the bottom sediments are "good - moderate".

In the area of mixed waters of the Ukrainian shelf in the upper layer of water on the Central region of NWBS Zernov's *Phyllophora* Field (St.16) were found the compounds in small quantities somewhat inhibited the reproduction of algae, and in higher concentrations stimulated this test function (Fig. 6).

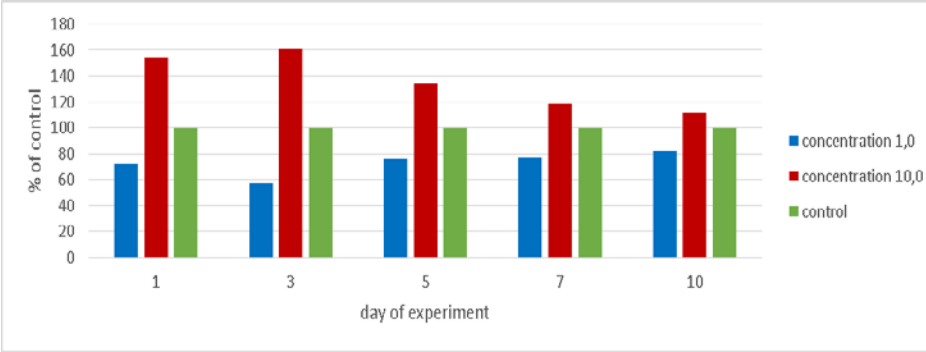
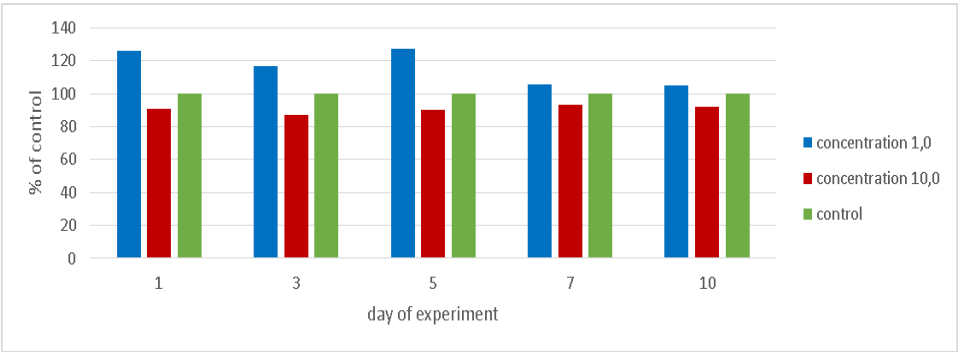


Fig. 6. Change in the number of *D. communis* cells (in % of control) during the biotesting of the surface waters in the mixed waters region (Zernov's *Phyllophora* Field, St.16, 31.08.2019)

On the border of mixed waters and the central part of NWBS, Zernov's *Phyllophora* Field (St.17) (near-bottom water) substances from the bottom layer (depth 41 m), on the contrary, stimulated the reproduction of algae in low concentrations and inhibited it in higher concentrations (Fig. 7).



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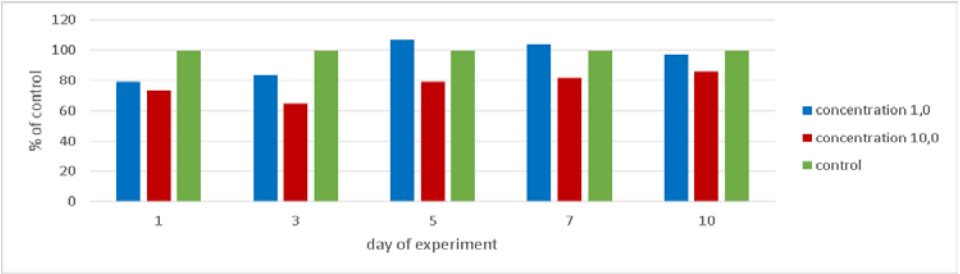
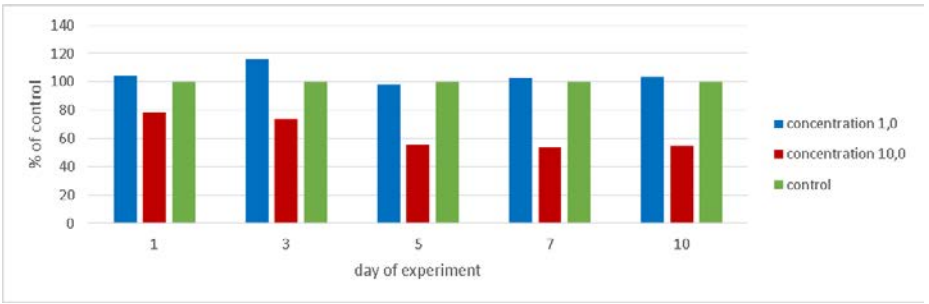


(b)

Fig. 7. Change in the number of *D. communis* cells (in % of control) during the biotesting of the surface (a) and near bottom (b) waters in the mixed-central region (Zernov's *Phyllophora* Field, St. 17, 31.08.2019)

The results of our study show the absence of acute toxicity of substances in the water bodies of mixed waters region and central region of the Ukrainian part of the Black Sea in the summer of 2019. The surface waters of mixed waters and central region can be characterized as those belonging to the “good” and “high” ecological status classes, and near-bottom waters as “good” ecological status class.

In autumn 2019, the surface and near-bottom (depth 65 m) seawaters at the Romanian part of the shelf in minimum experimental concentration contained substances that have a stimulating effect on algal reproduction. In the maximum concentration, the samples of the surface layer showed a toxic effect on the reproduction of test-object (the number was 54.79% of the control). In the bottom sediments, there were no substances that showed a pronounced effect on the reproduction of test object algae cells (Fig.8, Annex 1,Table 1).



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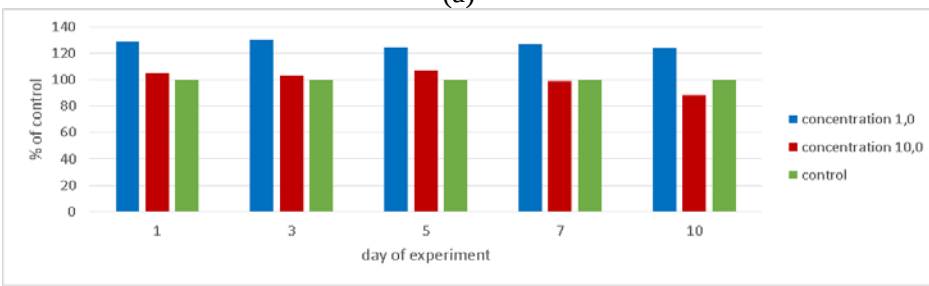

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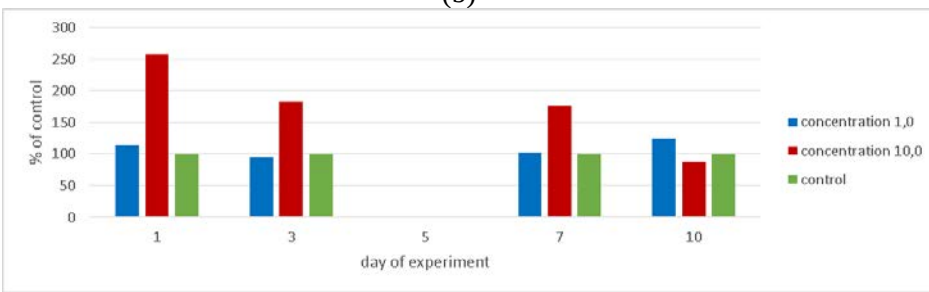

(c)

Fig.8. Change in the number of *D. communis* cells (in % of control) during the biotesting the surface water (a), near-bottom water (b) and bottom sediments (c) taken in the Romanian waters (St. RO-5, 02.10.2019)

The surface layer of water, near-bottom waters from 41 m depth and bottom sediments at a depth of 43 m in Bulgarian waters also, did not contain toxic substances, respectively. In the experimental samples, the number of cells of the experimental object was close to control, or slightly exceeded (Fig. 9, Annex 1, Table 1).

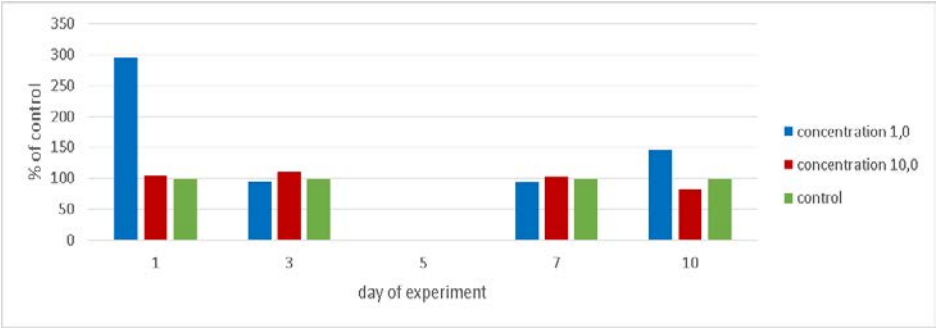

Fig. 9. Change in the number of *D. communis* cells (in % of control) during the biotesting the bottom sediments, taken in the Bulgarian waters (St. BG-1, 03.10.2019)

In autumn 2019, the surface and near-bottom (depth 65 m) layers of Romanian waters can be described as belonging to the "high" ecological status class, while the samples with the maximum concentration of the surface layer of water can be classified as ecological class of waters "moderate". Surface and bottom (depth 41 m) layers of Bulgarian waters can be described as those belonging to the "high - good" ecological status class. The bottom sediments of Romanian and Bulgarian waters belong to "good" ecological status class.

During the experiments, the obtained values of pH changes of the control and experimental media, where the studied object was cultivated, are insufficient for conclusions. Indicators of changes in the alkalinity of the experimental environment are due to many factors: the processes of algae life and changes in pollutants.

CONCLUSIONS

In the summer and autumn of 2019, biotesting of water and sediments of Ukrainian, Romanian and Bulgarian water bodies with different effects of anthropogenic factors and anthropogenic load showed that in the vast majority of the studied areas there are no toxic substances for planktonic algae *D. communis*. The ecological status of NWBS shelf waters is better than coastal water bodies.

During summer 2019, the surface waters of the coastal waters of the Dniester and Dnieper-Bug zones and the waters of the mixed and central zone of the Ukrainian part of the Black Sea can be characterized as belonging to the class of ecological status "good" and "high". The

bottom sediments (except for the Dniester region - "moderate") were "good" - "high". In autumn 2019, the surface layer of water in the Ukrainian coastal reservoirs of the Danube region belonged to the class of "good" ecological status, while the bottom sediments "good - moderate" and "toxic."

In autumn 2019, the surface and bottom layers of Romanian (depth 65 m) and Bulgarian waters (depth 41 m) are characterized as belonging to the class of ecological status of waters "high", except for samples with the maximum concentration of the surface layer of Romanian waters, which can be classified as "moderate". The bottom sediments of Romanian and Bulgarian waters belong to the class of ecological status - "good".

The ecological condition of the Black Sea studied areas requires comprehensive monitoring studies.

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Annex 1

Table 1. The results of biotesting on microalgae *D. communis* and ecological status class of the sampling waters and bottom sediments

Date	Station	Type of the sample	Sampling depth	Concentration $g \cdot l^{-1}$	Number of the cells at the end of the experiment, % of control	Ecological status class
09.11.2019	St.2	bottom sediments	10 m	1,0	74.11	Moderate
09.11.2019	St.2	bottom sediments	10 m	10,0	66.22	Moderate
09.11.2019	St.2	seawater	0 m	1,0	96.7	Good
09.11.2019	St.2	seawater	0 m	10,0	89.14	Good
14.11.2019	St.3	bottom sediments	5 m	1,0	84.93	Good
14.11.2019	St.3	bottom sediments	5 m	10,0	84.9	Good
14.06.2019	St.3	seawater	0 m	1,0	93.54	Good
14.06.2019	St.3	seawater	0 m	10,0	92.83	Good
14.06.2019	St3	bottom sediments	1 m	1,0	70.69	Moderate
14.06.2019	St.3	bottom sediments	1 m	10,0	79.54	Good
14.09.2019	St.4	seawater	0 m	1,0	91.46	Good
14.09.2019	St.4	seawater	0 m	10,0	84.87	Good
18.06.2019	St.6	bottom sediments	5,1 m	1,0	98.68	Good
18.06.2019	St.6	bottom sediments	5,1 m	10,0	133.5	High
18.06.2019	St.6	seawater	0 m	1,0	107.76	High
18.06.2019	St.6	seawater	0 m	10,0	101.61	High
31.08.2019	St.17	seawater	0 m	1,0	104.75	High

Date	Station	Type of the sample	Sampling depth	Concentration $g \cdot l^{-1}$	Number of the cells at the end of the experiment, % of control	Ecological status class
31.08.2019	St 17	seawater	0 m	10,0	92.24	Good
31.08.2019	St.17	seawater	41 m	1,0	97	Good
31.08.2019	St.17	seawater	41 m	10,0	86.09	Good
31.08.2019	St.16	seawater	0 m	1,0	82.45	Good
31.08.2019	St.16	seawater	0 m	10,0	111.55	High
30.09.2019	St. 15	Seawater	0 m	1,0	98.02	Good
30.09.2019	St.15	seawater	0 m	10,0	89.59	Good
30.09.2019	St.15	bottom sediments	26 m	1,0	88.48	Good
30.09.2019	St.15	bottom sediments	26 m	10,0	70.8	Moderate
02.10.2019	St. RO-5	bottom sediments	65 m	1,0	124.02	High
02.10.2019	St. RO-5	bottom sediments	65 m	10,0	88.21	Good
02.10.2019	St. RO-5	seawater	0 m	1,0	103.75	High
02.10.2019	St. RO-5	seawater	0 m	10,0	54.79	Moderate
02.10.2019	St. RO-5	seawater	65 m	1,0	124.02	High
02.10.2019	St. RO-5	seawater	65 m	10,0	88.21	Good
03.10.2019	St. BG-1	bottom sediments	43 m	1,0	144.67	High
03.10.2019	St. BG-1	bottom sediments	43 m	10,0	82.87	Good
03.10.2019	St. BG-1	seawater	9 m	1,0	91.74	Good
03.10.2019	St. BG-1	seawater	9 m	10,0	94.24	Good
03.10.2019	St. BG-1	seawater	41 m	1,0	106.59	High
03.10.2019	St. BG-1	seawater	41 m	10,0	81.28	Good