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INTERNATIONAL JOURNAL ON ALGAE

AIMS & SCOPE

The algae are heterogeneous assemblage of phytosynthetic organisms, one of the most vast and diverse groups of ancient photoautotrophic pro- and eukaryotic organisms (about 30 000 known species). They are micro- and macroscopic, unicellular, colonial, or multicellular, mobile and immobile, attached and free-living. Algae are widespread in water and soil habitats, at different geographic latitudes, and on all continents. They occur in waters with different degrees of salinity, trophicity, organic matter, and hydrogen ions, and at various temperatures. They include planktonic, periphytonic and benthic organisms. Algae are unique model organisms in evolutionary biology and also are used in various genetic, physiological, biochemical, cytological, and other investigations. Algae have practical significance as edible or poisonous plants, as indicator organisms in the monitoring of ecological systems, as agents of self-purification of polluted waters and in the purification of sewage, as the primary producers in the trophic chains of hydrobionts in marine and freshwater, and also as organisms for biotechnology.

The quarterly *International Journal on Algae* (*IJA*) publishes selected papers translated from the first Russian language phycological journal, *Algologia*, founded in 1991 in the former Soviet Union. The aim of *Algologia* is to present recent advances in algology. The journal covers both fundamental and applied aspects in algology, including papers based on the results of wide range of field and experimental studies, as well as reviews and surveys and procedure papers. The journal is intended for specialists in theoretical, experimental, and applied algology, hydrobiology, microbiology, all scientists using algae as a model organisms for research, and all those interested in general problems of biology.

The aim and scope of *IJA* is to inform the western scientific community, especially algologists, about original studies by scientists of the former Soviet Union and Eastern Europe in the following subjects: General Problems of Algology; Morphology, Anatomy, Cytology; Reproduction and Life Cycles of Algae; Genetics; Physiology, Biochemistry and Biophysics; Ecology, Cenology and Conservation of Algae and their Role in Nature; Flora and Geography; Fossil Algae; Systematics, Phylogeny and Problems of Evolution of Algae; New Taxa and Noteworthy Records; and Applied Algology.

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Amino Acid Content of Benthic Macroscopic Growths of Algae and Sediments in Hypersaline Water Bodies*

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ABSTRACT

The amino acid content of benthic growths of algae and sediments (peloids) in hypersaline water bodies has been investigated and analyzed for Berdiansk Spit and Arabatskaya Spit (northwestern coast of the Azov Sea, Ukraine). The content of amino acids of benthic macroscopic growths of algae correlates with amino acids of corresponding peloids within 93–98%. Accumulation of amino acids in peloids depends on the reductive-oxidative conditions in hypersaline water bodies, including the amount of biomass and species of benthic macroscopic growths of algae.

KEYWORDS: amino acids, benthic growths of algae, hypersaline water bodies, peloid.

INTRODUCTION

The territory of the northwest coast of the Azov Sea is characterized by the formation of a significant number of small water bodies, the salinity of which corresponds to the hypersaline content according to the Venice system (Venice system ..., 1959).

Salt content in the water is a limiting factor for the existence of plant and animal organisms. Algae are the core group of producers in hypersaline water bodies.

Biologically active substances of algae (Sirenko & Kozitskaya, 1988) during the process of life and destruction of their organic matter may accumulate in sediments. Amino acids are characterized by significant participation in metabolic processes. Most of the algae are investigated from the point of quality and sometimes in the quantitative content of the different amino acids (Barashkov, 1972; Sirenko & Kozitskaya, 1988; Tartarotti et al., 2001; McClelland & Montoya 2002; Daume et al., 2003; Kalashnikova et al., 2004; Kolmakova et al., 2004; Tartarotti & Sommaruga, 2006; El-Sheekh et al., 2011; Hassan et

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al., 2012; Koch et al., 2012; Jegan et al., 2013). There is a lot of literature on dissolved amino acids in the thickness of seawater and marine sediments (Blackburn & Henriksen, 1983; Gonzalez et al., 1983; Aufdenkampe et al., 2001; Keil, 2001; Boyle et al., 2004; Nunn & Keil, 2006; Teira et al., 2006; Tesdal et al., 2013). At the same time, the amino acid composition of algae forming benthic macroscopic growths in a hypersaline water body with a non-regular hydrological regime within a depth of up to 1 m, where they are the only producers, has not been studied. Organic substances in the bottom sediments of peloids in this case are formed by algae mortmass.

The aim of this article is to specify the amino acid composition of benthic growths of algae and peculiarities of amino acid accumulation in peloids of hypersaline water bodies of the northwest coast of the Azov Sea.

Research objectives:

– the specification of biomass of macroscopic benthic growths of green and blue-green algae;

– the analysis of the qualitative and quantitative composition of amino acid macroscopic growths of algae and peloids;

– the revelation of the peculiarities and similarity degree of the amino acid composition of algae and peloid macroscopic growths in hypersaline water bodies.

MATERIALS AND METHODS

Material for this research was represented by samples of algae and peloid benthic macroscopic growths in hypersaline water bodies of the northwest coast of the Azov Sea (Ukraine): on the Berdiansk Spit near the Red Lake and on Arabatskaya Spit near the Zjablovskoye Lake.

Algological material and samples of peloids were selected for analysis in 2011 in the spring-summer period. The collection of algae was carried out according to generally accepted hydrological techniques in three replicates from the surface of the bottom of the water body by means of a scraper (Zilov, 2009). For the study of algae, light microscopes "Biolam R-14", "EC XY series" and stereoscopic microscope MBS-1 were used. Identification was carried out according to the appropriate identification literature (Komárek & Anagnostidis, 1998, 2005). Biomass of algae was determined by the countable-volume method (Algae ..., 1989).

The samples of peloids were selected under benthic macroscopic growths of algae by means of a tube sampler in three replicates with a depth of 0–10 cm.

Specification of the amino acid composition was performed in the A.V. Palladin Institute of Biochemistry by the method of ion-exchange liquid-column chromatography on an auto-analyzer of amino acids T 339 (produced by "Microtechna" Czechia).

Acid hydrolysis was carried out by chloride acid for the educing of amino acids linked in proteins and peptides. The hydrolysis was held according to the following scheme: the thoroughly weighted amino acids within the accuracy of 0.001 g sample was placed on the bottom of tubes made of heat-resistant glass (Pyrex). The corresponding amount of 6 N chloride acid was added to a dry sample in the tube. The tube was cooled in liquid nitrogen. After the contents of the tube got frozen, the air was pumped with the help of a vacuum pump to prevent oxidation of amino acids as a result of hydrolysis. Then the tube was sealed. A sealed tube was put to a thermostat with a constant temperature +106 °C for 24 hours. At the end of hydrolysis, the test tube precooled to room temperature and then opened. The content was quantitatively transferred to a glass beaker and CL type acid was evaporated in a water bath. Then the sample was dissolved in a 0.3-normal lithium-citrated buffer (pH 2.2) and was put on an ion-exchange column of the amino acid analyzer.

Ninhydrin was used for detection and registration of amino acids in eluates. Ninhydrin reagent was added to a liquid, which was washed out of the column. Then the mixture was heated at 100 °C in the reaction bath.

In order to calculate the amount of amino acids in the sample, a standard mixture of amino acids with a known concentration of each amino acid was added to the column of the automatic amino acid analyzer. The qualitative composition of the mixture of amino acids was determined by comparing the chromatograms of the standard and tested mixtures of amino acids (Kozarenko, 1975).

RESULTS AND DISCUSSION

In the hypersaline water bodies of the Berdiansk Spit green algae *Cladophora siwaschensis* C. Meyer forms benthic growths (Fig. **1**) and on the Arabatskaya Spit the blue-green algae *Lyngbya aestuarii* (Mertens) Liebm. and *Microcoleus chthonoplastes* (Fl. Dan) Thuret (Fig. **2**) were found.

The salinity of hypersaline water bodies of the Berdiansk Spit was 160–190%, and on the Arabatskaya Spit it was 140-170 PPT. The biomass of benthic growths of algae *L. aestuarii* and *M. chthonoplastes* revealed changes in the analyzed period from 30 to 55 g/m^2, and *C. siwaschensis* – from 175 to 180 g/m^2.

Under the growths of algae in the studied water bodies a peloid of black color with a smell of hydrogen sulfide and width of 10–20 cm was noticed at the Berdiansk Spit, and with a width of 50–100 cm on the Arabatskaya Spit. These peloids belong to highly mineralized light-sulfide and mid-sulfide chloride-magnesium-sodium ones (Solonenko, 2012).

Fig. **1**. Benthic macroscopic growths of *Cladophora siwaschensis* in hypersaline water bodies of the Berdiansk Spit

Fig. **2**. Benthic growths of *Lyngbya aestuarii* and *Microcoleus chtonoplastes* in hypersaline water bodies of the Arabatskaya Spit

It is known that the spectrum of amino acids for each species of algae is very specific (Barashkov, 1972; Sirenko & Kozitskaya, 1988). For example, green algae are characterized by a prevalence of lysine, arginine, and glutamine acid (Barashkov, 1972), and blue-green alga *Phormidium uncinatum* (Agardh) Gomont, *Anabaena cylindrica* Lemmerm., *A. flos-aquae* (Lyngb.) Bréb. by a prevalence of arginine (Sirenko & Kozitskaya, 1988). Depending on the type of algae, the second place in the spectrum can be occupied by alanine – *Phormidium uncinatum*, valine – *A. cylindrical*, alanine – *A. flos-aquae* (Barashkov, 1972). As for *Aphanizomenon flos-aquae* (Lyngb.) Ralfs the first place is occupied by histidine, the second – by arginine (Barashkov, 1972). Studies revealed 17 amino acids in benthic macroscopic growths of algae and peloids (Table 1).

In benthic macroscopic growths of the green algae *Cladophora siwaschensis* and blue-green algae *Lyngbya aestuarii* and *Microcoleus chthonoplastes* glutamic and aspartic acid, glycine prevailed in quantitative terms. These acids took the first places in the corresponding spectra.

TABLE 1. Amino acid content of benthic growths of algae and peloids in hypersaline water bodies of Berdjansk Spit and Arabatskaya Spit, %

Amino acids	Berdjansk Spit			Arabatskaya Spit		
	Place in the spectrum by number in biomass of algae	Algae, %	Peloids, %	Place in the spectrum by number in biomass of algae	Algae, %	Peloids, %
Glutamic acid	1	0.157±0.005	0.098±0.005	1	0.035±0.003	0.061±0.003
Aspartic acid	2	0.123±0.004	0.085±0.005	2	0.032±0.003	0.035±0.002
Glycine	3	0.074±0.003	0.059±0.004	3	0.024±0.002	0.029±0.003
Leucine	4	0.072±0.003	0.042±0.003	5-6	0.020±0.002	0.030±0.003
Alanine	5	0.072±0.003	0.051±0.004	4	0.021±0.002	0.028±0.002
Valine	6	0.066±0.003	0.040±0.003	5-6	0.020±0.002	0.026±0.002
Lysine	7	0.053±0.003	0.032±0.003	12	0.009±0.002	0.017±0.002
Threonine	8	0.049±0.003	0.036±0.003	7-8	0.017±0.002	0.022±0.002
Serine	9	0.047±0.003	0.037±0.003	7-8	0.017±0.002	0.021±0.002
Phenylalanine	10	0.045±0.003	0.031±0.002	9	0.014±0.002	0.027±0.002
Tyrosine	11	0.044±0.003	0.027±0.002	14	0.005±0.001	0.010±0.001
Arginine	12	0.042±0.003	0.035±0.003	10-11	0.011±0.002	0.016±0.002
Isoleucine	13	0.041±0.003	0.026±0.002	10-11	0.011±0.002	0.019±0.002
Proline	14	0.030±0.003	0.027±0.002	13	0.006±0.001	0.018±0.002
Histidine	15	0.014±0.002	0.006±0.001	15	0.003±0.0005	0.005±0.001
Methionine	16	0.013±0.002	0.003±0.0005	17	0.001±0.0005	0.002±0.0005
Cystine	17	0.010±0.001	0.003±0.0005	16	0.002±0.0005	0.005±0.001
Total	~	**0.95**	**0.64**	~	**0.25**	**0.37**

Generally, the range of amino acids by the amount of green and blue-green algae in the biomass of benthic macroscopic growth is mostly similar. The exceptions are lysine and tyrosine.

The amino acid content of the benthic growths formed by green algae *Cladophora siwaschensis* is higher than the same indicator for *Cyanophyta* – 0.95% and 0.25%, respectively.

In peloids of hypersaline water bodies of Berdiansk Spit under the benthic macroscopic growths of green algae aspartic and glutamic acids dominate, in a smaller amount – glycine, alanine.On the Arabatskaya Spit under the growths of blue-green algae, in addition to these amino acids, there also are leucine, valine, phenylalanine, serine and threonine in sufficient quantities.

The coefficient of correlation between the content of amino acid algae growths and corresponding peloids is: on the Berdiansk Spit +0.98, on Arabatskaya Spit +0.93.

Total amino acid content in peloids of hypersaline water bodies of Berdiansk Spit is higher in comparison with Arabatskaya Spit (Table 1). However, the ways of amino acid accumulation in peloids are essentially different.

The accumulation of amino acids in peloids of hypersaline water bodies of Arabatskaya Spit occurs, and in peloids of Berdiansk Spit "the effect of combustion" is observed, which is reflected correspondingly in excess or reduction of the absolute number of amino acids in peloids compared with a biomass of algae. However, the biomass of algae on Berdiansk Spit exceeds the similar indicator of Arabatskaya Spit by 5–6 times, which results, as a whole, in a greater content of amino acids in peloids (0.64% against 0.37%).

The difference in the accumulation of amino acids in peloids of Arabatskaya Spit occurs due to the mutual influence of many factors, including redox conditions in hypersaline water bodies. On Arabatskaya Spit more expressed reduction conditions are observed (Table 2); they prevent the rapid oxidation of organic substance and are conducive to its accumulation in bottom sediments. Regenerative processes' development and decomposition slowdown of organic substance is connected with the accumulation of hydrogen sulfide. It was found out, that the content of hydrogen sulfide in peloids of Arabatskaya Spit is almost twice more than in peloids of Berdiansk Spit (Table 2).

The accumulation of separate amino acids in peloids of Berdiansk Spit and Arabatskaya Spit compared with their initial content in the macroscopic growths of algae is different (Tables 1, 3). There are three groups of amino acids according to the peculiarities of their accumulation in peloids. The first one is characterized by the slow process of destruction, the second – by the active process of destruction, and the third – by the relative balance of processes of destruction and accumulation (Table 3). This classification, in general, reflects the accumulation and destruction of amino acids of green and blue-green algae under certain redox conditions in hypersaline water bodies.

TABLE 2. Physical and chemical characteristics of peloids of hypersaline water bodies

Index	Berdiansk Spit	Arabatskaya Spit
Humidity, %	50±1	43±1
Average density, g/cm^3	1.483±0.005	1.568±0.005
The content of sulfides FeS, % on the damp mud	0.26±0.02	0.49±0.03
including H_2S, % on the damp mud	0.10±0.01	0.19±0.01
Iron (II), mg/100 g of the dry substance	300.8±0.5	536.0±0.5
Iron (III), mg/100 g of the dry substance	140.4±0.6	467.0±0.8
The correlation Fe^{3+}/ Fe^{2+}	0.47±0.03	0.87±0.04
Redox potential, mV	-170±5	-205±5

TABLE 3. Peculiarities of accumulation of amino acids in peloids of hypersaline water bodies

Amino acids	Berdiansk Spit		Arabatskaya Spit	
	Place in the spectrum	Content of amino acids in peloids of hypersaline water bodies relative to their amount in macroscopic growths of algae, %	Place in the spectrum	Content of amino acids in peloids of hypersaline water bodies relative to their amount in macroscopic growths of algae, %
Proline	1	90.0	1	300.0
Arginine	2	83.3	11	145.5
Glycine	3	79.7	16	120.8
Serin	4	78.7	15	123.5
Threonine	5	73.5	14	129.4
Alanine	6	70.8	12	133.3
Aspartic acid	7	69.1	17	109.4
Phenylalanine	8	68.9	5	192.9
Isoleucine	9	63.4	8	172.7
Glutamic acid	10	62.4	7	174.3
Tyrosine	11	61.4	3-4	200.0
Valine	12	60.6	13	130.0
Lysine	13	60.4	6	188.9
Leucine	14	58.3	10	150.0
Histidine	15	42.9	9	166.7
Cystine	16	30	2	250.0
Methionine	17	23.1	3-4	200.0

Irrespective of redox conditions in the hypersaline water bodies, there are a number of amino acids, which have similar regularities. So, proline, isoleucine, and phenylalanine are characterized by slow destruction, and aspartic acid, glutamic acid, valine, and leucine – by the active destruction.

In the conditions of hypersaline water bodies of Berdiansk Spit prolin, arginin, serine, threonine, phenylalanine, and isoleucine destruct slowly; the balance between the processes of accumulation and destruction is peculiar for glycine, alanine, tyrosine, histidine, cystine, and methionine; the active process of destruction is characteristic for aspartic and glutamic acids, valine, lysine, and leucine.

In the conditions of hypersaline water bodies of Arabatskaya Spit proline, cystine, tyrosine, methionine, phenylalanine, lysine, histidine, and isoleucine tend to show a slow process of destruction for amino acids of *Cyanophyta* growths; the state of balance is peculiar for arginine; the active process of destruction – for aspartic and glutamic acid, glycine, alanine, serine, threonine, valine, and leucine.

CONCLUSIONS

In benthic macroscopic growths of algae and peloids there are 17 amino acids. Amino acid content of algae growths correlates with amino acids of their corresponding peloids on Berdiansk Spit at 98%, on Arabatskaya Spit – at 93%. Accumulation of amino acids in peloids depends on the species of algae, their biomass, and redox conditions in the hypersaline water bodies. The nature of the accumulation of separate amino acids in peloids of Berdiansk Spit and Arabatskaya Spit is different in comparison with their initial content in benthic macroscopic growths of algae.

REFERENCES

Algae … 1989. *Algae: Reference Book /* Eds. S.P. Wasser. Nauk. Dumka Press, Kiev. [Rus.]

Aufdenkampe, A.K., Hedges, J.H., Krusche, A.V. et al. 2001. Sorptive fractionation of dissolved organic nitrogen and amino acids onto fine sediments within the Amazon Basin. *Limnol. and Oceanogr.* **46**(8): 1921–1935.

Barashkov, G.K. 1972. *Comparative biochemistry of algae.* Pishch. Prom. Press, Moscow. [Rus.]

Blackburn, T.H. & Henriksen, K. 1983. Nitrogen cycling in different types of sediments from Danish waters, *Limnol. and Oceanogr.* **28**(3): 477–493.

Boyle, K.A., Kamer, K. & Fong, P. 2004. Spatial and temporal patterns in sediment and water column nutrients in an eutrophic southern California estuary. *Estuaries* **27**(3): 378–388.

Daume, S., Long, B.M. & Crouch, P. 2003. Changes in amino acid content of an algal feed species (*Navicula* sp.) and their effect on growth and survival of juvenile abalone (*Haliotis rubra*). *J. Appl. Phycol.* **2–3**(15): 201–207.

El-Sheekh, M.M., El-Otify, A.M. & Saber, H. 2011. Alterations in proteins and amino acids of the Nile cyanobacteria *Pseudanabaena limnetica* and *Anabaena wisconsinense* in response to industrial wastewater pollution. *Braz. Arch. Biol. Technol.* **4**(54): 810–820.

Gonzalez, J.M., Grimalt, J.O. & Albaigés, J. 1983. Amino acid composition of sediments from a deltaic environment. *Mar. Chem.* **14**: 61–71.

Hassan, S.H., Hameed, M.S.A., Hammouda, O.E. et al. 2012. Effect of different growth conditions on certain biochemical parameters of diferent cyanobacterial strains. *Malays. J. Microbiol.* **8**(4): 266–272.

Jegan, G., Mukund, S., Rama Raja Valli Nayagam, S. et al. 2013. Amino acid content and biochemical analysis of the methanolic extract of *Oscillatoria terebriformis. Int. J. Pharm. Res. & Develop.* **5**(7): 22–27.

Kalashnikova, O.M., Shapovalova, E.N., Barkhutova, D.D. et al. 2004. Determination of amino acid composition of microbial mats in aquatic ecosystems of the Baikal region using thin-layer chromatography. *Vestn. Moscow Univ.* Ser. Chem. **45**(6): 393–398.

Keil, R.G. 2001. Reworking of amino acid in marine sediments: Stable carbon isotopic composition of amino acids in sediments along the Washington coast. *Limnol. and Oceanogr.* **46**(1): 14–23.

Koch, U., Creuzburg, D.M., Grossard, H.P. et al. 2012. Differences in the amino acid content of four green algae and their impact on the reproductive mode of *Daphnia pulex. Fund. Appl. Limnol.* **181**(4): 327–336.

Kolmakova, A.A., Kalacheva, G.S., Gladyshev, M.I. et al. 2004. Comparative study of seasonal dynamics of amino acids in water two small Siberian reservoirs. *Vestn. Krasnoyarsk. Univ.* (7): 106–120.

Komárek, J. & Anagnostidis, K. 1998. *Cyanoprokaryota.* 1. Teil: *Chroococcales.* Gustav Fischer, Jena, etc.

Komárek, J. & Anagnostidis, K. 2005. *Cyanoprokaryota.* 2. Teil: *Oscillatoriales.* Elsevier/Spektrum, Heidelberg.

Kozarenko, T.D. 1975. *Ion-exchange chromatography of amino acids.* Nauka Press, Novosibirsk. [Rus.]

McClelland, J.W. & Montoya, J.P. 2002. Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology* **83**: 2173–2180.

Nunn, B.L. & Keil, R.G. 2006. A comparison of non-hydrolytic methods for extracting amino acids and proteins from coastal marine sediments. *Mar. Chem.* **98**: 31–42.

Sirenko, L.A. & Kozitskaya, V.N. 1988. *Biologically active substances of algae and water quality*. Nauk. Dumka Press, Kiev. [Rus.]

Solonenko, A.M. 2012. Physico-chemical characteristics of peloids of amfibial sites of Arabatsaya Spit and Berdiansk Spit. *Dop. NAS Ukraine* (1): 171–173. [Ukr.]

Tartarotti, B. & Sommaruga, R. 2006. Seasonal and ontogenetic changes of mycosporine-like amino acids in planktonic organisms from an alpine lake. *Limnol. and Oceanogr.* **51**: 1530–1541.

Tartarotti, B., Laurion, I. & Sommaruga, R. 2001. Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient. *Limnol. and Oceanogr.* **46**: 1546–1552.

Teira, E., Aken, H.V., Veth, C. et al. 2006. Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic. *Limnol. and Oceanogr.* **51**: 60–69.

Tesdal, J.-E., Galbraith, E.D. & Kienast, M. 2013. Nitrogen isotopes in bulk marine sediment: linking seafloor observations with subseafloor records. *Biogeosciences* **10**: 1–18.

***Venice* … 1959.** Venice system. The final resolution of the symposium on the classification of brackish waters. *Arch. Oceanogr. Limnol.* **11**: 243–248.

Zilov, E.A. 2009. *Hydrobiology and aquatic ecology* (*Organization, functioning and pollution of the aquatic environment*). Irkut. State Univ. Publ., Irkutsk. [Rus.]