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[1]Bayraktar V. N., [2]Polukarova L. A.

SUMMER SEASONAL ENZYME ACTIVITY, MACRO- AND MICROELEMENTS IN MACROPHYTES ISOLATED FROM LITTORAL AQUATORIES OF THE TILIGUL ESTUARY

[1]Odessa Mechnikov National University;
[2]University Clinic of Odessa National Medical University

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In the article given results of the summer seasonal enzyme activity, concentration of macro-and microelements, some parameters of cellular metabolism in macrophyte isolated from littoral aquatories of the Tiligul estuary. It was indicated enzyme activity in the summer.

Investigation of qualitative and quantitative composition of macrophyte and their biochemical parameters on example of enzyme activity, concentration of macro-and microelements, some metabolic parameters allow us better and fully to assess ecological status of coastal waters of small rivers [1, 4]. Green and red algae living in littoral aquatories significantly act to the common ecological condition and furthermore, red algae also are indicators of environmental situation in water ecosystem [1, 6]. Hydrochemical parameters of water and soil also needs to be monitored for timely assessment of possibly changes. Environmental monitoring is an integrated observation system the environmental situation of the ecology which allows to evaluate and forecast the state of the environment under the influence of natural and anthropogenic factors. Problem of ecological monitoring due to the increasing anthropogenic and industrial impact on the environment are actual. When water pollution changes the mactophyte species composition, their biomass production, morphological abnormalities occur. There is a change of dominant species that allow to determine features of cenosis [2, 11].

Phosphorus limitation in phytoplankton has been studied using a variety of methods including nutrient incubation experiments and direct measurements of phosphorus uptake, cellular phosphorus content, nutrient ratios, and the presence of alkaline phosphatase activity (APA) [8, 9, 10]. The phosphatases are enzyme responsible for liberation of inorganic phosphate from organic phosphate esters. The algal samples were inoculated in medium contains p-nitrophenyl phosphate and if phosphate was liberated from p-nitrophenyl phosphate color changes which is positive test for phosphatase enzyme. Phosphatase enzymes are believed to have an essential function in nutrients dynamics in aquatic habitats since they promote the degradation of complex phosphate compounds into orthophosphate and organic moieties. After 24 hours of incubation macrophyte

samples Ulva (Enteromorpha) and mixed partially death algae were found to be phosphatase positive [3, 5, 7].

The aim of this study was to determine biochemical parameters of macrophyte species obtained from the Tiligul estuary in summer, and then for further to monitor changes in the aquatic macrophytes in the Tiligul estuary. To achieve these goals, we formulatied several tasks: to identify the macrophyte species growing in the littoral waters in the Tiligul estuary. To determine such biochemical parameters as: enzyme activity, and the concentration of macro- and micro-elements in macrophyte homogenates. This research was carried out in the summer season. Mactophyte homogenate enzyme activity was investigated for such cellular enzymes as: lactate dehydrogenase, aspartate aminotransferase, amylase, alkaline phosphatase, and alanine aminotransferase.

MATERIAL AND METHODS

The samples of macrophytes for investigation were taken from waters the littoral aquatories of the Tiligul estuary. Biochemical parameters of macrophytes were tested in the summer. Over this period of time, we investigated each of 5 littoral aquatories in the recreation areas of the Tiligul estuary, in addition to identifying parameters of macro- and micro- element concentration in macrophyte homogenates based on a principle of photometric analysis, carried out using a biochemical analyser Respons-920 (DiaSys Diagnostic Systems GmbH, Germany). All tests were conducted using reagents, and specific test kits for each tested parameter, incuding: calcium, phosphorus, magnesium, iron, and chloride. The kits were produced by the BioSystems Company (S.A. Costa Brava, Spain). Test kits for investigation of Chloride were produced by the Pliva Company, (Lachema Diagnostika, Brno, Czech Republic). Investigation of electrolyte, such as sodium and potassium, were carried out using ionometric determination by a biochemical analyser ILyte Na/K with ionselective block (produced by Instrumentation Laboratory Inc., Bedford, MA, USA). The most important cations for macrophytes investigation are: sodium, potassium, calcium, magnesium, and iron. The most important anions for investigation of macrophytes are: chloride and phosphorus. Macrophyte species collected from littoral aquatories of the Tiligul estuary. Algal tissue homogenized in the universal laboratory homogenizer, type MPW-309, laboratory tissue homogenizer cell disrupter (produced in Poland). Statistical deviation and significance were evaluated by Student's t-test with P-value: $P < 0.1$; $P < 0.05$; $P < 0.01$.

RESULTS AND DISCUSSION

In the summer period, littoral waters are not always clear, with signs of foaming at the edges of coastal waters from the surface-active substances. According to our investigation of coastal aquatic flora in the Tiligul estuary, conclusions can be reached concerning the processes of eutrophication. As a result of these processes, the estuary is formed from the excess of inorganic nutrients, through flushing them with coastal soils as a result of rainfall [1]. The

compounds nitrogen, phosphorus, iron, potassium, and sulphur, are necessary for the normal development of aquatic plants as well as macrophytes. In the littoral aquatories, we noted rich grow of Zostera noltei Hornemann, 1832, which is not algae, but is an aquatic plant. Their abundance on the estuary water surface is associated in summer with cyanobacteria and increased growth of micro- organisms that consume all of the adjacent oxygen dissolved in the water, leading to the rotting seaweed that can be observed washed ashore. This manifests in the marked overgrowth of watercourse aquatic vegetation, and in the presence of macrophytes indicating the ecological status of Tiligul estuary coastal waters, such as: Cladophora laetevirens (Dillwyn) Kützing, 1843. An abundant influx of nutrients comes about due to human activities; in particular with the presence of human recreational bases in the village Koblevo, in the Berezan district of the Nikolaev region. We indicated the following species of macrophytes Cladophora laetevirens (Dillwyn) Kützing, 1843, Ceramium rubrum (C. Agardh, 1811), Ulva intestinalis Linnaeus, 1753, Ulva prolifera O. F. Müller, 1778, and Rhizoclonium tortuosum (Dillwyn) Kützing, 1845, and some others. Macrophyte species isolated from Tiligul estuary were identified in appearance, and microscopic analysis. In the Fig.1-2 shown two species of macrophyte examples, genus Polysiphonia: P.sanguinea and P. elongata.

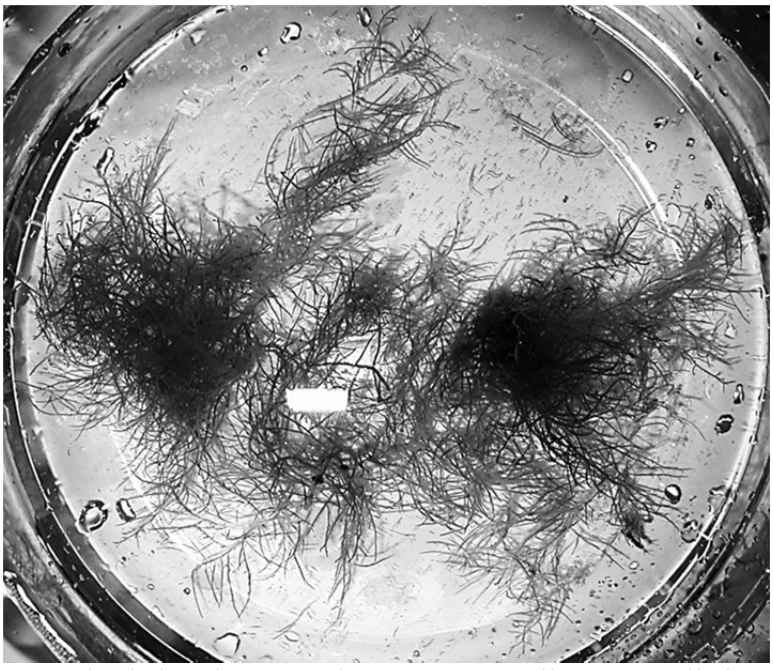

Fig.1. Polysiphonia sanguinea (C.Agardh) Zanardini, 1840

Polysiphonia sanguinea, has red unstable pigment. In the natural conditions, aquatories of Tiligul Estuary bloody red hue of macrophyte Polysiphonia sanguinea, but enough to place samples of Polysiphonia sanguinea in bottle for transportation for 15-20 minutes and bluddy red pigment at once will be disappeared.

Fig.2. Polysiphonia elongata (Hudson) Sprengel, 1827

Anthropogenic inputs of nutrients to coastal waters have rapidly restructured coastal ecosystems. Macrophyte biomass increased as nitrogen loads increased, but the response of individual taxa varied. Specifically, biomass of *Cladophora albida*, *Cladophora latevierens* and *Polysiphonia elongata* increased significantly as nitrogen loads increased. The biomass of other macroalgal taxa tended to decrease with increasing load, and the relative proportion of these taxa to total macrophyte biomass also decreased. The seagrass, Zostera noltei, disappeared from the higher loaded estuaries, but remained abundant in the estuary with the lowest load. Summer seasonal changes in macroalgal standing stock were also affected by nitrogen load, with larger fluctuations in biomass across the year and higher minimum biomass of macroalgae in the higher loaded estuaries. Macroalgal biomass was not related to irradiance or temperature, but Zostera noltei biomass was highest during the summer months when light and temperatures peak. Irradiance might, however, be a secondary limiting factor controlling macroalgal biomass in the higher loaded estuaries by restricting the depth of the macroalgal canopy. The relationship between the bloom-forming macroalgal species, *Cladophora albida*, *Cladophora latevierens* and *Polysiphonia elongata*, and nitrogen loads suggested a strong connection between development on watersheds and macroalgal blooms and loss of seagrasses. The influence of watershed land uses largely overwhelmed seasonal and inter-annual differences in standing stock of macrophytes in these temperate of the Tiligul estuary.

Table 1.

Enzyme activity in the summer in macrophyte homogenate collected from littoral aquatories of the Tiligul estuary

Enzyme activity (μmol/min x 10^{-2}L)	Macrophyte species						
	Bryopsis plumosa	**Cladophora albida**	**Ulva compressa**	**Chondria tenuissima**	**Polysiphonia elongata**	**Ulva intestinalis**	**Ulva flexuosa**
Alanine aminotranspherase	400±13,5	207,5±2,6	119,5±1,1	104,5±0,9	220,7±1,1	114,5±0,9	232,4±0,4
Amylase	3,8±0,7	5,2±0,3	7,9±0.3	2,3±0,1	4,2±0.2	3,5±0,6	4,9±0,3
Aspartate aminotranspherase	732,8±3,0	152,7±1,9	227,4±2,9	86,3±0,4	195,8±0,4	743,6±2,6	358,5±5,8
Lactate dehydrogenase	142,7±3,6	99,6±2,2	78,0±0,4	109,5±0,9	169,3±0,9	36,5±1,1	87,9±0,2
Phosphatase (alkaline)	8,3±0,2	66,4±1,9	**1,6±0,05	6,6±0,3	4,9±0,2	*6,6±0,1	5,7±0,6
Cholinesterase	66,4±0,3	43,6±0,9	43,8±0,9	3,3±0,2	6,6±0,2	**3,3±0,04	*11,6±0,1

*Note: *P≤0,1; **P≤0,05; The standard deviation, and the statistical significance of differences evaluated by Student t-test (P≤ 0.05 and P≤ 0.01).*

Table 2.

Concentration of macro- and microelements in the autumn in macrophyte homogenate collected from littoral aquatories of the Tiligul estuary

Macro- and microelement concentration (mmol/L)	Macrophyte specie						
	Bryopsis plumosa	**Cladophora albida**	**Ulva compressa**	**Chondria tenuissima**	**Polysiphonia elongata**	**Ulva intestinalis**	**Ulva flexuosa**
Potassium (mmol/L)	**7,84±0,01	6,83±0,07	5,9±0,07	14,0±0,2	*7,95±0,05	38,3±1,2	5,91±0,1
Sodium (mmol/L)	52,8±0,7	64,3±0,2	54,7±0,2	136,0±0,2	103,2±0,4	49,3±0,8	122,4±0,8
Calcium (mmol/L)	*2,2±0,05	**0,86±0,01	**0,1±0,007	7,7±0,2	3,84±0,08	3,01±0,2	4,39±0,1
Phosphorus (mmol/L)	**0,36±0,02	**0,24±0,01	**0,07±0,006	**0,56±0,01	0,45±0,02	*0,38±0,02	*0,23±0,02
Magnesium (mmol/L)	**2,24±0,02	*1,1±0,05	**1,02±0,003	*3,61±0,02	**1,62±0,04	*1,36±0,04	1,61±0,09
Iron (μmol/L)	**0,9±0,02	1,0±0,1	*0,5±0,05	4,5±0,3	4,3±0,2	6,0±0,2	8,0±0,4
Chloride (mmol/L)	66,4±0,3	37,7±0,4	14,5±0,1	183,1±1,4	133,4±3,2	67,6±1,1	140,8±1,4

*Note: * P≤0,05; ** P≤0,01; The standard d eviation, and the statistical significance of differences evaluated by Student t-test (P≤ 0.05 and P≤ 0.01).*

Table 3.

Metabolism parameters in the autumn in macrophyte homogenate collected from littoral aquatories of the Tiligul estuary

Parameter of Metabolism	Macrophyte species						
	Bryopsis plumosa	**Cladophora albida**	**Ulva compressa**	**Chondria tenuissima**	**Polysiphonia elongata**	**Ulva intestinalis**	**Ulva flexuosa**
Protein (total) (g/L)	*0,3±0,02	0,09±0,3	0,2±0,5	*0,6±0,03	**0,05±0,01	*0,06±0,02	**0,08±0,002
Glucose (mmol/L)	*0,2±0,02	*0,01±0,04	**0,02±0,002	**0,14±0,01	*0,16±0,02	*0,01±0,04	**0,01±0,001
Trigliceride (mmol/L)	*0,12±0,02	*0,32±0,02	**0,06±0,01	*0,42±0,03	*0,34±0,02	*0,25±0,03	**0,33±0,01
Notrogen (mmol/L)	**1,48±0,01	**0,93±0,01	**0,95±0,04	**0,57±0,01	*0,81±0,02	*0,36±0,02	**0,11±0,01

*Note: * P≤0,05; ** P≤0,01; The standard deviation, and the statistical significance of differences evaluated by Student t-test (P≤ 0.05 and P≤ 0.01).*

The effects of temperature on enzyme activity are complex and include the effect on stability of the Phophatase alkaline, the effect on the actual velocity of breakdown of the complex and the effect on the enzyme-substrate affinity. In the benthic algae tested, the optimum temperature for Alkaline Phosphatase is usually higher than temperatures likely to occur in nature, often with values ranging from 25 to > 30 °C . This has a clear implication for the so-called optimum conditions for the enzymatic assay. Values are generally lower than, as occurs typically in many enzymes in macrophyte species.

Although a rise in assay temperature for material sampled at a particular time probably leads in most cases to an increase in Alkaline Phosphatase, this does not mean that activity for a particular species is necessarily highest at the time of year when field temperature are highest. The Alkaline Phosphatase of benthic intertidal macroalgae is affected by salinity. Enzymatic activity has been shown to be very low under low salinities and to increase with increasing salinity up to 45–50‰, where maximum Alkaline Phosphatase was found. However, several days of exposure under low salinity caused an enhancement of Alkaline Phosphatase in Cladophora albida and Ulva rigida by the hyposaline stress and a decrease in tissue. The effect of salinity includes both an osmotic component and one which increases with concentration of particular ions. The two effects can be distinguished if some of the main salts contributing to changes in salinity are replaced with a non-electrolyte. The effect of salinity on Alkaline Phosphatase seems to be attributable not only to the ionic strength, but to a specific effect of particular cations as Na+ or Mg2+. As the intertidal zone may be subject to a broad range of salinity conditions during various combinations of the tidal cycle and climatic conditions, ranging from almost fresh water after heavy rain to high salinity at high irradiance and strong desiccation, the effect of these different salinity conditions on Alkaline Phosphatase requires further investigation. Phosphatase activity of benthic macroalgae usually shows an optimum alkaline pH for activity, mostly between 8.7–9, a value likely in the field only in shallow pools of the upper littoral zone, where high pH values are often reached.

CONCLUSIONS

The macrophyte species that demonstrated high fermentative activity and high macro- and micro-element concentrations, as well as indicating a high correlation between enzyme activity, macro- and micro-elements and some parameters of cellular metabolism.

Comparison of cellular enzyme activity in macrophyte showed high levels of fermentative activity for Lactate Dehydrogenase, Alanine aminotransferase, Aspartate aminotransferase, Cholinesterase. At the same time determined low levels of Phosphatase (alkaline), and Amylase.

This analysis of macroalgal biomass enzymatic activity and canopy height in the Tiligul estuary, as well as suggest that peak macroalgal accumulation may be spurred by increased nitrogen supply, but ultimately the canopy is limited by

light availability. Since macroalgal biomass in the Tiligul estuary falls within the range, it seems that similar processes may be occurring elsewhere. We would therefore anticipate that macroalgal biomass would increase gradually and then as self-shading restricts the rate of photosynthesis deeper in the macroalgal canpopy may be more frequent, or the specific macroalgae involved may have more efficient photosynthetic abilities.

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[1]Байрактар В. Н., [2]Полукарова Л. А.

ЛЕТНЯЯ СЕЗОННАЯ АКТИВНОСТЬ ФЕРМЕНТОВ, МАКРО - И МИКРОЭЛЕМЕНТОВ У МАКРОФИТОВ ВЫДЕЛЕННЫХ ИЗ ПРИБРЕЖНЫХ АКВАТОРИЙ ТИЛИГУЛЬСКОГО ЛИМАНА

Ключевые слова: *макрофиты, активность ферментов, макро- и микроэлементы, метаболизм.*

В статье даны результаты сезонной активности ферментов,

концентрации макро- и микроэлементов, некоторые показатели клеточного метаболизма у макрофитов выделенных из прибрежных акваторий Тилигульского лимана. Была установлена активность ферментов в летний период.

[1]Байрактар В. М., [2]Полукарова Л .А.

ЛІТНЯ СЕЗОННА АКТИВНІСТЬ ФЕРМЕНТІВ, МАКРО- І МІКРОЕЛЕМЕНТІВ У МАКРОФІТІВ ВИДІЛЕНИХ З ПРИБЕРЕЖНИХ АКВАТОРІЙ ТИЛІГУЛЬСЬКОГО ЛИМАНУ

Ключові слова: *макрофіті, активність ферментів, макро- і мікроэлементів, метаболизм.*

У статті дано результати літньої сезонної активності ферментів, концентрації макро- і мікроелементів, деякі показники клітинного метаболізму у макрофітів виділених з прибережних акваторій Тилігульського лиману. Була встановлена активність ферментів в літній період.