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Peculiarities of Growth and Metabolism in Japanese Kelp in Habitats Exposed to Chronic Contamination

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A higher content of toxic metals, particularly cadmium and arsenic, was detected in *Laminaria japonica* (Rudnaya Bight, The East (Japan) Sea). These seaweeds were found to have anatomic alterations in the vegetative tissue, inhibition of development of the generative tissue and stronger pigmentation. It was also established that *Laminaria japonica* (in Rudnaya Bight) contained more monovalent metals, like potassium, and less divalent ones, calcium and magnesium, as compared to seaweeds from an ecologically clean area (Cape Mayachnyi, The East Sea). Content of iodine and fucose in seaweeds that grew in Rudnaya Bight was lower than that in seaweeds collected near Cape Mayachnyi. Changes of chemical composition in Japanese kelp from Rudnaya Bight proved to be most evident in seaweeds in the first year of their growth, and the greatest morphological and anatomical changes in vegetative and generative tissues were in the second year.

Key words: toxic elements; Japanese kelp; cortical tissue; parenchyma; conductive tissue; sporiferous tissue; chemical composition

Brown seaweeds are frequently used as indicators of ecological burden on the marine environment. Chronic exposure of marine waters to pollution with toxic metals may alter species composition of vegetation (Klochkova, Berezovskaya, 2001) and age structure in colonies of some brown seaweed species (Parchevsky, Prazukin, Popov *et al*., 1985). Macroalgae's responses to significant contamination of environment are manifested as alterations in their morphometric characteristics and anatomical structure of the lamina (Gusarova, Ivanova, Shaposhnikova, 2005). This agrees well with literature data on the impact that heavy metal pollution of environment exerts on various physiological functions in macrophytes (Zolotukhina, Gavrilenko, Burdin, 1987). Studies of biochemical changes in algal cells caused by toxicity of heavy metals are less represented in literature. First, an impact of heavy metals on chlorophyll synthesis is observed both in higher plants and algae (Ready, Prasad, 1990). In this case fermentative activity in algae gets changed, and chloroplasts and mitochondria destroyed. In areas subjected to a technogenic pollution, content of dry substances in seaweeds decreases (Chmykhalova, Korolyova, 2005). The influence of higher concentrations of toxic elements in the environment on chemical composition of seaweeds is studied poorly.

The goal of this work is to study chemical composition of the Japanese Kelp (*Laminaria japonica*), morphological and anatomical structure of its lamina and generative tissue depending on the conditions in seaweed's habitat.

Materials and Methods

In this work, specimens of *Laminaria japonica* Aresh. were studied according to the classification of the division Phaeophyta by M. J. Wynne (1981). Japanese kelp specimens of the first and the second year of vegetation were collected in August, in the northern part of Rudnaya Bight, close to the Rudnaya River mouth, as well as in near-shore waters around Cape Mayachnyi, East (Japan) Sea (Fig. 1). Length and width of lamina and weight of entire seaweed were measured. Age of kelp specimens was defined by a complex of signs.

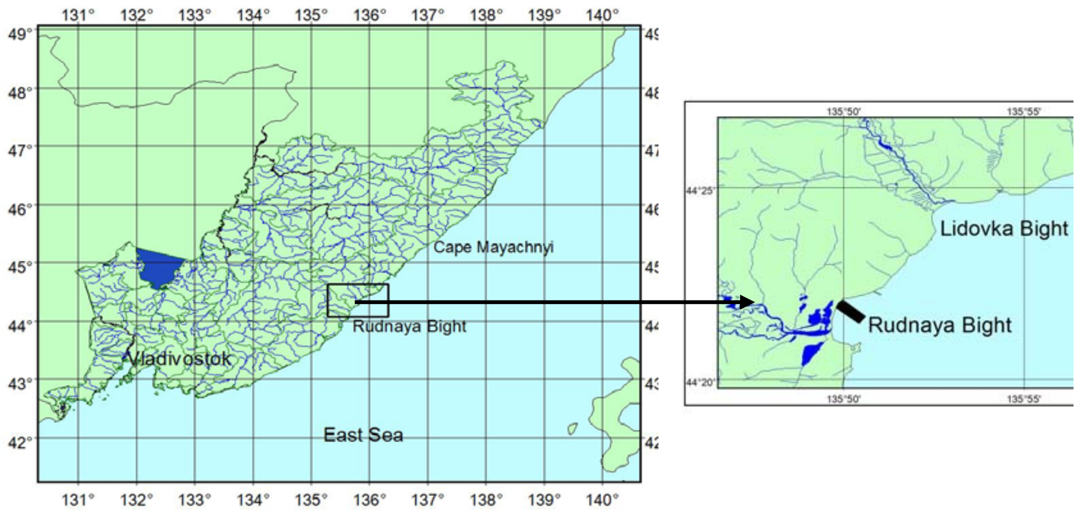

Fig. 1. Map of the studied area

For the histological analysis of the lamina, rectangles 1 cm by 2 cm were cut out in 10 of 25 measured specimens, at the distance of 50 cm from the base of the lamina in the second-year seaweeds and 20 cm in the first-year seaweeds. For the histological analysis of the sporiferous tissue, several areas were cut out of the largest sori. The material was fixed according to the conventional technique (Jensen, 1965).

After the morphometric analysis, seaweeds' thalli were dried at the ambient temperature, grinded and their chemical composition was analyzed. Standard techniques were used in the study (GOST 26185-84. 1984). Macro- and microelemental composition was determined by atomic-absorption spectroscopy with an atomic-absorption flame-emission spectrometer Nippon Jarrell Ash AA-855. Samples were prepared according to the "Methodological recommendations for preparation of samples of environmental objects and fishery products to atomic absorption determination of heavy metals" (Kovekovdova, 1987).

The total content of nitrogenous substances in raw materials was measured by the Kjeldahl method with the FOSS Kjeltec 2300 Analyzer (Sweden). Total lipid content was determined according to the Bligh and Dyer method (Bligh, Dyer, 1959). The content of fucose in algal biomass was measured with the spectrophotometer by the color reaction with L-cysteine and sulfuric acid (Usov, Smirnova, Klochkova, 2001). The total content of carbohydrates was measured by the colorimetric method at the wavelength of 620 nm (Krylova, Lyaskovskaya, 1965).

Results and discussion

On the northern coast of Primorsky Krai, Rudnaya Bight, adjoining the Dalnegorsk ore mining region, is characterized with a higher content of heavy metals in dissolved mineral and organic forms in water, as well as in suspension and soil (Shul'kin, 2004). As concentration of heavy metals in the environment grows, they not only accumulate but also change contents of other elements in seaweeds. It was recorded that a presence of manganese, for instance, stimulates accumulation of iron in proteins of membrane and cytoplasm in Japanese kelp (Tropin, Zolotukhina, 1997). When absorbing heavy metals, seaweeds are known to emit potassium ions (Ready, Prasad, 1990).

We found that elemental composition in Japanese kelp specimens collected in waters with various degree of contamination varied significantly (Table 1).

Table 1. Macro- and microelemantal composition in Japanese kelp from different habitats (in % of dry substance)

Place of collection	Ca	Na	K	Mg	n•10^{-3}							
					Mn	Fe	Zn	Cu	Sr	Cd	Pb	As
Rudnaya Bight	0.7	1.1	7.5	0.6	1.5	112.4	8.9	0.9	30.0	0.095	0.49	18.8
Cape Mayachnyi	1.2	1.1	5.9	0.7	1.7	117.0	4.9	0.7	25.0	0.014	0.22	3.8

Seaweeds from Rudnaya Bight contained higher concentrations of potassium and less cations of divalent metals like calcium and magnesium. Seaweeds from this area showed higher concentrations of zinc, copper, strontium as compared to the specimens collected near Cape Mayachnyi. Significant concentrations of toxic elements, particularly cadmium and arsenic, were also recorded. Higher content of elements like those affects physiological condition of seaweeds that can alter not only their morphological characteristics but also metabolism.

Near Cape Mayachnyi, the laminae of first-year seaweeds were lance-linear in shape, from a light-olive to a dark-olive color, and the second-year ones were linear and brown. Cross sections of laminae of both ages are shown in the Fig. 2.

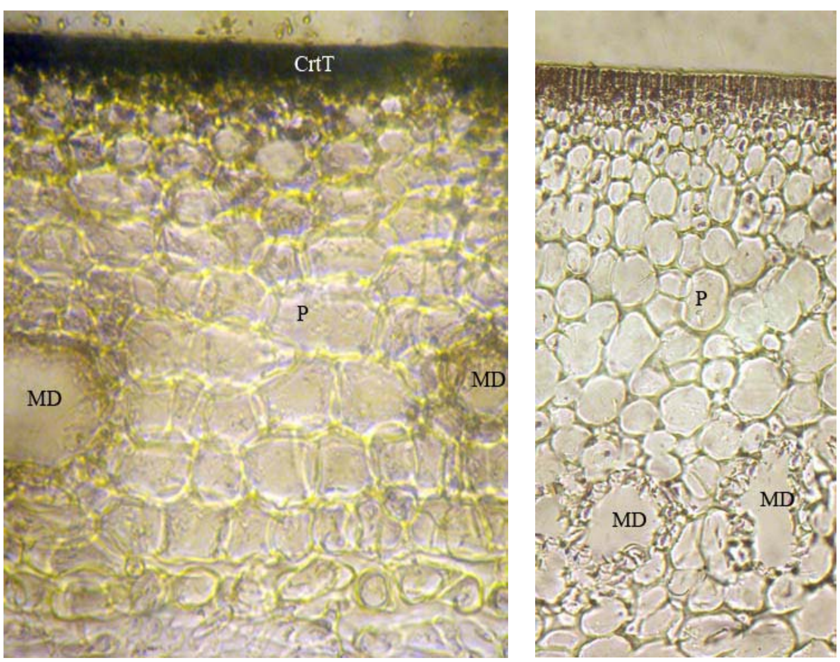

Fig. 2 Cross section of the laminae of first-year and second-year Japanese kelp collected near Cape Mayachnyi (magnification150x). CrtT—cortical tissue; P—parenchyma; MD—mucous ducts

In Rudnaya Bight, the first-year seaweeds featured a saturated dark olive color, and those of the second year were dark brown. An intensive coloration of superficial meristem cells (cuticle, cortical tissue) was found in cross-sections of laminae at various ages (Fig. 3).

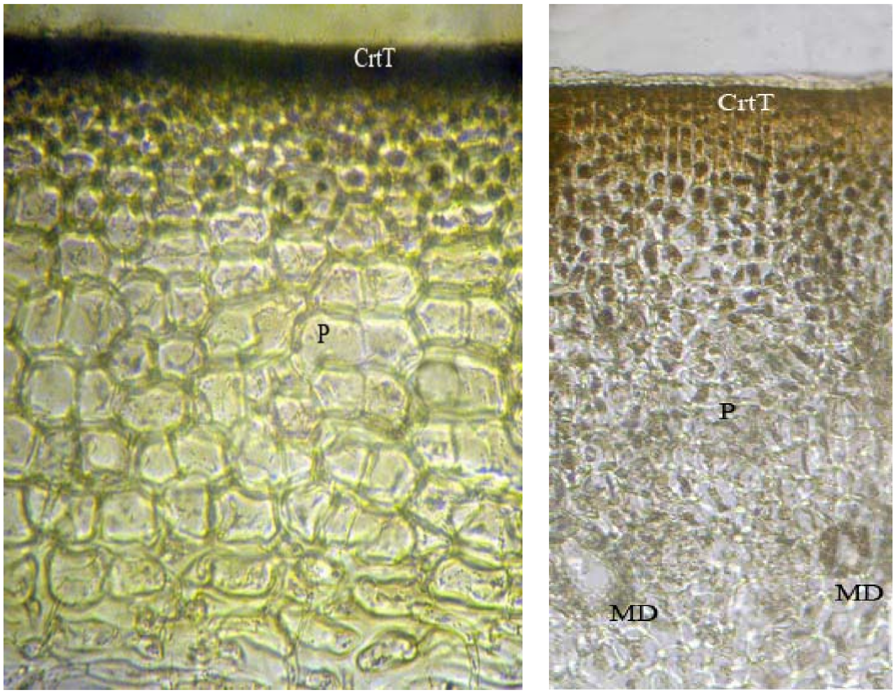

Fig. 3 Cross section of laminae of the first-year and second-year Japanese kelp collected in Rudnaya Bight (magnification 150x). CrtT—cortical tissue; P—parenchyma; MD—mucous ducts

Cortical cells had thick membranes and granular protoplasm. In the second-year laminae, membranes of heterotrophic cells, secretory cells that line mucous ducts, as well as sieve tubes and hyphae of the conductive tissue were colored intensely. Thalli in Japanese kelp of both ages got darker, probably, because of a greater quantity of pigments that evidenced an intensive photosynthesis process, as if they experienced a lack of light (Titlianov, Zvalinsky, 1971).

Laminae of both ages from Rudnaya Bight had thickening of cortical and conductive tissues as a result of increased number of cell rows. At the same time, thickness of the ground parenchyma tissue was found to be reduced that could be related to decreasing the size of heterotrophic cells. In the second-year seaweeds, manifestation of these signs was more obvious than that in the first-year ones. The ratio of functional tissues (cortical : parenchyma : conductive) in the laminae of first-year seaweeds collected near Cape Mayachnyi was 17.8 : 71.7 : 10.5, while that in seaweeds of the same age from Rudnaya Bight – 17.2 : 67.1 : 15.7. In the second-year seaweeds, this ratio was 3.2 : 85.3 : 11.5 and 7.3 : 79.8 : 12.9, respectively. Thickening of the cortical and the conductive tissues in the lamina can be related to activity of photosynthetic processes, which promote seaweeds' adaptation to pollution (Zolotukhina, Gavrilenko, Burdin, 1987). Then the process of accumulation of nutritive substances slows down that causes the parenchyma layer to get thinner.

We did not detect any substantial variations in amount of main organic compounds in second-year Japanese kelp that grew in waters with different levels of contamination (Table 2). However the content of iodine in seaweeds from Rudnaya Bight was much lower than that in seaweeds near Cape Mayachnyi. This was typical both for the second-year and the first-year Japanese kelp, though the latter usually accumulates over 0.2% more iodine in summer.

Table 2. Chemical composition in Japanese kelp of various ages, collected in different habitats (in % of dry substance)

Age, location	Mineral substances	Carbo-hydrates	Lipids	Protein N*6.25	Mannitol	Iodine
First-year kelp, Rudnaya Bight	32.8	15.3	4.8	7.8	13.8	0.019
Second-year kelp, Rudnaya Bight	27.6	14.0	1.1	8.06	14.7	0.127
Second-year kelp, Cape Mayachnyi	26.3	17.3	1.5	8.48	14.5	0.25

Chemical composition in the first-year and the second-year Japanese kelp collected in Rudnaya Bight differed greatly. In total, the first-year seaweeds had 16% less organic substances, 4 times as much lipids and 6.5 times as little iodine as the second-year specimens did (Table 2). Although chemical composition of Japanese kelp of both ages that grew in the conditionally clean area did not differ much (Aminina, Podkorytova, 1992). Thus, growing in contaminated waters substantially influences seaweeds' metabolism that finally has an effect on elemental composition and also on content of some organic compounds, particularly during the first year of seaweeds' growth.

One of organism's biochemical mechanisms for protection against the toxic impact of heavy metals is their removal from main metabolic pathways by binding them in insoluble complexes. A great share of heavy metals in brown algae is supposed to be bound by acidic polysaccharides – fucoidan and alginic acid, and the latter manifests a high sorption activity towards divalent and polyvalent metals (Saenko, 1992).

The analysis showed that content of alginic acid in Japanese kelp from different habitats varied insignificantly. At the same time, the seaweeds near Cape Mayachnyi had 40% more fucoidan (Fig. 4) than those from Rudnaya Bight. After extraction of fucoidans, wastes of Japanese kelp from Rudnaya Bight contained 3 times as much fucose (the main monomer unit of fucoidan) as those from the background area (Fig. 5). Probably the fractional structure of fucoidans in seaweeds from various habitats may vary significantly.

Higher concentrations of heavy metals in the environment are known to affect reproduction in some of seaweed species (Gusarova, Ivanova, Shaposhnikova, 2005). And disturbances in polysaccharide synthesis can be one of the main causes of this. Particularly, a dependence between beginning of sporification and higher content of fucoidan was established in brown algae (Imbs, 2010).

Our studies showed that all the Japanese kelp specimens collected near Cape Mayachny had sporiferous tissue. In cross sections of large sori of second-year seaweeds, sporiferous tissue had a mosaic structure (Fig. 6a), in which sporangia 50–55 µm in length and 9–10 µm in width, with a granular content and mucous covers on tops prevailed. Sporangia in first-year seaweeds were at least 30–35 µm in length and up to 9 µm in width, with a homogenous content and flat mucous thickening in the upper part (Fig. 6b).

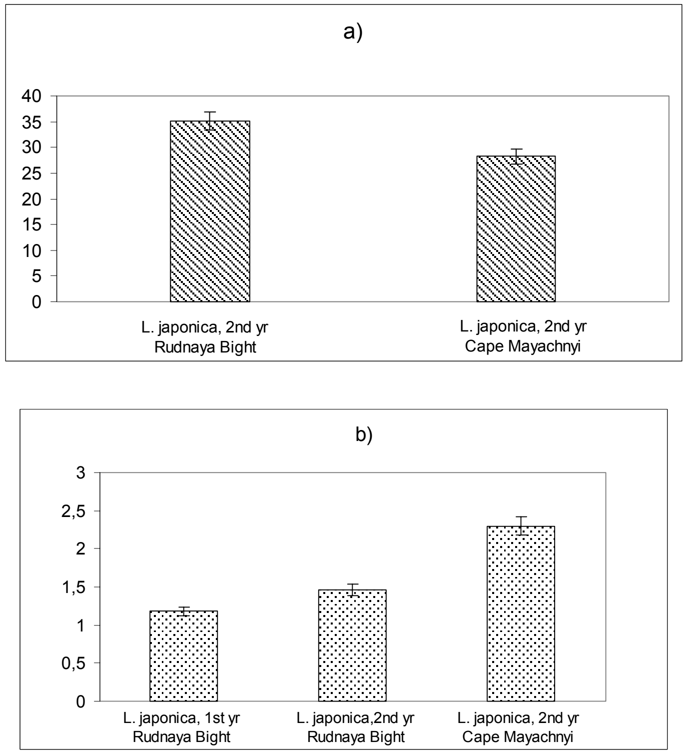

Fig. 4 Content of alginic acid (a) and fucoidan (b) in Japanese kelp from different habitats (in % of dry substance)

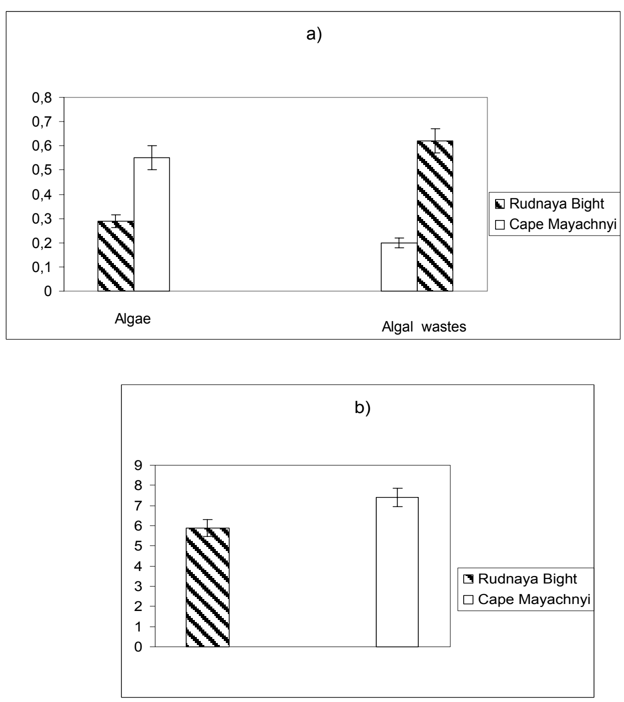

Fig. 5 Content of fucose in Japanese kelp from different habitats after polysaccharide extraction (a) and in polysaccharides (b) (in % of dry substance)

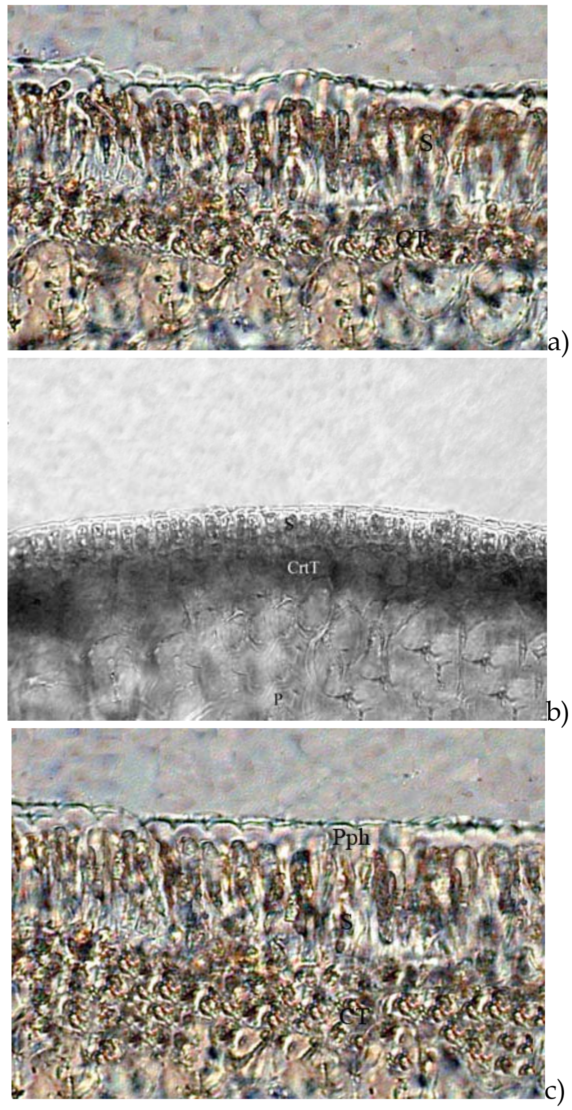

Fig. 6 Sporiferous tissue in the second-year (a) and first-year (b) Japanese kelps collected near Cape Mayachnyi and sporiferous tissue in the second-year Japanese kelp collected in Rudnaya Bight (c) (magnification 250x). S—sporangia; Pph—paraphyses; CT—cortical tissue; P—parenchyma

In Japanese kelp from Rudnaya Bight, sporangia with homogenous content prevailed in the second year of vegetation (Fig. 6c). The sporiferous tissue in the first-year plants was at various phases of sporangium maturity. In such a way, we observed a delay in maturation of sporiferous tissue both in the first-year and the second-year seaweeds.

Conclusion

Growing in polluted waters influences metabolism in seaweeds significantly that has an effect both on their elemental composition and content of some organic compounds. Amount of iodine and fucose in Japanese kelp from the environment exposed to chronic contamination (Rudnaya Bight, East Sea) is lower as compared to seaweeds from conditionally clean waters (Cape Mayachnyi, East Sea).

Chronic pollution of the environment causes morphological and anatomical alterations in the vegetative tissue that are manifested in thicker cortical and conductive tissues and a thinner layer of the ground parenchyma tissue. Stronger pigmentation is also observed. Forming of the generative tissue in seaweeds slows down both in the first and the second year of vegetation.

Changes in chemical composition of Japanese kelp that grows in Rudnaya Bight become most obvious in first-year seaweeds, and morphological and anatomical changes in the vegetative and generative tissues – in second-year ones.

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