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FUCOXANTHIN AND HEAVY METALS IN BROWN ALGAE OF GENUS *CYSTOSEIRA* C. AGARDH FROM WATER AREAS WITH DIFFERENT ANTHROPOGENIC INFLUENCES (BLACK SEA)

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Brown algae are recognized as bioindicators of heavy metal contamination in coastal waters. Comparison of morphological and functional characteristics of algae living in different environmental conditions is essential for understanding mechanisms of marine organism adaptation to anthropogenic environmental impact. The aim of this study is to determinate concentration of fucoxanthin and heavy metals in branches of brown seaweeds *Cystoseira barbata* (Stackhouse) C. Agardh and *Cystoseira crinita* Duby in water areas with different anthropogenic pressures. The content of fucoxanthin in the samples is determined by means of thin layer chromatography, and heavy metals are quantified using atomic absorption spectrophotometry. The maximum concentrations of fucoxanthin (3 $mg \cdot g^{-1}$ of dry weight), lead (48.5 $\mu g \cdot g^{-1}$), zinc (62.6 $\mu g \cdot g^{-1}$), and cadmium (3.2 $\mu g \cdot g^{-1}$) are found in branches of 2- to 3-month-old seaweeds. The content of fucoxanthin in the branches of *C. barbata* is 1.5 times higher than that in *C. crinita*. The *Cystoseira* seaweeds living in a eutrophic bay have higher concentrations of the pigment and zinc than the macrophytes from open waters. The elevated levels of fucoxanthin in brown algae of this genus found in eutrophic and heavy-metal-polluted water areas demonstrate the important role of this carotenoid in the adaptation of the algal photosynthetic apparatus to anthropogenic environmental changes.

Ключевые слова: brown algae, *Cystoseira*, age, heavy metals, fucoxanthin, Black Sea

Heavy metal ions are among the most severe environmental contaminants, which pose serious threat to the land and water ecosystems, as well as to the human health. It is important in such investigations to include different systematic groups of organisms, such as brown algae, which are a the subject of the presented study. Brown algae *Cystoseira* abundantly grow along the shores of the Black Sea, where they are among prevailing species in benthic assemblages of the upper sublittoral zone. They are also good bioindicators of heavy metal contamination of coastal seawater. Accumulation of heavy metals in brown algae has been repeatedly discussed in scientific publications [1, 12]. However, some authors of monitoring studies did not take into consideration the age of the branches they used [1] whereas others just differentiated between young and old branches, or referred to the age of the whole thallus [6]. Little is known about responses of the brown algae to the increase in heavy metals in their natural environment. Seasonal cycles of metals in these macrophytes can depend on the amount of metals in the sea water and on vegetation processes typical for the season.

Fucoxanthin (Fc), the major pigment in brown algae, chrysophytes, and diatoms, contributes over 10 % to the integral content of carotenoids in natural conditions. Together with chlorophyll *a* (Chl a), this pigment forms Fc-Chl *a*-protein complexes in thylakoids where it participates in light harvesting and energy transfer. The pharmacologically active Fc has demonstrated its remarkable antioxidant, cytostatic, anti-inflammatory, hypotensive and antidiabetic properties [11]. More recently, brown algae of the genus *Cystoseira* from the Black Sea were studied in this respect as an interesting natural raw material [15]. Virtually no information is available on Fc stored in *Cystoseira*. The original data concerning the integral xanthophyll content (fucoxanthin, violaxanthin and neofucoxanthin *A*) were obtained half a century ago from determination of carotenoids in different parts of thallus of this alga [9]. It should be kept in mind that neither any techniques for determining the age of algal branches nor reliable methods of pigment structure determination (HPLC, NMR, etc.) were known at that time. Taking this into consideration, we aim in this work at establishing whether Fc content in branches of the Black Sea brown algae *Cystoseira barbata* (Stackhouse) C. Agardh 1820 and *Cystoseira crinita* Duby 1830 depends on their age and on anthropogenic pressure on the habitats.

MATERIAL AND METHODS

Surveys were made since December 2013 till December 2014, at two stations in the coastal zone of the Black Sea, one near Cape Foros and the other in Karantinnaya Bay, Sevastopol (N44.39°, E33.79° and N44.61°, E33.49°, respectively) (fig. 1).

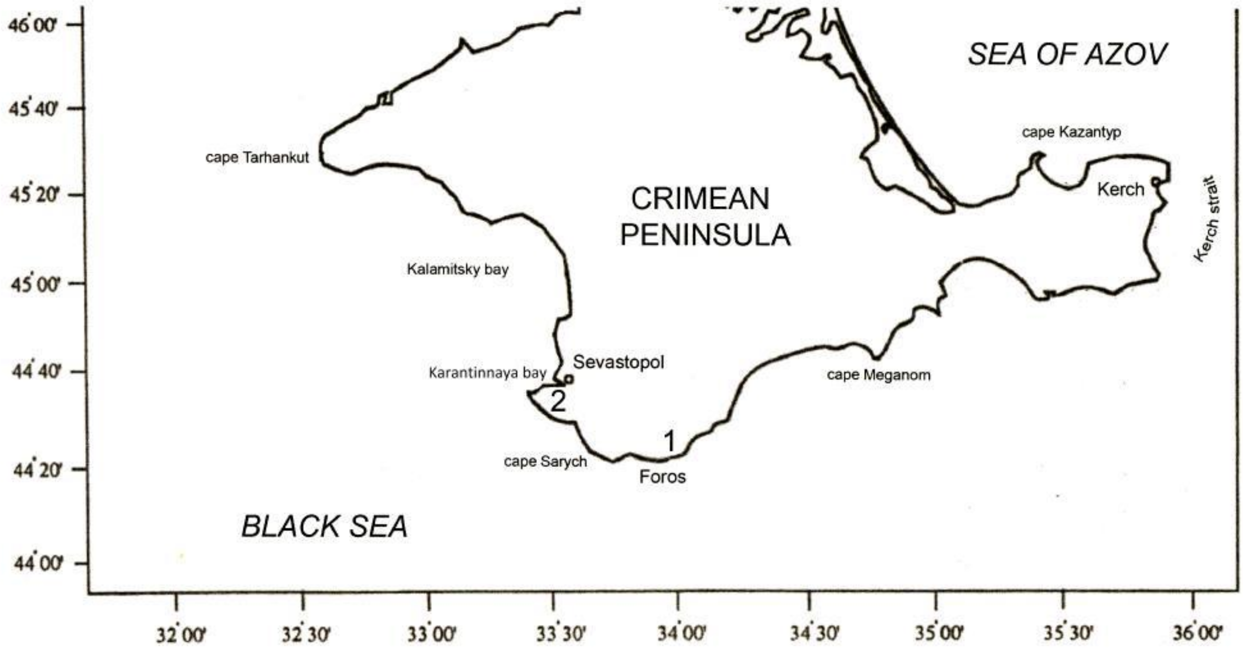

Рис. 1. Zones of sampling in the Black Sea: 1 – Cape Foros, 2 – Karantinnaya Bay

Fig. 1. Места отбора проб в Черном море: 1 — мыс Форос, 2 — Карантинная бухта

Samples of *Cystoseira* were collected monthly from 0.5- to 1.0-m depths and separated into stems and branches; their age was determined as described earlier [5]. The branches were divided into five age groups: younger than 1.9 months old; 2–3 months old; 3–5 months old; 5–6 months old; and older than 6 months. Branches were cleansed from visible epiphytes, rinsed with distilled water and dried at 105 °C. Samples collected for determination of heavy metals were prepared using wet mineralization procedure [2]. Lead, cadmium, zinc and copper were determined with an atomic absorption spectrophotometer *SpectrAA 5* (Varian, Australia) using standard samples.

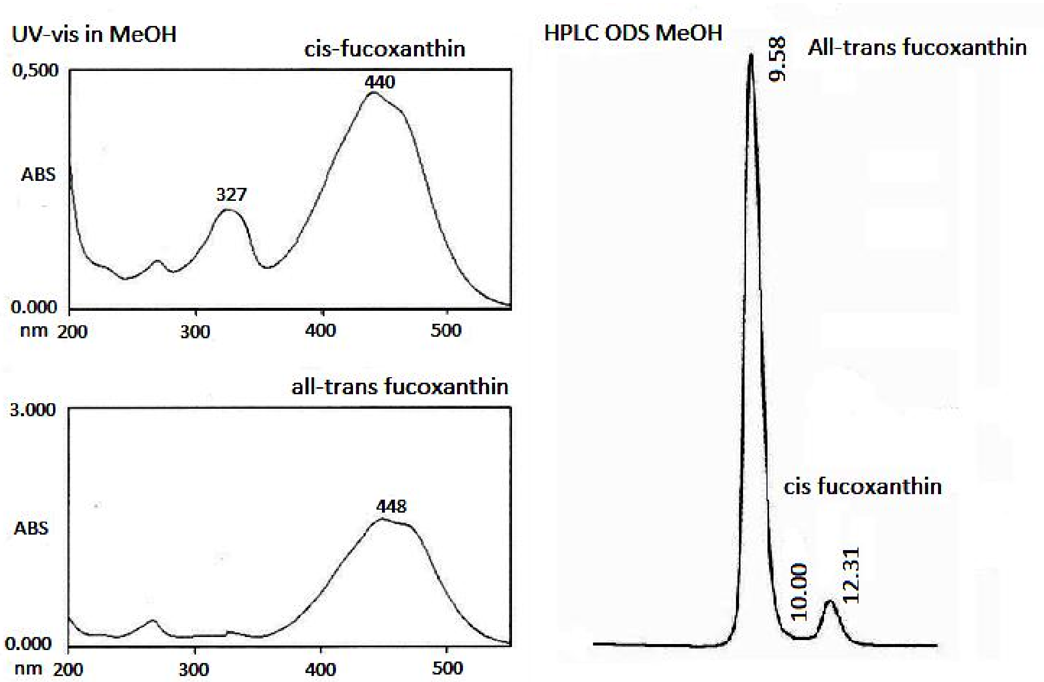

Рис. 2. The UV-VIS spectra and HPLC of fucoxanthin

Fig. 2. Спектры фукоксантина в УФ-видимой области и HPLC

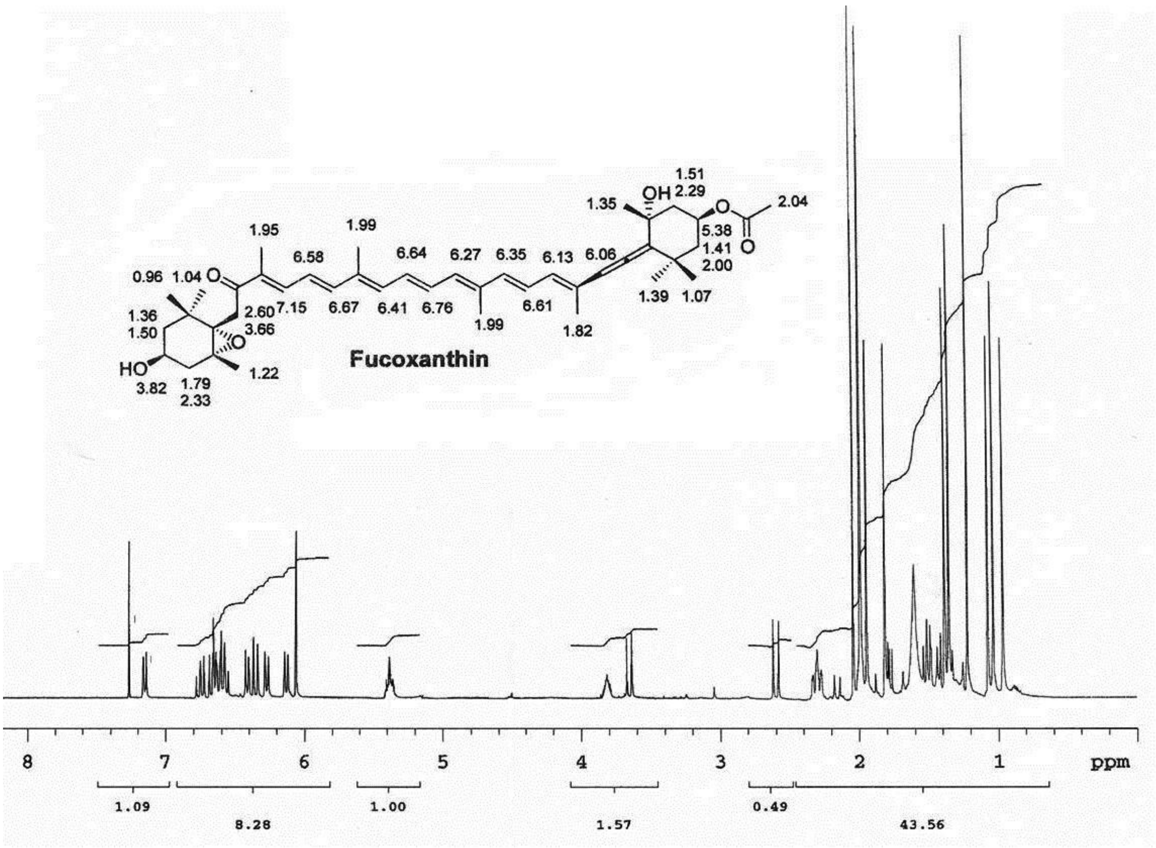

Рис. 3. The ^{1}H-NMR spectrum of fucoxanthin

Fig. 3. ^{1}H-ЯМР спектр фукоксантина

Simultaneously, algal samples for determination of Fc were prepared. Branches of each age group were cut into 8–15-mm pieces and 15 ml of ethanol was added to each of the 5.0±0.1-g sample. Fc from the sample was extracted twice at room temperature during 2 days; the extracts were merged together. The total extract was separated using thin-layer chromatography (TLC) on glass plates (20×20 cm) coated with 0.5-mm-thick silica gel. The eluent was 3 : 7 acetone : *n*-hexane. The extract and Fc standard were evenly applied on the glass plate; in the end, the Fc fraction was carefully removed from the plate and dissolved in ethanol.

Spectra of Fc were recorded on spectrophotometer SF-2000 (Russia) and its content (Fc, mg) in the ethanolic extract was calculated from the equation [10]:

$$F = (D \times V \times 10)/E^{1\%}_{1cm}, \tag{1}$$

where D is the absorbance at 448 nm wavelength; V is the volume of extract, ml; and is the extinction coefficient equal to 1280 [13]. The measurements were made in four replications.

UV-VIS, HPLC and ^{1}H-NMR techniques were used for determining physicochemical characteristics of crystalline Fc used as a standard (figs. 2–3). HPLC was performed on reversed phase columns (ODS-UG-5; 150×4.6 mm, Nomura Chemical Co., Aich, Japan) on the liquid chromatography system Hitachi L-7100 (Tokyo, Japan) with methanol as eluent. ^{1}H-NMR spectra (500 MHz, Varian UNITY INOVA 500) were registered in $CDCl_3$ with TMS as an internal standard.

RESULTS AND DISCUSSION

Karantinnaya Bay in Sevastopol is one of the urban bays which are rated as heavily polluted. The south-western nearshore currents prevailing in the adjacent seawater area [4] transfer pollutants from Karantinnaya Bay to nearby Streletskaya, Kruglaya and Kamyshovaya bays and further to Kazachaya Bay, thereby deteriorating their environmental status. Cape Foros, another sampling location, is in a special tourism-and-recreation zone stretched along the Southern coast of Crimea, where there large are no industrial plants and seaports. Sea water in this zone has only minor amounts of pollutants, including heavy metals, and is rated as relatively pure [8].

The trophic level in Karantinnaya Bay differs from that in the sea water at Cape Foros. The eutrophication index E-TRIX, which acquires growing popularity among researchers assessing marine environment quality, is calculated according to equation:

$$E - TRIX = log_{10}([Chl\ a] \times [D\%O_2] \times [PT] \times [DIN] \times 1.5)/1.2, \tag{2}$$

where Chl *a* is chlorophyll a concentration in the sea water, μg/l; $D\%O_2$ is a deviation of dissolved oxygen from 100 % saturation, absolute units; PT is total phosphorus, μg/l; DIN is total dissolved inorganic nitrogen, μg/l [17]. In the seawater quality grading, the eutrophication index values below 4 mean low trophic level, whereas the values 4–5, 5–6, and 6–10 indicate medium, high, and very high levels, respectively. This criterion has shown that the sea water in some Crimean bays is gravely contaminated with nutrients, is turbid and promotes near-bottom hypoxia development [3]. Values of E-TRIX index below 4 are typical for seawater areas with well-aerated water column, high transparency and low nutrient concentrations. The observations we conducted for 20 months indicate that the eutrophication index estimates range from 2.42 to 4.18 in polluted Karantinnaya Bay and from 1.40 to 2.88 in the conventionally pure sea water. The values increase in summer, reaching a peak in July, and decrease in colder seasons to reach minimum in October. The values below 4 measured in the relatively pure sea water suggest that, outside the bays, the trophic level in the Crimean coastal sea water can be rated as low.

For determination of heavy metals and Fc stored in the brown algae *C. barbata*, 1st-order branches at the age of 0.3–7.0 months are used (fig. 4). Copper in the branches is found to monotonously decrease with the age. Average concentrations of zinc, lead and copper are 62.6, 48.5 and 3.2 μg/g_{dry}, respectively. The concentrations of these contaminants, as well as Fc content (3 mg/g_{dry}), are largest in 2–3-month-old branches (fig. 5). The amounts of these metals and Fc are considerably lower in the juvenile branches (younger than 1 month) and in those older than 6–7 months. After the discovery of the branches of the age group II (2–3-month-old) actively accumulating heavy metals and Fc, it is essential to gain an insight into the seasonal regularities, i. e., whether the content of these substances depends on season.

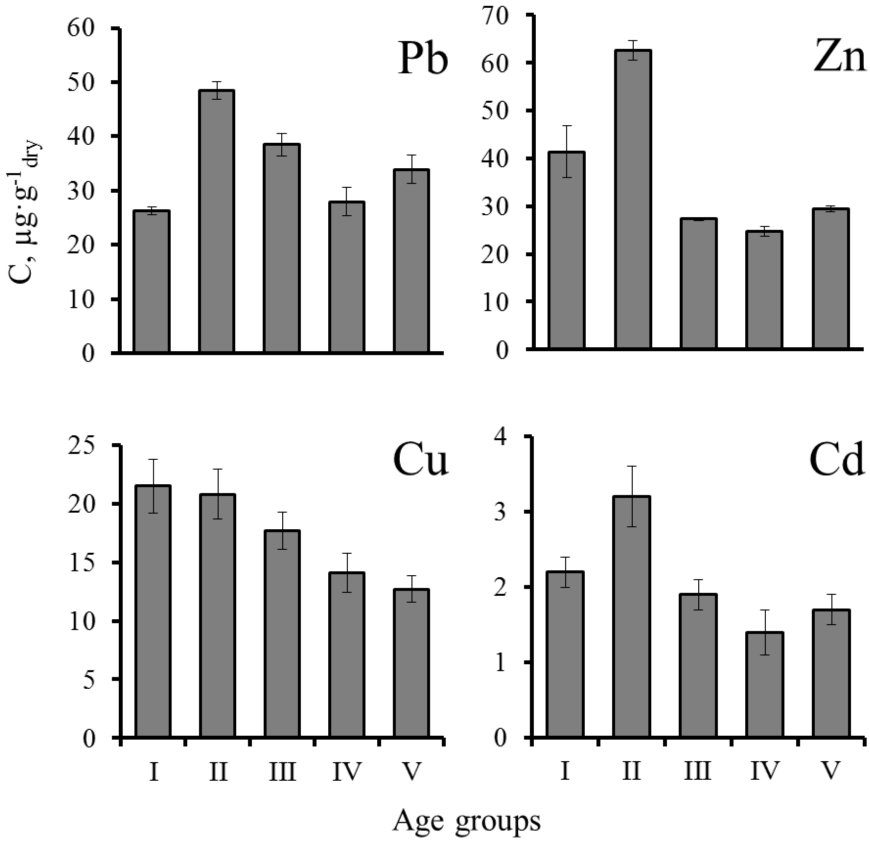

Рис. 4. Concentrations of heavy metals (C) in the 1st-order branches of *Cystoseira barbata*. The age groups of the branches are classified as follows: I (younger than 1.9 months), II (2–3 months), III (3–5 months), IV (5–6 months), and V (older than 6 months)

Fig. 4. Концентрации тяжелых металлов (C) в ветвях *Cystoseira barbata* первого порядка. Возрастные группы ветвей: I (< 1.9 мес.), II (2–3 мес.), III (3–5 мес.), IV (5–6 мес.), V (> 6 мес.)

C. barbata and *C. crinita* from Karantinnaya Bay are found to store considerably greater amounts of zinc than the macrophytes near Foros (fig. 6). The zinc concentrations throughout the year are higher in *C. barbata* than in *C. crinita*, which can be a species-specific indicator. Regardless of the sampling locality, the highest content of this metal in *C. barbata* is registered in January, and in *C. crinita* it is found in September — October. An interesting tendency in the accumulation of this pollutant in *C. barbata* has been observed as zinc concentration increases to its maximum while lead concentration decreases to minimum, and *vice versa*. In winter and spring, the lead concentration increases to the largest values in both algae.

C. barbata and *C. crinita* from the seawater area under higher anthropogenic pressure are significantly richer in Fc (fig. 7). In both macrophytes, regardless of the sampling area, the accumulation of this pigment is larger in autumn and winter. In spring it decreases, and in summer, when the sea warms up to the highest temperatures, it drops to its minimum. Compared to *C. crinita*, *C. barbata* has 1.5-times higher concentration of this carotenoid in its branches.

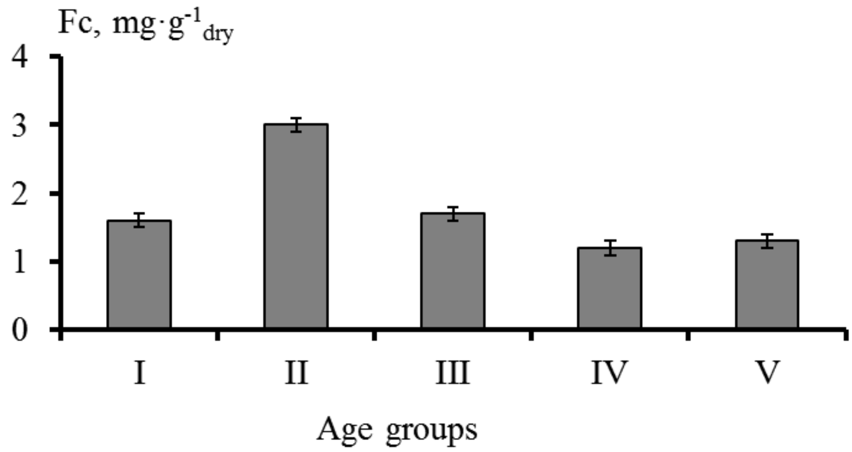

Рис. 5. Concentrations of fucoxanthin (Fc) in the 1st-order branches of *Cystoseira barbata*. The age groups of the branches are classified as follows: I (younger than 1.9 months), II (2–3 months), III (3–5 months), IV (5–6 months), and V (older than 6 months)

Fig. 5. Концентрации фукоксантина (Fc) в ветвях *Cystoseira barbata* первого порядка. Возрастные группы ветвей: I (< 1.9 мес.), II (2–3 мес.), III (3–5 мес.), IV (5–6 мес.), V (> 6 мес.)

The accumulated amounts of heavy metals and Fc vary with the age of the branches. The concentration of these substances is higher in 2- to 3-month-old branches and significantly lower in other age groups. The most plausible explanation is that the juvenile, younger than one-month-old branches have not finished growing and those older than 4 months have used up their photosynthetic potential for reproduction and play negligible role in biosynthesis of thallus. As evidenced in an earlier work, two-month-old branches show higher photosynthetic rate than other parts of thallus [7].

The increased zinc concentrations observed in young algal branches can be due to enhanced activity of zinc finger proteins. These proteins actively participate in DNA transcription and translation, RNA transport and chromatin remodeling [11]. Zinc contained in these proteins works as a catalyst of metabolic processes. In the algae, zinc is accumulated due to its presence in enzymes which have zinc finger domain in their structure and form complexes with polysaccharides.

Compared with the seaweeds from the conventionally pure seawater area, *C. barbata* and *C. crinita* from eutrophic Karantinnaya Bay contain larger amount of zinc (fig. 6). Possibly, this can be explained by a growth aberration or/and by a greater zinc pollution in the sea area. According to some earlier works, microelement composition of the macrophytes growing in polluted and in relatively pure habitats is different; besides, increased concentrations of metals in the sea water trigger their accumulation in the seaweeds [1]. Presently, brown algae are among commonly recognized bioindicators of marine pollution by heavy metals.

Sea currents changing with season, which are a phenomenon typical for the open-sea areas, can appreciably influence hydrochemical composition of the habitat. In some periods of the year, the terrigenous runoff drastically enhanced by seasonal storms and cloudbursts brings markedly greater amounts of heavy metals into the sea, especially near the shore. The records show that *C. barbata* and *C. crinita* have the highest content of lead in winter and spring, whereas the largest accumulation of zinc is in winter and autumn, and that of copper is in spring, concurrently with the onset of intensive growth of the branches.

Lead and zinc in the branches of *C. barbata* demonstrate quantitatively opposite trends: the higher lead concentration, the lower zinc accumulation. This tendency can result from biological antagonism between these metals. By competing with lead in molecular binding and removing it from the living cell pool, zinc decreases lead content in the plant. With the onset of the inhibited growth phase, the contrary is seen. Hypothetically, the pendulum-like imbalance between zinc and lead can be explained by growing adaptation of the seaweeds to the heavy-metal pollution of environment.

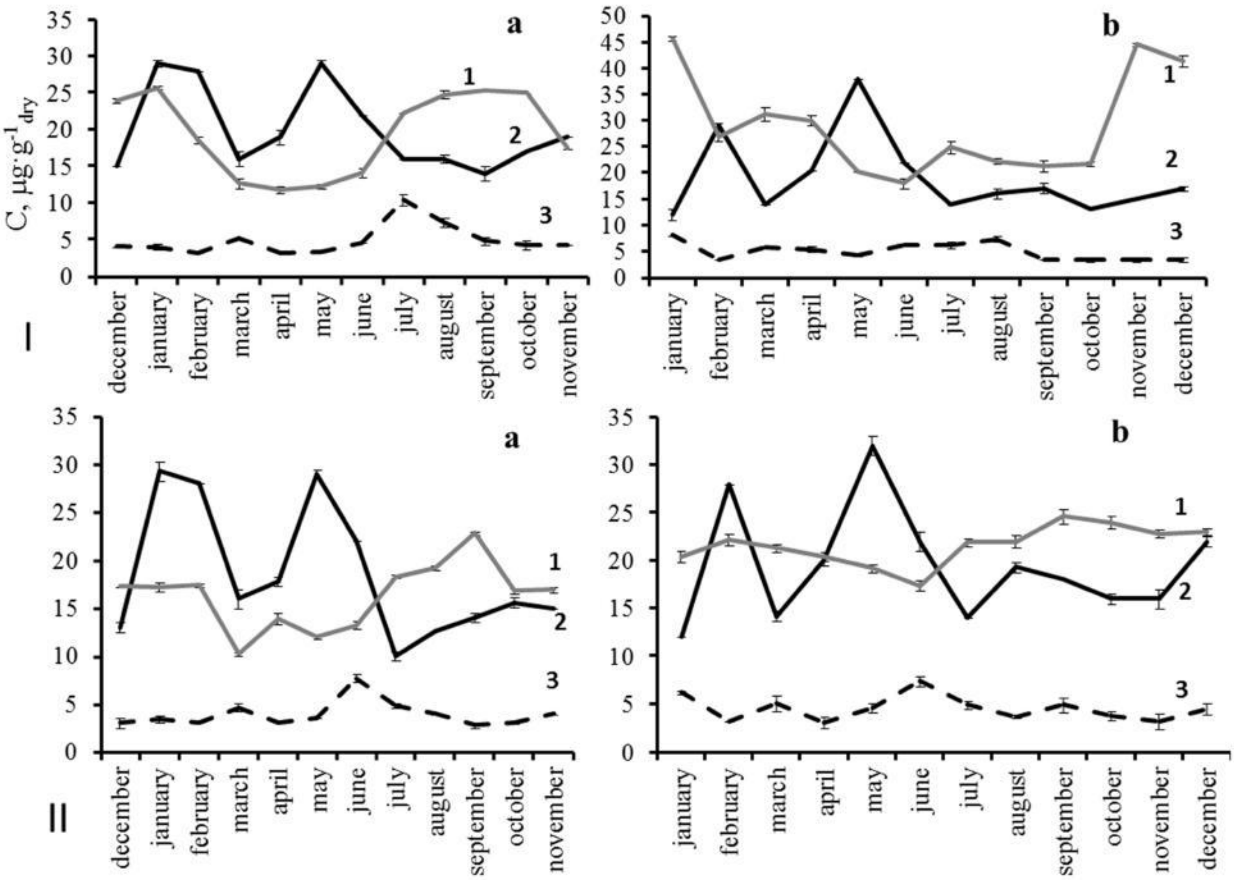

Рис. 6. Concentrations (C) of zinc (1), lead (2) and copper (3) in the 2- to 3-month-old branches of *Cystoseira barbata* (I) and *C. crinita* (II) from the seawater area at Cape Foros (a) and from Karantinnaya Bay (b)

Fig. 6. Концентрация (C) цинка (1), свинца (2) и меди (3) в 2–3-месячных ветвях *Cystoseira barbata* (I) и *C. crinita* (II) из акватории мыса Форос (a) и Карантинной бухты (b)

Biological role of the lead accumulation in *Cystoseira* branches is obscure. Yet, it was reported that in the sea water containing up to 1 mg Pb per 1 litre, photoresistance of chlorophyll increased, as concluded from the longer latent period [1]. With higher concentrations of lead, the latent period shortened, completely vanishing at 10 mg/L. The increased photoresistance concomitant with the low lead content can be due to the influence of this metal on protein-pigment complexes or to the temporal stimulation of recovery processes in the pigment apparatus.

Similarly, accumulation of cadmium can be related to the period of active growth of younger algal branches. In proteins, ions of cadmium can replace zinc and iron. The replacement of zinc disturbs normal functioning of zinc finger proteins which, in its turn, inhibits cell division and growth of the seaweeds. The displacement of iron triggers cytochrome-*C*-oxidase complex malfunction and results in oxidative stress. By replacing magnesium in chlorophyll, cadmium decreases light-harvesting ability of the vegetation [16]. Moreover, cadmium is a toxicant which endangers almost all living organisms.

Autumn and winter are the seasons when Fc peaks in *C. barbata* and *C. crinita*. Taking into account that this is the time when the thallus plentifully shoots out new branches, the winter Fc maximum can be associated with active photosynthesis, growth and reproduction. This agrees with the summer decline of xanthophylls in the thalli and with the accumulation of carotenoids in autumn and winter [9]. It is worthwhile noting that the content of Fc in *C. barbata* and *C. crinita* from the Black Sea is comparable with that in some other brown algae.

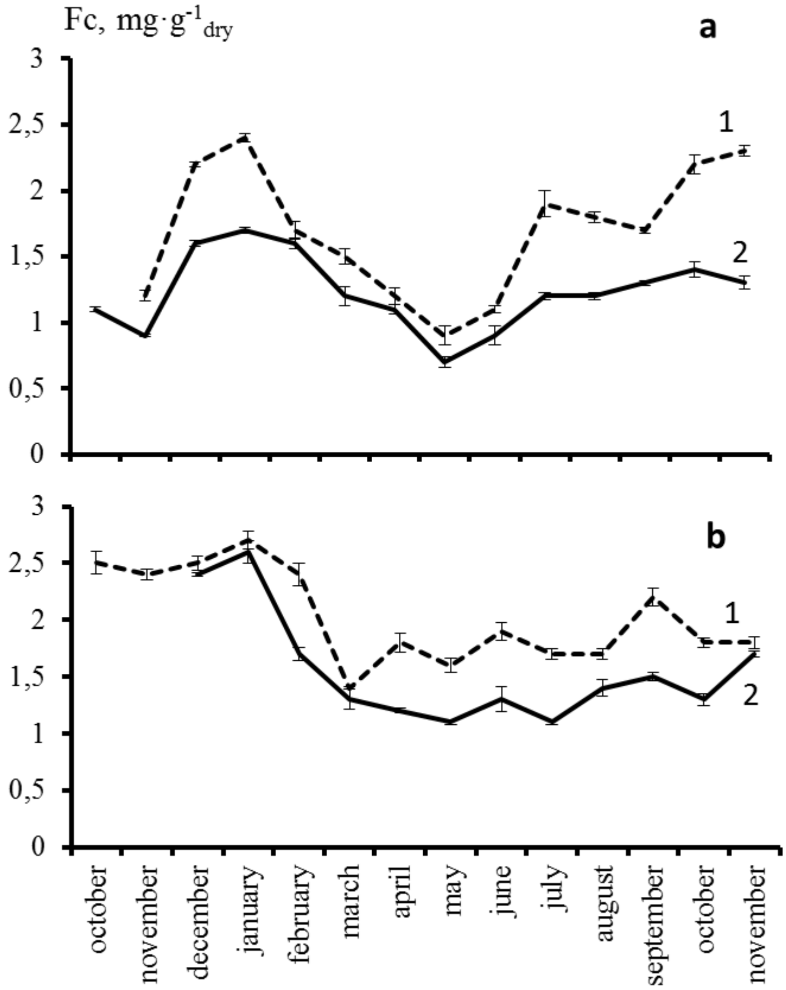

Рис. 7. Seasonal dynamics of fucoxanthin (Fc) in 2- to 3-month-old branches of *Cystoseira barbata* (1) and *C. crinita* (2) collected near Cape Foros (a) and in Karantinnaya Bay (b)

Fig. 7. Сезонная динамика содержания фукоксантина в 2- и 3-месячных ветвях *Cystoseira barbata* и *C. crinita*, собранных у мыса Форос (a) и в Карантинной бухте (b)

According to our observations, the two *Cystoseira* species differ in their preferences for biotopes. Unlike *C. barbata*, *C. crinita* rarely inhabit semi-closed bays, giving preference to open-sea areas. Both *C. barbata* and *C. crinita* have higher concentration of Fc in eutrophicated Karantinnaya Bay than in the open-sea locations (fig. 7).

The decrease of carotene and the appearance of its free-radical metabolites seen in the *Cystoseira* vegetation from the polluted seawater areas can be interpreted as activation of antioxidative processes protecting photosynthetic apparatus of the algae against the induced oxidative stress and photodestruction [3].

Conclusion. To summarize, the larger Fc content, which is characteristic of the brown algae *C. barbata* and *C. crinita* from eutrophicated or heavy-metal-polluted habitats, suggests that this carotenoid plays a key role in developing adaptation of algal photosynthetic apparatus to the human-induced changes in the marine environment.

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ФУКОКСАНТИН И ТЯЖЁЛЫЕ МЕТАЛЛЫ В БУРЫХ ВОДОРОСЛЯХ РОДА *CYSTOSEIRA* C. AGARDH ИЗ АКВАТОРИЙ С РАЗЛИЧНЫМ АНТРОПОГЕННЫМ ВОЗДЕЙСТВИЕМ (ЧЁРНОЕ МОРЕ)

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Бурые водоросли являются признанными биоиндикаторами загрязнения прибрежных морских вод тяжёлыми металлами. Сравнение морфологических и функциональных характеристик водорослей, обитающих в различных экологических условиях, необходимо для понимания механизмов адаптации гидробионтов к антропогенным изменениям окружающей среды. Цель исследования — определение количества фукоксантина и тяжёлых металлов в ветвях бурых водорослей *Cystoseira barbata* (Stackhouse) C. Agardh и *Cystoseira crinita* Duby в акваториях с различным уровнем антропогенной нагрузки. Содержание фукоксантина в пробах определяли методом тонкослойной хроматографии, тяжёлых металлов — атомно-абсорбционной спектрофотометрии. Максимальная концентрация фукоксантина (3 мг·г$^{-1}$ сухой массы), свинца (48.5 мкг·г$^{-1}$), цинка (62.6 мкг·г-^{1}) и кадмия (3.2 мкг·г$^{-1}$) содержится в ветвях водорослей возрастом от 2 до 3 месяцев. Концентрация каротиноида в ветвях *C. barbata* в 1.5 раза выше, чем у *C. crinita*. Цистозиры, обитающие в эвтрофной бухте, содержат большее количество пигмента и цинка, чем макрофиты из открытых акваторий. Повышенное содержание фукоксантина в бурых водорослях, обитающих в эвтрофных и загрязнённых тяжёлыми металлами акваториях, свидетельствует о важной роли каротиноида в адаптации фотосинтетического аппарата водорослей к условиям антропогенных изменений окружающей среды.

Keywords: бурые водоросли, *Cystoseira*, возраст, тяжёлые металлы, фукоксантин, Чёрное море