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RESEARCH ARTICLE

Catalase activity and lipid peroxidation process of the Black Sea macroalgae dominants (epiphytes and lithophytes) under different environmental conditions

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Abstract

Catalase activity (CA) and the lipid peroxidation process (LPO) in *Cystoseira* phytocoenoses of the Black Sea under different environmental conditions were studied. Eleven dominants of the Black Sea macroalgae of different life forms (epiphytes and lithophytes) were investigated. It was shown that the LPO level of macroalgae changed in the conditionally clean water area over the range from 13.29±0.56 to 40.56±4.15 nM MDA/g, whereas CA level changed from 30.24 ± 5.1 to 108.67 ± 6.8 mcg H_2O_2/g·x min. The intensification of the LPO process by 27% on average was found in epiphytes macrophytes, as compared with the lithophytes. The maximum intensification was recorded in *Polysiphonia subulifera*, and the minimum intensification was recorded – in *Callithamnion corymbosum* (47 and 8.5%, respectively). The CA values of epiphytes macrophytes CA increased by 42.7% on an average as compared with lithophytes. The maximum difference between the CA indices of the epiphytes and lithophytes was recorded in *C. corymbosum* (59%) and the minimum one was recorded in *Ceramium virgatum* (10%).

Keywords: Catalase activity, lipid peroxidation process, macroalgae, epiphytes and lithophytes, household pollution, Black Sea

Introduction

The study of metabolic peculiarities of macrophytes growing under different conditions is an important environmental task. The study of macroalgae metabolic processes is a significant step towards the preservation of biodiversity, as they are biochemical processes that provide the adaptation of organisms to the dynamic environment. The *Cystoseira* phytocoenoses are generally known to be a key part of the Black Sea inshore vegetation and particularily of the Sevastopol inshore area (Kalugina-Gutnik 1975;

Chernyshova 2008). The *Cystoseira phytocoenoses* occupy the widest area of the phytal zone in the Crimean open shores, from 0.5 to 15 m, forming a zone type of vegetation (Kalugina-Gutnik 1975; Milchakova 2003). The increase in anthropogenic load on the offshore water areas and their eutrophication have lead to the degradation of *Cystoseira* communities. This phenomenon is accompanied by the considerable intensification of the epiphytation process in the communities of Black Sea macroalgae, of *Cystoseira* in particular, both in polluted and in conditionally clean water areas (Milchakova 2003; Kovardakov and Firsov 2007; Chernyshova 2008). The study of the response of *Cystoseira crinita* (Desf.) Bory and *Cystoseira barbata* C. Ag. and their epiphytes makes it possible not only to estimate the adaptive potential, but also to perform the prognostic assessment of the probable transformation of vegetable coenosis under complex environmental conditions.

The state of macroalgae is known to be related to their antioxidant system (AOS). It is activated under conditions that require starting the metabolic adaptive mechanisms, which allow the hydrobionts to adapt to the impact of a wide range of environmental factors. The AOS activation manifests itself by stimulating the activity of the enzymes (dismutase superoxide, catalase (CAT), ascorbate- and glutathione-peroxidases and reductases) that block the spread of free-radical processes. In case of excessive appearance of free-radical forms of oxygen, the self-accelerating process of lipid peroxidation (LPO) leads to the destruction of unsaturated lipids ensuring the integrity of cell membranes, to the disturbance of the structure and functions of protein and other biologically significant macromolecules and, consequently, to the apoptosis (Menchikova *et al.* 2006). The integrated study of the LPO process and CAT makes it possible to reveal the mechanisms of the marine macroalgae adaptation to the conditions of household sewage pollution in the near-shore zone.

The purpose of this work is to study the variability of the LPO process and catalase activity (CA) of the dominants of the Black Sea macroalgae of different life forms under the phytocoenoses conditions of *Cystoseira*, with a range of depths (from 1 to 5 m) under the influence of household sewage pollution. The findings, cited in the present work, have been obtained for the first time as similar research has never previously been conducted.

Materials and Methods

The objects of study were eleven dominants of the Black Sea macroalgae: *Chaetomorpha aerea* (Dillw.) Kütz., *Cladophora albida* (Nees) Kütz., *Ulva rigida* C. Agarch, *Cladophoropsis membranacea* C. Agarch, *Callithamnion corymbosum* (J.E. Smith) Lyngb., *Ceramium diaphanum* (Lightfoot) Roth, *Ceramium virgatum* Roth, *Gelidium crinale* (Hare ex Turner) Gaillon, *Gelidium spinosum* (S.G. Gmelin) P.C. Silva, *Laurencia coronopus* J. Agarch, *Polysiphonia subulifera* (C. Agarch) Harvey.

The research was conducted at two stations of the Sevastopol near-shore area differing in the degree of household sewage pollution and amount of incoming flow during the autumn of 2008-2009 (Figure 1). The mature, developed thalli of algae - lithophytes and their epiphytes were sampled for analysis at the depths of 1, 3 and 5m.

The LPO level was defined by the accumulation of the final product of lipid oxidation-malondialdehyde (MDA) with the help of thiobarbituric acid. The method is based on the reaction between malondialdehyde and thiobarbituric acid, which proceeds with the formation of coloured trimetine complex containing the MDA molecule and two molecules of thiobarbituric acid (Stalnaya and Garishvili 1977) with high temperature and acid pH.

Method for determination of LPO
Macroalgae thalli (1mg) was triturated with cold 9% saline solution on the ice. We worked with 10% solution of the thalli. Then the samples were centrifuged at 3000 rpm for 15 min. We worked with the supernatant. In reaction sample added 0.5 ml of supernatant, 0.5 ml distilled water and 1 ml 17% trichloroacetic acid for denaturation of proteins. After that the samples were centrifuged at 3000 rpm for 15 min. In 2 ml supernatant added 1 ml of 0.8% thiobarbituric acid. The samples was boiled for 10 minutes at the water bath. The blank sample contained 2 ml of distilled water and 1 ml of 0.8% tiobarturic acid. Appeared pink color samples of malondialdehyde (MDA) measured on the spectrophotometer (SF-2000) at a wavelength of 532 nM. Calculation produced using a molar extinction coefficient equal $1.56 \cdot 10^5$ M^{-1} l^{-1}. Date was counted as nM MDA/(g thalli wet weight).

Method for determination CA
The algae CA was determined by the Bach and Zubkova method (Beryozov 1976), adapted for macrophytes (Milchakova and Shakhmatova 2007). All analyses were conducted in 30 minutes or in one hour after macroalgae sampling. The CA method is based on the ability CAT to disintegrate H_2O_2 on the oxygen and water. Catalase activity was determined by the amount of decomposed hydrogen peroxide and was counted as mkg/(g thalli wet weight x min). Supernatant was prepared by the method described above. In reaction sample added 10 ml of distilled water, 1 ml of supernatant, 2 ml of 3% solution of H_2O_2 and left for 30 min for the reaction. For the blank sample was used 1 ml of supernatant, boiled for 10 minutes at 100°C, 10 ml of distilled water, 2 ml of 3% solution of H_2O_2. After 30 min the reaction was stopped, adding 10% sulfuric acid. Than the samples was titrated with 0.1 N solution $KMnO_4$. Calculation was performed on the difference of volumes $KMnO_4$, which went on titration of the blank and experience sample. Date was counted as mcg H_2O_2/(g thalli wet weight x min).

The data of lithophytes were taken for 100% for calculation percent in the main. The number of measurements for each species is equal to three. The inaccuracy is presented by the standard deviation.

On the basis of analysis of published data (Ovsyaniy *et al.* 2001; Pavlova *et al.* 2001; Gubanov *et al.* 2002; Mironov *et al.* 2003) the sampling water areas were conditionally rated as polluted: Quarantinnaya Bay; and lightly polluted (conditionally clean): Kruglaya Bay (Table 1).

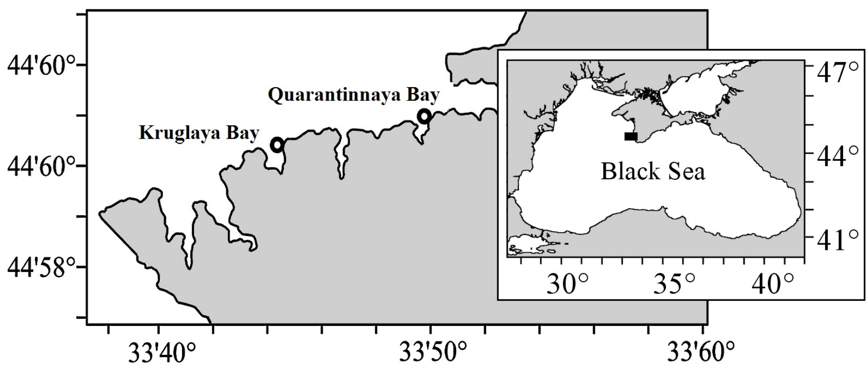

Figure1. Locations of sampling points

Table 1. General description of the sea-water and pollution of bottom sediments in the water area of the Sevastopol region according to Ovsianiy *et al.* (2001), Pavlona *et al.* (2001), Gubanov *et al.* (2002)

Area	**Amount of untreated household sewage flow** (thousand m^3/year)	**Content in bottom sediments** (mg/100g)		**Content in water, mcg/l**			
		Achl*	OH	NO_2^-	NO_3^-	NH_4^+	PO_4^{-3}
Quarantinnaya Bay	547**	0.12	34.0	0.04	0.92	0.44	0.14
Kruglaya Bay		0.01	traces	0.04-0.07	0.02 -0.6	0.11-0.33	0.4

*Achl-content of bitumoid, extracted chloroform; OH-content of oil hydrocarbons in the bottom sediments; dash-no pollution.
**The amount of untreated household sewage flow was calculated by municipality.

Results

The LPO data for lithophytes and epiphytes under different environmental conditions are presented in Figures 2 and 3.

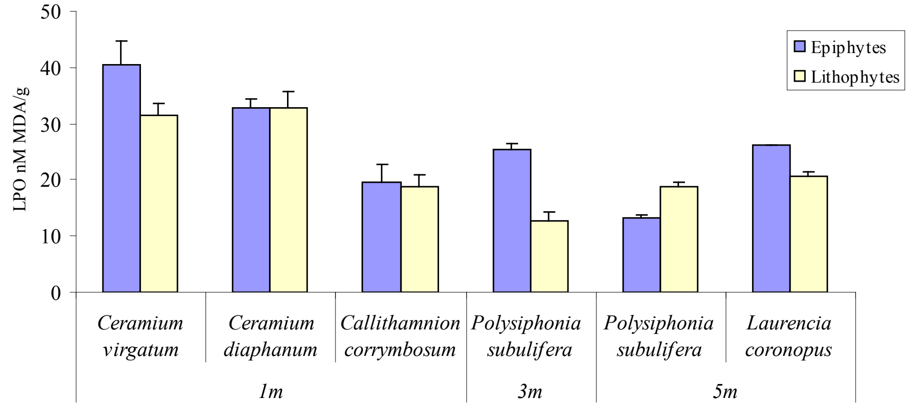

Figure 2. Change in the content of malondialdehyde in Black Sea macroalgae of different life forms (epiphytes- and lithophytes) with corresponding depths in Kruglaya Bay (summer and autumn of 2009)

The LPO value in the macrophytes, fastened to the bottom in the lightly polluted water area, ranged from 12.68±1.67 in *P. subuliphera* to 32.82±2.79 nM MDA/g in *C. diaphanum,* respectively*,* in epiphytes -13.29±0.56-40.56±4.15 nM MDA/g with minimum values for *P. subuliphera* and maximum ones for *C. virgatum.* The increase in LPO index by 27% on average, in comparison with lithophytes, was revealed in four epiphyte species. The LPO maximum increase by 47% was recorded in epiphytes *P. subuliphera* at a depth of 3 m, whereas the minimum increase by 8.5% was recorded in epiphytes *C. corrymbosum* at a depth of 1 m as compared with lithophytes. The decrease of MDA content was found only in the epiphyte *P. subuliphera,* as compared with the lithophyte of the same species at a depth of 5 m (from 18.8±0.8 in lithophyte to 13.3±0.6 nM MDA/g in epiphyte) (Figure 2). The varied response of LPO to the change of the depth of growing was found in the epiphyte *P. subuliphera.* Thus, at a depth of 3 m the lipid oxidation process in the epiphytes of this species increased by 47%, and it decreased by 30% at a depth of 5 m, as compared respectively with lithophytes.

Under the influence of household sewage pollution in Quarantinnaya Bay the LPO index ranged in lithophytes from 10.56±0.45 in *G. spinosum* to 60.13±14.43 nM MDA/g in *C. virgatum,* and epiphytes ranged from macroalgae -18.39±0.54 to 49.65±2.71 nM MDA/g respectively with minimum values for *G. spinosum* and maximum ones for *C. membranacea* (Figure 3).

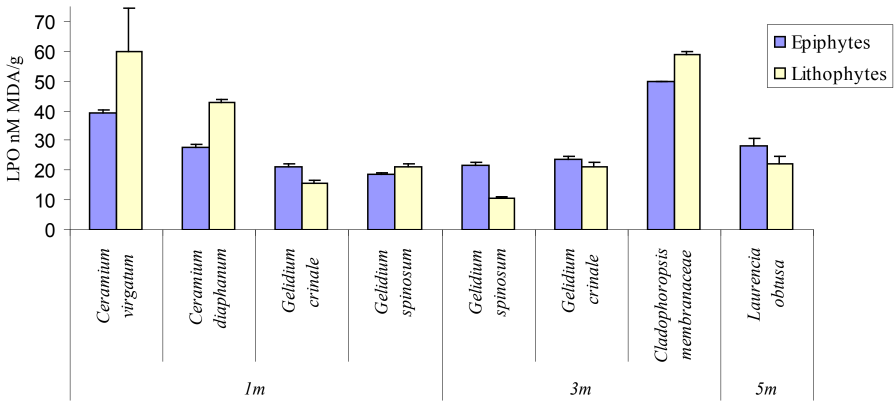

Figure 3. Change in the content of malondialdehyde in Black Sea macroalgae of different life forms (epiphytes- and lithophytes) with depths in Quarantinnaya Bay (summer and autumn of 2009)

The lithophytes algae reacted to the pollution more clearly than epiphytes. Thus, The LPO process in the lithophytes *C. virgatum* and *C.diaphanum* intensified by 48% and 18%, respectively, in the polluted water area, in comparison with the conditionally clean one, (if we take to 100% LPO in conditionally clean water area). The tendency of the LPO level, increased in epiphytes in the lightly polluted water area, as compared with lithophytes, does not become as unambiguous under household sewage pollution conditions. The MDA content decrease was recorded by 12-45% in epiphytes, as compared with lithophytes, in four out of eight studied species (*C. virgatum, C. diaphanum, G.spinosum* and *C. membranacea*).

The change of growing depth had different impacts upon the change of the LPO index in lithophytes and epiphytes. The same tendency of the LPO increased between 9-14% at depths of 1 and 3 m, respectively and it was recorded for *G. crinale* species of both lithophytes and epiphytes. At the same time, *G. spinosum,* under pollution conditions at different depths, showed that the LPO index both increased in epiphytes, as compared with lithophytes, and decreased (by 15% and 53%) at depths of 1 and 3 m, respectively.

The data on the activity of the epiphytic and lithophytic CAT in the conditionally clean water areas of Kruglaya Bay are shown in Figure 4. As for lithophytes, the range of changes in CA totalled from 14.38±1.5 in *P. subulifera* to 73.11±3.27 mcg H_2O_2/g·min in *C. virgatum.* As for epiphytes, CA changed over the range of 30.24± 5.1 to 108.67±6.8 mcg H_2O_2/g·min with the minimum values for *P. subulifera* and maximum ones for *C. corymbosum.* The CA increased by 42.7% on average was recorded in the epiphyte macrophytes, as compared with lithophytes. The maximum difference between the CA values for lithophytes and epiphytes was recorded in *C. corymbosum* (59%), the minimum one-in *C. virgatum* (10%).

As the depth increases from 1 to 5 m, the CA decreases from 54.81 ± 5.0 in the surf zone to 30.24 ± 3.2 mcg H_2O_2/ g·min at a depth of 5 m was observed in the epiphyte thalli of *P. subulifera*, and from 22.68 ± 3.27 at a depth of 1m to 14.38 ±1.52 mcg H_2O_2/ g·x min in lithophytes of the same species at a depth of 5 m. In *L. coronopus* the epiphytes CA, increased by 44-46%, was recorded at a depth from 1 to 3 m, respectively.

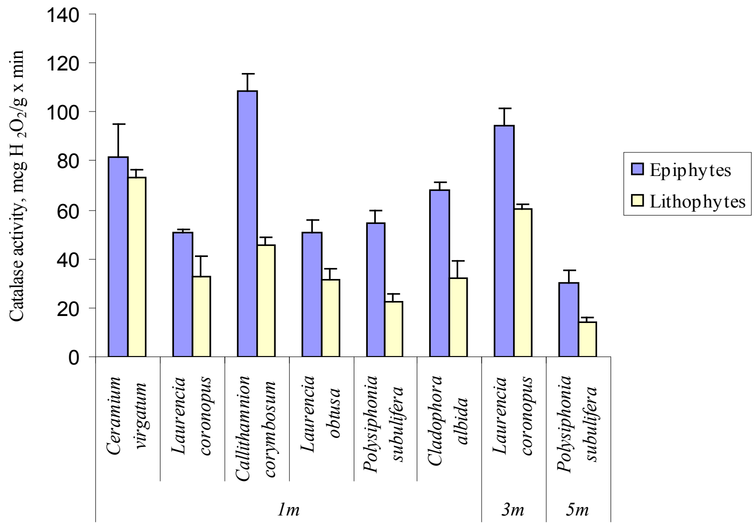

Figure 4. The CA in the Black Sea macroalgae of different life forms (epiphytes and lithophytes) with various depths in Kruglaya Bay (summer and autumn of 2008)

The data on the CAT of the lithophytes and epiphytes under the influence of household sewage pollution are shown in Figure 5. The tendency to increase CA in epiphytes, as compared with lithophytes, was recorded under the conditions. CA of the macroalgae, fastened to the substrate, changed from 26.46±1.89 to 304.12±69.23 mcg H_2O_2/g·min in *C. diaphanum* and *G. crinale,* respectively. The changes in epiphytes CA under these conditions ranged from 24.57±1.89 to 330.75±34.0 mcg H_2O_2/g·min with the minimum values for *C. diaphanum* and maximum ones for *G. crinale.* The CA increase in epiphytes macroalgae, as compared with lithophytes, totalled 8-79% on the whole for six out of seven studied species, though the maximum difference among these indices was recorded in *L. obtusa* (79%), and the minimum difference was in *G. crinale* (8%). The CA decreased by 8% in epiphytes, as compared with lithophytes, was recorded only in *C. diaphanum.*

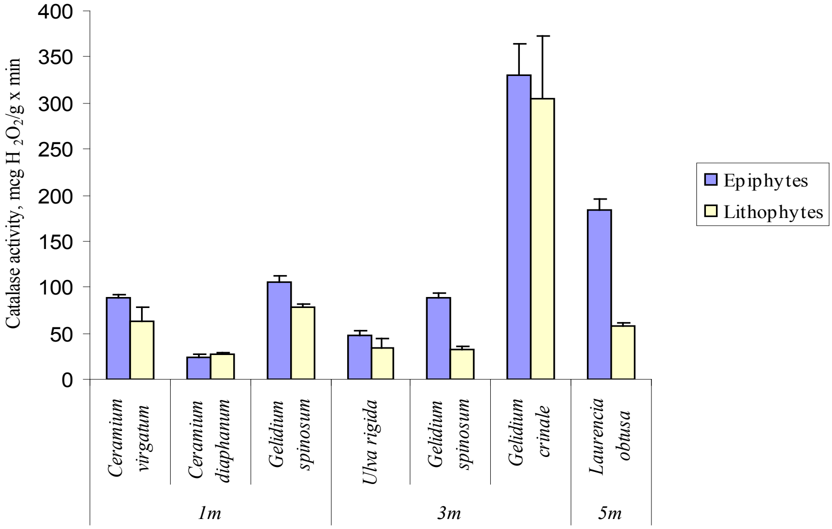

Figure 5. The CA in Black Sea macroalgae for different life forms (epiphytes and lithophytes) with various depths under household sewage pollution conditions for Quarantinnaya Bay (summer and autumn of 2008)

Discussion

The epiphytism phenomenon is widely spread both among land plants and water plants. The majority of Black Sea algae can grow, like epiphytes, on other macroalgae, most frequently on Cystoseira (Morozova-Vodyanitskaya 1940; Kovardakov and Firsov 2007). The algal epiphytism is regarded as a primitive symbiosis form, in the presence of which very unstable and short-time relations are formed among the plants. Vinogradova (1989) suggests understanding any plant growing on the other plants, irrespective of their spatial connections, by the term "epiphyte", and considers the connections among them as indifferent, commencing symbiotic. Nevertheless, some authors (Zhigadlova 2011) record the sparing effect of epiphytes for example, on representatives of the genus *Palmaria*. It was established that the epiphytes form rhizoids penetrating into a phorophyte thallus, at the same time the interpenetration of metabolites is not generally recorded (Turnur and Evans 1977). However, the comparison study of macroalgae, growing on the pondweed *P. pectinatus* and on the plastic plates, revealed the absence of difference in biomass and species product, but the reduced activity of acid phosphatase was recorded in epiphytes. That permitted the authors to make a supposition that it is possible for epiphytes to get phosphorus compounds from a phorophyte plant (Rawlence 1972). The additional inflow of phosphorus compounds from a phorophyte results in the intensification of any epiphyte's metabolic processes and creates more favourable conditions for its growth and development. The effect of the LPO and CA increase, revealed in this study, in the epiphyte macrophytes by 27-42.7%, respectively, in the conditionally clean water area, as compared with lithophytes, can be explained by this phenomenon. In addition, insolation intensification is known to produce an increment of the concentration of active forms of oxygen in the cells of practically all species of algae, which favours of the photosynthesis process, activation, as well as LPO and CA (Pinto *et al.* 2005). Lithophytes are at the bottom level of the phytocoenotic community, and *Cystoseira* as the case in question, receive much less solar radiation than epiphytes. Thus, it is possible that the recorded intensification of the LPO process (by 27% on average) and intensification of CA (by 42.7% on average) can be caused in most studied epiphyte macroalgae by the additional flow of free radicals resulting from the influence of the solar radiation in the upper levels of the *Cystoseira* phytocoenoses.

It is possible to provide another explanation of the revealed intensification of the LPO and CA processes in epiphytes, as compared with lithophytes, which is related to the interspecific interactions. The ability of algae to synthesize the substances that depress the development of the organisms is the indirect consequence of the evolutional adaptation to generate toxic substances against consumer animals. The inhibitory effect of the substances, secreted by the cells of a certain species of algae, was established on the growth of the cells of the other species, and these interactions were recorded among different species of phytoplankton and among sea macrophytes (Salovarova *et al.* 2007). It was found that in case of the interspecific interactions algae secrete free radicals or active forms of oxygen (Labas *et al.* 2010), which can result as well in intensification of the lipid oxidation and CA processes in epiphytes (Menchikova *et al.* 2006).

Under household sewage pollution conditions it was found that the algae, fastened to the bottom, reacted to the pollution differently than epiphytes (Figures 2 and 3). Therefore, when studying the LPO process in the lithophytes *C. virgatum* and *C.diaphanum,* the intensification of the lipid oxidation process by 48 and 18%, respectively, in the polluted water area compared with the conditionally clean water areas, is completely explicable, as the ground, to which the lithophytes directly fasten, actively absorbs contaminants (Table 1). It causes toxic oxidizing stress in macrophytes, under which the products of xenobiotic oxidation, would turn into free radicals, and intensify the chain reaction of peroxidation of lipid fatty acids and CA related to it. The reduction of the malondialdehyde concentration in epiphytes by 3-70%, respectively, as compared with lithophytes, was observed under pollution conditions. Epiphyte species may be protected better from the toxic influence in connection with the stimulation of metabolic processes in epiphytes, as compared with lithophytes, which was previously recorded (Rawlence 1972).

Conclusion

Differences in the level of LPO and CA in the Black Sea macroalgae of different life forms (epiphytes-and lithophytes) have been found. It has been shown that the LPO and CA level was on average 30% higher in epiphytes macrophytes compared lithophytes. In the conditions of household pollution, this trend was changed. In determining the LPO in species *C. virgatum, C. diaphanum,* reverse phenomenon was observed of the increase in LPO in lithophytes compared with epiphytes. In the study of CAT a decrease in the difference between the values of CA of epiphytes and lithophytes has been observed. He same has been observed in all species studied except *L. obtusa* at the depth of 5 m. This is probably due to the stress felt by macroalgae when exposed to household sewage pollution. Species of *C. virgatum* and *C. diaphanum* can be offered as indicators for monitoring coastal waters, upon exposure household sewage pollution.

This finding can be used both during the long-term monitoring of macrophytobenthos reserves and for conducting the express analysis of the state of macrophytes in the near-shore zone of the Sevastopol coastal waters in instances of volleys of sewage, as well as for the prognostic assessment of the long-term changes of bottom vegetation under the persistent anthropogenic impact.

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