

**VI International Conference  
ADVANCES IN MODERN PHYCOLOGY**



**BOOK OF ABSTRACTS**

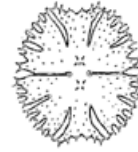
**15-17 May 2019, Kyiv, Ukraine**



**National Academy of  
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**M.G. Kholodny  
Institute of Botany**



**Ukrainian Botanical Society  
Phycological Section**

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## CONTENTS

Andrusyshyn T.V., Halyniak O.V. Pigmental composition of <i>Chlorella vulgaris</i> Beijer. for actions of metals and non-metals.....	9
Apego Gianina Cassandra May B., Dungog Ma. Rica Teresa B., Yñiguez Aletta T. Phytoplankton assemblage in whale shark capital Donsol, Sorsogon .....	10
Berezovska V.Yu. Algal floristic studies of reservoirs of Kyiv Upland (Forest-Steppe Zone, Ukraine) .....	11
Bilous O.P., Barinova S.S., Tsarenko P.M. Algae as indicators of environmental conditions in water bodies of Ukraine.....	13
Bodnar O.I. Lipids biosynthesis in <i>Chlorella vulgaris</i> Beijer. under the influence of some trace elements.....	15
Boldina O.N. Green monads sharing their habitat with macroorganisms.....	17
Borysova O.V., Gromakova A.B. Distribution and ecology of <i>Chara papillosa</i> Kütz. (Charales, Charophyta) in Ukraine .....	18
Bren O.G., Solonenko A.M. Algae of salt reservoirs on the Berdyansk Spit .....	20
Bryantseva Yu.V., Sergeeva A.V. Taxonomic composition of microalgae of the Sevastopol coastal area (Black Sea, Crimea) .....	21
Bryantseva Yu.V., Sergeeva A.V. Ukraine microalgae database.....	23
Bukhtiyarova L.N. Functional morphology of the diatom frustule in systematics of Bacillariophyta .....	24
Cepoi L.E. The influence of oxidative stress on the quality of phycological biomass ...	26
Davydov O.A. Ecological and morfological structure of microphytobenthos in Verbnoe Lake (Ukraine).....	28
Demchenko E. Planozygotes of flagellated volvocales (Chlorophyta) inhabiting ephemeral water bodies and their role in life cycles of these algae.....	29
Dobrojan S.N. Characteristics of morphological indicators of cyanophyte algae <i>Calothrix gracilis</i> F.E. Fritsch cultivated on drew and z-8 nutritive medium ....	31

Dobrojan S.N., Şalaru V.V., Jigău Gh.V., Ciobanu E.D. Utilisation biomass of <i>Nostoc linckia</i> Bornet ex Bornet et Flahault algae as biofertilizer for cultivation sunflower ( <i>Helianthus annuus</i> ) .....	33
Ennan A.A., Shichalyeyeva G.N., Gerasimiuk V.P., Kiryushkina A.N., Tsarenko P.M. Algal flora of the Kuyalnik Estuary: the history of study and current state .....	34
Garkusha O.P. Effect of decomposition of the seaweed wrack on microalgae growth on sandy and rocky supralittoral of northwestern part of the Black Sea.....	36
Genkal S.I., Trifonova I.S. To the morphology and taxonomy of <i>Aulacoseira granulata</i> (Bacillariophyta) .....	38
Gerasimiuk V.P. Microscopic algae of benthos of rivers of the north-western Black Sea (Ukraine) .....	39
Glaser K., Van A. Diversity of diatoms in biological soil crusts.....	40
Gol'din E.B. Cyanobacterial action and histopathology in insects with a different type of nutrition.....	41
Gottschling M., Owsiany P.M., Kretschmann Ju. The importance of the epitype concept for reliable species determination in protists such as dinophytes .....	43
Grubinko V.V. Regulation of metabolism in algae for the production of lipids and biologically active substances .....	44
Hisoriev H. Algae diversity of the Central Asia water bodies.....	46
Holzinger A., Rippin M., Pichrtová M., Arc E., Kranner I., Becker B. Transcriptome analysis and metabolite profiling reveal a vertical differentiation within a <i>Zygnema</i> sp. (Zygnematophyceae, Charophyta) mat from the High Arctic .....	47
Kalashnik K.S. Morphofunctional organization of the “basiphyte-epiphyte” algosystem of the Gulf of Odessa .....	48
Kapustin D.A. The Genus <i>Synura</i> Ehrenb. (Chrysophyceae) in Ukrainian algal flora .....	50
Khudjaev M., Jusupova F., Kurbonova P., Boboev M., Hisoriev H. Algae biodiversity of some water reservoirs of Central and South Tajikistan .....	51
Kirpenko N.I., Leontieva T.O. Growth intensity of <i>Desmodesmus communis</i> Hegew. and <i>D. subspicatus</i> Hegew. et Schmidt in various environments .....	52

Komaristaya V.P., Bilousova K.M. Beta-ionone as a stimulant of carotenogenesis in <i>Haematococcus pluvialis</i> Flotow .....	54
Kondratyuk S.Ya., Hur J.-S. Phycobiont and mycobiont switching in lichen symbiotic association .....	56
Konishchuk M.O., Borysova O.V., Konishchuk V.V., Pankovska H.P. Algae of water bodies in the National Natural Park Pivnichne Podillia (Lviv Region, Ukraine) .....	57
Kovalchuk N.A. Assessment of macroalgal species diversity on water area of the PA “Kurgalsky” (the Gulf of Finland, Baltic Sea) .....	59
Kovalchuk N.A., Hop H. Some quantitative characteristics of the population of <i>Alaria esculenta</i> (L.) Grev. from Kongsfjord (the Western Spitsbergen, 79° N).....	60
Kryvosheia O.M. Diatoms of the Sula River (Ukraine).....	61
Kurbonova P.A. Distribution and ecology of <i>Closterium</i> species (Streptophyta) in Tajikistan .....	63
Maltsev Y.I., Maltseva S.Y., Kulikovskiy M.S. Molecular and morphological investigation of cosmopolitan diatom <i>Hantzschia amphioxys</i> (Ehrenb.) Grunow (Bacillariophyceae).....	64
Maltseva I.A., Maltsev Y.I., Bren O.G., Yarova T.A., Pavlenko O.M., Yakoviichuk O.V. Algae as indicators of the ecological state of marine ecosystems in the coastal part of the Azov Sea .....	65
Mienasova A.Sh. Most ancient Podolia’s algae as trigger for the formation of phosphorites.....	67
Mikhailyuk T., Tsarenko P., Glaser K., Holzinger A., Demchenko E., Karsten U. <i>Dictyosphaerium</i> -like morphotype in terrestrial algae: what is <i>Xerochlorella</i> (Trebouxiophyceae, Chlorophyta)? .....	69
Minicheva G.G., Tretiak I.P. Long-term restoration of the Zernov’s Phyllophora Field .....	70
Minicheva G.G., Tsetskhladze M.S. Macroalgae of Georgian coast as indicator of ecological status .....	70

Mykhailenko N.F., Zolotareva O.K. Growth rates and photosynthetic energy transduction efficiency of <i>Chlorella vulgaris</i> Beijer. grown in the presence of copper and selenium nanocitrates .....	74
Nikonova S.E. Cyanoprokaryota of the hyperhaline Kuyalnik Estuary (Ukraine) in terms of supplying seawater from the Black Sea .....	76
Novakovskaya I.V., Egorova I.N., Kulakova N.V., Patova E.N., Shadrin D.M. Morphological and genetic characteristics of <i>Coelastrella</i> species from the Urals and Khentey Mountain Systems .....	78
Nyporko S.O., Demchenko E.N. The record of <i>Diplosphaera chodatii</i> Bial. emend. Vischer on moss <i>Homalothecium philippeanum</i> (Spruce) Schimp.....	80
Olshytynska O.P., Tymchenko Yu.A. <i>Cymatopleura</i> W.Sm. s.l. (Bacillariophyta) in the Black Sea bottom sediments.....	81
Pirko Ya.V., Postovoitova A.S., Rabokon A.M., Bilonozhko Yu.O., Kalafat L.O., Korkhovoy V.I., Borysova O.V., Tsarenko P.M., Blume Ya.B. Molecular genetic algae profiling of the Selenastraceae family .....	83
Rachynska O.V. Microphytobenthos algae of mussel shells from the Odessa coastal zone as bioindicators of marine environment .....	84
Raida O.V., Burova O.V. The macrophytic algae of Dzhurynskyi and Divochi Sliozy waterfalls (Ternopil Region, Ukraine) .....	86
Romanenko P.O., Vynogradova O.M., Romanenko K.O., Ivannikov R.V., Babenko L.M. Interesting representative of genus <i>Brasilonema</i> Fiore et al. (Nostocales, Cyanobacteria) growing on tropical plants in the greenhouse of the M.M. Grishko National Botanical Garden (Kyiv, Ukraine).....	88
Sadogurskiy S.Ye., Belich T.V., Sadogurskaya S.A. Floristic diversity of macrophytes in marine water areas of nature reserves in the Crimean Peninsula .....	90
Salaru V.V., Semeniuc E.N. Rare species of algae of Moldova.....	92
Sapozhnikov Ph.V., Kalinina O.Yu., Snigirova A.A. Phytoperyphyton of the marine plastic (pet) near the Crete coast.....	93
Semeniuk N.Ye. Assessing stability of Dnipro epiphytic algal communities' taxonomic and quantitative diversity (case-study of Kyiv Water Reservoir) .....	95

Shalygina R.R., Shalygin S.S., Redkina V.V., Gargas C.B., Johansen J.R. <i>Stenomitos kolaensis</i> , a new species of cyanobacteria from Kola Peninsula, Russia .....	95
Shelyuk Yu.S. Phytoplankton development in small reservoirs .....	97
Shevchenko T.V. Oligocene Zmiiv algal flora of the Subparatethys (Northern Ukraine) .....	97
Shevchenko T.F., Klochenko P.D., Dubnyak S.S. Epiphyton under conditions of unstable hydrological regime of a cascade plain reservoir .....	100
Shyndanovina I.P. <i>Gonatozygon aculeatum</i> W.N. Hastings and <i>Pleurotaenium simplicissimum</i> Grönblad - new taxa of rare desmids (Zygnematophyceae, Streptophyta) for Ukraine .....	102
Snigirova A.A., Kurakin A.P. Microalgae on the plastic substrates in the coastal area of the Gulf of Odessa (the Black Sea) .....	103
Stepanov S.S. Influence of methanol and H <sub>2</sub> O <sub>2</sub> on lipid bodies accumulation by <i>Chlamydomonas reinhardtii</i> Dang. ....	105
Stepanov S.S. The way to increase the efficiency of production H <sub>2</sub> by <i>Chlamydomonas reinhardtii</i> Dang.....	107
Stepanova V.A. Rare species of diatoms (Bacillariophyta) of the coast of the Gulf of Finland (Leningrad Region, Russia) .....	107
Terenko G.V. New invasions of alien species of planktonic microalgae into the North-Western part of the Black Sea (Ukraine) .....	110
Tkachenko F.P. Algae of mineralized stream of the slopes of the Tiligul Estuary of the Black Sea .....	112
Trofim A., Bulimaga V. Biochemical composition of Cyanobacterium <i>Calothrix marchica</i> Lemm. isolates from Moldovan soils and perspectives in biotechnological applications.....	113
Tsarenko P.M. Algal flora of Ukraine – floristic-geographical and biotechnological aspect.....	115
Tsarenko P.M., Borysova O.V., Konishchuk M.O. Microalgae strains of the IBASU-A collection as a basis for biotechnological studies .....	117



Usenko O.M., Konovets I.M., Mardarevych M.G. Effect of hydroxycinnamic acids on green algae and cladocerans.....	119
Van A. A taxonomic study of strains from the genus <i>Stichococcus</i> Nägeli based on ecophysiological, morphological, and molecular data .....	121
Vynogradova O.M., Mikhailyuk T.I., Gromakova A.B. New and interesting records of Cyanobacteria in biological soil crusts from chalk outcrops of Kharkiv Region (Ukraine) .....	122
Wojtal A.Z., Pocięcha A., Ciszewski D., Cichoń S., Cieplak A. Response of diatoms to the mining of the Zn-Pb ore (South Poland) .....	123
Yaremych A.V., Karamushka V.I. Phycocyanin content assessment by the spectral response of <i>Arthrospira platensis</i> Gomont biomass .....	124
Zotov A.B. The influence of nitrogen to phosphorous ratio on inter-annual variability of phytoplankton structure of the Odessa Region (the Black Sea, Ukraine).....	126

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**PIGMENTAL COMPOSITION OF *CHLORELLA VULGARIS* BEIJER.  
FOR ACTIONS OF METALS AND NON-METALS**

The influence of stressful factors on algae causes them to change the physiological processes, including the photosynthetic activity. Of particular interest to study is the reaction of photosynthetic pigments – chlorophylls a and b, carotenoids, pheophytin (Sun et al., 2014). The state of the pigmental system is largely determined by the concentration of metals in the environment (Pasichna, 2016). It is known that the insignificant increase of individual elements in the growth medium of plants can create a stimulating effect on living organisms. However, the exceeding of the threshold values reveals their toxic properties. It is established that certain amounts of selenium compounds have the ability to reduce the toxic effect of individual metals (Bodnar et al., 2016). In this case, it is also important to remember the range of concentrations that exist between the amount of selenium necessary for an organism and its toxic effect. It is very limited and depends on the degree of oxidation of selenium, the type of selenium compound, the type of organism and other factors (Myhailenko, 2016).

There was determined the content of pigments in micro-populations of an algal-pure culture of green freshwater algae *Chlorella vulgaris* Beijer. in the action of sodium selenite (10.0 mg Se (IV)/l) separately and in association with metal ions and nonmetals. The algae culture was grown on Fitzgerald's medium (modified by Zehnder and Gorham №11) at a temperature of 22-25°C and illumination of daylight (2500 lux) for 16 hours a day. The biomass of living cells was taken after 7 days of cultivation. As a control there was the culture of algae, which was grown without the addition of compounds of selenium and zinc ions, chromium and iodine. The content of pigments was determined spectrophotometrically. The extraction was performed with a 90 % solution of acetone. The colour intensity of the extract was measured by the wave lengths which correspond to the maximum absorption of carotenoids and chlorophylls a and b, the calculations were carried out according to the methodology.

It was found that in comparison with the control (158.98 µg/l), in the cells of *Ch. vulgaris* there was an increase in the content of: chlorophyll a in 1.2 times

under the influence of Se (IV); in 1.7 times under the action of Se (IV) + Zn<sup>2+</sup>; in 1.1 times under the action of Se (IV) + Cr<sup>3</sup>. There was a decrease in the content of pigment under the action of Se (IV) + I<sup>-</sup>.

Regarding the content of chlorophyll b, then its increase in cells *Ch. vulgaris* concerning to control (88.04 µg/l) was: in 1.0 times under the influence of Se (IV); in 2.6 times under the influence of Se (IV) + Zn<sup>2+</sup>; in 1.1 times under the influence of Se (IV) + Cr<sup>3</sup>. There was an insignificant decrease in the content of pigment under the influence of Se (IV) + I<sup>-</sup>.

The correlation of chlorophylls a/b increased concerning to control (1.80): in 1.2 times under the influence of Se (IV); in 1.0 times under the influence of Se (IV) + Cr<sup>3</sup>; in 1.1 times under the influence of Se (IV) + I<sup>-</sup>. There was a decrease in the index in 1.5 times under the influence of Se (IV) + Zn<sup>2+</sup>.

The content of carotenoids concerning to control (50.14) increased: in 1.3 times under the action of Se (IV); in 1.5 times under the action of Se (IV) + Zn<sup>2+</sup>; in 1.2 times under the action of Se (IV) + Cr<sup>3</sup>. The content of carotenoids is reduced in 1.2 times under the action of Se (IV) + I<sup>-</sup>.

Consequently, under the influence of sodium selenite separately and in association with zinc and chromium ions, there is an increase in the content of pigments in cells. The highest values of chlorophyll a, b and carotenoids were observed under the action of Se (IV) + Zn<sup>2+</sup>. The introduction of Se (IV) + I<sup>-</sup> into the environment leads to a decrease in the content of pigments in cells. The identified effects can be used to regulate the photosynthesis activity and chlorella performance *in vitro*.

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## **PHYTOPLANKTON ASSEMBLAGE IN WHALE SHARK CAPITAL DONSOL, SORSOGON**

Donsol, Sorsogon of the Bicol province of the Philippines experiences an annual pilgrimage of the whale sharks *Rhincodon typus*, supporting a prolific

tourism industry. This has been associated with the productivity of the water brought about by high abundance of plankton, though the factors driving this productivity are not understood. These whale shark sightings were categorized into the "off-season", spanning September to November, and "season", covering December to May. Here we present preliminary results investigating the relationship of biological, chemical and physical parameters covering the months of September 2017, January 2018, and May 2018 in 12 sampling stations along the coast of Donsol. Phytoplankton were optically analyzed to reveal their composition, density and distribution across the sampling stations. Nutrient concentrations of nitrate + nitrite, ammonia, silicate and phosphate were analytically determined for both near-surface and near-bottom depths. Current speed, direction, and associated physical characteristics of the water were recorded real-time during sampling to characterize the hydrographic conditions of the area. Data were statistically analyzed to investigate the interplay of the various measured parameters and the seasonal sightings of whale shark. From this, a substantial amount of environmental and biological data will be contributed to the limited information on biophysical dynamics in this unique area. The initial results on the relationships between the physical factors, phytoplankton assemblage and whale shark occurrences will be presented.

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**ALGAL FLORISTIC STUDIES OF RESERVOIRS OF KYIV UPLAND  
(FOREST-STEPPE ZONE, UKRAINE)**

Summary and comprehensive information about algae diversity of central part of Forest-Steppe zone - Kyiv Upland Section was absent in the literature for a long time. The Kyiv Upland area has a well-developed hydrological network with a large number of different types of reservoirs (small rivers, lakes, ponds, fish-ponds, granite quarries). At the same time, the territory is continually undergoing significant transformation and anthropogenic pressure on biota. This work presents results of the study of different type of reservoirs of Kyiv Upland.

Samples were taken from different waters bodies during summer expeditions of 2015–2017 according to routine procedure (Vodorosli ..., 1989).

During our investigation of the reservoirs we found 638 algae species (671 int. taxa). Our study recorded about 263 (285 int. taxa) new species for the Kyiv Upland. Of these, *Anabaenopsis circularis* (G.S. West) Wolosz. et V.V. Mill., *Phacus tropidontus* Conrad, *Achnantheidium saprophilum* (Kobayasi et Mayama) Round et Bukht., *Encyonema neomesianum* Krammer, *Encyonopsis minuta* Krammer et E. Reichardt and *Navicula microrhombus* (Cholnoky) Schoeman et Archibald are for the first time given for the flora of Ukraine. *Acutodesmus javanensis* (Chodat) P. Tsarenko, *Brachysira microcephala* (Grunow) Compère, *Cosmarium turpinii* var. *podolicum* Gutw., *Encyonopsis subminuta* Krammer et E. Reichardt, *Eunotia crista-galli* Cleve, *Heteronema acus* (Ehrenb.) F. Stein, *Mallomonas striata* Asmund, *Mastogloia elliptica* (C. Agardh) Cleve in A.W.F. Schmidt, *Tribonema ulotrichoides* Pascher are rare for algal flora of Ukraine (for these species no more than 5 localities are known). The first locations about 15 species are recorded for the Forest-Steppe of Ukraine: *Achnantheidium eutrophilum* (Lange-Bert.) Lange-Bert., *Amphora inariensis* Krammer, *Botryococcus terribilis* Komárek et Marvan, *Caloneis lancettula* (Schulz) Lange-Bert. et Witkowski *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya et Subba Raju, *Craticula subminuscula* (Manguin) Wetzel et Ector, *Diploneis elliptica* (Kütz.) Cleve, *Encyonopsis falaisensis* (Grunow) Krammer, *Eunotia faba* (Ehrenb.) Grunow in Van Heurck, *Navicula erifuga* Lange-Bert., *Pinnularia subgibba* Krammer, *Stephanodiscus neoastraea* Håkansson et Hickel, *S. parvus* Stoermer et Håkansson, and *Radiococcus planktonicus* J.W.G. Lund. Noteworthy records for this region include *Acutodesmus regularis* (Svirenko) P. Tsarenko, *Centrtractus africanus* F.E. Fritsch et M.F. Rich, *Cyclotella atomus* Hustedt, *Dicellula geminata* (Printz) Korschikov, *Discostella pseudosteliger* (Hustedt) Houk et Klee, *Diploneis oculata* (Bréb.) Cleve, *Encyonopsis microcephala* (Grunow) Krammer, *Eunotia exigua* (Bréb. ex Kütz.) Rabenh., *E. naegelii* Migula, *Fallacia subhamulata* (Grunow) D.G. Mann, *Gloeotaenium loitlesbergianum* Hansg., *Gomphonema laticollum* E. Reichardt, *G. vibrio* Ehrenb., *Granulocystopsis decorata* (Svirenko) Tsarenko, *Navigeia decussis* (Østrup) Bukht., *Planktolyngbya contorta* (Lemmerm.) Anagn. et Komárek, *Pseudanabaena redekei* (Goor) B.A. Whitton, *Pseudostaurosira subconstricta* (Grunow) Kulikovskiy et Genkal, *Platessa conspicua* (Ant.Mayer) Lange-Bertalot, *Stauroneis kriegeri* R.M. Patrick, *Scenedesmus bacillaris* Gutw., *Staurosira binodis* (Ehrenb.) Lange-Bert., *S. subsalina* (Hustedt) Lange-Bert., *S. venter* (Ehrenb.) Cleve

et J.D. Möller, *Stephanodiscus delicatus* Genkal, *Staurosirella pinnata* (Ehrenb.) D.M. Williams et Round, and *Surirella librile* (Ehrenb.) Ehrenb.

We are recorded localities of the 2 species which listed in the Red Data Book of Ukraine (2009) – *Nitellopsis obtusa* (N.A. Desvaux) J. Groves and *Thorea hispida* (Thore) Desvaux. Two endangered species (*Lychnothamnus barbatus* (Meyen) Leonhardi and *Nitella confervacea* (Bréb.) A. Braun ex Leonhardi) are proposed to include in next edition of Red Data Book of Ukraine and IUCN Red List.

As follows, according to the original and literary data algal diversity of Kyiv Upland represented by 936 species (1013 int. taxa), which belong to 11 divisions, 17 classes, 56 orders, 121 families, 305 genera. Taxonomic structure is formed by the representatives of the divisions: Cyanoprokaryota – 120 sp. or 125 int. taxa (12.3 %), Euglenophyta – 116 sp. or 134 int. taxa (13.2 %), Dinophyta – 25 sp. or 26 int. taxa (2.6 %), Cryptophyta – 7 sp. (0.7 %), Chrysophyta – 21 sp. or 24 int. taxa (2.4 %), Bacillariophyta – 341 sp. or 363 int. taxa (35.8 %), Xanthophyta – 29 sp. or 30 int. taxa (2.9 %), Eustigmatophyta – 10 sp. or 11 int. taxa (1.1 %), Rhodophyta – 2 sp. (0.2 %), Chlorophyta – 210 sp. or 233 int. taxa (23.1 %), Charophyta – 55 sp. or 58 int. taxa (5.7 %).

Species diversity of algae of investigation area comprised about 30 % of Forest-Steppe zone diversity and 15 % of Ukrainian algal flora.

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**ALGAE AS INDICATORS OF ENVIRONMENTAL CONDITIONS IN WATER BODIES OF UKRAINE**

At present, Ukraine's algal flora is considered as one of the richest in Europe and has been thoroughly and thoroughly studied for some groups of algae. This

flora (including freshwater, marine, soil and aerophytic forms) combines 5498 species and 6583 infraspecies taxa belonging to 15 divisions: Cyanoprocarvota, Euglenophyta, Chrysophyta, Haptophyta, Xanthophyta, Eustigmatophyta, Raphidophyta, Phaeophyta, Bacillariophyta, Dinophyta, Cryptophyta, Glaucocystophyta, Rhodophyta, Chlorophyta and Charophyta. It represents more than 42 % of the species composition of the global continental algae flora or about 10 % of the Earth's algal flora (Tsarenko et al., 2006).

The basis of the species richness of the flora of algae of Ukraine's is formed by green (Chlorophyta), diatom (Bacillariophyta) and charophyte (Charophyta) algae, which unite over half (about 58 %) of the species composition of national flora. Low species diversity in the flora of Ukraine is common for cryptophytes (59 species), haptophytes (45 species) and eustigmatophytes (9 species), as well as raphidophytes (8 species) and glaucocystophytes (3 species). However, general poverty of the species composition in the global flora and an insufficient level of study characterize these groups.

Proposed indicator characteristics of species is divided according to the main systems of ecological preferences: biotopical distribution, water mass dynamics and oxygenation, temperature regime, pH, salinity, organic pollution according to Sládeček, organic pollution according to Watanabe, nutrition type and trophic state.

From the known Ukrainian flora and considering mentioned preferences, the indicator species may be quantified as 2858 species and 3299 infraspecies taxa.

Of the total known for Ukraine flora amount, 2801 taxa may characterize biotopical distribution (according to Sumita, 1986; Barinova et al., 2006, in Rus.). The water mass dynamics and oxygenation indicators due to works of Cholnoky, 1968; Van Dam et al., 1994; Barinova et al., 2006, composed 1274 taxa. As the indicators of temperature regime for Ukraine, it was formed by 293 taxa (according to Patrick, 1971; Barinova et al., 2006, in Rus.). For the characteristics of water pH the list for Ukraine were formed by 1422 taxa (Hustedt, 1938–1939, Meriläinen, 1967; Battarbee, 1984; Davis, 1987; Van Dam et al., 1981; Holmes, 1986; Kharitonov, 1981, 2010, in Rus.; Dixit, Dickman, 1986; Kovács et al., 2006; Stastny, 2010; Coesel, 2014). The indicators of salinity were formed by the list of 1181 taxa (according to Kolbe, 1927; Hustedt, 1957; Ehrlich, 1995;) Stoermer, Smol, 1999; Barinova et al., 2006, in Rus.). Indicators forming the group of organic pollution according to

Sládeček, were formed by 2354 taxa and the list was formed with the help of works (Kolkwitz, Marsson, 1902, 1909; Pantle, Buck, 1955; Sládeček, 1973; DePauw, Hawkes, 1993; Barinova et al., 2006, in Rus.). For the characteristics of organic pollution according to Watanabe 339 indicators are known for our country (according to Watanabe et al., 1986; Dell'Uomo, 1996). Of the Ukrainian flora amount, for nutrition type preferences, 305 taxa were investigated (making this list was possible due to works: Van Dam et al., 1994; Lenzenweger, 2003; Coesel, Meesters, 2007; etc.). Trophic state was formed by 1301 taxa (according to Van Dam et al., 1994, Kharitonov, 2010, in Rus.; Lenzenweger, 2003; Coesel, Meesters, 2007; <http://hydro.chmi.cz/isarrow/taxontable.php?agenda=POV&lng=eng&lng=eng>; Barinova, 2017; Barinova, Fahima, 2017).

From the known Ukrainian flora and considering mentioned preferences, the indicator species may be quantified as 2858 species and 3299 infraspecies taxa. The proposed ecological preferences of algae characterize state of aquatic environment in Ukraine and can be used for similar floras. This information is presented in the monography "Algal indication of water bodies in Ukraine: methods and perspectives" (Barinova, Bilous, Tsarenko, 2019).

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### **LIPIDS BIOSYNTHESIS IN *CHLORELLA VULGARIS* BEIJER. UNDER THE INFLUENCE OF SOME TRACE ELEMENTS**

The natural high content of lipids in cells of many microalgae became the primarily reason for obtaining lipid compounds with a varied range of practical applications - from biologically active additives to biofuels (Michalak, 2017). The chemical composition and physical factors changes of the culture medium can modify the chemical composition of algae as well as the ratio of some organic compounds.

As for lipids, the main goal in biotechnological systems is their amount, the formation rate and the ratio of separate classes that can be regulated by various



external and internal factors (Hu, 2004). The most common strategies for enhancing lipid biosynthesis is the influence of chemical factors of growth medium: concentration of CO<sub>2</sub>, change in the ratio of N and P, addition of phytohormones and trace elements, etc. At the same time, the high ability of microalgae to bioaccumulation of chemical elements and formation of their biocomplexes with intracellular macromolecules *in vitro* opens up the prospective for obtaining biologically active additives that are enriched with necessary microelements, for example, Se and ions of certain biogenic metals (Doucha, 2009, Grubinko, 2018).

So, the aim of the study was to investigate the direction and intensity of general metabolism and biosynthesis of lipids in *Chlorella vulgaris* Beijer. under specified conditions after the inclusion of labeled precursors.

Inclusion of <sup>14</sup>C-bicarbonate into carbohydrates, proteins and lipids of *Ch. vulgaris* significantly differed as in a control group and under the action of Se, Zn and Cr salts. Under these all conditions, the predominance of the label inclusion in lipids was 2-3 times higher in relation to the intensity of its inclusion in carbohydrates and 9-12 times in relation to the intensity of its inclusion in proteins. In addition, during the action of all trace elements, one third of the <sup>14</sup>C-bicarbonate label were stably incorporated into proteins, while in the lipids, the inclusion of H<sup>14</sup>CO<sub>3</sub><sup>-</sup> exceeded the benchmarks by 1.5 after adding Se and Se + Zn, by 1.6 times after adding Se + Cr for conditional control in carbohydrates. It is found that in *Ch. vulgaris* under the actions of Se the process of bicarbonate inclusion into phospholipids (PL) is separately activated, after adding Se + Zn we can observe the decrease of its inclusion into the triacylglycerols (TAG) and its increase in PL and nonetherified fatty acids (NEFA), and after adding of Se + Cr we observed the modification of the label inclusion into TAG and diacylglycerols (DAG).

It should be noted that the lipogenesis activation by the action of the Se, Zn, Cr compounds was confirmed in the majority of cases with the increase in the intensity of <sup>14</sup>C-oleate inclusion into different classes of chlorella cells' lipids and the increase of glycerol-3-phosphatacyltransferase (G-3-FAT) activity. This enzyme is key for lipid biosynthesis, which activity was evaluated by the inclusion of <sup>14</sup>C-oleate into lipids.

By the intensity of the inclusion of <sup>14</sup>C-oleate we established that the general tendency under the action of Se, Zn, Cr salts was a significant increase in the

biosynthesis of PL and to a lesser extent in the TAG and a decrease in the biosynthesis of DAG and partly of NEFA in *Ch. vulgaris*.

At the same time, the high activity of the enzyme G-3-FAT in *Ch. vulgaris* correlated with the maintenance of relatively stationary content of TAG and PL in cells.

Thus, the metabolic peculiarities of lipid metabolism regulation in *Ch. vulgaris* that were studied in a direction of increasing the formation and accumulation of lipids and their separate classes using sodium selenite together with Zn (II) and Cr (III).

These data can be used to obtain lipidous biologically active drugs enriched with essential micronutrients.

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## **GREEN MONADS SHARING THEIR HABITAT WITH MACROORGANISMS**

During the study of green blooms in the North-West Russia the five cases of green monads coexisting with other organisms were detected.

Four different algal taxa were isolated from the eggs of *Rana* L. species. These monads were easily allocated from contents around a germ to agar medium. Later they were identified as *Edaphochlamys debaryana* Pröschold & Darienko, *Chlamydomonas asymmetrica* Korshikov, *Gloemonas* sp. and *Chlorococcum* sp., using light microscopy (LM), transmission electron microscopy (TEM) and sometimes 18S rRNA sequencing. Their morphological characters coincided mostly with that of earlier diagnosed taxa from free-living green monads.

Only one taxon was occasionally observed on dark-green colored *Sphagnum* sp., which was preliminary identified as *Microglena* sp., based only on LM and TEM.

All examined green monads differ markedly not only in morphology but also in contrasting cellular ultrastructure.

It seems that these algae are not obligate to corresponding associations with macroorganisms, since they were met as free chlamydomonads in different

biotopes of the same territory. The predominance effect of each of the studied algal taxa inside or on the surface of coexisting macroorganism is probably due to their mutualistic relationships.

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## **DISTRIBUTION AND ECOLOGY OF *CHARA PAPILLOSA* KÜTZ. (CHARALES, CHAROPYTA) IN UKRAINE**

*Chara papillosa* Kütz. (= *Chara aculeolata* Kütz. sensu Hollerb. et Krassavina, *C. intermedia* A. Braun ex A. Braun, Rabenh. et Stizenb.) is one of the large calcicolous and alkaliphilic species known in Europe, Asia, North and South America. It is widely distributed in Eurasia but everywhere rather scarce. The distribution of the species is restricted by hard fresh calcium enriched or slightly salinity waters and associated to lakes, freshwater, brackish and mesohaline continental water bodies, as well as inland hyposaline seas. Under favorable conditions it can form rush own phytocenosis or in an association with other charophytes, sometimes occupying vast area of large lakes or bays of seas, e.g. phytocenosis of *C. papillosa* in Italian Volcanic lakes and other European hard water deep lakes (Azzella, 2014) and in northern part of Caspian Sea between the Volga and Ural deltas (Zivoglad, Krivonosov, 1980, in Rus.). At the same time, *C. papillosa* is considered as endangered species in Balkans (Blaženčić et al., 2006), Great Britain (Stewart, Church, 1992), Czech Republic (Caisová, Gąbka, 2009), Poland (Siemińska et al, 2006, in Polish), Switzerland (Auderset Joye, Schwarzer, 2012); near threatened in Nordic countries (Langangen, 2007); vulnerable in Germany (Korsch et al., 2012).

*Chara papillosa* occurs in different physiogeographic regions of Ukraine (Ukrainian Polissia, broad-leaved-forest, forest-steppe and steppe zones) in

corresponding water habitats. There is only site (Shatsk National Nature Park (NNP), Volyn Region) in Ukrainian Polissia where it grows and forms phytocenosis together with *C. virgata* Kütz. and rarer with *C. aspera* Dethard. ex Willd. in eight lakes (Svitiaz, Luky, Liutsemer, PISOCHNE, Pulemetske, Karasynets, Ozertse, Somynets) of Shatsk group and four other Volyn lakes (Bile, Okhotun, Prybych, Velyke Zgoranske). Its own phytocenosis has been found in broad-leaved-forest zone in land-improvement canals on the carbonate swamps near Brody-city (Lviv Region). Two sites of *C. papillosa* are known in forest-steppe zone: oxbow-lake Shaparnia in the vicinity of Kyiv-city (Holosiivsky NNP) and two lakes belonging to the Oskil River basin in the neighborhood of chalky outcrops (Dvorichansky NNP, Kharkiv Region).

Until 1980-s this species thrived in shallow coastal waters of the Black and Azov seas covering large area of their bays (Karkinitzkyi, Dzharylgachskyi, Tendrovskyi Yagorlytskyi and Syvash) and produced tons of biomass (Kalugina-Gutnik, 1975; Pohrebniak, Ostrovchuk, Yeremenko, 1973, in Rus.). However, then its growth area was sharply reduced in connection with increasing anthropogenic press leading to eutrophication (Tkachenko, Maslov, 2002; Borysova, Tkachenko, 2008, in Rus.). *C. papillosa* (= *C. intermedia*) was included in Red List of *Charales* in Ukraine with status of conservation as vulnerable (Palamar-Mordvintseva, Tsarenko, 2004).

In spite of domination of *C. papillosa* in the Black sea - Azov region its localities in inland water bodies of steppe zone were not revealed for long time. In 2013-2014 years the first findings were made during our exploration of small steppe river Bereka, tributary of the Siversky Donets (Kharkiv Region). Overall, five sites of permanent phytocenosis formed by *C. papillosa* together with *C. contraria* and *C. vulgaris* have been revealed in the river located in Lozova and Barvinkove districts. It should be noted that due to hot dry climate of steppe zone the river has unstable hydrological and hydrochemical regimes and partly dries up in summer. It caused of dying out charophytes which appeared next year as annual species indicating the high adaptive abilities of Charales species.

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### **ALGAE OF SALT RESERVOIRS ON THE BERDYANSK SPIT**

The reservoirs with brackish and saline water are considered a valuable recreational resource. A group of shallow reservoirs on the Berdyansk spit (Zaporizhzhja Region, Ukraine) has high recreational potential. Specificity of the hydrochemical composition, temperature and water regime determines a growth of algae in these water bodies. Algae often can be the primary producers in these water bodies; they can affect the chemical composition of water and form the conditions for peloid genesis. There is a need to investigate algae as an important component of these reservoirs.

The data on algae of the Berdyansk spit are very limited (Yarovoi et al., 2007, 2008) and only concerns the algae of salt marsh at the Krasne Lake. Other reservoirs of the Berdyansk spit have not yet been studied in terms of the diversity of algae.

Specific water regime causes significant changes in the size and depth of these reservoirs, even to possible partially or complete evaporation. That is why we have studied planktonic and benthic algae and algae from the areas of evaporation of these reservoirs. We have obtained information on the species composition, dominant families and species for the salt lakes of the northern part of Berdyansk Spit named Dovge, Seredne, Krasnoper and Krugle. In addition, we have supplemented data on algae of the Krasne lake.

As a result of investigations, we have found 52 species of algae from Cyanoprokaryota – 31 species (59.6 % of the total species number), Bacillaryophyta – 13 (25 %), Chlorophyta – 7 (13.5 %) and Rhodophyta – 1 (1.9 %). Identified species belong to 30 families and 38 genera. Peculiar features of the studied lakes algoflora are prevalence of Cyanoprokaryota representatives, the presence of some marine algae (for example, *Polysiphonia opaca* (C.Agardh) Moris & De Notaris) and absence of Xanthophyta species.

The most numerous genera are *Nostoc* – 4 species; *Leptolyngbya*, *Lyngbya*, *Calothrix*, *Trichormus* – 3 species; *Pseudanabaena*, *Oscillatoria*, *Schizothrix*, *Cocconeis*, *Phormidium* – 2 species. The average number of species in families – 2.0. The leading families in algal flora of investigated water bodies having a level of

species richness above this value are Nostocaceae (8 species), Oscillatoriaceae (7), Leptolyngbyaceae (3) and Rivulariaceae (2).

The largest number of algae species was recorded in the Krasne Lake (23 species). Lakes Krasnoper (21) and Dovge (19) rank next in species richness followed by Krugle (16) and Seredne (15) lakes.

The most common species of investigated reservoirs were cyanoprokaryotes *Spirulina tenuissima* Kützing and *Lyngbya aestuarii* Liebman ex Gomont, green alga *Cladophora siwaschensis* C.J. Meyer and diatom *Amphora coffeaeformis* (C. Agardh) Kützing.

*Cladophora siwaschensis* and *Lyngbya aestuarii* formed macroscopic algal growths in studied lakes (these two species were prevailing in biomass). The growths covered the bottom of the reservoirs and formed solid algal mats on the bottom of dried up water bodies. *C. siwaschensis* formed felt-like green coating, while *L. aestuarii* – dark green slimy films. Such macroscopic algal growths are widely distributed along the North-Western Coast of the Sea of Azov. Occasionally, *Leptolyngbya perelegans* (Lemm.) Anag. & Kom. formed green spots on the dried up bottom of reservoirs.

Thus, in conditions of changing water, temperature and salt regimes, in the amphibious saline waters the most adapted to existence is a group of algae with prevailing of *Cladophora siwaschensis* and *Lyngbya aestuarii* that are capable of forming macroscopic algal growth.

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## **TAXONOMIC COMPOSITION OF MICROALGAE OF THE SEVASTOPOL COASTAL AREA (BLACK SEA, CRIMEA)**

Apart from the most common groups of microalgae: diatoms and dinoflagellates (investigated by authors earlier), Sevastopol coastal area phytoplankton is represented by 7 other divisions of phytoplankton, that we

conditionally called "others". Increasing taxonomic diversity of microalgae, "others" increase the stability of marine ecosystems.

Identification of these algae groups during routine sample processing can be difficult since most species have relatively small sizes. However, the abundance of some species can reach a concentration of hundred millions of individuals per cubic meter that can cause negative changes in marine communities.

In phytoplankton samples collected within monthly environmental monitoring of the Sevastopol coastal area (conducted by IBSS NASU biophysical ecology department in 2008-2016) 41 taxa were identified. 21 species belonged to 19 genera, 15 families, 14 orders, 9 classes, 7 divisions and 4 kingdoms. The most abundant species were representatives of the classes Coccolithophyceae (*Emiliania huxleyi* (Lohmann) W.W. Hay et H.P. Mohler (maximum abundance  $722,4 \cdot 10^6$  cells/m<sup>3</sup>), Chrysophyceae (*Dinobryon sertularia* Ehrenb. and *Dinobryon baltica* (Schütt) Lemmerm. –  $28 \cdot 10^6$  cells/m<sup>3</sup> and  $22,4 \cdot 10^6$  cells/m<sup>3</sup>), as well as unidentified species forms of Cryptophyceae, Cyanophyceae classes и Flagellates group.

In the study we combined our own results with analyses of the literature sources for over 117 years period and obtained the annotated checklist of phytoplankton taxa of "others" group for the Sevastopol coastal area. Check-list includes 79 species (including one subspecies) related to 47 genera, 29 families, 19 orders, 12 classes, 7 divisions, 4 kingdoms of two Empires. The actual number of microalgae species in the region, however, is clearly in dispute. In the first known study for the region (Minkevich, 1899) the information on the 7 Euglenozoa и Cryptophyta species was published. However, none of them, apart from the one species – *Cryptomonas erosa*, were reported for the region in the subsequent period. Until the mid-20th century, there was an information "gap" about "other" microalgae species in the area and only in the work of N.V. Morozova-Vodyanitskaya (1954) the number of species reported increased to 10. Phytoplankton review was published in 1965 by A.I. Ivanov where 51 species were mentioned for the Sevastopol coastal area. The same number of species were found by M.I. Senicheva (2008). Our results coincide with corresponding I.G. Polikarpov et al. (2003) studies where 41 taxa were identified for the region (only 20 taxa were identified to species level).

Analysis of the species list by the number of citations in various sources showed that the real number of species existing in the region of Sevastopol more

than two times less. Thus, 26 species were unique – cited only by one from 7 literature sources. Their identification requires additional confirmation. 11 species we can consider reliable and 20 unquestionable (mentioned in 3-7 sources).

If we exclude "doubtful" species from the list, the species richness of the phytoplankton of the Sevastopol seashore is appeared to be significantly lower: 27 genera, 20 families, 16 orders, 11 classes, 8 divisions, 5 kingdoms of two Empire, which is closer to the average values from 4 main publications (25, 20, 15, 10, 7 and 4 respectively). Our studies confirm the need for detailed taxonomic investigations and use of modern molecular methods in the identification of species.

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### **UKRAINE MICROALGAE DATABASE**

There is a large list of literature references which provide information on the taxonomic composition of Ukraine's (including the adjacent seas: the Sea of Azov and the Black Sea) microalgae. Describing a new species or preparing summary reports with species checklists require the analyses of all previous publications. The amount of time and effort required to perform such an analysis is tremendous. Moreover, it should be redone every time by each specialist. Even the reviewing of literature for research can be consider as rather complicated task as a large number of literature sources belong to "grey literature", which is difficult to discover and access. There is a need for development of Ukraine microalgae database that is updated on regular basis and can be accessed online.

In 2008, the IT group of Institute of Biology of Southern Seas NAS Ukraine together with leading scientists in the field of marine phytoplankton research started the development of the Black Sea marine phytoplankton check-list as well as an open access repository - the storage of published by IBSS NASU materials.

On the basis of created resource, a number of works with microalgae checklists were published. In 2014 the online resource with the database was frozen. Starting from 2018, in M.G. Kholodny Institute of Botany of NAS of Ukraine



the similar resource for the creation of Ukraine microalgae checklist (database) is being developed.

The web resource is developed on the basis of Mediawiki engine - free and open source software for wiki creation. Pages with species description include the main taxonomic information, links to literature, geographic distribution in Ukraine (marine regions gazetteer is used), ecological characteristics. Introduced AlgaeBase identifier enables the link to an internationally accepted standardized name and associated taxonomic information, and make it possible to present the most accurate and actual information on the species taxonomy. It is also decided to establish the repository of Ukraine microalgae literature and the gallery of original images obtained using optical microscopy and SEM. In addition the biovolume calculation formulas will be included to database with the reference to the European guidance on the estimation of phytoplankton biovolume (EN16695) to ensure interoperability within routine monitoring.

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## **FUNCTIONAL MORPHOLOGY OF THE DIATOM FRUSTULE IN SYSTEMATICS OF BACILLARIOPHYTA**

The diatom frustule (DF) morphology is extremely diverse, genetically determined, species-specific and is the basis for the systematics of the division Bacillariophyta. The data on the studying of the DF functions in the field of nanotechnology have allowed to make the conclusion about its taking part in many vital processes of the diatom single-cell organism. The *concept of the diatom frustule functional morphology* is grounded on the following theses (Bukhtiyarova 2009).

1. All structural elements of the DF are divided *on the basic elements* (= db-elements) and *functional units* (= df-morphs).
2. The functional units of the DF form a hierarchical system: df-converter (= velum) – areola – stria – valve – frustule.

3. The functional units of the DF has own evolution in accordance with environmental conditions in which every species of Bacillariophyta inhabits for the historical time.

4. Each functional unit of the DF performs several functions in the vital activity of the diatom algae.

The suggested concept has allowed for the first time to find out of the functional morphology for the "horseshoe spot" in epiphytic diatom genus *Planotidium* F.E. Round et Bukht.: lighting of the chloroplast side that is turned to the substrate and transformation of light energy to the thermic one. The adequate term for this functional unit of the DF – *lensoid*, was proposed (Bukhtiyarova, Lyakh, 2009) and classification of the lensoid types has been developed (Bukhtiyarova 2017).

Presence of the lensoids in the DF corresponds to the *species rank of taxonomy* as this df-morph belongs to the *unique* ones which were found in the frustule of some species as only one unit (sometimes as several ones). The main functions of the unique df-morphs are in adaptation to certain environment but not every species even within the same genus has them. For instance, many species of *Planotidium* have not the lensoids. In the same time different types of the lensoids has been found in species of other genera: *Ctenophora* (Grunow) Williams et F.E. Round, *Fragilaria* Lyngbye, *Psammothidium* Bukht. et F.E. Round, *Eucoconeis* Cleve ex F. Meister. The species of all mentioned genera, as well as of *Planotidium*, are usual in epiphitic communities, i.e. inhabit in the similar environment that is an evident of the adaptation functions of the unique df-morphs.

The other example of the unique df-morph is *catinus* – paired pores fields on the poles in the diatoms with bipolar frustules, has been the only character used to create the genus *Geissleria* Lange-Bert. et Metzeltin. As a result, the species with very different frustule morphology have been united under the same generic name. Studying of fine frustule morphology of the genus *Geissleria* had required the taxonomical revision and description of the new genera: *Baikalia* Bukht., *Navigeia* Bukht., *Placogeia* Bukht., *Grachevia* Bukht., *Goldfishia* Bukht., *Nadiya* Bukht., *Cymbelgeia* Bukht. (Bukhtiyarova, Pomazkina 2013).

Natural or phylogenetic classifications of the organisms are hierarchical, therefore specification of the DF hierarchy shows the directions of its evolution and thus evolutionary relationships in the Division Bacillariophyta. In our opinion, the basis for the diatom *taxa of higher ranks* than species can be the *universal* df-morphs

– morphologically similar and consistently many times repeated functional units that are presented in the frustule of every diatom species. Universal df-morphs perform vitally important functions in the diatom unicellular organism: absorption and excretions of the matters in cell metabolism, focusing and filtering of light energy, selective concentration of the matters which particles reach the cell membrane, biological and chemical protection. The examples of universal df-morphs are areolae and striae which fine morphology was used to divide the genus *Geissleria* on several new genera.

Functional morphology implies the study of the morphological units in the organism context, with determination their position and functions in the hierarchical structure of the organism in whole. This approach will contribute to elaboration of the natural system of Bacillariophyta.

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## **THE INFLUENCE OF OXIDATIVE STRESS ON THE QUALITY OF PHYCOLOGICAL BIOMASS**

Oxidative stress in living cells (excessive accumulation of reactive oxygen species) is characteristic practically for all intensive biotechnological processes, including phycological production. *Arthrospira platensis* Gomont (= *Spirulina platensis* (Gomont) Geitler), one of the most used cyanobacterium and valuable source of protein and antioxidants, is grown industrially in many countries and under very different conditions. Biomass production of *Spirulina* is constantly growing. In pursuit of high productivity, it is very important to keep the high quality of the biomass.

The influence of thermal, salt, illumination and chemical stress on strain *Arthrospira platensis* CNMN-CB-11 (*Spirulina*) under laboratory and production conditions was investigated. The obtained results suggest that production conditions were stressful compared to laboratory ones. Under optimal condition during accelerating growth phase in laboratory, the level of malondialdehyde

(MDA) in *Spirulina* significantly decreased, whereas industrial conditions stimulated MDA accumulation.

High temperature was a stress factor for *Spirulina* under both laboratory and production conditions. The amount of malondialdehyde in the biomass grown at high temperature (40 °C) was 3 times higher than at optimal temperature. Phycobiliprotein content in the first half of exponential growth phase dropped by 30-33 % compared to optimal conditions, both in laboratory and production conditions, but at the end of the life cycle, the amount of phycobiliproteins in biomass grown at different temperatures was approximately equal. Under laboratory conditions, lipid content in *Spirulina* biomass decreased practically twice and carbohydrates increased by 35 % at high temperature compared to the optimal temperature conditions. In the same time, the difference in lipid content was minimal, and the content of carbohydrates was lower under industrial production conditions.

*Arthrospira platensis* CNMN-CB-11 is a technological strain with short life cycle selected for industrial cultivation under continuous illumination. Thus, photoperiodism (12 h/L: 12 h/D) has determined a state of stress, leading to an increase of antioxidant enzymes (superoxiddismutase, catalase and peroxidase) activity up to 1.5 times. The protein content in *Spirulina* biomass was significantly lower under periodic illumination compared to continuous light (by 12.1-25.8%). The quantity of phycobilins and carbohydrates was higher (by 32% and up to 1.8 times, respectively) under light stress condition compared to continuous illumination.

*Spirulina* biomass under salt stress conditions contained 3 times more MDA compared to control. High concentrations of NaCl (40 and 50 g/L) substantially decreased the antioxidant activity down to 50%. Due to salt stress, biomass produced in a growth cycle decreased substantially. The amount of proteins and phycobilins decreased and lipid and polysaccharide content increased compared to control.

*Spirulina* responded to the chemical stress, induced by copper by increasing the amount of carbohydrates and lipids. Also, the quantity of phycobilin pigments in biomass of *Spirulina* significantly decreased under stress conditions.

All types of investigated stress are associated with increasing MDA amount in biomass and with the modification of the antioxidant enzymes activity and biomass composition. Depending on the type of stress and its intensity, the values

of these parameters may increase or decrease. Under moderate stress conditions, certain technological advantages such as increasing *Spirulina* biomass production and high levels of phycobilins and carbohydrates in biomass can be achieved.

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## **ECOLOGICAL AND MORFOLOGICAL STRUCTURE OF MICROPHYTOBENTHOS IN VERBNOE LAKE (UKRAINE)**

Describing and studying of aquatic organisms community structure of different grade and its structural elements are the urgent task for sanitary hydrobiology, as in a modern period due to the complex multivariable impact on water ecosystems in description and estimation of its synbioindication state is of primary importance (Oksiyuk, Davydov, 2012). Informative synbioindicators in the structure of microphytobenthos include ecological and morphological groups, combining algae with the similar ecological and morphological characteristics (Oksiyuk, Davydov, Karpezo, 2009).

The aim of the work comprised the investigation of basic ecological and morphological groups of algae in the microphytobenthos of the human-modified water body (Verbnoe Lake, Kyiv).

As a result of the studies conducted in summer 2013-2014 in the microphytobenthos of Verbnoe Lake littoral zone 8 ecological and morphological groups of algae (EMG) was distinguished. These groups related both to benthic organisms (B) – the autochthonous components of algae community, and to plankton and periphytic organisms (A) – the allochthonous forms.

Benthic organisms were represented by EMG of littoral eurytopic diatoms, large diatoms, small and rather large diatoms, filamentous blue-green algae, individual and cenobial green algae (Chlorococcales) and charophyte algae.

Benthic organisms were dominated by small and rather large diatoms (EMG B<sub>3</sub>) forming 35.4 % of microphytobenthos species richness. They were mainly

represented by obligatory benthic organisms (78.2 %), and the amount of facultative benthic organisms was substantially lower (21.8 %).

Littoral eurytopic diatoms (EMG B<sub>1</sub>) were formed both by obligatory (85.7 %) and by facultative benthic organisms (14.3 %). The percentage of EMG B<sub>1</sub> comprised 10.8 % of microphytobenthos species diversity.

The large diatoms (EMG B<sub>2</sub>) included only obligatory benthic organisms. The filamentous blue-green algae (EMG B<sub>5</sub>) included obligatory and facultative benthic organisms in equal ratio. The quantity of EMG B<sub>3</sub> and EMG B<sub>5</sub> in microphytobenthos specific richness made up 3.1 % each.

Ecological and morphological group of individual and cenobial green algae (Chlorococcales) (EMG B<sub>6</sub>) was represented by facultative benthic organisms, and ecological and morphological group of charophyte algae (EMG B<sub>8</sub>) – by obligatory benthic organisms. Among all EMG their shares in microphytobenthos species richness were the smallest – 1.5 % each.

Among allochthonous forms periphytic organisms is most representative – 32.3 % of microphytobenthos species richness. The share of planktonic organisms did not exceed 12.3 %.

Thus, it was shown, that in microphytobenthos species diversity the main role among benthic organisms belong to ecological and morphological group of small and rather large diatoms, and the main role among allochthonous components belongs to ecologic and morphological group of periphytic organisms.

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## **PLANOZYGOTES OF FLAGELLATED VOLVOCALES (CHLOROPHYTA) INHABITING EPHEMERAL WATER BODIES AND THEIR ROLE IN LIFE CYCLES OF THESE ALGAE**

Majority of algae inhabiting ephemeral water bodies (rain puddles) are movable flagellated organisms belonging to phylogenetic lineage Volvocales (Chlorophyta). Living conditions in temporal water bodies are quite extreme

because of limited time of their existence and changing conditions (salt concentration in the water, depth etc., Demchenko 2011). The fact of disappearance of water body as a result of complete drying raises a requirement to inhabiting organisms: presence of a rest stage in the life cycle which provides surviving during dry period to the moment of puddle formation after next rain. Green flagellated algae are characterized by sexual reproduction and formation of stable to drying zygote. Sexual reproduction of different species of volvoclean algae inhabiting in the same water body occurs usually simultaneously. It is a reaction of algae on increasing of salt concentration in the water caused by drying of water body. It is interesting that vegetative algal cells of some species exist during quite short period. It is known that *Chlamydomonas gelatinosa* Korshikov is characterized by planozygote formation after extremely short vegetative period. Therefore it is even difficult to find and observe vegetative cells of the alga (Korschikov, 1938). Gametes formed by alga copulate, but join partially and form unusual organism (planozygote) with duplicated organelles (including nuclei). Algae are able to long existence in planozygote state (about 20 days); they move, photosynthesize and grow like vegetative cells. Actually it is two gametes covered by common cell wall. Nuclei of planozygote join fast and form a zygote in the moment when conditions in the puddle start to be critical. Planozygote is represented gamete association or union. Therefore planozygote is typical state of some species of green flagellated algae, in which they exist during bulk of the life. Planozygote is a key stage in the life cycle of these algae and it provides surviving of species in rapidly changing conditions of ephemeral water bodies. It is possible to compare planozygotes with dicaryon stage of higher fungi, because of presence of two nuclei inside a cell. Sometimes planozygotes have fanciful shape, completely differed from vegetative cells of the species. Therefore some mistakes during identification of volvoclean algae are possible since considerable amount of known taxa of green flagellated algae have duplicated organelles (flagella, stigmas, pyrenoids, chloroplasts etc., Ettl 1983). Therefore planozygotes may be described as independent species or genera.

We observed and investigated morphology of planozygotes of 23 species of volvoclean algae (from genera *Chlamydomonas* Ehrenb., *Chloromonas* Gobi, *Polytoma* Ehrenb., *Dysmorphococcus* Takeda, *Cephalomonas* Higinbotham, *Chlorogonium* Ehrenb. and others) inhabiting ephemeral water bodies of Ukraine.

This stage of life cycle was unknown for 5 of these species. Analysis of our results showed that taxonomic status of some genera and species of volvoclean algae characterized by duplicate organelles should be revised.

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## **CHARACTERISTICS OF MORPHOLOGICAL INDICATORS OF CYANOPHYTE ALGAE *CALOTHRIX GRACILIS* F.E. FRITSCH CULTIVATED ON DREW AND Z-8 NUTRITIVE MEDIUM**

The research of morphological indicators of algae is very important, especially to identify and determine the species, as well to establish phenotype, physiological state, the influence of the environmental factors, the stage of their development and their cultivation etc.

This research was focused on the analysis of dynamical changes in dimensional morphological indicators of the cyanophyte algae *Calothrix gracilis* F.E. Fritsch, cultivated on the Drew and Z-8 nutritive medium.

The cyanophyte algae *C. gracilis* was selected in pure culture and is deposited in the collection of the Scientific Research Laboratory Vasile Şalaru, of the Moldova State University. The algae was cultivated under laboratory conditions, with the periodic method, providing continuous illumination of 3000 lux, temperature of 21-22 °C, without stirring the samples. During the experiment under monitoring were examined the morphological dimensions of vegetative cells, of heterocysts and spores through their length and width analysis. Experiments were performed during 15 calendar days, with measurements being performed over a 3-day interval. The obtained results were statistically processed using the Microsoft Office 2017 computerized program, determining the arithmetic mean and the standard error.

As a result of the research it was found that vegetative cells of algae *C. gracilis* cultivated on Drew nutritive medium had maximum values of the length of  $11.55 \pm 0.50 \mu$  and the minimum –  $6.50 \pm 3.25 \mu$  and the widths of  $6.87 \pm 0.31 \mu$  and



3.92±0.19 μ, respectively. Heterocysts had a maximum length of 5.18±0.25 μ and a minimum of 3.75±0.18 μ and a width of 5.41±0.29-4.00±0.20 μ. The spores had maximum length of 10.00±0.50 μ and a minimum of 8.12±0.41 μ and the width was 7.50±0.37 – 6.25±0.32 μ.

The cultivation of algae *C. gracilis* on the Z-8 nutritive medium, the vegetative cells had a maximum length of 9.00±0.42 μ and a minimum of 7.50±0.30 μ and the widths were between 5.62±0.22 – 2.50±0.10 μ. Heterocysts had maximum lengths of 8.37±0.43 μ and a minimum of 5.18±0.25 μ and the widths were 10.85±0.52-5.41±0.29 μ. Spores were maximum lengths of 12.5±0.51 μ and a minimum of 7.51±0.31 μ and a width of 11.25±0.48 – 5.50±0.25 μ. It is noteworthy that all of them: the vegetative cells, the heterocysts and the spores had the tendency for dimensional growth followed by decreases, which were repeated continuously. This process is similar in both cases cultivated algae *C. gracilis* on the Drew medium and Z-8 nutritive medium.

As a result of these researches, it was found that cultivation of algae *C. gracilis* on the Z-8 nutritive medium (which is rich in nutrients) has smaller vegetative cells, but the heterocysts and spores are bigger. Which shows that the lack of some micro and macro elements from the nutritive medium of the algae, contribute to the appearance of morphological changes, which are manifested especially by increased size of the vegetative cells and diminished size of the heterocysts and spores. Regardless of the composition of the nutritive medium of cultivation *C. gracilis*, the size of the cells and spores are self-regulated, they grow and then decrease, a process which is repeated continuously. The same process was noticed by us on other cyanophyte algae *Nostoc flagelliforme*, *N. gelatinosum*, *Cylindrospermum licheniforme*, *Spirulina platensis*, *N. punctiforme*, *Anabaena minima* and *Calothrix elenkinii*. The results obtained allow us to conclude that the investigated morphological indicators characteristics of *Calothrix gracilis* obey to the Biological Law of the Dimensional Grow, common practically to all living organisms, including the human species.

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**UTILISATION BIOMASS OF *NOSTOC LINCKIA* BORNET EX BORNET et FLAHAULT ALGAE AS BIOFERTILIZER FOR CULTIVATION SUNFLOWER (*HELIANTHUS ANNUUS*)**

The use of cyanophyte nitrogen-fixative algae biomass as a biofertilizer for growing crop plants is of considerable interest in the positive way of how algae effect the soil, crop plants and their ability to contribute to the restoration and improvement of natural biota, to the degradation of pesticides, herbicides, insecticides and other substances widely applied in both classical and organic farming. Among the nitrogen-fixing cyanophyte algae group is *Nostoc linckia* Bornet ex Bornet et Flahault, which inhabits intensively varied soil types, and at the same time its biomass is rich in multiple biologically active substances.

Thus, in order to highlight the influence of the *N. linckia* manifested in the cultivation of the sunflower, experiments were made in the open field, the results of which are presented in this thesis. The research was done in 2018, during May-August, in Maleiești Village, Orhei District of the Republic of Moldova within the company Vindex Agro LLC. In the experiments was used live biomass of the *N. linckia* at a dose of 3 kg/ha – considered a batch of algae, and as a control served a nearby batch with the same area, where no algal biomass was administered. Experiments were mounted on an area of 90 m<sup>2</sup> for each batch, each variant being exposed in three iterations. Algal biomass was given by sprinkling with potable water at the surface of the soil when sunflower plants had 13 leaves.

During the experiments the total nitrogen content and soil pH, the growth of the seedling and the amount of harvest have been monitored.

In variants with algal biomass administration was attested positive changes in physicochemical properties of soil. The pH of the soil changed into low alkaline direction (in the biomass variants the pH ranged between 7.48-7.65 and in the control groups 6.85-7.53) and the total nitrogen content of the soil in the algal biomass groups was higher (in the algal biomass variants the total nitrogen was between 0.46 %-0.51 % and in the control groups – 0.42 %-0.48 %). It is worth

mentioning that in algal biomass variants we observed oscillations of the total nitrogen content of the soil during the experimental period, characterized by quantitative increases followed by decreases, after which this process was repeated, which allows us to find out that the biomass of the experimental algae contributes to quantitative self-regulation of soil nitrogen.

Administration of the *N. linckia* algae biomass has had a positive impact on the height growth process of the plants throughout the monitored period. If initially the seedlings of the algal biomass groups were  $34.20 \pm 1.70$  cm high then at the end of the experiments they reached –  $258.00 \pm 2.52$  cm and in the initial control version the seedlings had a height of  $42.00 \pm 1.60$  cm, and at the end of the experiments –  $250.80 \pm 3.40$  cm.

At the same time, in algal biomass variants there was a quantitative increase of the sunflower harvest ( $3.08$  t/ha in the algal biomass and  $3.0$  t/ha in the control).

As the result of this investigation we can conclude that the application of the biomass of *N. linckia* as a biofertilizer for cultivation of sunflower contributes to the improvement of the soil properties and the increase of the yield obtained.

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## **ALGAL FLORA OF THE KUYALNIK ESTUARY: THE HISTORY OF STUDY AND CURRENT STATE**

The history of the formation, development, and functioning of the resort "Kuyalnik" (the outskirts of the Odessa city) since its formation by E.S. Andrievsky (1834) and scientific algological research in this area for almost two centuries of time is analysed in detail. The 3-period gradation of algofloristic investigations in Kuyalnik Estuary and waterbodies of its basin, as well as soil communities of a

coastline, is conducted. First period (the second half of the 19th century – the beginning of the 20th century) is characterized by intermittent mentioning of the presence of algae in the water column and in the benthos of this water body (representatives of Cyanoprocyota, Euglenophyta and Chlorophyta divisions, 4 species as a whole) (I. Shmakov, G.N. Buchinsky, E.M. Brusilovsky). Second period (first half of the twentieth century) – an attempt to introduce the ecological, floristic and hydrobiological approaches in the study of the species composition of algae during the period of changes in the hydrological and hydrobiological regime of Kuyalnik Estuary. In addition, the impact of mentioned regimes on formation of its biocenosis, as well as the nature of the distribution of the composition of algae in the water area and the territorial characteristics of the formation of algal biomass with their possible participation in the formation of peloids – creation of the foundation for the systematic study of the algae of the estuary at the Odessa I.I. Mechnikov National University (I.S. Rays, V.I. Pidlisky, N.L. Zagorovsky, I.I. Pogrebnyak). Third period (the second half of the twentieth century - the beginning of the twentieth century) – compilation of data on the floristic diversity of algae and patterns of its manifestation and changes in the period of variability of the salinity level in the reservoir, seasonal fluctuations of their taxonomic and species composition, in particular macrophyte species as the main producers of biomass during desalination, the nature of influence of individual environmental factors and the identification of patterns of development algal communities, as well as the genesis of their composition and, accordingly, the conclusion that, under the conditions of eulimene, ultrahaline species of algae do not belong to marine forms, and to forms of freshwater genesis (I.I. Pogrebnyak, N.E. Guslyakov, V.P. Gerasimiuk, D.A. Nesterova, F.P. Tkachenko).

Our investigations of the late XX – early XXI century (1983–2018) are characterized by an integrated approach to the study of species diversity and identification its specificity and patterns of distribution of algae in Kuyalnik Estuary and the waters of its basin (rivers, streams, lakes, periodic waterbodies, canals), allowed to establish the composition of these organisms according to different types of water bodies and some soils of adjacent territories (278 species, presented by 280 species and infraspecies taxa, belonging to 108 genera, 60 families, 31 orders, 10 classes and 7 divisions) and carry out its floristic and taxonomic analysis, reveal its general specificity and originality of certain types of basin water

bodies, as well as characteristic coenotic and typological complexes of algae, substrate confinement and to form their general ecological characteristics, determine the algafloristic uniqueness of the Estuary as well as to describe 3 new species for science. Studies on the Kuyalnik Estuary algae with varying salinity from 50 to 347 ‰ have shown that the salinity of the algae is the limiting factor determining the development of algae. Due to the changes in environmental conditions over the past 60 years (increasing salinity as well as pollution), the algal species composition of the Estuary has undergone significant changes. Algal flora of the studied reservoir was noticeably poorer, and significant changes occurred in the ratio of environmental groups. The marine species complex that previously existed has now changed to brackish-water and hypergalin, while the number of marine algae has decreased from 37.5 to 28.4 %, the number of brackish-water increased from 31.3 to 41.6 %, and freshwater species has not changed. The abundance of benthos microscopic algae in Kuyalnik Estuary has changed to 4.2 in the area the Korsuntsovsky ponds to 390.55 mln cells/m<sup>2</sup> near the village Stara Emetivka. Benthos microphyte biomass Kuyalnik Estuary was insignificant and ranged from 0,003 nearby Korsuntsovsky ponds to 3.457 g/m<sup>2</sup> near the village Stara Emetivka. The average biomass of phytomicrobenthos in the water area of this reservoir composed 0.523 g/m<sup>2</sup>, and phytomacrobenthos around 2000 g/m<sup>2</sup>.

This information is presented in the monography of the series "Encyclopedia of Kuyalnik Estuary" (ed. A.A. Ennan), vol. 2: Algae (2019).

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### **EFFECT OF DECOMPOSITION OF THE SEAWEED WRACK ON MICROALGAE GROWTH ON SANDY AND ROCKY SUPRALITTORAL OF NORTHWESTERN PART OF THE BLACK SEA**

The processes of decomposition of storm wrack hydrobionts and their contribution to the functioning of coastal ecosystems have been studied in both natural and laboratory conditions (Smith, Foremann, 1984; Mews, Zimmer, 2006).

In particular, it was found that a whole complex of organisms — from bacteria to macrofauna — is involved in the processes of decomposition of storm wrack (Koop, Newell, 1982). Such comprehensive studies for the conditions of the Black Sea were started in the Institute of Marine Biology of the National Academy of Sciences of Ukraine. The main results of laboratory experiments on the effect of decomposition of different origin wrack (seaweed, seagrass and mussel) on sand interstitial community are presented in the works of the authors (Aleksandrov, Dyadichko, Garkusha & al., 2013, 2016; Garkusha 2017). The purpose of this work was to study the effect of decomposition of macrophyte algae wrack on the growth of microalgae of northwestern part of the Black Sea sandy and rocky supralittoral in natural conditions.

During 2013 and 2014 field experiments of the decomposition of wrack on the sandy and rocky supralittoral in two areas of northwestern part of the Black Sea coast were studied. The first is the coast of Dofinovsky Estuary was studied from April 22 to May 14, 2013. The second is the coast of Grigorievsky Estuary was studied from May 20 to June 19, 2014. In the first experiment on sandy supralittoral sand samples were taken from vertical sections on horizons 0–10 cm (surface), 20 cm (middle layer), 40–45 cm (level of pore water) and pore water. On the Grigorievsky Estuary coast samples were taken without making vertical cuts, just in wrack and below them in the surface layer of sand. In all cases, control surveys were conducted in places where wrack was absent. Algae and remnants of seagrass, which were raised by a diver from the sea bottom, were used as wrack in the first field experiment. In 2014, the experiment used algae as wrack, which was collected in the coastal zone of the Grigorievsky Estuary. Macrophytes from the genus *Enteromorpha* were prevailed in the wrack. Wrack was laid out on a sandy beach and on rocks with a diameter of 10–20 cm at a distance of 20–30 cm from the water's line.

As a result of the field experiment, it was found that on the sandy littoral with an increase in the exposition period of the decomposition have been decrease the quantitative indicators of microalgae epiphyton seaweed. In addition, the growth of microalgae were in the layers of sand, which are located directly below the wrack. The maximum biomass  $22.00 \cdot 10^{-3} \text{ mg} \cdot \text{cm}^{-3}$  is fixed in a 20 cm layer of sand. In the experiment, the biomass of microalgae in the pore water was 2 to 4 times higher compared to the control. On the 7th day of the exposition, the abundance of microalgae in the field experiment on the sandy and rocky littoral was, accordingly, 2.8 and 1.5 times higher than in the control, the biomass was 1.75

and 1.78. In general, with an increase in the period of exposition there is a general trend: first an increase with a maximum on the 7th day and then a decrease in the quantitative indicators of microalgae. Such a phenomenon can be explained by the degree of decomposition of wrack and their gradual drying.

Thus, in the natural conditions under the influence of the decomposition of seaweed wrack on the sandy littoral was fixed the microalgae growth in layers of sand that are located directly below wrack and the biomass of microalgae in pore water have increased 2–4 times as compared to control. With an increase in the exposition period for the sandy and rocky littoral have been established the general tendency: first increasing with a maximum on the 7th day and then reducing the quantitative indicators of microalgae.

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**TO THE MORPHOLOGY AND TAXONOMY OF *AULACOSEIRA GRANULATA* (BACILLARIOPHYTA)**

This scanning electron microscopy study investigates the morphology of *Aulacoseira granulata* valves from a number of Volga reservoirs, lakes (Sestroretsky Razliv, Baikal, Khanka, Kurilskoye) and the Amur River. A high variability of the following quantitative features is revealed: valve diameter and its height, number of areola rows and areolae in 10 µm on the valve mantle, number and length of spines on the separation valves. Low values of the valve height-to-diameter ratio and a high number of areolae in 10 µm typical for *A. muzzanensis* are also recorded in *A. granulata* populations. A certain correlation is shown between some size characteristics: as the valve diameter increases, the valve height-to-diameter ratio decreases. This correlation is true for other representatives of this species (*A. baicalensis*, *A. islandica*, *A. subarctica*). The results of this study justify the reference of *A. muzzanensis* to the synonymy of *A. granulata*. Based on the original and literature data, it is suggested to extend the diagnosis of *A. granulata*.

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### **MICROSCOPIC ALGAE OF BENTHOS OF RIVERS OF THE NORTH-WESTERN BLACK SEA (UKRAINE)**

The rivers of the North-Western Black Sea region are not well understood in the algological relation. The works of N.K. Sredinsky (1872-1873) is widely known in this plan, B.N. Axentyev (1926), D.O. Svirenko (1926), P.P. Shirshov (1928), J.V. Roll (1937, 1961), A.I. Ivanov (1982, 1987), L.V. Skoryk (1992), P.D. Klochenko, T.I. Mitkovskaya, A.I. Sakevich (1993), O.A. Davydov (1997), O.S. Tarashhuk (2004), D.A. Nesterova, L.M. Terenko (2007), O.P. Tsarenko-Belous, P.D. Klochenko (2008), A.N. Mironyuk, F.P. Tkachenko (2012, 2013), etc., which are devoted to the study of microphytobenthos, periphyton and phytoplankton of rivers of the North-Western Black Sea region. However, summarizing the work on microscopic algae of benthos, which would have raised the issues of modern systematics, ecology and biogeography of microphytics, unfortunately, still do not.

The researches were carried out in the rivers of the North-Western Black Sea region (Danube, Dniester, Southern Bug, Baraboy, Big Kuyalnik, Kamenka, Kodyma, Kuchurgan, Mertvovod, Neteka, Sinyuha, Tiligul, Yagorlyk) during 1997-1998 and 2001-2018. Samples were selected in the fouling of macroscopic algae and higher aquatic plants, stones, on the surface of soft soils (sands and silt), ice. During this period 473 samples were collected and processed at 55 stations and 215 permanent and 473 temporary preparations were manufactured.

As a result of the research carried out in the rivers of the North-Western Black Sea region 438 species of microscopic algae were revealed, which belong to the 161 genus, 75 families, 38 orders, 12 classes, 8 divisions, 4 kingdoms and 2 empires. The total number of species belonged to Bacillariophyta (273 species), Cyanoprokaryota (60), Chlorophyta (57), Euglenophyta (21), Streptophyta (11), Chrysophyta (11), Dinophyta (4) and Xanthophyta (1) algae. The largest number of species of algae was observed in the following rivers: Danube (288 species), Southern Bug (206), Dniester (166), Big Kuyalnik (139), Kodyma (104), Kuchurgan (97), Baraboy (84) and Yagorlyk (54).



New species (*Spirulina albida* Kolkwitz, *Anabaena sedovii* Kossinsk., *Chrysamoeba scherffelii* (Pascher) Matv., *Mallomonas apochromatica* Conrad, *M. genevensis* Chodat, *Pseudopolyedriopsis skujae* Hollerb., *Melosira undulata* var. *normanii* Arn., *Gomphonema subclavatum* (Grunow) Grunow, *Luticola goeppertiana* (Bleisch) D.G. Mann, *Geissleria ignota* (Kraske) Lange-Bert. et Metzeltin, *Navicula alinae* Lange-Bert., *Stauroneis tackei* (Hust.) Kramer et Lange-Bert, *Amphora subacutiuscula* Schoeman, *Iconella curvula* (W.Sm.) Ruck et Nakov, *Tetracystis intermedia* (Deason et H.C. Bold) R.M. Br. et H.C. Bold and *Cosmarium horomuiense* Hirano) for water bodies of the North-Western Black Sea region were discovered in rivers.

In the rivers were also found new species of algae for the territory of Ukraine: *Anabaena sedovii*, *Mallomonas apochromatica*, *M. genevensis*, *Geissleria ignota*, *Navicula alinae*, *Stauroneis tackei*, *Amphora subacutiuscula*, *Iconella curvula*.

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### **DIVERSITY OF DIATOMS IN BIOLOGICAL SOIL CRUSTS**

Biological soil crusts (biocrusts) are formed by living organisms and their by-products, creating a microecosystem on top of the soil. Biocrusts conduct important ecological functions like soil nutrient enrichment and stabilization. Terrestrial diatoms are abundant in biocrusts, but systematic research on their biodiversity and biogeography is missing up to now. In general, terrestrial diatoms are largely ignored in research, although their aquatic counterparts are the main marine primary producers worldwide.

To close this gap of knowledge, we collected biocrusts from different locations in Germany and made permanent slides for morphological diatom identification. The slides were scanned at 630x magnification; morphometrics was done with the automated image processing software SHERPA.

The diatom composition in northern German coastal sand dunes appeared to be dominated by four genera: *Achnanthes* Bory, *Pinnularia* Ehrenberg, *Hantzschia* Grunow and *Luticola* Mann. Similar, in inland locations, *Hantzschia* and *Pinnularia* were among the most frequent diatoms. But in inland, we frequently found also the genus *Stauroneis* Ehrenberg. The species *Achnanthes coarctata* Grunow, *Hantzschia amphioxys* Grunow and *Luticola mutica* Mann are well-known terrestrial diatoms, but this is one of the few studies that shows them present in epipsammic environments. Less frequently, we also found small diatoms difficult or impossible to identify. This indicates that the biocrusts research holds the potential to find new, yet unknown diatom species.

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## **CYANOBACTERIAL ACTION AND HISTOPATHOLOGY IN INSECTS WITH A DIFFERENT TYPE OF NUTRITION**

Biocidal activity of cyanobacteria can cause various, selective and many-sided manifestations in insect organisms in dependence on their type of nutrition. We evaluated a number of histopathological abnormalities in the test objects possessing different ways of feeding (e.g. polyphagous fall webworm *Hyphantria cunea* Drury and oligophagous Colorado potato beetle *Leptinotarsa decemlineata* Say) in model assays. Leaves of typical host-plants (ash-leaved maple *Acer negundo* L. for fall webworm and potato *Solanum tuberosum* L. for Colorado potato beetle) were sprayed by aqueous suspension of dried cyanobacterial powder (*Microcystis aeruginosa* Kütz. emend. Elenk. from Dnipro habitats). Nutrition, growth, metamorphosis and viability of the second stage larvae were observed, and histopathology was examined during 7 days. Cyanobacterial biocidal action caused general inhibitory specific effect, including mainly deterrent and toxic components,

and induced irreversible changes first of all in digestive tract and then in other organs. Comparative analysis of cyanobacterial action revealed some differences in histopathological picture of test objects. In particular, in the first days of observation processes of the midgut epithelium desquamation had pronounced effect in Colorado potato beetle more than in fall webworm. This is because of superior defensive system in polyphagous organisms, as evidenced by the abundance of bottle cells in fall webworm. On the other hand, the peritrophic membrane is absent in Colorado potato beetle, so cyanobacterial biologically active substances are in direct contact with the midgut epithelium. Besides the morphology of fat body, have visible differences in fall webworm and Colorado potato beetle. In the first case, we observed necrobiotic aberrations, as well as sudden loss of its volume; this process is in a progress, especially during five-seven days of assay. Fat body of Colorado potato beetle has necrobiotic and dystrophic degeneration, but its reduction is significantly less than in fall webworm. Interestingly, the status of fat body is a very sensitive and important indicator of cyanobacterial biological activity or/and toxicity. Firstly, degradation of fat body is the typical morphological character of deterrent effect and nutritional disease, and decrease of its size is an evidence of intestinal adverse event. Meanwhile dystrophy and necrosis of this substance are the results of penetration of cyanobacterial biological active or/and toxic compounds. We observed common vacuolization of cytoplasm, blurring of distinctions between cells, and cytolysis consequentially. In most cases, we revealed these abnormalities in fall webworm as very intensive and rapid, more frequently than in Colorado potato beetle; the destructive effect took place during five days. In some individuals fat body disintegrated in total, in others it remained as a group of very small islets. Degradation of digestive tract produces irreversible abnormal changes in excretory system of the both test objects, *e.g.* in Malpighian tubes (karyolysis, desquamation of epithelial cells, degradation and necrosis, especially in fall webworm), as the adverse after-effects of general intoxication. In a parallel, we observed disintegration of muscle fibers, including homogenization of cytoplasm in their cells, pathological thinning and vasculum. These processes can be a part of the general solution of insect organism continuity and its vital rhythms. In despite of predominantly intestinal mode of cyanobacterial action (MoA) on insect organism, external application of suspension to the larvae

adversely affected the cuticle, superficial part of respiration system, and fat body in subcuticular zone.

As a result, possible reason of insect mortality is not only in toxic effect, but also in trophic factor, and cyanobacterial biocidal characteristics can be determined more likely as deterrent than toxic; and hereby fall webworm is more susceptible to cyanobacterial influence than Colorado potato beetle.

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**THE IMPORTANCE OF THE EPITYPE CONCEPT FOR RELIABLE SPECIES DETERMINATION IN PROTISTS SUCH AS DINOPHYTES**

Solid knowledge about ecosystem functioning and community dynamics during seasonal or longer periods, as well as conservation strategies and the impact of invasive species, essentially relies on precise original data about the spatial and temporal occurrence of the inhabiting species. For unicellular organisms such as dinophytes, the biodiversity assessment did not started until the late 18<sup>th</sup> century using light microscopy. Type material, particularly of older taxa, consists of specimens mounted permanently on glass or mica slides or of illustrations only. In many cases, type material is ambiguous and makes reliable species determination problematic because of various sources of error, including imperfect documentation of subcellular diagnostic traits or insufficient morphological differentiation within cryptic species complexes. For a correct application of such ambiguous scientific names, the Shenzhen ('botanical') Code (ICN) provides a tool for designation of interpretative epitypes. In our ongoing research, we clarify the taxonomic identity of dinophytes (such as *Durinskia oculata* from Prague, Czech Republic; *Palatinus apiculatus* from Berlin, Germany; *Parvodinium mixtum* and

*Spiniferodinium limneticum* from Tatra Mountains, Poland) by collecting samples at corresponding type localities. After establishing living strains, species are DNA-barcoded using rRNA sequences and investigated using contemporary light and scanning electron microscopy. Strains that are morphologically consistent with corresponding protologues are used for designation of interpretative epitypes in form of permanent slides for light microscopy. The significant difference from the historical types is that fully documented epitypes correspond to living material enabling DNA sequencing as well as experiments in ecology. Thus, epitypification is a key tool for a stable taxonomy and reliable species determination ensuring an unambiguous link between a scientific species name, its protologue, morphology, ultrastructure, genetic characterisation and spatial distribution, all of which are of great importance especially for character-poor, unicellular organisms such as dinophytes. Species with clarified names can be studied also in terms of spatial and temporal distributions as well as of specific habitat requirements, with the potential of being reliable bioindicators. The resulting predictability of protist occurrences has also importance for applications such as Water Framework Directives as well as for the consideration of the ongoing environmental changes.

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## **REGULATION OF METABOLISM IN ALGAE FOR THE PRODUCTION OF LIPIDS AND BIOLOGICALLY ACTIVE SUBSTANCES**

The progress of algae evolution was possible due to the ions of various metals, including so-called heavy metals in toxic concentrations in their habitat environment. As a result, there formed mechanisms of toxin resistance that support their optimal level in cells. Ions of many metals in some concentrations are effective regulators of metabolism, which is an ecological factor in the regulation of their population.

We investigated the structural and functional features of membranes at absorption of  $Mn^{2+}$ ;  $Zn^{2+}$ ;  $Cu^{2+}$ ;  $Pb^{2+}$ ;  $Cr^{3+}$  by unicellular green algae *Chlorella vulgaris*

Beij. The research of the absorption and influence of the ions of metals was conducted:  $Mn^{2+}$  (0.1; 0.2; 0.5 mg/l);  $Zn^{2+}$  (1.0; 2.0; 5.0 mg/l);  $Cu^{2+}$  (0.001; 0.002; 0.005 mg/l);  $Pb^{2+}$  (0.1; 0.2; 0.5 mg/l),  $Cr^{3+}$  (1.0; 2.0; 5.0; 10.0 mg/l) during 1-7 days on the intensity, direction and localization of lipid biosynthesis in *Chlorella* in order to develop technologies improving the biosynthesis of lipids. It is established that absorption of ions of  $Mn^{2+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Pb^{2+}$  and  $Cr^{3+}$  by cells of *Chlorella* is fluctuating and carried out in four stages: the stage of protective self-isolation of cells (stress-reaction), the stage of the active absorption of metals, the stage of secondary inhibition of the absorption, and the stage of restoring active absorption, that correspond with structural and functional reconstruction of cell. For the first time it is showed that investigated factors contribute to the accumulation of lipids of around 15-113 %, including triacylglycerols – 36-181 %, dyacylglycerols – 6-190 %, phospholipids – 1,6-10,5 %, nonetherified fatty acids – 49-257 % in comparison with control. Here with, there are synthesized first of all polar phospholipids, also triacylglycerols and dyacylglycerols with residues of unsaturated fatty acids. Ratio of major classes of lipids (TAG:DAG:PL:FFA, %) in the control amounted to 22:16:47:15, for the actions of:  $Mn^{2+}$  – 27:25:28:20,  $Zn^{2+}$  – 26:16:33:25,  $Cu^{2+}$  – 22:22:39:17,  $Pb^{2+}$  – 21:21:37:21,  $Cr^{3+}$  – for the actions of I during the 7th day – 431:11:39:19. Substrates for lipid synthesis are glycerol-3-phosphate, formed by the phosphorylation of glycerol and acetyl-CoA, formed from amino acids, and providing with energy of this process happens due to activation of the citric acid cycle, as evidenced by increased activity of 2-oksoglutarate dehydrogenase (by 5 % and 35 % compared with control for actions of  $Zn^{2+}$  and  $Cr^{3+}$  respectively) and succinate dehydrogenase (in 2.8-5.6 times compared with control for actions of all investigated metal ions).

For the first time, it is established that biotechnologically effectiveness for the intensification of the biosynthesis of lipids by *Chlorella* are triacylglycerols – by 6 % is effect of  $Zn^{2+}$  (5.0 mg/l, 7 days), and phospholipids – by 18-34 % are effects of  $Cu^{2+}$  (0.002 mg/l, 3 days),  $Pb^{2+}$  (0.5 mg/l, 7 days),  $Cr^{3+}$  (5.0 mg/l, 7 days). It is established that the regularities biosynthesis of individual classes of lipids (phospholipids and triacylglycerols) can serve as a basis for the development of technology of industrial cultivation of algae in order to obtain industrial perspective lipid biomass.

In the process of incubation of an algal pure culture of *Chlorella* during 7 days in the presence of sodium selenite (10.0 mg/l) in conjunction with Cr (III) (5.0 mg/l) in lipids an increase of the content of selenium and chromium against the control indicators was found. In the process of study in lipid metabolism, the growth of the content of common lipids was noted, and it was discovered that the accumulation of selenium and chromium in their complexes occurs most in the diacylglycerols and phospholipids. It was selected the lipid and selenium chrome lipid complex, per introduction to healthy rats at a dose of 1.85 µg selenium, 1.1 µg of chromium and 0.5 mg of lipids per ml of 1 % water-starch suspension, they didn't caused by endogenous intoxication in the body, the prooxidant processes in the liver and blood serum of animals were oppressed, activated antioxidant status, which are used in the formation of components of antioxidant system. The obtained results open the prospect of using selenochromlipid complex as a biologically active additive for the prevention and improvement of metabolic disorders in diabetes type 2.

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### **ALGAE DIVERSITY OF THE CENTRAL ASIA WATER BODIES**

A brief survey of literature and original data, and also comprehensive results on study of algae diversity in Central Asia water bodies are given. There are about 3000 species and infraspecific taxa of different taxonomic groups were found in Central Asia (Tajikistan, Uzbekistan, Kirgizstan, Turkmenistan and south part of Kazakhstan) water bodies. Algae biodiversity of water reservoirs in Tajikistan and in Uzbekistan studied better then in all other Central Asian countries. All 3000 species and infraspecific taxa of algae are belonging to 423 genera, more than 100 families and 11 divisions: Cyanophyta (Cyanobacteria), Euglenophyta, Cryptophyta, Dinophyta, Chrysophyta, Xanthophyta, Bacillariophyta, Chlorophyta, Streptophyta, Glaucophyta and Rhodophyta. Among them Bacillariophyta, Chlorophyta, Cyanophyta and Streptophyta more abundant in their species quantity (representing more than 300-500 species and infraspecific taxa), and their

distribution area in different water bodies of Central Asia. This region is divided on several botanical-geographical regions: Pamir-Alay, Syrdariynskiy, Amydariynskiy, Aral-Kaspiyskiy, Aral Sea, Kyzyl-Kumskiy, Karakumskiy and Tien-Shanskii. The greatest number of algae – more than 2500 species and infraspecific taxa was found in Pamir-Alay, Syrdariynskiy and Amydariynskiy regions. Flora of the Central Asia algae in botanical-geographical analysis demonstrates to be genetically dissimilar and involves different elements of flora: boreal, boreal-mountain, nemoral, ancient Mediterranean and multiregional. Slightly expressed endemism of algae (1.5 %) and prevalence of boreal and multiregional elements (24-35 %), testify that allochthonous elements had penetrated from Siberia and China play an important role in the origin of algae flora of the Central Asia.

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## **TRANSCRIPTOME ANALYSIS AND METABOLITE PROFILING REVEAL A VERTICAL DIFFERENTIATION WITHIN A *ZYGNEMA* SP. (*ZYGNEMATOPHYCEAE*, *CHAROPHYTA*) MAT FROM THE HIGH ARCTIC**

The conjugate green alga *Zygnema* sp. thrives in extreme habitats and was collected in the high arctic near Longyearbyen, Svalbard. From the algal mat two different layers were extracted, the bottom layer contained dark green algal filaments and a top layer contained pale filaments, directly irradiation exposed. These layers were separately analyzed by a combined metatranscriptomic and metabolite profiling approach. Quality filtered transcriptomic reads were assembled to 122,089 genes with N50 of 764 bp. 6,569 genes were significantly up-regulated in the upper layer, 149 down-regulated. Amongst the up-regulated



genes, compounds of PS I and II, light-harvesting complexes and chlorophyll synthase, and early light-inducible proteins (ELIPs), carbohydrate metabolism including starch degrading enzymes and cell wall metabolism were found. Furthermore amino acid-, ascorbate- and thioredoxin metabolism, ROS scavengers and genes involved in DNA repair were up-regulated. A total of 173 metabolites were identified by a GC-MS approach, in the top layer the strongest differentially accumulated compounds were glucose, maltose, mannose and amino acids proline and alanine. Starch degradation was observed by transmission electron microscopy. These findings lead to the conclusion that the top layer of a natural *Zygnema* sp. mat protects the bottom layers and is highly activated by the irradiation input.

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## **MORPHOFUNCTIONAL ORGANIZATION OF THE "BASIPHYTE-EPIPHYTE" ALGOSYSTEM OF THE GULF OF ODESSA**

The "basiphyte-epiphyte" algosystem is an integral autotrophic community in which macro- and microalgae with different ecological activity create a dynamic system. The ratio of components of the algosystem depends on the intensity of the primary production process.

The morphofunctional organization of the "basiphyte-epiphyte" algosystem of the Gulf of Odessa was investigated depending on the season, type of substrate (artificial, natural), depth and level of eutrophication, during the period 2007-2018.

During the research, the following morphofunctional indices were analyzed: specific surface ( $S/W$ ,  $m^2 \cdot kg^{-1}$ ) and surface index (SI, units), which quantitatively express the ecological activity and the intensity of the autotrophic process.

The value of the specific surface of the macro- and microcomponent of the "basiphyte-epiphyte" algosystem depends on the season and has a different dynamics. For basiphyte, there is a decrease in the  $S/W$  value from winter to summer and a slight increase in the autumn months. For epiphytes, on the contrary,

increase in the value of the surface area from the winter months to the elderly and a slight decrease in the autumn is observed. Thus, epiphytes with high ecological activity receive the advantage of developing in seasons with an increased intensity of autotrophic process.

The seasonal correlation of the contribution of basiphytes and epiphytes to the active surface of the algosystem during the year is determined. The largest contribution to the active surface of the community of microepiphytes is traced in spring (75 %), the smallest – in summer (35 %). The winter season is characterized by the same contribution of the basiphytic and epiphytic component of the algosystem, in the the autumn season the predominance of basiphytes is investigated.

On the natural substrate, the surface index value of the basiphytic component of the algosystem was higher in 1.5 times, and the epiphytic – almost twice according to the values of the SI of these components on the artificial substrate. The obtained results show that the natural substrate in the coastal zone creates prevailing conditions for the development of the "basiphyte-epiphyte" algosystem in comparison with the artificial substrate.

The dependence of the surface index of components of the "basiphyte-epiphyte" algosystem on the depth of growth was established. The development of components of the algosystem at depths of 0 and 0.5 m sets the maximum values – the SI was equal to 45 units, which is twice higher than the SI at depths of 1 and 1.5 m.

Eutrophication determines the development of components of the "basiphyte-epiphyte" algosystem, which allowed it to be proposed as a holistic object for assessing the ecological status of the aquatic environment in accordance with the standards of the EU Water Framework Directive. Indicators of the algosystem – the coverage of basiphyte by epiphytes ( $P_{(b/e)}, \%$ ) and the ratio of the surface of the basiphyte and epiphyte ( $SI_b/SI_e$ , units) are characterized by convenience, accuracy and high sensitivity to the level of eutrophication of marine coastal ecosystems.

By indicator  $SI_b/SI_e$  status classes of sections of the Gulf of Odessa were determined, differing in the level of man-made load. It was shown that the waters near the Cape Pivnichnyy and Velykyy Fontan, attributed to the status of the class "High" and "Good", respectively. This is due to the fact that they are located away from industrial enterprises and have intensive water exchange. The coastal area of

the Cape Malyy Fontan, which is located in a zone with a large recreational load and with insufficient water exchange, is rated by the category "Moderate". The water area of the Odessa seaport is classified as "Bad". In general, according to the indicators of the "basiphyte-epiphyte" algosystem, the coastal waters of the Gulf of Odessa are rated "Moderate" as a status class, which is confirmed by the results of other studies (Minicheva, 2013).

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## **THE GENUS *SYNURA* EHRENB. (CHRYSOPHYCEAE) IN UKRAINIAN ALGAL FLORA**

The genus *Synura* Ehrenb. comprises colonial chrysophytes with cells covered with siliceous scales. The prominent Ukrainian phycologist A.A. Korshikov was the first who made a revision of this genus based on the scales structure visible under the light microscope (Korshikov 1929). Current taxonomy of the genus *Synura* is based exclusively on the ultrastructure of scales and the correct identification is possible under the electron microscope only (Kristiansen, Preisig, 2007; Jo et al., 2016).

During our studies of silica-scaled chrysophytes from the water bodies of Ukrainian Polissya and Male (Lesser) Polissya we revealed 13 taxa from the genus *Synura* (Kapustin, Tsarenko, 2013; Kapustin, Gusev, 2015, 2016; Kapustin, Gusev, Lilitskaya, 2016). Most of them are reported for the first time in Ukraine and one species was described as new to science.

Below we give the synopsis of the genus *Synura* in Ukrainian algal flora.

*Synura* Ehrenb,

Section *Synura*

*Synura uvella* Ehrenb. emend. Korshikov, *S. splendida* Korshikov.

Section Curtispinae

*Synura curtispina* (J.B. Petersen et J.B. Hansen) Asmund, *S. echinulata* Korshikov, *S. korshikovii* D. Kapustin et E.S. Gusev, *S. leptorrhabda* (Asmund)

K.H. Nicholls, *S. sphagnicola* (Korshikov) Korshikov, *S. spinosa* Korshikov, *S. synuroidea* (Prowse) Pusztai et al.

Section *Peterseniae* Petersen & Hansen ex Balonov

*Synura conopea* Kynčlová & Škaloud, *S. glabra* Korshikov emend. Kynčlová & Škaloud, *S. macropora* Škaloud & Kynčlová, *S. petersenii* Korshikov emend. Škaloud & Kynčlová, *S. petersenii* f. *columnata* Siver

Among the species of *Synura* which had previously been recorded in Ukraine (Dogadina, 2006) we didn't find only two ones, *S. adamsii* G.M. Smith and *S. splendida* Korshikov. *Synura lapponica* Skuja currently belongs to the genus *Neotessela* Jo et al., however its record in Ukraine should be verified.

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## **ALGAE BIODIVERSITY OF SOME WATER RESERVOIRS OF CENTRAL AND SOUTH TAJIKISTAN**

There are several water reservoirs were built in Central and South Tajikistan: Roghun, Norak, Boigozi, Sangtuda-1, Sangtuda-2, Muminobod, Selbur, and Sarband. Due to the literature data and original investigation, 350 species (394 species and infraspecific taxa) were found in these water reservoirs. They belong to 8 divisions of algae, 15 classes, 38 orders, 72 families, and 147 genera. Bacillariophyta and Chlorophyta are dominant due to their species abundance and their taxonomic structure. They represent 42 and 22 % respectively from all algal species of mentioned water reservoirs. Species diversity of Cyanoprokaryota is near 9 %, Chrysophyta – 8 %, Streptophyta – 8 %, and Euglenophyta – 7 % from all algal species founded in water reservoirs. Algal flora of each water reservoirs has been identified, and algae species diversity in Muminobod and Norak Reservoirs were the highest (175 species and infraspecific taxa, or 45 %; and 162 or 45 % respectively), lesser in Selbur (137 or 38 %) and in Sarband (110 species and infraspecific taxa or

34 % from all algae species founded in water reservoirs). The main role on algal flora formation in all investigated water reservoir belong to Bacillariophyta and Chlorophyta, which take respectively the first and second places in species and interspecies diversity. Species diversity of Cyanoprokaryota was higher in Sarband in comparative to other reservoirs, but they have lesser species diversity in Muminobod, Norak and Selbur, keeping the fours place due to systematic abundance of algae divisions. Increasing role of Chrysophyta algae was marked in Norak Reservoir (25 species and 27 infraspecific taxa), but they represent the less species abundance in other reservoirs. Among Chlorophyta and Streptophyta species, which were found in explored water reservoirs, a number of species are interesting and new floristic findings for Tajikistan algal flora: *Pedinomonas minor* Korschikov, *Haematococcus pluvialis* Flot. emend. Wille, *Chlamydomonas conferta* Korschikov, *Ch. gloeocystiformis* Dill, *Ch. steinii* Gorosch., *Ulothrix aequalis* Kütz., *U. limnetica* Lemmerm., *U. subtilissima* Rabenh., *U. variabilis* Kütz., *Chlorhormidium rivulare* (Kütz.) Starmach, *Chaetophora elegans* Hazen., *Stigeoclonium stagnatile* (Hazen) Collin., *Draparnaldia plumosa* (Vaucher) C. Agardh, *Penium cylindrus* (Ehrenb.) Bréb. ex Ralfs, *Closterium libellula* Focke ex Ralfs, *C. tumidum* Johns., *Cosmoastrum punctulatum* (Bréb.) Pal.-Mordv. и др.

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### **GROWTH INTENSITY OF *DESMODESMUS COMMUNIS* HEGEW. AND *D. SUBSPICATUS* HEGEW. ET SCHMIDT IN VARIOUS ENVIRONMENTS**

Microalgae are of considerable interest as active producers of biomass for a variety of purposes. In this connection, the researches directed at finding promising species, determining the optimal conditions for their cultivation and the possibility of increasing yields continue.

One of the important areas of such researches is the choice of nutrient medium. The purpose of this work is to determine the peculiarities of growing

cultures of two representatives of the *Desmodesmus* genus, depending on the composition of the culture environment.

The intensity of growing green algae *Desmodesmus communis* Hegew. and *D. subspicatus* Hegew. & Schmidt (Chlorophyta) was investigated. Pure algae of cultures were grown under extensive conditions at a temperature of  $25 \pm 0.5^\circ\text{C}$ , a light intensity of 2.5 kcal and periodic mixing. The experiments used Fitzgerald environments (modified by Zehnder and Gorham), Tamiya and Bold. The concentration of algae cells was determined by counting in Horyayev chamber (Metody fiziologo-biokhimicheskogo issledovaniya vodorosley ..., 1975) and calculating the daily average gain ( $k = \frac{N_t - N_0}{N_0 * t}$ , where  $N_0$  and  $N_t$  - cell concentration, ml / ml, at the initial and final period,  $t$  is the study time interval, days).

The selected culture environments are slightly different in composition of anions and cations. However, the most significant differences between the environments are the difference in the content of the main nutrient elements. The Tamiya environment has a smaller amount of macro-nutrients, but contains 10-20 times more nitrogen (0.69 g/l) and significantly higher phosphorus concentration (0.285 g/l) compared to two other media. The Bold environment prevails over Fitzgerald environment by the amount of phosphorus phosphates (0.053 g/l versus 0.007 g/l). Thus, the Bold environment is the poorest in nitrogen content, and the Fitzgerald environment is the content of phosphorus, while the Tamiya environment is the richest in the main biogenic elements.

It has been established that in these nutrient media under extensive conditions the intensity of growing the investigated species of green algae is marked by distinct differences.

The highest growth rates for the investigated types of algae were observed on the Fitzgerald medium: the concentration of *D. communis* cells increased by 4.5 times, *D. subspicatus* increased by 11.2 times over 33 days, which is, according to the daily average growth rate, equal to 0.11 and 0.31 respectively. At the same time *D. communis* during this period practically went into a stationary stage of growth, while *D. subspicatus* continued to intensively grow.

In the Bold nutrient media, the intensity of algae growth was much lower, with accelerated output of all crops to the stationary stage. At the same time, for

the Tamiya nutrient media, the lowest indicators of the intensity of algal growth were recorded.

On analyzing the results obtained, we can conclude that in extensive conditions, high concentrations of nutrients do not contribute to the intensive growth of green algae and shorten its duration.

The intensity of growing algae essentially depends on the composition of the nutrient medium, but its formation is species-specific. In extensive cultivation conditions, investigated algae prefer environments with moderate concentrations of nitrogen and phosphorus. When using the Fitzgerald environment, *D. subspicatus* has a greater productive potential.

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**BETA-IONONE AS A STIMULANT OF CAROTENOGENESIS IN  
*HAEMATOCOCCUS PLUVIALIS* FLOTOW**

Beta-ionone is a carotenoid derivative often found in plant essential oils. It can also be synthesized chemically. In the fermentation culture of mold *Blakeslea trispora* Taxter for beta-carotene production, beta-ionone has a long history of use as an effective stimulant of beta-carotene synthesis and accumulation (Anderson et al., 1958). In *B. trispora* beta-ionone is considered an analog for trisporic acid (Rao, Modi, 1977) – fungal sex hormone involved in “plus” and “minus” strains mating and stimulant of beta-carotene synthesis and conversion into trisporic acid itself (Werner et al., 2012). Fungal trisporic acid belongs to a wider class of biochemical compounds called apocarotenoids that includes other signal molecules, such as vitamin A in animals and abscisic acid in plants (Liang et al., 2018). Abscisic acid was shown to stimulate astaxanthin synthesis in green alga *Haematococcus pluvialis* Flotow (Gao et al., 2013), an industrial source of this valuable carotenoid for aquaculture and functional food (Shah et al., 2016).

The aim of this study was to check whether relatively cheap and easy to handle synthetic beta-ionone can stimulate accumulation of astaxanthin in

*H. pluvialis* and beta-carotene in *Dunaliella salina* Teodor., another important algal industrial source of carotenoids for food and feed (Packer et al., 2016).

The algae were cultivated under the full factorial design, 16 experimental samples, 4 factors each at 2 levels: 3 of them were light (2 or 8 klx), nitrogen source ( $\text{KNO}_3$  for *D. salina* and  $\text{NaNO}_3$  for *H. pluvialis*, not added or 80 mg/l), and phosphorus source ( $\text{K}_2\text{HPO}_4$ , not added or 10 mg/l). The 4<sup>th</sup> factor was salinity for *D. salina* (NaCl, 58 or 232 g/l) and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  concentration for freshwater *H. pluvialis* (not added and 25 mg/l). Cultures were grown for 43 days in the fed-batch regime, nitrogen and phosphorus supplied in half-dose every 3-4 days. Beta-ionone (0.005 %) was added to the culture medium at the end of cultivation, as it is usually applied to *B. trispora*, 3 days before separating cells from the medium. The 16 experimental samples of the full factorial scheme without beta-ionone addition served as control. On the 43<sup>rd</sup> day, cell yield in each sample was counted using hemocytometer. Cells were separated from the medium by centrifugation and pigments were extracted with ethyl acetate; for *H. pluvialis* having a thick cell wall, the cells were ground with a mortar and pestle during extraction. The absorbance of pigment extract from each sample was measured at 440 nm and total carotenoid content calculated according to the reference formula  $E^{1\%}_{1\text{cm}} = 2500$  (IARC, 1998). Cellular carotenoid content was expressed as pg per cell. The experiments were repeated in triplicate. Distribution of the data was assessed by Shapiro-Wilk's normality test. ANOVA was used to analyze the data, and Fisher's LSD test was applied to compare the samples.

Microscopic examination of the cultures and cell counts showed that in *D. salina* beta-ionone caused substantial color loss and death of most cells. Presumably, beta-ionone acted as an organic solvent damaging cell membranes and penetrating naked cells of this species. In *H. pluvialis* beta-ionone stimulated the transition from motile cells to thick-walled aplanospores but did not influence the total cell counts in the cultures. In *H. pluvialis* among all the samples in the experimental design, beta-ionone stimulated carotenoid accumulation more than twice under nitrogen starvation and high light. These factors themselves are known to be the most potent inducers of carotenogenesis in *H. pluvialis* (Fábregas et al., 2003). The observed effect of beta-ionone suggests that apocarotenoids could play the signal role in carotenogenesis induction by nitrogen deficiency and high light in *H. pluvialis*. Beta-ionone could be potentially used to stimulate carotenogenesis in *H. pluvialis* industrial culture.



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### **PHYCOBIONT AND MYCOBIONT SWITCHING IN LICHEN SYMBIOTIC ASSOCIATION**

A phenomenon of photobiont shift in lichen symbiotic association was an object of special study since the dual nature of lichens was open in XIX century. It is now well accepted that photobiont switching in lichen association is rather common, it has a number of ecological advantages, but mostly it does not have taxonomic consequences.

Evidences of mycobiont switching in lichen symbiotic association found to be documented only in molecular era of investigation of lichens and especially during last decade. A phenomena of presence of 'extraneous mycobiont DNA' in lichen association is proposed to describe cases when results of DNA extraction belong to another mycobiont, and do not belong to either 'own [=expected]' mycobiont, endophyte or endolichenic fungi.

Illustrations of such phenomenon were obtained within special study of molecular characters of the Teloschistaceae (Kondratyuk et al. 2013 a, 2014 a, b, 2015 a, b, c, 2016 a, b, 2017 a, 2018 a, b), as well as special study of molecular characters within the '*Lichen Flora of Korea*' project (Hur et al. 2016, 2018; Kondratyuk et al. 2013 b, c, 2014 c, 2015 d, e, 2017 b, c, 2019 a).

Cases of the presence of nuclear and mitochondrial DNA of *Ivanpisutia oxnerii* in the thalli of *Rinodina xanthophaea*, the presence of DNA of widely distributed in Eastern Asian region *Biatora longispora* in thalli of *Oxneriopsis oxneri*, *Agonimia pacifica*, as well as the presence of DNA of *Phyllopsora* species in thalli of *Agonimia pacifica* and *Verrucaria marginata*, etc. will be illustrated and discussed. Published results of the presence of 'extraneous' mitochondrial DNA in thallus of *Sedenkikovaea baicalensis*, or in *Oxnerella safavidiorum* as well as members of the subfamily Brownlielloideae of the Teloschistaceae published in previous years will be shown too.

Conclusion that a phenomenon of presence of 'extraneous mycobiont DNA' in lichen symbiotic association may illustrate the possibility of mycobiont switching in lichen thalli is done. Taxonomic consequences of this phenomenon will be also discussed.

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## **ALGAE OF WATER BODIES IN THE NATIONAL NATURAL PARK PIVNICHNE PODILLIA (LVIV REGION, UKRAINE)**

The National Natural Park (NNP) Pivnichne Podillia created in 2010 is located in the Brody, Bug and Zolochiv districts (Lviv Region, Ukraine) with total area 15587.92 hectares (Konishchuk, Batochenko, Pankovska, 2017). According to algofloristic zoning of Ukraine (Palamar-Mordvintseva, Tsarenko, 2015) its territory belongs to the Middle Dnipro Algofloristic Subprovince and Left-Bank-Dnister Algofloristic District. Previously, an algal diversity of this territory was practically unexplored. Therefore, in 2017 during of the field investigation in the Zakhidniy Buh and Seret river basins some samples of plankton, periphyton and benthos were collected from such main water bodies as rivers, ponds, bogs and streams. They were studied by routine methods (Vodorosli..., 1989) using light microscopes Amplival and PrimoStar Carl Zeiss Jena.

A total of 111 species (114 infraspecific taxa) have been revealed in the water bodies of the NNP Pivnichne Podillia. They belong to 69 genera, 42 families, 29 orders and 15 classes from 8 divisions: Cyanoprokaryota, Euglenophyta, Chrysophyta, Xanthophyta, Bacillariophyta, Dinophyta, Chlorophyta, and Charophyta. Bacillariophyta (33.3 % of total species number) and Chlorophyta

(30.7 %) lead in species diversity. They are followed by Euglenophyta (12.3 %). A variety of Cyanoprokaryota (7.9 % of total species number), Charophyta (7.9 %), Xanthophyta (4.4 %), Chrysophyta (2.6 %) and Dinophyta (0.9 %) is represented rather lower.

Among them 17 species (14.9 % of total species number) are the most widely distributed within the NNP Pivnichne Podillia and found in all types of water bodies. These species are *Microcystis wesenbergii* (Komárek) Komárek ex Komárek (Cyanoprokaryota); *Euglena pavlovskoënsis* (Elenkin et Poljanski) T.G. Popova, *Trachelomonas oblonga* Lemmerm., *T. volvocina* (Ehrenb.) Ehrenb. (Euglenophyta); *Aulacoseira italica* (Ehrenb.) Simonsen, *Epithemia gibba* (Ehrenb.) Kütz., *Gomphonema acuminatum* Ehrenb., *G. coronatum* Ehrenb., *Melosira varians* C. Agardh, *Meridion circulare* (Greville) C. Agardh (Bacillariophyta); *Coelastrum microporum* Nägeli, *Desmodesmus opoliensis* (P.G. Richter) E. Hegew., *Enallax acutiformis* (Schröd.) Hindák, *Mucidosphaerium pulchellum* (H.C. Wood) C. Bock, Pröschold & Krienitz, *Monoraphidium griffithii* (Berk.) Komárk.-Legn., *Pseudopediastrum boryanum* (Turpin) E. Hegew. (Chlorophyta); and *Coleochaete irregularis* Pringsh. (Charophyta). The majority of them is also widely distributed species in Ukraine in whole. The greatest algal species diversity is revealed in rivers (59.6% of total species number), less in bogs (40.3 %) and the least in streams and ponds (16.7 % and 9.6 %).

In addition, two localities of rush phytocenosis *Charetum vulgaris* Corillion 1957 have been found in the NNP Pivnichne Podillia (Brody District, near Verkhobuzh Village, Seret River and near Zvyzhen Village, bog, canal, V.V. Konishchuk 16.07.2017; area of revelé ca. 2 m<sup>2</sup>, projective cover 100 %). This association is one of the most widely distributed in Ukrainian Forest-Steppe and Steppe; however, for the Lviv Region it is recorded for the first time.

Thus, the first study on algae of the NNP Pivnichne Podillia has showed their sufficient species diversity that is important recommendation of this territory for regional nature conservation as well as the necessity of its further investigation and protection.

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### **ASSESSMENT OF MACROALGAL SPECIES DIVERSITY ON WATER AREA OF THE PA "KURGALSKY" (THE GULF OF FINLAND, BALTIC SEA)**

Regional complex sanctuary "Kurgalsky" is situated in the Kingisepp District of the Leningrad Region, on the Kurgalsky Peninsula and adjoining water area of the Gulf of Finland up to 10 m isobath. Total area of the sanctuary is 59950 hectares, including 38400 hectares (64 % of area of the PA) of water area of the Gulf of Finland. The construction of the "Nord Stream – 2" gas pipeline passed through the territory and water area of this PA. Therefore, we believe that it is important to publish data on the assessment of the species diversity of macroalgae of the PA "Kurgalsky", which we performed earlier, before the construction of the gas pipeline, in 2007 and 2008. The Sanctuary has status the Ramsar Site. Shallow water areas, bays and straits between the islands serve as feeding sites for masses of waterfowl during their seasonal migrations. On the PA coastal shallow waters have status of specially protected objects. Macroalgae play an important in the formation of bottom vegetation of coastal waters in the sanctuary. Macroalgae flora of water area of The Gulf of Finland devoted to the PA Kurgalsky was studied in 2007–2008. All samples were taken by algologist-SCUBA-diver. The flora of macroalgae of water area of the Gulf of Finland related to regional complex sanctuary "Kurgalsky" presented by 19 species. 9 species from them related to green macroalgae (Chlorophyta). Charophyta are presented by the two species only. Brown algae (Phaeophyceae) are presented by 5 species and Rhodophyta are presented by 3 species. 4 species of macroalgae (*Tolypella nidifica* (O. Müll.) A. Braun, *Pseudolithoderma subextensum* (Waern) S. Lund, *Fucus vesiculosus* L. and *Hildenbrandtia rubra* (Sommerf.) Menegh.) presented on waters of the PA are included in the Red Data Book of Nature of the Leningrad Region. Data obtained as a result of the floristic composition investigations of protected area "Kurgalsky" allows to correct existing conception about ecology of a number of macroalgae

species and to specify data on depth and horizontal distribution of macroalgae in the Gulf of Finland.

Very important spawning area of Baltic herring is situated on this water region of the Gulf of Finland (Ostov, 1971, in Rus.). It was shown that appearance of drifting algal mats in different parts of investigated area may have negative influence on benthic organism environments and Baltic herring reproduction conditions.

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**SOME QUANTITATIVE CHARACTERISTICS OF THE POPULATION OF *ALARIA ESCULENTA* (L.) GREV. FROM KONGSFJORD (THE WESTERN SPITSBERGEN, 79° N)**

*Alaria esculenta* (L.) Grev. is perennial brown alga from the family *Laminariaceae* Bory. This high-boreal arctic species is recorded in many regions around Svalbard (Gulliksen, 1999). However, data on quantitative characteristics of populations of this species are very scarce. In the present work some data about density of thalli and biomass of the *A. esculenta* population are given. Analysis of

the data obtained allows concluding that the most part of *A. esculenta* thalli is concentrated in outer and middle zones of the fjord. The largest values of thalli density in *A. esculenta* population (absolute values vary from 50 up to 210 specimens /m<sup>2</sup>) are recorded in outer and middle zones of the fjord at the depth about 2.5 m. In outer zone the population has a high values of thalli density at the total depth interval, where Laminariaceae algae dominated (from 2.5 m to 15 m).

At rocky bottoms in surf ecotopes of the outer fjord zone at the depth more than 11 m *A. esculenta* forms large spots of monodominant communities, composed from gigantic thalli till 13 years age.

In the middle zone of the fjord population hasn't high values of density deeper than 10 m. In the inner fjord zone thalli density of this species is very low and thalli are not presented in quantitative samples (but they rarely occur in Laminarian forest growths).

The highest biomass values (about 24 kg / m<sup>2</sup>) are formed by the *Alaria esculenta* population in the outer part of the fjord in the depth range of 11–15 m. Very large biomass values (5–13 kg / m<sup>2</sup>) are characteristic for the outer and middle parts of the fjord in the depth interval of 5–11 m.

Thus, the data obtained allow us to conclude that *A. esculenta* plays an important role in the formation of a belt of laminarian forests in the outer and middle parts of Kongsfjord, and with the advancement into the inner part of the fjord, the role of this species in the formation of laminarian forests sharply decreases.

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### **DIATOMS OF THE SULA RIVER (UKRAINE)**

The Sula River is a left-bank tributary of the Dnipro River and located on the territory of the Poltava and Sumy regions. During the over 100th years period of

studying the algae species composition of the Sula River, today only 130 species of diatoms for this water body are known (Plutenko, 1871; Alexenko 1894; Lytvynova, 1967, 1972; Pugach, 1979; Pugach, Zhuravlyova, 1980; Shcherbak, 1996, 1997, 2014; Semeniuk, 2014). However, these data do not show the general picture of the diatom flora of this river, because the most of the last studies were predominantly hydro-biological and related to the river's mouth. It was the main reason why we have started to study diatoms of the down-, mid- and upstream of the Sula River (from the Romny City to the Nyzhniosulsky National Nature Park).

As a result of the original research, 288 diatoms species (290 intraspecific taxa (i.t.)) were found in the Sula River. It has been shown, that the diatoms species composition is represented by the three classes, 16 orders, 30 families and 63 genera. The ratio of the number of diatom taxa from the Sula River is 1:2.1:9.6. The average number of species in families is 9.6, but only 10 families have species diversity more than 10 species: Gomphonemataceae, Naviculaceae, Bacillariaceae, Fragilariaceae, Stauroneidaceae, Cymbellaceae, Surirellaceae, Pinnulariaceae, Achnanthidiaceae, Sellaphoraceae. The ratio of the number of families and genera is 2.1, but majority families have 1-2 genera (70 %). Also, this distribution is inherent of the genera structure: the average number of species in genera is 4.6, but almost half of the genera are represented by the 1-2 species (42.9 %). The leading genera in number of species are *Navicula* (32), *Gomphonema* (26), *Nitzschia* (26) and *Cymbella* (11). The increased of species numbers in families and genera can be related to the study of various diatoms habitats (periphytic, benthic, planktonic), the difference in the hydrochemical condition between downstream (soda-sulphate salinity of soils), mid- and upstream of the Sula River (predominance of calcium bicarbonate in soils and sediments), in addition, the increase of the total mineralization of the Sula River waters in the direction from the streamhead to the river's mouth (Vynarchuk, Hilchevskyi, 2010). In general, for the Sula River 328 (331 i.t.) diatoms species were found in total with literary data. The data of species composition were increased on 61.6 %.

Also, high originality of the algae flora investigated river is noted. 17 species of *Bacillariophyta* are new for the Ukrainian Forest-Steppe (Middle-Dnipro algofloristic subprovince) and 10 for the Left-Bank Forest-Steppe (Left-Bank-Dnipro algofloristic region). Moreover, 19 species are new for the flora of Ukraine and most of them was published as new findings from the territory Nyzhniosulsky National Nature Park (Kryvosheia, Kapustin, 2019 a, b): *Achnanthidium eutrophilum* (Lange-

Bert.) Lange-Bert., *Amphora neglectiformis* Levkov et Edlund, *Aneumastus balticus* Lange-Bert., *Caloneis biconstrictoides* Levkov, *Cymbopleura florentina* var. *brevis* Krammer, *Gomphonema angusticephalum* E. Reichardt et Lange-Bert., *G. microlaticollum* Kulikovskiy, Kociolek et Solak, *G. olivaceoides* Hust., *G. calcareum* Cleve, *Hippodonta neglecta* Lange-Bert., Metzeltin et Witkowski, *Karayevia kolbei* (Hust.) Bukht., *K. suchlandtii* (Hust.) Bukht., *Luticola hlubikovae* Levkov, Metzeltin et A. Pavlov, *Placoneis abiskoensis* (Hust.) Lange-Bert. et Metzeltin, *P. paraelginensis* Lange-Bert., *Sellaphora blackfordensis* D.G. Mann et S. Droop, *Stauroneis balatonis* Pantocsek, *S. separanda* Lange-Bert. et Werum, *Tryblionella salinarum* (Grunow) Pantocsek. New findings are distributed (Algosozology, 2008): 9 – very rare (1.9-3.8 % from the total number of samples), 7 – rare (5.7-7.5 %) and 3 – relatively rare (11.3-13.2 %). The presence of a large variety of species composition, new and rare species in the Sula River evidence about value investigated water body in total and the need for its preservation and further study.

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## **DISTRIBUTION AND ECOLOGY OF *CLOSTERIUM* SPECIES (STREPTOPHYTA) IN TAJIKISTAN**

Genera *Closterium* Nitzsch ex Ralfs counts approximately 300 species in all continental water bodies of the world and belongs to 10 greatest genera of the Streptophyta division of algae. Unfortunately, species diversity and ecological features of their distribution in different water bodies of Central Asia in general, and in Tajikistan in particular, have not been investigated yet. Thus far 34 species and 51 infraspecific taxa (including species) of *Closterium* were found in different water bodies of Central Asia. Most of *Closterium* species were found in Uzbekistan and Tajikistan water bodies (27 and 21 species respectively). In Kazakhstan and Turkmenistan this genus is represented by 17 and 18 species respectively. In Kyrgyzstan water bodies this genus is represented only by 8 species. As known, most of the Streptophyta species adapted in soft mountainous water bodies, where pH value lower than 7. But *Closterium* species ecologically more tolerant to different



habitat to comparison with other genera of the Streptophyta division, and prefer acidic (pH<7) as well as neutral (pH=7) and alkalic habitats. *Closterium* species distributed inequally in Tajikistan territory. Most of species (15) were found in South Tajikistan Depression (Boboev, 2016, in Rus.), less of them (13 species) – in Pamir water bodies (Kurbonova, 2012, 2017, in Rus.), and the lowest number of *Closterium* species (8) were found in North Tajikistan water bodies. All identified in Tajikistan *Closterium* species also inequally distributed depending on the ecological group of algae and type of habitat. Most of species equally occur in plankton, as well as in benthos (*Closterium acerosum* Ehrenb. ex Ralfs, *C. acutum* (Lyngb.) Bréb., *C. diana* Ehrenb. ex Ralfs and others), but *C. lanceolatum* Kütz., *C. moniliferum* (Bory) Ehrenb. ex Ralfs and others prefer benthic associations, or grow epiphytic in vascular plants' leaves. Some *Closterium* species are selective to oligotrophic (*C. cornu* Ehrenb., *C. didymotocum* Ralfs, *C. kuetzingii* Bréb., *C. lunula* (Müll.) Nitzsch и др.) or eutrophic water bodies (*C. acerosum*, *C. leibleinii* Kütz. ex Ralfs). Thus they can be used as a reliable bioindicators for water quality evaluation of the fresh water bodies.

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## **MOLECULAR AND MORPHOLOGICAL INVESTIGATION OF COSMOPOLITAN DIATOM *HANTZSCHIA AMPHIOXYS* (EHRENB.) GRUNOW (BACILLARIOPHYCEAE)**

Until now, the diversity of representatives from the genus *Hantzschia* Grunow inhabiting of soils in Ukraine and European Russia was limited by the species *Hantzschia amphioxys* (Ehrenb.) Grunow, *H. elongata* (Hantzsch) Grunow and *H. vivax* (W. Sm.) Grunow with some ifraspecific taxa (Aleksakhina and Shtina 1984; Kostikov et al. 2001; Tsarenko et al. 2009; Maltseva 2009). At the same time, the adoption of a narrow species concept by many researchers led to the description of a number of new species within the genus *Hantzschia*. Thus, 17 new

taxa were identified by H. Lange-Bertalot with co-authors (Lange-Bertalot et al. 2003) mainly from samples from Sardinia island, 1 taxon is from springs in Germany (Werum and Lange-Bertalot 2004) and 5 new taxa from fresh water bodies and soil of the South Atlantic islands (Zidarova et al. 2010). Until recently, many of the described species were identified with *Hantzschia amphioxys*, which is positioned as a cosmopolitan species.

We have studied morphology, ultrastructure and phylogeny of 13 soil diatom strains, which belongs to *H. amphioxys* s.l. using 18S rDNA, 28S rDNA and *rbcL*. We show that our strains contain five different species of *Hantzschia*, including three new for science. Five strains we identified as *H. abundans* Lange-Bertalot. We indicated an insignificant curvature of the raphe near its external central ends. Four of the examined strains were represented by different populations of *H. amphioxys* and their morphological characteristics are fully corresponded with accepted isolectotype and epitype. The main specific features of this species include 21-25 striae in 10  $\mu\text{m}$ , 6-11 fibulae in 10  $\mu\text{m}$ , 40-50 areolae in 10  $\mu\text{m}$  and internal central raphe endings bent to opposite directions. Three new species were described based on differences with shape of the valves, a significant excess of the dimensional characteristics, a smaller number of striae and areolae in 10  $\mu\text{m}$  and the position of the internal central raphe ends. Based on the study of morphological variability and phylogeny of soil *Hantzschia*-species from different geographical locations we concluded that some sympatric populations of pseudocryptic taxa are exist in Holarctic.

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## **ALGAE AS INDICATORS OF THE ECOLOGICAL STATE OF MARINE ECOSYSTEMS IN THE COASTAL PART OF THE AZOV SEA**

The Sea of Azov is socially and economically important for Ukraine. It provides the development of fishing, transportation, recreation and other industries. In general, the ecological situation of the Sea of Azov corresponds to the

global tendencies of anthropogenic transformation of marine ecosystems. At the same time, it is characterized by its own peculiarities such as shallowness, continentality, significant inflow of river waters, and poor connection with the Black Sea.

Algae are used worldwide for the assessment of anthropogenic changes in marine ecosystems (Mocenni and Vicino, 2006; Gharib and Dorgham, 2006; Gharib et al., 2011; Raveh et al., 2015; Wells et al., 2015; Arroyo and Bonsdorff, 2016). Potential of algae used as indicators is much greater than that of physical-chemical methods. For this reason, their use is obligatory according to the requirements of Water Framework Directive (WFD), Marine Strategy Framework Directive (MSFD) (European Commission, 2008).

Research of algae in the Sea of Azov has a long history. The first information about algae of the Sea of Azov and its bay Syvash is represented in the works of K.I. Meyer and V.M. Arnoldi and is dated within the beginning of the 20<sup>th</sup> century. The works were later complemented by both native and foreign scientists (Kovaleva, 2016). Different parts of the Sea of Azov are explored unequally. The algae in the western and southwestern Sea of Azov are the least explored. The latest data go back to the beginning and the middle of the 20<sup>th</sup> century and concern Molochnyi Estuary (Proshkina-Lavrenko, 1950; Vladimirova, 1960 a), Sivash lake (Meyer, 1915; Proshkina-Lavrenko, 1940, 1962; Vladimirova, 1960 b; Ivanov, 1960) and Henichesk Strait (Merezhkowsky, 1902).

In summer 2017, the excessive growth of macrophytic algae was observed in waters of the Sea of Azov in this coastal area. It produced uncomfortable conditions for tourists and residents of the coastal area. The algae proliferated on the surface of water 10-20 meters away from the water's edge. They were thinning out further off the coast. Their samples were taken during the expeditions to 5 stations of the coastal waters of the Sea of Azov (Berdiansk spit (the end of the spit), the area of Lysiacha clough (Berdiansk district), village Botievo, Stepanivka spit, island Biriuchy (border "Chynka")). The object of research was macrophytic and microscopic algae from algal blooms. The material was gathered in accordance with accepted procedures in hydrobiology (Topachevsky and Masyuk, 1984). Nomenclature of representatives is given in accordance with the identification guide of national data collection (Tsarenko et al., 2006).

The main producer of macroscopic proliferations on island Biriuchy, in the area of Lysiacha clough and on Berdiansk spit was *Cladophora albida* (Nees) Kütz., a green alga from an order *Siphonocladales*. On Berdiansk spit there was an

accumulation of filamentous algae with higher aquatic plants (*Zostera marina* L., *Ruppia maritima* L.). On Stepanivka spit, the main producer of macroscopic proliferation was another species from a genus *Cladophora* Kütz. – *Cladophora siwaschensis* K.J. Meyer. However, filaments of this species were not numerous in the area of Lysiacha clough. A great number of discovered diatoms were attached to macrophytes. Only single members of division Rhodophyta were discovered among filamentous algae.

During the research 18 algae species that were a part of proliferation of macroscopic algae were found at different points. They were representatives of the following divisions: Bacillariophyta – 10 species, Chlorophyta – 6 species, Rhodophyta – 2 species.

Thus, information about different algae characteristics is an integral part of understanding and predicting changes in marine ecosystems. It is valuable for both current assessment of ecosystems and long-term monitoring programs.

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## **MOST ANCIENT PODOLIA'S ALGAE AS TRIGGER FOR THE FORMATION OF PHOSPHORITES**

The most ancient and numerous imprints of algae in Podolian Middle Dnister area are found and described from Kalyus Beds. It is Nagoryany Formation of Upper Vendian.

Kalyus Beds are represented by homogeneous, dark-grey, thin-bedded mudstones. Characteristic feature of the beds is the occurrence of phosphorite concretions and two levels with algae remains (Sokolov, Fedonkin, 1985 a, b).

Three species of Vendetenian algae make up the Metaphyta complex – *Vendotaenia antiqua* Gnil., *Fusosquamula viasovi* Ass., *Pilitella composite* Ass. In the rock, the algae are non-mineralized elastic brown ribbons, which occur either singly or completely cover of the bedding surface. Vendetenida tapes have a tissue-like structure, but without signs of a conductive system. This proves their algal nature. *Vendotaenia* are among the most ancient Metaphyta. According to the general

appearance of thalli, the nature of sporangia and the type of metabolism, they are referred to as brown algae, which were adopting a benthic lifestyle. Algal ribbons have good preservation and are bent in the same plane, which indicates that they were buried *in situ*. (Gnilovskaya, 1971; Aseeva, 1976, 1988 a).

Kalyus Beds includes an assemblage of microphytofosills – acritarchs and filamentous algae too. They are mainly represented by two species – *Leiothrichoides typicus* Herm. и *Ljadovia exasperate* Herm., although in some sections they are described *Eoholynia longa* A. Istch., *E. capillaria* A. Istch., *Kalusina compacta* A. Istch., *Fusosqamula vlasovi* Ass. (Aseeva, 1988 b).

Phosphorite concretions, phosphate mineralization of mudstones and numerous algae remains in the Kalyus Beds point at the existence of a certain connection between them.

The black color, the presence of globular pyrite, the bitumen interlayers and the value of the protoxid module - 1.32-1.83 (Sokur, 2011) indicate the conditions for sedimentation recovery. Algal textures say that sedimentation occurred in the euphotic zone, that is, at the depth of penetration of light. This is confirmed by the ecology of modern brown algae, which live from the low-water line to a depth of 20-30 m.

In the Late Vendian, there was no terrestrial vegetation, therefore sloping substrates eroded much faster and the land was a vast plain almost at the level of the water's edge. The coastline (in the modern sense) didn't exist, it constantly migrated and this led to the fact that in the "coastal zone" formed numerous gulfs, overgrown with algae.

Phosphorus compounds entering the marine basin were digested by cyanobacteria, eukaryotic microorganisms and Vendotaenian algae and accumulated in living cells in the form of polyphosphoric acids.

Cyanobacterial communities had multidirectional vectors of their life activity, so ones created various biochemical barriers. Acidic medium was for dissolving apatite and/or francolite, and then alkaline one, which was necessary for phosphorus precipitation (Frolov, 1993). In addition, phosphorus could be precipitated as a result of seasonal fluctuations in temperature, which led to departure of the chemical equilibrium (Sokur, 2011). Also, as I think, algal films and microbiofilm could hold phosphorus-containing pelitic particles on their surfaces.

Land vegetation cover was absent, therefore the coastline constantly migrated so the primary structure of algal mats and biofilms were periodically disturbed. They were fragmented – their rolling, sticking, etc. took place. As a result,

thrombolytics (nonlayered clot structures) were formed. Further lithification takes place under reducing conditions at the bottom and at the top of the sediment (Malyonkina, 2015; Morov, 2016). If the concentration of phosphorus is high in sludge waters, phosphorite concretions can form from thrombolites then. We can see that in Kalyus Beds.

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### **DICTYOSPHAERIUM-LIKE MORPHOTYPE IN TERRESTRIAL ALGAE: WHAT IS XEROCHLORELLA (TREBOUXIOPHYCEAE, CHLOROPHYTA)?**

Several strains of terrestrial algae isolated from biological soil crusts of Germany and Ukraine were identified by morphological methods as widely distributed species *Dictyosphaerium minutum* Petersen (= *Dictyosphaerium chlorelloides* (Nauman) Komárek et Perman). Investigations of the phylogeny showed their position, despite our expectations, outside of Chlorellaceae (Trebouxiophyceae) and distantly from *Chlorella chlorelloides* (Naumann) C. Bock, Krienitz et Pröschold, to which this taxon was attributed after revision of the genus *Chlorella* Beyerinck based on an integrative approach (Bock et al., 2011). SSU rRNA phylogeny determined the position of our strains inside a clade recently described as a new genus of cryptic alga *Xerochlorella olmiae* Fučíková, P.O. Lewis et L.A. Lewis (2014) isolated from desert biological soil crusts of USA. Investigation of the morphology of the authentic strain of *X. olmiae* showed *Dictyosphaerium*-like morphology, as well as some other characters, common for our strains and morphospecies *D. minutum* (= *D. chlorelloides*). *D. minutum* was described as terrestrial alga (Petersen 1932) and was later united with the earlier described

aquatic representative *D. chlorelloides* (basonym: *Brachionococcus chlorelloides* Naumann (1921)) because of their close morphological relationship (Komárek & Perman 1978). The revision of *Chlorella* mentioned above provided only one aquatic strain (*D. chlorelloides*), which determined its position in the genus (Bock et al. 2011). But terrestrial strains of the morphospecies were not investigated phylogenetically. Our study showed that the terrestrial *D. minutum* is not related with the morphologically close *D. chlorelloides* (= *Chlorella chlorelloides*, Chlorellaceae) instead represented a separate lineage in Trebouxiophyceae, recently described as genus *Xerochlorella*. Another strain isolated from biological soil crusts of maritime sand dunes (Baltic Sea coast, Germany) was preliminary identified as *Dictyosphaerium dichotomum* Ling et Seppelt (1998) described from Antarctic soils. SSU rRNA phylogeny showed the position of this strain also inside the *Xerochlorella*-clade in close relationship to other strains identified as *D. minutum*. But ITS-2 secondary structure of both species showed considerable differences, which are sufficient to justify assignment to two separate species of the genus *Xerochlorella*. Therefore respective revision of *Xerochlorella* was proposed, including nomenclatural combinations, epitypifications and emendations of two species. New characters of the genus based on investigation of morphology and ultrastructure of the respective algae were determined. Some nomenclatural inaccuracy for *Chlorella chlorelloides* was also corrected.

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**LONG-TERM RESTORATION OF THE ZERNOV'S PHYLLOPHORA FIELD**

Zernov's Phyllophora Field (ZPF) known as a unique object of accumulation of the red algae from the genus *Phyllophora* Grev. It is located in the central part of the north western shelf of the Black Sea (NWBS) at depths from 15 to 50 meters.

The bottom communities on the Black Sea shelf are interesting not only in terms of algal diversity, but also as a biological indicator: their state can manifest the trends of the general ecological state of the Black Sea ecosystem. The task of monitoring the Black Sea ecosystem according to the requirements of the EU Marina Strategy standards (MSFD, 2008/56 / EC) makes it possible to use the indicators of ZPF algae communities to assess the Ecological Status Class (ESC) of the Black Sea shelf.

We analyse the long-term changes in the structure of ZPF algae communities using empirical data obtained in March 2008 (expedition on RV "Poseydon") and May 2016 (expedition on RV "Mare Nigrum") at the same point in the northern part of the field. From 22 macrophytobenthos indicators recommended for the Black Sea Monitoring (Minicheva et al., 2015), nine include the complex of morphofunctional indicators (Minicheva, 2013).

The analysis of the long-term changes of the floristic composition of the macrophytes communities of ZPF showed that it reflects four stages of eutrophication of NWBS (Aleksandrov et al., 2017): I – natural state; II – intensive eutrophication; III – immobility; IV – steady trend of de-eutrophication. The long-term trend of the number of species in the floristic composition of the ZPF's algal communities showed that it has a directly opposite relationship with the level of eutrophication of the NWBS. During the periods of the maximum level of eutrophication of NWBS (II - intensive eutrophication - 1980th), the floristic diversity of ZPF's algal communities was reduced to 8 species (Kalugina-Gutnik & Evstigneeva, 1993). At present (2016-2017), the floristic diversity of ZPF macrophytes (30 species) has practically reached the level of 1964 (31 species).

Currently, the phytocenoses of the ZPF consist of two main components: red algae from the genus *Phyllophora* (*Coccotylus truncatus* (Pallas) M.J.Wynne et J.N.Heine and *Phyllophora crispa* (Hudson) P.S.Dixon), which belong to the sensitive species ( $S/W_p \leq 25 \text{ m}^2 \cdot \text{kg}^{-1}$ , *k*-species) and a complex of the filamentous red, brown and green algae representing tolerant species ( $S/W_p > 25 \text{ m}^2 \cdot \text{kg}^{-1}$ , *r*-species). It is well known, that the improvement of ecological situation in marine ecosystems caused restoration of sensitive macrophytes species; vice versa, development indices of tolerant species reduce. During the period from 2008 to 2016, in the northern part of the ZPF, the increase of the structural-functional indicators of sensitive species (*C. truncatus* and *Ph. crispa*) was recorded, as well as the decrease of the indicators of tolerant species (red, brown and green filamentous algae). For



example, the share in bottom cover of the *C. truncatus* population increased from 0.1 to 6 %, of the *Ph. crispera* population – from 0 to 4 %. Vice versa, the shares of populations of the filamentous algae (*Polysiphonia sanguinea* (C. Agardh) Zanardini, *Ectocarpus siliculosus* (Dillwyn) Lyngbye, *Cladophora vadorum* (Areschoug) Kützing) decreased from 70 to 50 %. Over the 8-year period for this area, the morphofunctional indicator  $S/W_x$  decied from 148 to 84 ( $m^2.kg^{-1}$ ). It corresponds to the increase of the ESC categories from "Poor" to "Moderate" and the Ecological Quality Ratio value doubling (from 0.42 to 0.81).

Presented data on the long-term restoration trend of the floristic composition, improvement of the morphofunctional organization of algal community and increase of the category of the ecological status of the ZPF in the last decade obtained with the financial and organizational support of the EMBLAS II project.

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**MACROALGAE OF GEORGIAN COAST AS INDICATOR OF ECOLOGICAL STATUS**

The international monitoring and achievement of the Good Ecological Status (GES) of the Black Sea ecosystem according to standards of the Marine Strategy (MSFD, 2008/56/EC), requires from all six Black Sea countries to implement the European environment standards at the regional level. Georgia, as a member of the Black Sea Ecological Commission and a partner of the EMBLAS project, has made significant steps towards the implementation of the requirements of the MSFD to assess the environmental quality of the national Black Sea coast based on the state of biological communities, in particular – macrophytobenthos.

The coastal macroalgae communities of the national coast of Georgia were used as an Ecological Quality Element on the basis of which it is possible to evaluate

the 5<sup>th</sup> categories (High, Good, Moderate, Poor, Bad) of Ecological Status Class (ESC), as well as to determine the Threshold Value (TV) of indicators that correspond to GES or NotGES. These indicators use the parameters of the floristic composition, biomass, bottom coverage of macrophyte communities and also the complex of morphofunctional indicators (Minicheva, 2013), which were used to express the Ecological Evaluation Index (EEI) (Orfanidis et al. 2011).

For the first time for the Georgian coast, at four monitoring stations (Sarpi, Green Cape, Batumi port, Tsikhisdziri) significantly differing in the intensity of anthropogenic load, in the period of 2016-2017 there were completed seasonal surveys of macrophytobenthos with the identification of classical and morphofunctional indicators. The list of 25 species of macroalgae of the Georgian coast was created and their ecological activity was determined on the basis of a morphofunctional indicator: the specific surface area of the population ( $S/W_p$ ). We selected sensitive species for which the morphological parameters and ecological status correspond to  $S/W_p \leq 25 \text{ m}^2 \cdot \text{kg}^{-1}$ , (*k*-species). Species with such ecological activity are typical GES indicators for the Georgia coast: *Cystoseira barbata* (Stackhouse) C. Agardh, *Gelidium spinosum* (S.G. Gmelin) P.C. Silva, *Nemalion lubricum* Duby, *Dermocorynus dichotomus* (J.Agardh) Gargiulo, M.Morabito & Manghisi. Based on empirical data on the spatial-temporal distribution of morphofunctional value of the three EEI:  $S/W_{3Dp}$  (Three Dominants Ecological Activity);  $S/W_x$  (Average Species Ecological Activity);  $S_{sp}$  (Percentage the sensitive species), national classification scales for ESC estimation, as well as TV for determining GES or NotGES of the Georgian coast were proposed.

The developed methods for assessing the ESC of the Georgian coastal waters based on the macrophytobenthos communities' state were used in the national monitoring. At the Green Cape station, in the floristic composition of macrophyte communities 40 % shared sensitive species with the  $S/W_p$  less than  $25 \text{ m}^2 \cdot \text{kg}^{-1}$ ; it corresponds to the ESC category "High". At the Sarpi and Tsikhisdziri stations, up to 30 % of sensitive species in the communities were observed, what corresponds to the ESC category "Good". In the phytobenthos community of the Batumi port station no sensitive species were find, what corresponds to the ESC category "Bad". In the community structure, green filamentous algae dominated, characterizing by high coefficients of ecological activity  $S/W_p$  (average about  $100 \text{ m}^2 \cdot \text{kg}^{-1}$ ). The use of the TV parameter for the integrated assessment of the monitoring areas allowed to

conclude that from the four Georgian monitoring stations, the three (Sarpi, Green Cape, Tsikhisdziri) correspond to the GES status. Only Batumi port station has a not satisfactory status: NotGES.

The presented data on the floristic composition, structure and morphofunctional organization of the macrophytobenthos of the Georgian coast are the first official national monitoring data according to the Maritime Strategy standards obtained with the financial and organizational support of the EMBLAS II project.

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**GROWTH RATES AND PHOTOSYNTHETIC ENERGY  
TRANSDUCTION EFFICIENCY OF *CHLORELLA VULGARIS* BEIJER.  
GROWN IN THE PRESENCE OF COPPER AND SELENIUM  
NANOCITRATES**

Copper (Cu) and selenium (Se) are essential microelements for green algae, however their high concentrations can exert toxic effects. Promising source of microelements are nanomaterials, not only colloid solutions of nanoparticles, but also so-called nanoaquachelates, the nanoparticles with the molecules of water and/or carboxylic acids as ligands. The ability of hydrated and/or carboxylated nanoparticles to penetrate easily the cell membranes and release the ligands thereafter form the prerequisite for their high biological activity. The toxicity of nanoaquachelates was found to be much lesser than that of respective inorganic salts (Kosinov, Kaplunenko, 2008; Borisevich et al., 2012). Currently the ultra-pure nanocarboxylates of principal biogenic and biocide elements are used in human and veterinary medicine, agriculture, food and cosmetic industry, municipal engineering etc. (Borisevich et al., 2012). Good prospects have nanocitrates, so far as the salts of citric acid are approved for use in food industry.

The aim of the research was to study the effects of copper and selenium nanoaquachelates carboxylated with citric acid (nCu-Citr or nSe-Citr, respectively) on biomass accumulation and photosynthetic photochemical performance of the

unicellular green algae *Chlorella vulgaris* Beijer. Nanocitrate preparations were obtained from the Ukrainian State Scientific Research Institute "Resource" (Kyiv). The concentrations of nanoparticles corresponded to those of respective ions occurring in natural aquatic environments, either polluted or not. The efficiency of the reactions of the light stage of photosynthesis was estimated by measuring modulated chlorophyll *a* fluorescence at room temperature.

The addition of 0.67–40.0 mg L<sup>-1</sup> nCu-Citr evoked the initial increase in *Ch. vulgaris* dry matter. If nCu-Citr concentration did not exceed 4 mg L<sup>-1</sup>, 20 % growth stimulation was retained up to the 24<sup>th</sup> day of experiment, the effect of the lowest applied concentration being the most sustainable. On the contrary, the addition of 20 to 40 mg L<sup>-1</sup> nCu-Citr proved to be toxic to *Ch. vulgaris* because it stopped the algal growth starting from the 12<sup>th</sup> day. nSe-Citr at 2–4 mg L<sup>-1</sup> concentrations induced 1.4-fold increase in biomass accumulation after 6 days of cultivation. The positive effect on growth retained for next 18 days, although weakening with time. The addition of nSe-Citr at smaller concentrations (0.07 or 0.2 mg L<sup>-1</sup>) at first caused the retardation of culture growth, but that effect disappeared after 18–24 days of cultivation.

Nanocitrates of copper (2–4 mg L<sup>-1</sup>) and selenium (0.4–4 mg L<sup>-1</sup>) caused the evident initial increase in such chlorophyll *a* fluorescence parameters as  $F_v/F_m$ , maximal quantum yield of Photosystem II (PSII) photochemistry, and  $F_v'/F_m'$ , the quantum yield of PSII photochemistry in the light-adapted state. The extent and duration of nSe-Citr positive effect on  $F_v/F_m$  and  $F_v'/F_m'$  values increased with the growth of their amount added. Photochemical fluorescence quenching coefficient ( $q_p$ ), estimating the proportion of light excitation energy captured by PSII that is used for electron transport, declined after 24 days of growth with nCu-Citr but increased after 6 days of the addition of 2–4 mg L<sup>-1</sup> nSe-Citr. Those alterations affected the net quantum yield of photosynthetic electron transport in PSII ( $\Phi_{PSII}$ ). Nonphotochemical quenching (NPQ) value did not change significantly in the presence of nCu-Citr. However 4 mg L<sup>-1</sup> nSe-Citr caused the reliable NPQ increase, so absorbed light energy exceeded the capacity of its utilization in photosynthetic processes.

Thus copper (0.67–4.00 mg L<sup>-1</sup>) or selenium (0.4–4 mg L<sup>-1</sup>) nanoparticles carboxylated with citric acid have positive effect on *Ch. vulgaris* growth and transiently improve the efficiency of PSII photochemical reactions. Therefore their

utilization may be recommended for further biotechnological investigations aimed to stimulate the accumulation of microelement-enriched algal biomass as the source of valuable nutrients or soil fertilizers.

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## **CYANOPROKARYOTA OF THE HYPERHALINE KUYALNIK ESTUARY (UKRAINE) IN TERMS OF SUPPLYING SEAWATER FROM THE BLACK SEA**

The Kuyalnik Estuary lies on the northwestern coast of the Black Sea. It is a valuable balneological and recreational object. Nowadays Kuyalnik Estuary is a hypohaline lake separated from the Black Sea by a broad sandbar 2 km long. Morphometric parameters of the estuary vary depending on hydrological and climatic conditions: length – within 17-28 km, width 2.5 km, surface area – 30-50 km<sup>2</sup>, average depth is about 1 m. The regulated runoff of the Big Kuyalnik River and other streams feeding the estuary has led to the drying out of the reservoir and extremal increase in its salinity. To solve this problem, it was decided to fill the estuary with seawater during the winter period (for 4 months a year) from 2014. Afterwards in 2017, for instance, the salinity of brine decreased from January until April from 205 to 162 ppt, then increased up to 270 ppt in November and again slightly decreased in December to 200 ppt.

Within the Cyanoprokaryota there are many species adapted to life in hyperhaline environments. Some can even grow at salt concentrations approaching NaCl saturation. Recently, Cyanoprokaryota of Kuyalnik Estuary has attracted close attention of researchers; however, their study covered Kuyalnik Estuary ecosystem (Gerasimiuk et al., 2011, Tsarenko et al., 2016), and the coastal solonets (Vinogradova, 2016). Our research concerns changes in the species composition of organisms with a decrease in salinity of brine (Nikonova, 2015). Here, we present the results of the study of Cyanoprokaryota diversity in the Kuyalnik Estuary during 2017 under salinity conditions up to 200 ppt (17 samples) and above 200 ppt

(43 samples). Samples of silt were taken monthly from five stations located in the north, central and south portions of the Kuyalnik Estuary. The samples were cultivated during one month; the native estuary water collected with the samples was used as a nutrient medium.

Totally, 27 species from 3 orders of Cyanoprokaryota were recorded: Synechococcales (15 sp., 55.6 %), Chroococcales (10 sp., 37.0 %) and Oscillatoriales (2 sp., 7.4 %). New records for Kuyalnyk Estuary are *Gloeocapsopsis crepidinum* (Thuret) Geit. ex Kom., *Rhabdoderma* cf. *bellyeense* Hortobágyi, *Romeria* sp., *Cyanosarcina* sp. In the salinity range from 162 to 200 ppt, the orders Synechococcales and Chroococcales were represented equally, in total 10 species were found. Eight species were recorded only once, while *G. crepidinum* and *Aphanotece utachensis* Tilden were more common: species frequency was 23.5 % and 17.6 %, respectively. In the salinity range from 200 to 270 ppt, 25 species of cyanobacteria were found: Synechococcales – 13 species (52 %), Chroococcales – 10 (40 %) and Oscillatoriales – 2 species (8 %). 12 species were rarely seen and were recorded no more than 2 times. The most frequent species were *G. crepidinum* (species frequency 60,5 %), *A. utachensis* and *Rhabdoderma* cf. *bellyeense* (both species had frequency quotients 18.6 %). A larger number of species found at high salinity, obviously due to the larger number of samples studied. The species that are more common reached higher quantitative values in cultures. The highest biomass values reached *A. utachensis* (117.4  $\mu\text{g}\cdot\text{cm}^2$ ), *Cyanosarcina* sp. (32.9  $\mu\text{g}\cdot\text{cm}^2$ ), *G. crepidinum* (24.7  $\mu\text{g}\cdot\text{cm}^2$ ). These species are also the basis of the metaphyton in the shallow water of estuary and they may be involved in the process of formation of peloids. When the samples of silt were cultured in a nutrient medium of lower salinity prepared from estuary brine diluted with seawater to a salinity of 60 ppt, 120 ppt and 180 ppt, an increase of species composition was noted: 47, 50 and 51 species were found, respectively. They include species of the genera *Lyngbya*, *Geitlerinema*, *Leptolyngbya*, *Spirulina*, as well as representatives of the order Nostocales – *Anabaena* and *Nodularia*.

It is obvious that at the highest salinities filamentous cyanoprokaryotes are rarely seen. Only unicellular species from the orders Synechococcales and Chroococcales were registered at salt concentrations approaching NaCl saturation.

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## **MORPHOLOGICAL AND GENETIC CHARACTERISTICS OF *COELASTRELLA* SPECIES FROM THE URALS AND KHENTEY MOUNTAIN SYSTEMS**

The genus *Coelastrella* Chodat includes free-living algae distributed in terrestrial and aquatic habitats. Species of this genus have a wide phenotypic variability. Morphological species identification is difficult because cells have simple morphology. Species can only be determined using the polyphasic approach. The aim of the work was to study morphological and molecular genetic characteristics of the species from *Coelastrella* genus isolated from the Urals and Khentey mountain systems by a polyphasic approach.

Strains of *Coelastrella* were taken from the collections of microalgae strains of the Institute of Biology, Komi Science Center, Ural Branch of the Russian Academy of Sciences (<http://ib.komisc.ru/sykoa>): SYKOA Ch-047-11, SYKOA Ch-045-09, SYKOA Ch-072-17 and Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of Russian Academy of Sciences (<http://sifibr.irk.ru/collection.html>): IRK-A 2, IRK-A 173. Strains were isolated from acidic (pH 4.28-6.67) and dry (moisture 11.3–20 %) soils of Ural mountain system (Polar, Subpolar and Northern Urals) and from samples of epilithic and epiphytic mosses collected in the Russian and Mongolian Khentey. Morphological features of the strains were studied for two years using Nikon Eclipse80i with a DIC system and Axio Scope A1 light microscopes. The length and width of cells (at least 100 cells) were measured once a week within two months. For species identification, essential monographs were used (Ettl, Gärtner, 1995; Andreyeva, 1998). DNA of

the strains was extracted using "FastDNASpinKit" (QBioGene, Canada). For the amplification of the 18S and ITS1-5.8S-ITS2 gene sequence, we used primers which are presented in the publications of A. Katana (Katana et al., 2001) and T.J. White (White et al. 1990). The fragments of 18S rDNA and ITS1-5.8S-ITS2 strain sequences were submitted to GenBank under MK480613, MK478814, MK450459, MK504637, MK504638 accession numbers.

Based on monographs of V.M. Andreyeva (1998) and H. Ettl et G. Gärtner (1995) we identified the strains as the *C. terrestris* (Reisigl) Hegewald & N. Hanagata because all of them have lemon-shaped and broadly ellipsoidal cells with 2 polar thickenings and 8 or more longitudinal ribs on the cell wall. Molecular analysis distributed the strains among several clades (with high bootstrap values). SYKOA Ch-045-09 и IRK-A 2 strains were close to *C. oocystiformis* (J.W.G. Lund) E. Hegewald et N. Hanagata, SYKOA Ch-047-11 – *C. aeroterrestrica* A. Tschalkner, G. Gärtner et W. Kofler, SYKOA Ch-072-17 – *C. rubescens* (Vinatzer) Kaufnerová et Eliás and IRK-A 173 were close to *C. terrestris* species. Long-term observation of their life-cycle allows us to identify a number of morphological features that separate the studied strains from *C. terrestris*. The strains closed to *C. aeroterrestrica* according 18S and ITS have high frequency of occurrence of cells with three polar thickenings and larger sizes – length 5-26  $\mu\text{m}$  and broad 5-19  $\mu\text{m}$ ; sometimes they have vacuoles; sporangia had 2-16 autospores. The strains close to *C. oocystiformis* have cells of 7-23  $\mu\text{m}$  long and 5-17  $\mu\text{m}$  broad; they have vacuoles; sporangia had 2-16 autospores (more often 4-8). Strains close to *C. rubescens* characterized by cells of 8-22  $\mu\text{m}$  long and 5-17  $\mu\text{m}$  broad, sometimes they have vacuoles; in comparison with the previous strains, the thickenings of the cell walls were observed to 1.5-2  $\mu\text{m}$  and were partially discarded in the old cultures; sporangia had 2-16 autospores. The obtained data fill up the literature information about species of this genus.

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**THE RECORD OF *DIPLOSPHAERA CHODATII* BIAL. EMEND. VISCHER ON MOSS *HOMALOTHECIUM PHILIPPEANUM* (SPRUCE) SCHIMP.**

The data on algae epiphytic on mosses are not numerous (Hoffman, 1989; Alfinito et al., 1998; Johansen, 1999; Van de Vijver et al., 2003, etc.). For Ukraine, 69 species of epibriophytic algae are known (Cyanoprokaryota – 2 species, Chlorophyta – 52, Streptophyta – 8, Stramenopiles – 7), which were identified on 7 species of mosses: *Pylaisia polyantha* (Hedw.) Schimp., *Ceratodon purpureus* (Hedw.) Brid., *Grimmia pulvinata* (Hedw.) Sm., *Pohlia nutans* (Hedw.) Lindb., *Polytrichum piliferum* Hedw., *Hypnum cupressiforme* Hedw., *Plagiothecium succulentum* (Wils.) Lindb. (Mikhailyuk et al., 2011). Here we present a new record: *Diplosphaera chodatii* Bial. emend. Vischer was found epibriophytically on the *Hypnum cupressiforme*, which grew on the north-western slope of the right bank of the Teteriv River.

*D. chodatii*, originally described from the lichen *Lecanora tartarea* (L.) Ach. (Vischer, 1960), was later revealed in 17 species of lichens (Voytsekhovich et al., 2011). It is also known as the common free-living terrestrial alga mostly found aerophytically on various substrates (Ettl, Gärtner, 1995; Algae ..., 2011). In addition, it was found epiphytically on lichen thalli (Polishchuk, Voytsekhovich, 2014).

*Homalothecium philippeanum* (Spruce) Schimp., a moss from the *Brachytheciaceae* Schimp family (Bryophyta), is remarkable by deeply longitudinally-folded, serrated on the whole edge of the leaves with a vein ending at the top of the leaf. It grows on rocks, mainly limestone, rarely on the ground (lime-bearing, marls), at the base of the trunks and tree roots. In Ukraine, it is quite common, especially in mountainous areas (Bachuryna, Melnychuk, 2003).

The moss turf are a favorable environment for the development of different groups of biotopes. Special environmental conditions (microhabitats) are formed inside of them that are different from the environment (water-holding capacity significantly increases within the moss turf, seasonal and daily extremes of

temperature equalize, organic substances accumulate, and the level of solar radiation significantly decreases (Ragulina, 2015).

*Diplosphaera chodatii* as an epiphyte of *Homalothecium philippeanum* was found on dolomites in the Glyboke Tract of the Rakhiv District, Transcarpathian Region, in shaded rock biotopes with a predominance of ferns.

As it was mentioned above, a peculiar feature of the studied moss is formation of deep longitudinal folds on the leaves. This microhabitat in terms of ecological conditions seems to be very favorable for the *D. chodatii*; it was abundant here in contrast to the smooth surface of the moss leaves both of the *H. philippeanum* and other mosses found in this ecotope.

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### **CYMATOPLEURA W.SM. S.L. (BACILLARIOPHYTA) IN THE BLACK SEA BOTTOM SEDIMENTS**

The genus *Cymatopleura* W. Sm. s.l. 1851 is included in the family *Surirellaceae* Kütz. 1844 to the alar canal raphed morphological group. *Cymatopleura* currently recognized as a distinct genus. The holotype species of genus is *Cymatopleura solea* (Brèb.) W. Sm. 1851. At present, there are 49 species names and 35 infraspecific names in the AlgaeBase database including in the genera. However, opinions on taxonomic validity of some species differ from author to author.

In fossils, representatives of the genus are known in the Pliocene of Armenia and Georgia, in the Holocene of Lithuania and Karelia, and in the Quaternary of Leningrad district (Russia), Poltava Region (Ukraine) and in the Black Sea bottom deposits. Species of the genus *Cymatopleura* are widespread in modern freshwater and saline environments. There are *Cymatopleura solea*, *C. solea* var. *apiculata*

(W. Sm.) Ralfs, *C. elliptica* (Brèb.) W. Sin. and *C. librile* (Ehrenb.) Pantocsek among recent diatom assemblages in the Black Sea basin. According to Guslyakov et al., Herasimyuk, Nevrova (1992), this species usually present in benthos of waters near the South-Western Crimea and in Sevastopol Bay. They have been reported among habitants of macroalgae, molluscs and stones on mud grounds in Dniester Estuary, Odessa, Tendra and Dzharylgach bays, and some limans.

In the Late Pleistocene-Holocene bottom deposits (during the last 8.5 ka) have been found such species, as *Cymatopleura solea*, *C. elliptica* та *C. librile* in diatom assemblages from top sediment layer (0-5 cm) to 450 cm in depth. These diatom containing deposits are extremely widespread in the Black Sea: they lie at different depths both the shallow coastal part, and the deep waters, where they can be redeposited. As far as we know, the most abundant finds of *Cymatopleura* frustules are in the North-Western shelf and Central deep waters, were *C. solea* is one of subdominant species.

On the base of our research, a new species of *Cymatopleura* – *C. euxinica* Tymchenko et Olsht., was described from the Upper Pleistocene (Upper Novoeuxinian) sediments of the Black Sea (Olshtynska & Tymchenko, 2018). This taxon differs from other constricted *Cymatopleura* species in the strongly transapically widened valves, a length-to-width ratio about 4:1 and valve face without reticulate thickenings or scattered silica granules.

Currently *C. euxinica* is defined as an extinct endemic representative of the genus *Cymatopleura*. We trace its distribution in the North-Western part of the Black Sea. According to our data, this species was found only in the top of the Upper Novoeuxinian sediments of the Black Sea shelf and continental slope. The Late Novoeuxinian sea-lake basin was characterized by lacustrine conditions with low salinity and shallow waters (Shimkus et al., 1973; Scherbakov, 1983). Since this species not found in sediments of other basins, we conclude it is an endemic and a specific component of the Novoeuxinian lacustrine diatom flora.

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## **MOLECULAR GENETIC ALGAE PROFILING OF THE SELENASTRACEAE FAMILY**

Species, and sometimes generic identification of family Selenastraceae representatives (Sphaeropleales, Chlorophyta) according to morphological characteristics remains still problematic and complicated. The search for new approaches to the study of the taxonomy of this group of coccoid green algae is relevant, and the determination of the affinity level and phylogenetic relationship and the appropriate place of specific taxa is a necessary argument in its comprehensive investigation. At the same time, high production characteristics and growth as well as fatty acid composition of some of them testify their importance as objects of bioconversion search and research. With this regard, it is involved modern molecular-biological approaches to studying of 4 species (7 strains) 3 genera of Selenastraceae (*Selenastrum gracile* Reinsch, *Monoraphidium griffithii* (Berk.) Komark.-Legn. and *M. minutum* (Nägeli) Komark.-Legn., as well as *Raphidocelis* spp.), that are stored in IBASU-A microalgae collection of M.G. Kholodny Institute of Botany of NAS of Ukraine.

Two different types of DNA markers were used to determine the difference between the microalgal samples tested at the molecular genetic level, namely Random Amplified Polymorphic DNA (RAPD) (Bardakci, 2001) and tubulin-based polymorphism (TBP) (Bardini et al., 2004). RAPD-method has long been used in the genetic analysis of various algae (Korkhovy et al., 2011; Ismail and Mohamed, 2017). TBP-method, which is based on the Exon-PrimedIntron-Crossing (EPIC-PCR), is based on the conservatism of the exon sequences of  $\beta$ -tubulin genes in all eukaryotic organisms and on the results obtained earlier (Pirko et al., 2019) may become a reliable tool for genetic profiling of different taxa of green algae.

According to the results of the study, it was found that about 42 polymorphic fragments were generated as a result of RAPD analysis, and 22 polymorphic amplicons. At the same time, using both methods, it is possible to differentiate all samples from each other. And when constructing UPGMA dendrograms on the basis of the similarity coefficients of Ney and Lee, obtained DNA profiles, the samples are distributed equally. Formed two major clades, of super-level rate *Monoraphidium* – *Selenastrum* – *Raphidocelis*, illustrating the affinity of *Monoraphidium* and *Selenastrum* species strains as well as heterogeneity of available strains of the genus *Raphidocelis*. Strains of the latter genus distributed between two clades (morphologically identified *R. subcapitata* demonstrates the affinity with the clade of the *Selenastrum* genus, as for *Raphidocelis* ssp. – with *Monoraphidium* clade) and require further investigations and determination of their proper place on the phylogenetic tree, as well as affinity with other representatives of Selenastraceae family. An additional study of morphological features *Raphidocelis* ssp. strains and their taxonomic belonging is required and in order to clarify the results of the molecular genetic profiling of algae in the future, it will be useful to develop a specific pair of primers to evaluate the polymorphism of the intron length of the  $\beta$ -tubulin genes in algae. In general, the results of the algae genotyping revealed significant differences between representatives of the super-genera level of the Selenastraceae family, and the SRT method can be successfully used in the molecular genetic analysis of algae.

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## **MICROPHYTOBENTHOS ALGAE OF MUSSEL SHELLS FROM THE ODESSA COASTAL ZONE AS BIOINDICATORS OF MARINE ENVIRONMENT**

The purpose of the work is to assess the quality of the marine environment of the Odessa coast and the Grigorievsky Estuary by the method of bioindication by systematic, quantitative, morphological, halobiont and saprobiological indicators of

the development of microphytobenthos. Object of research – microalgae from the surface of the mussel shells of the sea areas, different in terms of the level and nature of anthropogenic pressure. Samples were taken in a conditionally clean water area near the Cape Maly Fountain (Rachynska, 2007) and at the places of carrying out port operations – Odessa Port and Grigorievsky Estuary.

During 2018, the quantity of identified algae species varies from 41 to 50. Diatoms were dominated – 25-36 species. From summer to autumn, on mussel shells from vertical sides in the waters of Cape Maly Fountain and the port of Odessa, the number of microphytes species found increased 1.2 times at the expense of diatom algae. In the Odessa Port and Grigorievsky Estuary, the potentially toxic dinophyte algae *Prorocentrum micans* Ehrenb. has been intensively developed.

Abnormal diatoms were occurred separately. In the summer in the port of Odessa were found cavities of the valves *Cocconeis scutellum* var. *parva* (Grunow) Cleve, and in the autumn – *Stauroneis simulans* (Donk.) Ross ex Hartley, *Synedra crystallina* (C. Agardh) Kütz. (also abnormal in the Grigorievsky Estuary) and *Tabularia fasciculata* (C. Agardh) D.M. Williams et Round. The halobiont composition of the algae was mainly of poly- (13-20 species) and meso-halobes (10-16), and saprobiont – of  $\beta$ -mesosaprobies (13-21 species). The number of representatives of these ecological groups grew throughout the year. The fewest quantity of  $\alpha$ -mesosaprobies was in the summer on the coast of Cape Maly Fountain (4 species), the highest - in this time in Grigoryevsky Estuary – 8.

The basis of the number of algae of microphytobenthos on mussel shells was formed by blue-green algae of genus *Leptolyngbya*, *Lyngbya*, etc. Biomass algal flora was created mainly by large-cell diatoms *S. crystallina*, *T. fasciculata*, *Licmophora gracilis* (Ehrenb.) Grunow. Quantitative indicators of microphytobenthos development were minimal in the summer on the mussel valves from area of the Cape Maly Fountain ( $465.67 \cdot 10^6$  cells/m<sup>2</sup> and 161.56 mg/m<sup>2</sup>), maximum – in the autumn in the port of Odessa ( $2\,221.99 \cdot 10^6$  cells/m<sup>2</sup> and 1\,488.71 mg/m<sup>2</sup>). Throughout the year, both the number and biomass of algae on mussel shells in the Cape Maly Fountain increased in 1.2 times. In the port of Odessa, these quantitative indicators have increased in 3.3 and 3.4, and in the Grigorievsky Estuary – 3.0 and 3.2 times, respectively.

The retrospective analysis for the period 2009-2018 showed that the abundance of microphytobenthos was everywhere the lowest in 2018. In the area adjacent to the Cape Small Fountain, it was maximal in the summer of 2011, in the port of Odessa – in the summer of 2009, and in the Grigoryevsky Estuary – in the summer of abnormally hot 2010. Compared with the data of 2009 (Rachynska, 2010), at the Cape Small Fountain, it decreased by 6.4 times in the summer and at 4.1 in autumn, in the port of Odessa – by 18.9 and 1.2 times in the Grigorievsky Estuary – in 19.0 and 4.7 times in the summer and autumn. The biomass of microalgae over the last decade in the region of the Small Fountain Cape has increased by 1.6 and 1.5 times in the summer and autumn, while in the water areas of the Odessa Port and the Grigorievsky Estuary it has decreased slightly. During the decade, the number of saprobionts, in particular  $\alpha$ -mesosaprobies, increased everywhere.

Thus, the maximum systematic, quantitative and saprobiological indicators of microphytobenthos on the surfaces of mussel shells in 2018 and during 2009-2018 were recorded in the most anthropogenic pressured waters of the Odessa Port and the Grigoryevsky Estuary. This suggests that the algae of this ecological group are reliable bioindicators of the quality of the marine environment of the Odessa coastal zone.

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**THE MACROPHYTIC ALGAE OF DZHURYNSKYI AND DIVOCHI  
SLIOZY WATERFALLS (TERNOPIL REGION, UKRAINE)**

Ukrainian classification of habitats compatible with the EUNIS habitat classification, but a lot of already described and typified water habitats have incomplete information about algal component (National Habitat Catalogue of Ukraine, 2018). However, according to EUNIS habitat classification some water habitats (C2.12) can be differentiated with the characteristic algae species. We focused on the hard water bryophyte springs assigned as D:3.12 differentiated by

the bryophyte species. Logically, we expected to find characteristic algal species for the D:3.12 habitat especially because algae are the essential component of this habitat presented in waterfalls in different parts of western Ukraine. For our investigation two waterfalls of different types were chosen where algae are the important component of the plant diversity. Such studies will allow to detail water habitat characteristics, determine the features of algae distribution in different types of water habitats and modify their typification criteria taking into account algae component.

Dzhurynskyi Waterfall is the most powerful waterfall in flat part of Ukraine. It is 16 metres high and 20 metres wide, of semi-natural origin, consists of 3 cascades. It is located on the Dzhuryn River.

Waterfall Divochi sliozy geologically was formed on calcium-riched travertine bedrocks. From the ledge travertine rock spring water flows along the surface of the rock and falls on a moss-covered stalagmites. Both waterfalls located within Dniester Canyon in Zalishchyky District, Ternopil Region, Ukraine.

Species diversity of macrophytic algae in Dzhurynskyi and Divochi sliozy waterfalls was formed mainly by common for Ukraine species. A total of 8 species were recorded. They belong to *Chlorophyta* (7) and *Rhodophyta* (1). In both waterfalls *Cladophora glomerata* (L.) Kütz. and *Bangia atropurpurea* (Mertens ex Roth) C. Agardh are identified. The first one grew abundant in both waterfalls – near the Dzhurynskyi Waterfall and in the upper part of travertine rocks in Divochi sliozy Waterfall, where splashes of water moisturize the substrate.

The second one (*B. atropurpurea*) is a freshwater red alga. According to the literature data, it is rare in the Middle Dnipro Algofloristical Subprovince (Algae of Ukraine, 2006). In Dzhurynskyi Waterfall it was presented scarcely. But in Divochi sliozy Waterfall it was abundant (projective cover up to 50 %). It occurs in the lower part of travertine rocks, where water stream falls on its surface. It was noted that most of *B. atropurpurea* specimens had detached cellulose cell walls that is likely a result of mechanic damage.

On a flat part of the waterfall (between cascades) on the sandy ground *Aegagropila linnaei* Kütz. has been observed. This species is quite a rare one in Ukraine with only several locations known (in lakes of Rivne and Volyn regions) (Algae of Ukraine, 2011). This is an attached form of this species (whereas all previous records in Ukraine concerned about "lake ball" form). But all typical



features of *A. linnaei* (the aegagropiloid growth form, the irregular shape of basal cells, the formation of several branches at different poles of basal cells, thick cell walls, subterminal insertion of branches combined with delayed cell wall formation and serial insertion of branches) (Boedeker, Sviridenko, 2012) were observed.

In Dzhurynskyi Waterfall single specimen of *Cladophora crispata* (Roth) Kütz. and *Rhizoclonium hieroglyphicum* (C. Agardh) Kütz. were recorded. In Divochi sliozy Waterfall single specimens of *C. fracta* (O.F. Müller ex Vahl) Kütz. and *Rh. riparium* (Roth) Harvey were identified.

We concluded that algal species found in two waterfalls could be characteristic species of D:3.12 habitat but according to our results main part of algal species are common in the rivers, ponds and lakes in Ukraine and do not represent the D:3.12 habitat. Additionally we assumed that *Bangia atropurpurea* is a characteristic species (aerophitic and hydrophitic forms) of this habitat but it needs broad analysis of the Ukrainian hard water spring habitats involving numerical analysis.

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**INTERESTING REPRESENTATIVE OF GENUS *BRASILONEMA* FIORE ET AL. (NOSTOCALES, CYANOBACTERIA) GROWING ON TROPICAL PLANTS IN THE GREENHOUSE OF THE M.M. GRISHKO NATIONAL BOTANICAL GARDEN (KYIV, UKRAINE)**

Pantropical cyanobacterial genus *Brasilonema* Fiore et al. (Scytonemataceae) is known mainly from humid regions of Brazil, Mexico and USA. The genus, currently comprising 12 species, represents heterocytous false-branching cyanobacteria, mostly aerophytic or terrestrial in their ecology. Its type

species *B. bromeliae* was described after molecular and phenotypic evaluation of the populations forming mats on the leaves of various bromeliads including cultivated in a greenhouse of the Botanical Garden in San Paulo (Fiore et al., 2007).

In the greenhouse of the M.M. Hryshko National Botanical Garden (HNBG) in Kyiv filamentous cyanobacteria sharing main characters of *Brasilonema* morphotype formed macroscopic dark crusty mats on epiphytic tropical plants including bromeliads. Especially abundant they were on the aerial roots of orchids from the genera *Vanda* R. Br., *Cattleya* Lindl., *Trichocentrum* Poepp. & Endl., *Brassolaeliocattleya* and the stems and leaves of *Tillandsia usneoides* (L.) L. and *T. funckiana* Baker. Revealed morphotype did not coincide with any known species of the genus.

Here we present the results of the study of HNBG populations using light (Olympus BX53) and scanning electron (JEM-1230) microscopes. For cultivation, liquid and agarized N-free BG11 medium was used. The specimens from the fresh cyanobacterial mats and the cultures had some differences in thalli habitus, coloration and arrangement of filaments, frequency of false branching, trichome appearance, dimensional limits, etc. The following description is based on our observations of both natural and cultural material.

Thallus macroscopic, crusty and blackish in nature, wooly purple-brown mats in culture. In SEM, thallus has clear two-component structure: prostrate lower and cespitose upper portions; the latter is formed by ascending filaments conglutinating in anastomosing fascicles. Filaments long, densely and variously entangled, 11.4–16.8 (19.5)  $\mu\text{m}$  (in culture up to 30  $\mu\text{m}$ ) wide, cylindrical. False branching in nature occurs very rare, in culture frequent, mostly geminate, branches long. Sheaths colorless firm, thin or up to 2.5  $\mu\text{m}$  thick, in old filaments slightly lamellated. Trichomes (7) 8.1–11.9  $\mu\text{m}$  (in culture up to 27  $\mu\text{m}$ ) wide, not constricted or slightly constricted at cross-walls, usually not attenuated at the ends. In culture, the width of a trichome could vary within a single filament: thickened portions often observed in the middle part of trichomes (hormogonia formation?), sometimes trichomes with attenuated end. Cells 3.7–8.8  $\mu\text{m}$  long; cell content homogenous or slightly granular, greyish-violet, violet, blue-green, olive-green, emerald-green or yellowish; vacuole-like structures were not observed. End cells rounded. Heterocytes solitary, intercalary, yellowish, varied in shape: discoid, flattened-spherical, rounded, round-square, round-rectangular elongated or short

cylindrical, 9.6–13.3 (18.4)  $\mu\text{m}$  long, (6) 8–12.7 (17.1)  $\mu\text{m}$  wide, frequent both in nature and in culture. Hormogonia straight or curved, with rounded ends, 9.7–12.6  $\mu\text{m}$  wide; cells short, granulated. Preliminary molecular study confirmed close similarity of HN BG cyanobacteria to *Brasilonema*.

Cyanobacterial mats in the greenhouse of HN BG has been observed for many years. It could be brought to Kyiv with tropical plants collected by botanical garden scientists in Brazil in 1986.

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**FLORISTIC DIVERSITY OF MACROPHYTES IN MARINE WATER  
AREAS OF NATURE RESERVES IN THE CRIMEAN PENINSULA**

The Crimean Peninsula is washed by the sea waters from all sides. At the same time, the population and recreational infrastructure are concentrated along the coast, where the vast majority of the Natural Environment Protected Areas (NEPA) is localized as well. The conservation of the natural phytodiversity of the marine coastal zone is most effective in the boundaries of integral territorial-aquatic nature protected areas with a high rank of conservation. The basis of the NEPA and the ecological networks is formed by nature reserves (NRs). In Crimea, all five NRs are presented either by integral territorial-aquatic complexes or they have territorial-aquatic areas (including coastal lagoons). The structural and functional basis of most marine benthic biotopes is formed by macrophytobenthos. The nature and characteristics of the benthic vegetation of the NRs in Crimea are described in the reference report (Phytodiversity..., 2012). After this, no new information, changing ideas about vegetation cover, has been received. However, the existing information on the flora composition does not reflect the current situation. Firstly, new taxa were recorded. Secondly, the information about them is not always promptly published, and publications are not always taken into account in the final reports (which, in turn, accumulate inaccuracies and discrepancies). Thirdly, in recent years a number of important nomenclature-taxonomic changes have been adopted. Thereby a revision of the marine macrophytes flora of the

Crimean Peninsula NRs was carried out using our own materials and literature data. Nomenclature and taxonomy of macroalgae are given according to AlgaeBase, a global algal database (<http://www.algaebase.org/>), for vascular plants – according to Catalogue of Life, a global index of species (<http://www.catalogueoflife.org/>). It was found that the marine macroflora of the Crimean NR branch "Lebyazh'i ostrova" ("Swan Islands") (de facto now this Crimean NR has the status of a national park and the branch is allocated to an independent reserve) includes 97 species (hereinafter, including infraspecific taxa): Chlorophyta (Chl) – 30, Ochrophyta (Oh) – 11, Rhodophyta (Rh) – 47, Tracheophyta (Tr) – 7, Charophyta (Chr) – 2; taxonomic structure: 8 classes (C), 22 orders (O), 31 families (F), 51 genera (G). The flora of the Cape Martyan NR (de facto now it is in the status of a nature park) includes 147 species: Chl – 35, Oh – 29, Rh – 81, Tr – 2; 7C, 28O, 46F, 79G. The flora of the Kazantip NR includes 70 species: Chl – 31, Oh – 10, Rh – 25, Tr – 4; 7C, 18O, 27F, 36G. The flora of the Opuk NR includes 84 species: Chl – 21, Oh – 18, Rh – 44, Tr – 1; 7C, 22O, 33F, 50G. The flora of the Karadag NR includes 179 species: Chl – 44, Oh – 43, Rh – 92; 6C, 30O, 48F, 86G. Thus, in total 226 species are registered in the marine water areas of the NRs in the Crimea: Chl – 59, Ph – 52, Rh – 106, Tr – 7, Chr – 2; 9C, 33O, 61F, 116G. According to the modern views (Minicheva et al., 2014), it makes up 50 % of all macrophytes occur in the Azov-Black Sea basin. The rare fraction of flora includes 47 species (the maximum is in Cape Martyan NR and the Karadag NR – 37 and 36 species, respectively). Within these marine water areas, biotopes under the special protection of the EU Habitats Directive are located (Council Directive 92/43/EEC). All five of the mentioned NRs are structural elements of the Emerald Network (List of officially adopted Emerald sites, 2018), four of them are designated as Wetlands of International Importance (Ramsar List, 2019). The main threat to the natural phytodiversity of the marine water areas in the Crimean NRs is the anthropogenic transformation of biotopes due to the influence of adjacent recreational, urbanized and agrarian sites. This, among other things, once again raises the issue about the insufficiency of their size and the biotope representativeness. In addition, there is a problem associated with the change of the borders and the status of some protected objects. At the same time, the results show that the NRs of the Crimea remain a key element in the system for natural phytodiversity conservation and are important for maintaining the ecological balance in one of the most densely populated areas of Eastern Europe.

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### RARE SPECIES OF ALGAE OF MOLDOVA

With the increase in anthropogenic pressure, the expansion of economic and industrial activities is becoming increasingly important problems associated with the protection of natural ecosystems and the conservation of biological diversity, including algae. The latest edition of the Red Data Book of Moldova (2015) includes 208 species of higher and lower plants, as well as fungi. An important achievement in the protection of biodiversity can be considered the inclusion in the list of rare species of such a heterogeneous group of plant organisms as algae. The total species composition of the algae flora of Moldova is 1890 freshwater species and more than 800 soil species. The relevance of this work is related to the fact that the issues of protecting the species diversity of algae and their communities in our country are very poorly developed. Algological studies were related to the need to create a program aimed at protecting rare and endangered species of algae, using various criteria: floristical, phytogeographical, evolutionary, genetic, general biological, ecological, scientific, economic. Examination of the already designated protected areas in order to identify within their boundaries areas rich in rare species of algae. The latest edition of the Red Book of Moldova includes 8 species of algae that belong to 3 divisions, 4 classes, 7 families and 7 genera. The greatest number of rare species of algae was noted in the class Hormogoniophyceae – 4 species. These include: *Anabaena propinqua* Setch. et N.L. Gardner, *Nodularia harweyana* Thuret ex Bornet et Flahault, *Nostoc flagelliforme* Harvey ex Molinari-Novoa, Calvo-Pérez et Guiry, *N. gelatinosum* Schousb. ex Bornet et Flahault. To the endangered species of algae are 5 species of algae: *Didymosphenia geminata* (Lyngb.) M. Schmidt, *Compsopogon chalybeus* Kütz., *Nostoc gelatinosum*, *N. flagelliforme*, *Anabaena propinqua*. The smallest number of species is noted in the group of vulnerable algal species, namely 3: *Bangia atropurpurea* (Mertens ex Roth) C. Agardh, *Biddulphia laevis* Ehrenb., *Nodularia harweyana*. Most of the rare species are found in the southern regions of the Republic of Moldova, namely in the Cimislia area near the village of Bogdanovka, on saline soils. These include such soil algae as *Anabaena propinqua*, *Nodularia harweyana*, *Nostoc flagelliforme*, *N. gelatinosum*. The northern and central parts of the Republic are represented by

a smaller number of species. The habitat of algae is divided into 2 large ecological groups, these are freshwater algae and soil. Freshwater algae include species such as *Bangia atropurpurea*, *Compsopogon chalybeus*, *Didymosphenia geminata*, *Biddulphia laevis*. While the soil species are represented by such species as: *Anabaena propinqua*, *Nodularia harweyana*, *Nostoc flagelliforme*, *N. gelatinosum*.

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**PHYTOPERYPHYTON OF THE MARINE PLASTIC (PET) NEAR THE CRETE COAST**

The study of the growth and possible ways of biodegradation of marine macroplastics is relevant today. There are two reasons for this: 1. The formation of new communities for marine ecosystems that use plastic as a substrate (plaston cenoses); 2. Macroplastics` fragmentation into a numerous microplastic particles under the influence of hydroturbulence, sunlight, salinity and temperature fluctuations, but also the mechanical effect of populations of different periphytic species during the formation of a community mosaic. In addition, as we show by our experiments in natural and laboratory conditions, not all benthic macro- and microphytes are able to live on plastic, and communities differs on various types and brands of plastic that are in the same environment.

The remains of plastic bottles (PET), torn into large pieces with floated, finely torn edges, were found in the rocky coast of Crete in August 2018. They laid on the stone bottom of the pits, bordering the rocky shore, at a depth of about 3 m. The surface of the PET fragments was densely covered with whitish-brown-pink-crimson spots of cortical fouling. Macrophytes forming the community appearance on the PET surface were represented by two species: *Lithophyllum byssoides* (Lam.) Foslie and *Hydrolithon boreale* (Foslie) Y.M. Chamb. – cortical red algae, which

formed here an almost solid "shell" that occupied up to 80 % of the surface of the polymer substrate. For the first one, the usual biotope is the lower splash zone on the rocks, where the waves break even with a minimum groundswell and where it often forms an entire strip of densely folded calcareous thalli of a pink-scarlet color. For the second – the surface of sea grass *Cymodocea nodosa* (Ucria) Asch., growing at depths of 5-8 m. Here microcourtins of the cortical green *Ulvelia scutata* (Reinke) R. Niels., C.J. O'Kelly et B. Wyso, as well as red *Acrochaetium parvulum* (Kylin) Hoyt and *A. hlulekaense* Steg. that creep and rise above the surface of the substrate. Occasionally small stems of *Polysiphonia* and *Rhizoclonium* sp. (up to 0.7 cm in height) were noted. Large coenocytic spherical cells of the green alga *Blastophysa rhizopus* Reinke, or cells with irregular lobes 30–90 µm in diameter, were scattered singly or in small clusters both directly on PET and in cracks of calcareous red algae. Also in these breaks, the clusters of small (up to 200-300 microns) palm-shaped cyanobacterial colonies were localized: *Chroococcus varius* A. Braun and *Aphanocapsa litoralis* (Hansg.) Komárek et Anagn.

There were also rare trichomes of *Calothrix* aff. *fusca* Born. et Flah., *Spirulina meneghiniana* Zanardini ex Gomont, *Leptolyngbya lagerheimii* (Gomont ex Gomont) Anagn. et Komárek, *Oscillatoria crassa* (C.B. Rao) Anagn., *Pseudanabaena* sp., *Hyella tenuior* Erceg. and numerous thin trichome, with polarly vacuolated cells, species of *Limnothrix* spp.

In turn, on the open areas of the PET surface massive attached and free-living diatoms were noted: *Licmophora remulus* Grunow, *L. debilis* (Kütz.) Grunow, *Amphora tenuissima* Hust., *A. wisei* (Sal.) Simons., *A. ostrearia* Brèb. var. *vitrea* (Cleve) Cleve, *A. hyalina* Kütz., *A. securicula* Perag. & Perag., *Halamphora abuensis* (Fog.) Levk., *H. subholsatica* (Kram.) Levk., *Nitzschia nienhuisii* Sterr. & Sterr., *N. angularis* W. Sm. var. *affinis* (Grunow) Grunow, *N. spathulata* Brèb. ex W. Sm., *N. dissipata* (Kütz.) Rabenh., *Brachysira estonarium* Witk., *Cocconeis molesta* Kütz., *C. scutellum* Ehrenb., *C. distans* W. Greg. and rarely – three species of *Navicula* spp., including colonial. The most frequently and in the broadest spectrum of microhabitats were *Mastogloia* species, living on both cortical red algae and PET: *Mastogloia erythraea* Grunow, *M. crucicula* (Grunow) Cleve, *M. ovata* Grunow, *M. ovum-paschale* (A. Schmidt) A. Mann, *M. acutiuscula* Grunow var. *elliptica* Hust., *M. horvathiana* Grunow, *M. cribrosa* Grunow и *M. ovulum* Hust. Among the diatoms of other genera, *N. nienhuisii* formed short ribbon-like colonies, *C. molesta*, in some places created mono-type fields with almost regular cell arrangement, and *L. debilis* sparsely grew on most of the available surfaces.

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### **ASSESSING STABILITY OF DNIPRO EPIPHYTIC ALGAL COMMUNITIES' TAXONOMIC AND QUANTITATIVE DIVERSITY (CASE-STUDY OF KYIV WATER RESERVOIR)**

Assessing the algal communities' response to human impact is one of topical issues nowadays. Criteria of such assessment amongst other things include stability, that is a system's ability to return to the initial condition after disturbance. This term is close to "homeostasis" concept, in the aspect of which the community's diversity stability can be expressed as absence of any long-term increasing or decreasing trends in the community's qualitative or quantitative parameters.

The research aim was to assess stability of epiphytic algal communities' taxonomic and quantitative diversity.

Field studies of epiphytic algal communities were performed in the Kyiv Water Reservoir in summer seasons of 2008 and 2012–2016. To assess stability of algal communities' diversity in time we used the coefficient of variation ( $C_v$ , %), that is, the ratio of the standard deviation to the mean value.

Epiphytic algal communities of the Kyiv Water Reservoir were marked by high taxonomic diversity (between 144 and 187 species and infraspecies taxa were identified in different years). The interannual variation coefficient made up 9 % for the total taxonomic diversity, and from 10 % to 94 % for taxonomic diversity of separate divisions. The lowest of them (10–13 %) were recorded for Bacillariophyta and Chlorophyta, the highest (77–94 %) – for Euglenophyta and Xanthophyta. Thus, divisions represented by the largest number of species and forming the "nucleus" of epiphytic algal communities were distinguished by the smallest interannual variation, that is, by the highest stability of taxonomic diversity in time.

Shannon index varied within a wide range year over year (0.36–4.38 bit·mg<sup>-1</sup>). Variation coefficients of Shannon index made up from 6 to 52 %. Lower variation of this index (6–25 %) was recorded for communities, which maintained polydominant structure throughout the entire observation period. Higher variation coefficients of Shannon index (27–52 %) were noticed for communities, whose structure in certain periods was oligo- or monodominant. The correlation between the minimal



Shannon indices and variation coefficients of Shannon indices made up  $-0.94$  at  $p < 0.0001$ , and the correlation between the average Shannon indices and variation coefficients of Shannon indices was equal to  $-0.74$  at  $p = 0.002$ . It means that the higher is the average Shannon index, the smaller is the amplitude of its fluctuations. This can be explained by the fact that polydominant communities are more stable in time due to their higher resistance to the impact of environmental variables. Therefore, polydominant structure of epiphytic algal communities may be considered one of mechanisms sustaining their stability.

The epiphytic algal communities' biomass fluctuated between  $0.47 \pm 0.18 \text{ mg} \cdot 10 \text{ cm}^{-2}$  and  $3.09 \pm 1.18 \text{ mg} \cdot 10 \text{ cm}^{-2}$ . It is important that no persistent long-term increasing or decreasing trend in the algal biomass was observed during the observation period, and this may be indicative of the algal community's stability.

Thus, it has been proven, that at their present succession stage epiphytic algal communities of the Kyiv water reservoir are characterized by stability, which is an efficient mechanism sustaining their taxonomic and quantitative diversity.

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### ***STENOMITOS KOLAENSIS*, A NEW SPECIES OF CYANOBACTERIA FROM KOLA PENINSULA, RUSSIA**

*Stenomitos* Miscoe et Johansen is recently described cyanobacterial genus some species of which appears to be cryptic (Miscoe et al. 2016). Type species, *S. rutilans* Miscoe et J.R. Johansen isolated from the caves on Hawaii archipelago. Other species of that genus: *S. frigidus* (Fritsch) Miscoe et Johansen and *S. tremulus* (Johansen et Casamatta) Miscoe et Johansen were revised morphologically and

genetically. In our work, we describe a new member of genus *Stenomitos*, *S. kolaensis* sp. nov., isolated from the moderately acidic Al-Fe humic podzols with high concentration of the heavy metals of a boreal forest near Pechenganikel town, Murmansk region, Russia. We observed clear morphological autapomorphies in *S. kolaensis* separating it from all other species of the genus. 16S and 16S-23S ITS rRNA phylogenetic analyses was in congruency with morphology supporting elevation of the new species. Phylogenetic analysis of the 16S-23S ITS rRNA region resulted in *S. kolaensis* forming a separate supported clade distant from any other *Stenomitos* lineages including Antarctic strains of the misnamed taxon "*Leptolyngbya frigida*". Further, structure of the conserved ITS regions showed the same signal. *S. kolaensis* can be distinguished from other *Stenomitos* taxa by its geographical distribution, habitat preference, morphology 16S rRNA phylogeny, and differences in the secondary structure of the 16S-23S ITS region. Thus, using polyphasic approach we are describing *S. kolaensis* as a new species.

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### **PHYTOPLANKTON DEVELOPMENT IN SMALL RESERVOIRS**

Phytoplankton of small reservoirs (Denyshi Reservoir, Zhytomyr Reservoir on the Teteriv River, Berdychiv Reservoir on the Gnylopyat River, Myropil Reservoir and Novograd-Volynskiy on the Sluch River), and their hydrochemical regime were studied during 2004-2018 according to generally accepted methods (Methods ..., 2006).

During the research, 280 species were identified, represented by 304 intraspecific taxa, including nomenclature type of the species. Green algae (33.2 % of the total list) and diatoms (24.3 %) were the richest in floristic terms. Such ratio is typical for the specific plankton flora of each reservoir. The plankton of Zhytomyr Reservoir, which has the largest area and receives the flow of the Guiwa and Gnilopyat Rivers, was the most abundant. The larger the area and the volume of reservoirs are, the bigger is the blue-green algae's share: in Zhytomyr Reservoir and Denyshi Reservoir they occupy the third place according to their species diversity (13.8–14.2 %), in Berdychiv Reservoir they are slightly behind

Euglenophyta, and in the smallest reservoirs – Myropil Reservoir and Novograd-Volynskyi Reservoir the floristic share of Euglenophyta is more noticeable. The number of taxa in reservoirs closely and reliably correlates with the water area ( $r=0.80$ ,  $p=0.000001$ ).

Increased human impact causes a decrease in generic coefficients, and the proportion of monotypical species increases. This is confirmed by reliable correlation between the generic coefficient and the chlorides content ( $r = -0.82$ ,  $p<0.05$ ) and phosphates ( $r = -0.68$ ,  $p<0.05$ ). In addition, a correlation was found between the content of total nitrogen and the ratio of the number of species to the number of intraspecific taxa ( $r = 0.93$ ,  $p<0.05$ ). In reservoirs, the increase in the content of total nitrogen and phosphates causes a rise in the floristic share of small-cell centric diatoms, which leads to an increase in the ratio of the number of species of centric diatoms to pennate diatoms ( $r=0.28-0.83$ ;  $p<0.05$ ). Regarding time changes, over the past 15 years, the generic coefficients and species saturation with intraspecific taxa have slightly increased in reservoirs, whereas large reservoirs of the Dnipro and the Volga show a trend of phytoplankton taxonomic structure simplification with their age (Shcherbak, 2000, Korneva, 2009). This indicates a significant intensity of adaptation processes of algal groups in small reservoirs.

16 species had maximum occurrence in the reservoirs under study. Comparison of their composition showed a significant similarity (Sorensen similarity index was equal to 0,36-0,74). However, only one species of *Cyclotella meneghiniana* Thw. has a high occurrence rate in all reservoirs.

Phytoplankton quantitative diversity varied within a broad range. The highest average values of number (31.628-88.871 mln/dm<sup>3</sup>) and biomass (3.449-4.254 g/m<sup>3</sup>) were recorded in Berdychiv and Zhytomyr reservoirs. The factors that determine phytoplankton biomass include pH ( $r = 0.548$ ,  $p = 0.001$ ), color ( $r = -0.357$ ,  $p = 0.00001$ ), phosphorus content ( $r = 0.458$ ,  $p = 0.039$ ). In its turn, phytoplankton during its vegetation season affects the content of oxygen dissolved in water and the pH value, causing oxygen hypersaturation and pH shift to alkalinity. As the trophic levels increase in reservoirs, a shift in biomass maxima from summer to autumn is observed. Analysis of relation between the information variety and the influence of various environmental factors showed that the main abiotic parameter, which determined its value in the reservoirs, was the phosphate content ( $r = -0.733$ ,  $p = 0.00009$ ). Prevalence of the oligodominant phytoplankton structure in reservoirs indicates the specificity of human-disturbed reservoirs.

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### **OLIGOCENE ZMIIV ALGAL FLORA OF THE SUBPARATETHYS (NORTHERN UKRAINE)**

During the Oligocene period (about 30 million years ago), the territory of Northern Ukraine was covered by the shallow sea, which formed the northern margin of the Eastern Parathethys. This sea is called Subparathetys (Zosimovich, 1984). Dinoflagellates were components of plankton of the Subparatethys. We found their remains (dinoflagellate cysts) as a result of the standard palinological maceration (HF) of clays of the Zmiiv Formation (Upper Rupelian). The species of dinoflagellate cyst are: *Chiropteridium galea* (Maier) Sarjeant, *Membranophoridium aspinatum* Gerlach, *Apteodinium australiense* (Deflandre et Cookson) Stover et Evitt, *Pentadinium laticinctum* (Gerlach) Benedek et al., *Gerdiocysta* sp., *Rhombodinium draco* Gocht, *R. longimanum* Vozzhenn., *Deflandrea phosphoritica* Eisenack, *D. heterophlycta* Deflandre et Cookson, *Lingulodinium machaerophorum* (Deflandre and Cookson) Wall, *Selenopemphix nephroides* (Benedek) Benedek et Sarjeant, *Thalassiphora pelagica* (Eisenack) Eisenack et Gocht, *Homotryblium tenuispinosum* Davey et Williams, *H. floripes* (Deflandre et Cookson) Stover, *Distatodinium ellipticum* (Cookson) Eaton, *Spiniferites/Achomosphaera* group; the genus *Wetzeliella* is especially numerous (*Wetzeliella gochtii* Costa et Downie, *W. symmetrica* Weiler, *W. articulata* (O. Wetzel) Eisenack, *Wetzeliella* spp.). The freshwater and brackish-water species of the families Botryococcaceae (*Botryococcus braunii* Kütz.), Prasinophyceae (*Leiosphaeridium pusilla* Madler, *Tasmanites consinnus* (Cookson et Manum) Downie et al.), Zygnemataceae (*Ovoidites* sp.) and acritarchs (*Mycrstridium* sp., *Cymatiosphaera* sp.) were also found. It should be noted, fossils of *Pediastrum boryanum* (Turpin) Menegh. (Chlorophyceae), algae, which today lives exclusively in fresh water, are found in marine Zmiiv sediments. The genus *Pediastrum* extends to the Cretaceous, and the known Cretaceous and Paleogene forms very much resemble some modern ones. *Pediastrums* and other freshwater algae were transferred from the land to the marine paleobasins by water flows and buried in sediments together with dinoflagellate cysts.

The frequency of algal microfossils in Zmiiv section ranged from 5 % in the lower part of the section to almost 30 % in the upper part of the section. A general survey of the distribution of these microorganisms in the Zmiiv clay section (Kozak Mount, Kharkiv area) indicates the importance of the results for the environmental parameters of the Zmiiv geological time. Dinoflagellate cysts and freshwater algaeflora have in many cases provided excellent records of changes through time in salinity, temperature and nutrients, and have also been used reconstruct changes of hydrological regime in Parathethys sea. The algological record in the Kozak section shows a transgressive-regressive cycle in the Middle Oligocene. The ratio of marine and freshwater microalgae reflects fluctuations in the position of the coastline.

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### **EPIPHYTON UNDER CONDITIONS OF UNSTABLE HYDROLOGICAL REGIME OF A CASCADE PLAIN RESERVOIR**

The influence of hydrodynamic factors (current, water level fluctuations, and wind driven wave) on the structure of epiphyton occurring on macrophytes of various ecological groups was investigated on the example of the Kaniv Reservoir infracascade belonging to large plain reservoirs on the Dnipro (Ukraine). The reservoir is subdivided into the river and lake sections, including the transit and non-transit zones. The hydrological regime of the river section of the Kaniv Reservoir mostly depends on the operating conditions of the Kyiv Hydro-Electric Power Station located upstream. Its releases are responsible for intraday water level fluctuations up to 1.2 m. In the non-transit zones (bays, branches, etc.), fluctuations of water level are almost synchronous with those in the main riverbed. During the period of releases, discharge current velocity in the transit zone accounts for 0.3–1.0 m/s, whereas in the non-transit zone it is 0.05–0.10 m/s. The intensity of wind driven wave in the river section is not more than 0.1 m. In the lake section of the reservoir, discharge current velocity is mainly not more than 0.1–0.3 m/s.

The intraday water level fluctuations are about 0.05 m. Wave height in the transit zone is not more than 0.40 m, whereas in the non-transit zone – 0.10 m.

It has been found, that the most favourable conditions for the development of epiphyton are in the non-transit zone of the lake section of the Kaniv Reservoir, where macrophytes of all ecological groups had the largest number of epiphytic algal species in a sample, and they characterized by the highest numbers and biomass. In the bays of the river section, the intensity of epiphyton development was somewhat lower. In the main riverbed of the river section and in the transit zone of the lake section, intensive hydrodynamics significantly limited the development of epiphyton. Thus, the main factors inhibiting the development of epiphyton in the main riverbed of the river section included high current velocities and water level fluctuations, in the bays of the river section – water level fluctuations, whereas in the transit zone of the lake section – wind driven wave.

The performed investigations have shown that the increase in water movement conditioned by high current velocities, water level fluctuations, and wind driven wave caused qualitative (the decrease of epiphyton species richness, changes of its species composition, taxonomic structure, dominant complex, dimensional structure) and quantitative (values of numbers and biomass, quantitative indices of epiphyton development) changes. The average number of species in a sample and epiphyton numbers and biomass depended inversely on current velocity, water level fluctuations, and wind driven wave. This can result in the decrease in the amount of the released oxygen and organic matter produced by this community, and in the decrease in the intensity of the utilization of nutrients and accumulation of pollutants. Therefore, the role of epiphyton in the processes of water body self-purification can sharply decrease.

At the same time, we revealed that water movement variously influenced algal communities occurring on the substrates of different types. Significant water level fluctuations and intensive wind and wave inhibited the development of epiphyton on macrophytes of all ecological groups and stimulated the development of periphyton with a predominance of *Cyanoprokaryota* and *Bacillariophyta* inhabiting solid artificial inorganic substrates. This phenomenon can be considered as compensatory mechanism of homeostasis maintenance, due to which aquatic ecosystems are capable of withstanding an adverse effect of the consequences of hydrotechnical construction. The obtained data can be used in predicting the ecological state of the designed water bodies.

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### ***GONATOZYGON ACULEATUM* W.N. HASTINGS AND *PLEUROTAENIUM SIMPLICISSIMUM* GRÖNBLAD - NEW TAXA OF RARE DESMIDS (ZYGNEMATOPHYCEAE, STREPTOPHYTA) FOR UKRAINE**

Two remarkable desmid species new for flora of Ukraine were found in a group of ponds situated in the north of Ukraine (51° 57' 55.0"N 31° 10' 04.2"E) in Chernihiv Polissia. Originally these ponds were quarries for extracting sand used for glass industry. *Gonatozygon aculeatum* W.N. Hastings was so far found in 8 countries of Europe and *Pleurotaenium simplicissimum* Grönblad was previously reported only in 4 European countries (according to AgaeBase).

First these taxa were found in samples of 2010 and then fresh sampling of 2018 confirmed their occurrence.

*Gonatozygon aculeatum* (L: 64–67 µm, B (not apex): 7–8 µm, B (apices): 8–9 µm, L/B: 6.8–7.7). Cylinder shaped cell, length is 7 times breadth without spines (3 cells observed in samples of two different years), apices dilated and truncate, at the edge of apex the cell wall creates a thickened ring because the bottom of apex is somewhat indented. Ends are irregularly ornamented with rare granules that look like undeveloped spines (present only on apices), they are often visible on face view and present on some drawings of this taxon (Růžička, 1977; Kouwets, 1987; Lezenweger, 1999 etc.). Spines are rather short (2.5 µm) but making over 30 % of the cell diameter (7–8 µm) it seems worth considering that ratio in taxon identification knowing that cells size varies largely (in a similar way cell length to breadth ratio is indicated in most publications). Spines are scattered moderately densely, they are quite strong (broader at the basis) and have regular not very much variable length. Ukrainian specimen is very small comparing to other reports on this taxon and is closer in dimensions to report from Check Republic (Šťastný, 2010). This taxon is found in two ponds having the following ecological characteristics: 1) pH – 6.25, ORP – 45.0 mV, conductivity – 33.0 µS/cm; 2) pH – 6.71, ORP – 19.5 mV, conductivity 82.0 µS/cm.

*Pleurotaenium simplicissimum* (L: 600–625  $\mu\text{m}$ , B (basal): 31.5–34.0  $\mu\text{m}$ , B (ends): 25.5–29.7  $\mu\text{m}$ , L/B: 17.8–18.7 –( measured 2 cells so far). It is a very rare taxon (Růžička 1977; Šťastný, 2010). Long, rod shape cells with slightly marked basal swelling and nearly unwaved side lines. Truncate apices slightly expanded or straight with rounded angles and the crown of granules on the top, 7–8 small granules can be observed on face view. Cell walls are covered with small pores and gently punctate. Findings in Europe: first found in Finland (Grönblad, 1920), in 1923 J. V. Roll reports on *Pleurotaenium baculiferum* Roll (1923) found in Republic of Karelia (part of Russia bordering Finland) that was indicated as synonym by W. Krieger (1937), Germany (Krieger, 1937), Netherlands (Coesel, 1985), Czech Republic (Šťastný, 2009, 2010). Ukrainian specimen does not match the dimensional characteristics of samples from Finland, Russia and Germany (it is smaller). And it is within size limits reported from Netherlands and Czech Republic. It is found only in one pond (pH – 6.25, ORP – 45.0 mV, conductivity – 33.0  $\mu\text{S/cm}$ ).

In the last publication on *Pleurotaenium simplicissimum* for Europe (Šťastný, 2009) the author supposed that this extra rare in Europe taxon is "close to extinction" (Šťastný, 2009), fortunately our finding gives ground for optimism in this respect.

Flora of Ukraine is enriched with these two rare desmid species. Ecosystems in transformed environment (former sand quarry ponds) became habitats of extra rare desmid species; such habitats shall be given official protection status.

Publication giving extensive description of Ukrainian specimen with original diagnoses pictures and drawings shall be prepared as well.

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## **MICROALGAE ON THE PLASTIC SUBSTRATES IN THE COASTAL AREA OF THE GULF OF ODESSA (THE BLACK SEA)**

The work was done in the frame of the project "Study of the effect of plastic pollution on bottom marine assemblages" financed by Ukrainian Fund of Basic



Research in July-December 2018 (project No F83/88-2018). The 51 plastic bottles and bags with periphytic algae were sampled by diver along three transects on the depth of 1-2, 3-4 and 5-6 m in three locations in the Gulf of Odessa. The samples were cut into smaller fragments and fixed with formalin. To analyze the state of periphyton developing on the plastic surface, temporary alcohol-glycerol slides were made. For diatoms identification permanent slides were prepared. Separation of microalgae from plastic was done by ultra-sound (35 kHz).

The 46 species of microalgae have been identified at the plastic substrates found on the seabed in the study area: Bacillariophyta (38 species) and Cyanoprokaryota (8 species). Beyond the seaweeds 6 species were noted: *Ulvelia scutata* (Reinke) R. Nielsen, C. J. O'Kelly et B. Wysor та *U. lenz* P. Crouan et H. Crouan, *Ulva* sp., *Ceramium rubrum* C. Agardh, *Callitamnion corymbosum* (Smith) Lyngbye, *Melobesia* sp. The main representatives of microalgae were *Cocconeis* and *Amphora* genus, which formed a dense layer on the plastic surface. The most abundant were *Cocconeis scutellum* Ehrenb., *C. placentula* Ehrenb., *Amphora ovalis* (Kütz.) Kütz.

It should be noted that only a few species of diatoms, cyanobacteria and green macrophytes are attached directly to the plastic. Other species grow on the surface of seaweeds or on the layer of *Cocconeis* and *Amphora*. Species *Achnanthes brevipes* C. Agardh, *Diatoma elongata* (Lyngbye) C. Agardh, *D. vulgaris* Bory, *Melosira moniliformis* (O.F. Müller) C. Agardh., *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bert. formed colonies that can also become a substrate for microalgae.

The abundance of microalgae on plastic varied in the wide range from 10.0 cells cm<sup>-2</sup> to 1.6 million cells cm<sup>-2</sup>, on average 124.3 ± 38.3·10<sup>3</sup> cells·cm<sup>-2</sup>. On a transparent plastic bags the number was on average 134.2·10<sup>3</sup> cells·cm<sup>-2</sup>, on non-transparent it was twice less and made 58.5·10<sup>3</sup> cells·cm<sup>-2</sup>. On the surface of bottles the number of microalgae reached 146.2·10<sup>3</sup> cells·cm<sup>-2</sup> on average.

In general microalgae abundance was formed by diatom algae (up to 92 %). The number of cyanobacteria varied in the range from 75.0 to 24.0·10<sup>3</sup> cells·cm<sup>-2</sup>. The number of green algae *U. scutata*, which forms small colonies (25-90 mkm in diameter) was 5-154 cells·cm<sup>-2</sup>.

In our study we did not reveal any specific pattern of periphyton preference for substrate – bags or bottles. However, the diatom algae found on almost all studied plastics samples, whereas cyanobacteria were met on 50 % of bottles and 30 % of bags. We noted that cyanobacteria were more abundant on non-

transparent substrate, whereas diatom algae were 3.5 times less on such substrate than on transparent plastic.

Part of the samples contained a complex multi-tier fouling with components of macrozoobenthos, macrophytes and meiobenthic organisms, which may indicate that the plastic is under water for at least six months. In this case, a significant diversity of algae will be available. However, part of the plastic samples did not have any macro-fouling, probably, because of a rather short time in the marine environment. The distribution of such samples varies in depth due to high wave activity in the coastal zone.

Thus, we observe the complicated and diverse assemblages, which is common for the natural substrates of the Gulf of Odessa. The study of settlement process of plastics by marine organisms and their diversity is very new for the Black Sea (Sapozhnikov et al., 2018, in Rus.; Snigiriova, Portianko, 2018, in Ukr.).

In this regard, further researches should be directed to studies of periphyton processes formation on various plastic materials in marine environment combining it with the experimental studies in the laboratory.

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### **INFLUENCE OF METHANOL AND H<sub>2</sub>O<sub>2</sub> ON LIPID BODIES ACCUMULATION BY *CHLAMYDOMONAS REINHARDTII* DANG.**

Microalgae can be used as raw material for renewable energy. The challenge for biotechnological obtaining diesel fuel is to maximize the intracellular lipid content during cultivation of microalgae. It was confirmed that nitrogen starvation, adversely affecting microalgae growth, induces significant accumulation of neutral lipids in the cells of *Chlamydomonas reinhardtii* Dang. The total lipid yield can be increased due to growth promotion of culture by improving carbon supply. Methanol can be used by microalgae as a source of carbon and energy and stimulate growth of *Ch. reinhardtii*. It was found that the addition of methanol to the culture medium of *Chlorella sp.* accompanied by increased lipid content.

Methanol utilization accompanied with increasing the intracellular concentration of H<sub>2</sub>O<sub>2</sub>. There is evidence that oxidative stress caused by exogenous H<sub>2</sub>O<sub>2</sub> leads to accumulation of lipids in *Ch. reinhardtii*. The aim of our research was to determine how addition of exogenous methanol and H<sub>2</sub>O<sub>2</sub> affects accumulation of lipids in cells of *Ch. reinhardtii* provided nitrogen deficiency.

Autotrophic batch cultures of *Ch. reinhardtii* were used for research, which were grown as described previously, at room temperature under 100 μmol photons m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density (PPFD) of 24-h white fluorescent light on the surface of flasks. In the middle of the exponential growth phase (5 days) microalgae transferred to medium without nitrogen (-N environment). Simultaneously 50 mM methanol and (or) 100 mM H<sub>2</sub>O<sub>2</sub> was added.

Lipids content was quantitatively determined on spectrofluorometer QuantaMaster by the change in fluorescence Nile red at 590 nm, which corresponds to maximum of emission spectrum of the dye in complex with lipids. Measurements performed by the method of Kou. Lipid inclusion in *Ch. reinhardtii* cells were visualized using confocal scanning microscope LSM 510 META. Preparation of microalgae for confocal microscopy was performed by the method of Wang.

During the autotrophic growth of *Ch. reinhardtii* culture reached stationary phase at 10-14 day of growth. To study the induction of lipid accumulation we used culture at the exponential growth phase (5 days) with a concentration of 3 million cells × ml<sup>-1</sup>. During the experiment, the concentration of cells remained constant. After the first day cultivation on the medium without nitrogen observed the formation of lipid inclusions. Samples were taken in the dynamics immediately after transfer to the -N medium and through every day for 4 days. Induction of lipid inclusions by the nitrogen starvation can be considered as standard method because it is often used and gives the best result. First, after the removal of nitrogen from the medium lipid content in the cells did not change, and after 2, 3 and 4th day culturing increased by 61, 80 and 232 %, respectively. While growing in the medium without nitrogen with the addition of 50 mM methanol the dynamic of lipids accumulation in culture did not differ from controls. In the case with the addition of 100 mM H<sub>2</sub>O<sub>2</sub> increase in lipid content was only 87 % on the 4th day. It can be assumed that oxidative stress caused by H<sub>2</sub>O<sub>2</sub>, leads to dysfunction of the photosynthetic apparatus and therefore reduce the accumulation of lipids in stressful conditions. If was the combination of three factors - nitrogen starvation,

50 mM methanol and 100 mM H<sub>2</sub>O<sub>2</sub> addition then the lipid content was increased by 63 % by the first day of cultivation. At the end of the experiment lipid content exceeded the control one on 273 %. This combination of factors is best stimulated the accumulation of lipids in *Ch. reinhardtii*. This is probably due to accelerated oxidation and utilization of methanol in the presence of H<sub>2</sub>O<sub>2</sub>.

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### **THE WAY TO INCREASE THE EFFICIENCY OF PRODUCTION H<sub>2</sub> BY *CHLAMYDOMONAS REINHARDTII* DANG.**

The exhaustion of traditional energy sources (petroleum products, coal and gas) draws the public's attention to the use of alternative renewable energy sources. One of the prospective energy carriers of the future is the biohydrogen. Fuel obtained from H<sub>2</sub> is ecological since its use not accompanied by a greenhouse effect. Some green algae, in particular, *Chlamydomonas reinhardtii* Dang., in conditions of anaerobiosis and mineral nutrition deficiency, produce H<sub>2</sub>. During the aerobic stage, adaptation of the *Ch. reinhardtii* metabolism occurs, which further ensures the production of H<sub>2</sub> in the next anaerobic or production phase of hydrogen biotechnology. The task of biotechnology is to create cultivation conditions that reduce aerobic and prolong the producing stage. The reduction of the aerobic stage can be achieved by creating conditions that ensure the rapid removal of O<sub>2</sub> from the culture medium.

We investigated the effect of methanol and isoascorbate on the production of H<sub>2</sub> by *Ch. reinhardtii* under sulfur deprivation conditions in a closed system without access to air, as it is known that in the presence of these substances in the culture medium, the content of O<sub>2</sub> decreases.

Autotrophic batch cultures of *Ch. reinhardtii* were used for research, which were grown in TAP medium, at room temperature under 100 μmol photons m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density of 24-h white fluorescent light on the surface of flasks. In the middle of the exponential growth phase, culture concentrated by centrifugation at 3000 rpm. Then the cells transferred to growth medium without

sulfur (TAPS) and placed in closed reactors. 50 mM methanol or 100 mM isoascorbat was added to the TAPS medium. The determination of the H<sub>2</sub> content was performed amperometrically by means of a platinum Clark electrode in the gas phase of a glass cell at a temperature of 20 °C. The H<sub>2</sub> content was determined as a percentage to the pure H<sub>2</sub> obtained as result of the reaction of zinc with hydrochloric acid in the Kip apparatus.

If methanol or isoascorbat was added, the aerobic phase is reduced, and such cultures begin to produce H<sub>2</sub> earlier compared to the control. However, the presence of isoascorbate results in a reduction of duration of the productive phase of H<sub>2</sub> release. The volume of the gas phase in the reactors at the end of the experiment was the same in control and with the addition of 50 mM methanol. The percentage of hydrogen in the control was 95 %, with the addition of methanol - 90 % and isoascorbate - 62 %. Thus, the addition of methanol causes the reduction of the aerobic phase during the production of H<sub>2</sub>. Such an effect on the condition of microalgae culture is quite predictable since, as an activator of respiration, methanol reduces the concentration of O<sub>2</sub> in the culture medium.

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### **RARE SPECIES OF DIATOMS (BACILLARIOPHYTA) OF THE COAST OF THE GULF OF FINLAND (LENINGRAD REGION, RUSSIA)**

The Gulf of Finland is a brackish gulf of the Baltic Sea located in the north-eastern part of the sea. The eastern part of the gulf that belongs to the Russian Federation (Leningrad Region) is called the eastern part of the Gulf of Finland. Freshwater inflow into basin is very significant and water salinity is not high in the gulf, it is 0-4 ‰ in the gulf (average salinity in the sea is 8-10 ‰). At the same time, the salinity in the northern part is somewhat lower than that of the southern coast due to the desalinating influence of a rivers and the general circulation of water in the Gulf of Finland. This gulf is shallow enough, the maximum depth of the eastern part of the Gulf of Finland reaches 60 - 65 m in the area of the Gogland Island, in

the east direction there is a decrease in depth to 10-15 m. The southern shore of the gulf is mostly sandy, low. The coast is weakly cut, but forms three small bays: Narva Bay, Luga Bay, Koporsky Bay. The northern shore of the Gulf of Finland is composed of crystalline rocks, strongly indented and has numerous bays. The largest of the bays is the Vyborg Bay, among the islands are the Big, Western and Northern Birch Islands.

Samples of the diatom material were collected at a depth of 0-3 m from bottom along the shore of the gulf from various substrates: from the surface of sand, silt, stones, wooden and other artificial structures submerged in water, aquatic plants, macrophytes. The sediments of samples were obtained using light and scanning electron microscopes. The research was done using equipment of The Core Facility Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute RAS (St.-Petersburg, Russia).

In the study of the diatom communities of the Gulf of Finland, special attention was paid to the group of araphid diatoms. In the Gulf of Finland, 76 species (84 with intraspecific taxa) of araphid diatoms from 23 genera were found, of which 38 species from 16 genera were found in the eastern part of the Gulf of Finland (Intercalibr., 1993, 1994, 1995, 1996, Potapova 1999; Balashova et al., 2016; original data). The most widespread species on the coast of the eastern part of the Gulf of Finland are *Ctenophora pulchella* (Ralfs ex Kütz.) Williams et Round, *Diatoma moniliformis* Kütz., *Fragilaria vaucheriae* (Kütz.) Petersen, *Stauroforma atomus* (Hust.) Talgatti, Wetzel, Morales et Torgan in Talgatti et al., *Tabularia fasciculata* (C. Agardh) Williaams et Round and *T. tabulata* (C. Agardh) Snoeijs. Seventeen species are rare: *Diatoma polonica* Bak, Lange-Bert., Nosek, Jakubowska et Kielbasa, *D. mesodon* (Ehrenb.) Kütz., *Fragilaria amphicephaloides* Lange-Bert. in Hofmann, Werum et Lange-Bertalot, *F. inflata* var. *istvanffy* (Pant.) Hust., *F. mesolepta* Rabenh., *F. tenera* (W. Sm.) Lange-Bert., *Licmophora gracilis* var. *anglica* (Kütz.) H.Peragallo et M.Peragallo, *Meridion circulare* (Greville) C. Agardh, *Opephora krumbeinii* Witkowski, Witak et Stachura apud Lange-Bertalot et Genkal in Lange-Bertalot, *Pseudostaurosira elliptica* (K. Schum.) Edlund, Morales et Spaulding, *P. trainorii* Morales, *Stauroforma inermis* Flower, Jones et Round, *Staurosirella martyi* (Héribaud-Joseph) Morales et Manoylov, *Tabellaria floccuiosa* (Roth) Kütz., *Tetracyclus emarginatus* (Ehrenb.) Sm., *Ulnaria acus* (Kütz.) Aboal in Aboal et al., *U. ulna* (Nitzsch) Compère in Jahn et al. In the eastern part of the Gulf

of Finland, ten new species of araphid diatoms for the flora of the Leningrad region were found: *Fragilaria amphicephaloides*, *F. gracilis*, *F. inflata* var. *istvanffy*, *F. mesolepta*, *F. tenera*, *Licmophora gracilis* var. *anglica*, *Opephora krumbeinii*, *Pseudostaurosira trainorii*, *Stauroforma inermis*, *Tetracyclus emarginatus*.

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## **NEW INVASIONS OF ALIEN SPECIES OF PLANKTONIC MICROALGAE INTO THE NORTH-WESTERN PART OF THE BLACK SEA (UKRAINE)**

The distribution of alien species from different regions of the World Ocean into the Black Sea is ongoing process. Studies of biological invasions are particularly important under the conditions of global climate changes. We are facing the elimination of local native species and their replacement them by alien/invasive species.

Investigation of invasive alien plankton microalgae was conducted during 2011–2018. Nine invasive species of coastal phytoplankton belonging to 5 classes of microalgae were found.

*New for the Black Sea.* In January 2011 a freshwater species of dinoflagellate *Chimonodinium lomnickii* (Włosz.) Craveiro et al. with abundance of  $13.02 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of 0.05 g·m<sup>-3</sup> was noted for the first time (4.0°C; 13.3 psu). During the bloom, its abundance increased to  $1.43 \cdot 10^6$  cells·L<sup>-1</sup> and the biomass was 8.63 g·m<sup>-3</sup> (4.8°C; 13.1 psu) (Krakhmalny, Terenko, 2019). Two new mixotrophic forms of nanoplankton golden algae were noted (Terenko et al., 2013). One of them, phylose amoebae *Paulinella ovalis* (Wulff) Johnson et al. appeared in March 2013 with maximum abundance of  $48.92 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of 1.92 mg·m<sup>-3</sup> (3.1°C; 15.4 psu). Another one, freshwater *Ollicola vangoorii*

(W. Conrad) Vørs with maximum abundance of  $64.86 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of  $3.81 \text{ mg} \cdot \text{m}^{-3}$  (9.6°C; 12.7 psu) was found in April 2013.

*New for the northwestern part of the Black Sea.* In 2011, two new nanoplanktonic species of diatoms were noted in the summer plankton. *Chaetoceros thronsenii* (Marino et al.) Marino et al. achieved the maximum abundance of  $136.03 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of  $4.31 \text{ mg} \cdot \text{m}^{-3}$  of in June 2015 (21.0°C; 16.3 psu). The maximum abundance ( $36.66 \cdot 10^3$  cells·L<sup>-1</sup>) of *Chaetoceros minimus* (Levander) Marino et al. and biomass ( $0.71 \text{ mg} \cdot \text{m}^{-3}$ ) were registered in July 2015 (21.4°C; 17.0 psu). Though these diatoms were recorded in the northeastern part of the Black Sea previously (Pautova et al., 2007, 2013). The single-living marine chrysophyte flagellates *Dinobryon faculiferum* (Will.) Will. was first found off the coast of Turkey (Gülnur Özdemir, Orhan Ak, 2012). In Odessa Bay it was noted in March 2011 with the maximum abundance of  $107.69 \cdot 10^3$  cells·L<sup>-1</sup>, biomass of  $13.53 \text{ mg} \cdot \text{m}^{-3}$  (3.5°C; 7.4 psu). Two new species of choanoflagellates were recorded. The first one, *Calliacantha natans* (Grøntved) Leadbeater, was found in March 2011 with maximum abundance of  $16.39 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of  $1.42 \text{ mg} \cdot \text{m}^{-3}$  (3.1°C; 15.0 psu). The second one, *Diaphanoeca grandis* Ellis, was observed in October 2015 with maximum abundance of  $11.42 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of  $0.45 \text{ mg} \cdot \text{m}^{-3}$  (11.0°C; 16.9 psu). However, these species were recorded in the northeastern part of the sea and off the coast Bulgaria long before us (Valkanov, 1970; Moiseev, 1983). A new dinoflagellate *Peridinium quadridentatum* (F. Stein) Gert was observed in July, 2018 in the Odessa Bay with the maximum abundance of  $84.0 \cdot 10^3$  cells·L<sup>-1</sup> and biomass of  $0.56 \text{ g} \cdot \text{m}^{-3}$  (23.0°C; 17.9 psu). However, this species were recorded in the northeastern part of the sea before (Vershinin et al., 2005).

Most of alien species belong primarily to the warm-water complex, with the exception of *C. lomnickii*, which develops exclusively in the winter period. *C. lomnickii*, *C. thronsenii*, *O. vangoorii*, *D. faculiferum*, *P. ovalis* are common species in the annual phytoplankton community. While, *C. minimus*, *D. grandis*, *C. natans* are found in plankton less frequently.

Observations on the development of the alien species showed that each of these species occupied its ecological niche in the coastal microalgae community of this region.

The introduction of marine alien species invaders is primarily associated with ballast waters and has Mediterranean and, therefore Atlantic origin. Whereas freshwater species enter the north-western part with the flow of large rivers such as the Dnipro, Danube and Dniester.



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### **ALGAE OF MINERALIZED STREAM OF THE SLOPES OF THE TILIGUL ESTUARY OF THE BLACK SEA**

The reservoirs of the Regional Landscape Park "Tiligulsky" (RLPT) are represented by the estuary, salt lakes, lower reaches of the steppe rivers, ponds and mineralized streams. In total, according to the park staff, there are 24 streams. The active functioning of the vast majority of them is completed at the beginning of summer, and then they dry up. Only two adjacent streams function year-round in the upper headwater of the right-bank part of the estuary (near the Volkovo Village). Mineralized sources are a kind of ecotope, in which algae and higher aquatic plants are in the media with high mineralization and relatively low year-round water temperature. It was precisely the study of their aquatic vegetation in the natural chain of aquatic ecosystems of the park that our main attention was paid to. Floristic studies of these water bodies are a continuation of the inventory of the aquatic flora of RLPT (Tkachenko et al., 2017).

The investigated large stream about the Volkovo Village flows out from under the rocky Quaternary outcrops in the upper part of the right slope of the Tiligul Estuary, about 50 m above its level. The bed of the stream is rocky-silty-sandy. It flows into the ancient bed of the Tiligul River, flooded by the estuary. Its coordinates are: N 47 ° 02'68 'and E 30 ° 58 ''18'. The width of the watercourse is about 70 cm. The water flow rate is 7 dm<sup>3</sup>/s, the temperature, for example, was + 13° on 12/14/18 while the air temperature at this time is -4°. The water in the stream is clear, odourless, its pH was 8.16, electrical conductivity – 14.58 mS/cm, total mineralization – 7.69 g/dm<sup>3</sup>. Sulphates prevailed in the water of the stream – 4688.5 mg/dm<sup>3</sup>, chlorides – 1201.3, sodium ions – 985.8, calcium – 305.9 and magnesium – 247.5. Among biogenic, a noticeable content of nitrates was noted (21.3 mg/dm<sup>3</sup>). In 2017 (October), with more precipitation, water mineralization here was slightly less (5.55 g/dm<sup>3</sup>), but the nitrate content at this time was almost three times higher (86.6 mg/dm<sup>3</sup>) with MPC = 45 mg/dm<sup>3</sup>. Such physical and chemical properties of the water of the stream influence the composition of its aquatic vegetation.

In total, we collected 20 macrophytes samples in the reservoir under study. Among macroscopic algae 3 species of blue-green (*Nostoc punctiforme* Hariot, *Ocellularia beggiatoiformis* (Gom.) Strunecky, Komarek et J.R. Johansen, *O. tenuis* C. Agardh ex Gomont), 1 yellow-green (*Vaucheria dichotoma* (L.) C. Martius) were identified, as well as 8 green (*Cladophora glomerata* (L.) Kütz., *Microspora stagnorum* (Kütz.) Lagerh., *Rhizoclonium hieroglyphicum* Kütz., *Ulothrix tenerrima* (Kütz.) Kuütz., *U. zonata* (Web. et Mohr.) Kütz., *Ulva compressa* C. Agardh, *U. flexuosa* Wulfen, *U. intestinalis* L.), short-stem *Stuckenia pectinata* (L.) Borner developed on solid, silty-sandy soil in some parts of the stream, and water moss *Fontinalis antipyretica* L. ex Hedw. were found on large stones.

Certainly, both planktonic and benthic microscopic algae species were present in the water of the stream. In total, by analogy with the mineralized brooks of the Kuyalnik Estuary (Kiryushkina et al., 2008), there may be about 50 species with a predominance of diatoms and blue-green algae.

Thus, the aquatic vegetation of the investigated mineralized stream on the slopes of the Tiligul Estuary is quite diverse and is represented by 12 species of macroscopic algae and two species of higher aquatic plants. Its composition is not original and is similar to that in the Tiligul River.

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## **BIOCHEMICAL COMPOSITION OF CYANOBACTERIUM CALOTHRIX MARCHICA LEMMERM. ISOLATES FROM MOLDOVAN SOILS AND PERSPECTIVES IN BIOTECHNOLOGICAL APPLICATIONS**

The current development of phycobiotechnology is indispensably connected to the isolation of new strains of cyanobacteria, which are a source of biologically active substances for application in the development of organic farming, cosmetics, pharmaceuticals etc. It is known from literature that *Calothrix*

*marchica* var. *crassa* C.B. Rao has a high content of pigments in its biomass: chlorophyll, carotenoids, phycocyanin, allophycocyanin, phycoerythrin (Tiwari, et al 2005). According to Indian authors *C. javanica* De Wildeman is a producer of high amounts of phycoerythrin (80.9 µg/ml); another strain from the same region – *C. marchica* Lemmerm. – contains 16.6 µg/ml of chlorophyll *a* and 66.4 µg/ml of total carotenoids (Tiwari et al., 2015). The species *C. marchica* can be found over a very wide area: in the waters of Germany, Greece, India, Iraq, Iran and Israel (Vinogradova et al., 2000). In the Republic of Moldova *C. marchica* was found in steppe soils (Şalaru et al., 2012). Another strain of cyanobacteria, *C. elenkinii* Kossins., was also isolated from the soils of R. Moldova. This strain can be used as a source of biologically active substances, containing a high amount of carbohydrates and up to 20.0 % lipids (Trofim et al., 2013). The analyzed species, *C. marchica*, can also be used for the treatment of polluted waters. Researchers from Thailand have shown that *C. marchica* contributes to reducing the amount of lead ions, being a more effective bio-sorbent in intense illumination (Ruangsomboon et al., 2006). In global investigations, antitumoral research was carried out with the inclusion of this species. Thus, a group of Egyptian researchers have demonstrated that silver nanoparticles synthesized by *C. marchica* have shown very high antitumor activity (Khalifa et al., 2016). Another strain of *Calothrix* spp. was proved to be a producer of insecticidal sesquiterpenes (Höckelmann et al., 2009), which are very important for agriculture. The antimicrobial activity of *Calothrix* sp. extracts was established in other investigations (Sundaramanickam et al., 2015). It has been demonstrated that the chloroform and hexane extracts from *C. braunii* Bornet et Flahault biomass have a high antifungal and antibacterial activity (Malathi et al., 2015). The exploration of the valuable biochemical potential of *Calothrix* sp. is very relevant at present, because until now, it has not been fully studied and exploited as a source of bioactive substances. The aim of the present work was to isolate and study the cyanobacterium *C. marchica* that grows in the soils of the Republic of Moldova.

A series of cyanobacteria, which are of interest in the production of proteins, lipids, polysaccharides and pigments have been isolated recently by A. Trofim within the Phycobiotechnology SRL. One of the strains – *C. marchica* Lemm. CNMN-CB-18 – was isolated in pure culture. This strain grows in the soil of the Cogâlnic River meadow, Cimislia, Moldova. The cyanobacterium was isolated from a biocenosis containing many cyanobacteria – 15 species and varieties, followed by

6 species of chlorophytes and 3 diatom species. The most massive growth was recorded for the genera *Phormidium*, *Lyngbya* and *Hantzschia*. The strain was isolated by repeated inoculation on liquid and agar medium and developed on Drew mineral medium. Cultivation was carried out at the temperature of 23-30 °C, under the illumination of 1000-3000 lux. The cultivation period is 12-20 days.

The biochemical analysis of cyanobacterium *C. marchica* revealed high amounts of lipids – 33.7 % and carbohydrates – 32.9 %, followed by proteins – 14.66 %, and other components. In conclusion, the cyanobacterium *C. marchica*. CNMN-CB-18 can serve as a biotechnological object due to its content of bioactive substances for application in diverse fields such as: cosmetics, pharmaceuticals, agriculture, etc.

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## **ALGAL FLORA OF UKRAINE – FLORISTIC-GEOGRAPHICAL AND BIOTECHNOLOGICAL ASPECT**

Algal flora of Ukraine is one of the richest in Europe. It covers more than 5,200 species, represented by about 6,300 infraspecific taxa belonging to 967 genera and 14 departments, and only about 10-15 taxa are used as biotechnology objects. Flora of Ukraine reflects more than 42 % of the species composition of the world continental algal flora and about 10 % of the algal flora of the Earth. According to the results of an algofloristic and ecologo-geographical study and examination of algal flora of Ukraine its main features were established. The main of them are:

– the uneven distribution of the species composition of algae in this area by natural and climatic zones or algofloristical territories as well as geographic regions, which is due to zonal and azonal factors. The highest species diversity is typical for the reservoirs of the Forest-Steppe Zone (Middle-Dnipro algal floristic province – Palamar-Mordvintseva, Tsarenko, 2015). The diversity of algae in plain areas is much higher (more than 2.2 times) than mountainous;

– reducing the species diversity of Euglenophyta, Chlorophyta and Charophyta from northwest to southeast and increasing the role of Cyanoprokaryota and Bacillariophyta in mentioned direction. This ascribed to an increase in the degree of mineralization of the water and a decrease in the variety and number of reservoirs as well as the degree of wateriness of the territory;

– the territorial-taxonomic dependence in distribution is formed on the basis of the species composition of algae in the plain territory of Ukraine by Chlorophyta, Charophyta, Bacillariophyta and Cyanoprokaryota divisions. However, the value of these groups in the flora of mountain regions is sharply different from the plains, which is owing to the influence of physical and geographical factors, ecological and geographical features of the group and the genesis of the regional flora, as well as the degree of study groups;

– the ecological and taxonomic features of the regional algal flora of Ukraine as well as the differences in diversity, and, accordingly, in the place of a specific taxonomic group of algae in the general systematic series of leading groups. In particular, the most diverse (over a half of the Charophyte algae species composition) in Prypyat-Desna and Middle-Dnipro algal floristic provinces, as well as in the Carpathian-Danube, are desmidian algae. And in the Middle-Dnipro a high diversity is also characteristic for green (flagella and coccoid) algae. The sharp decrease in the composition of mentioned groups in mountainous regions is highlighted, while in Prypyat-Desna the richest species composition of filamentous (edogonian) algae, as opposed to the fact that diatoms and blue-green algae are the leading groups in their diversity in the Dnipro-Black Sea and Mountain Crimea provinces;

– presence of high percentage of boreal and boreal-arctic elements in the desmids flora of Ukraine and its alochtonic origin, as well as high content of non-moral and euridogolarctic species in the flora of green coccoid algae of Ukraine and its migratory and autochthonous origin. The low level of endemism of these groups of algae (3.2-4.6 %) and the features of their systematic structure probably is indicative of the significant transformations of this flora in the recent geological past, as well as the relative youth of the studied flora;

– from the territory of Ukraine 53 genera and 426 species (568 taxa of species and infraspecific rank) are described and recognized as independent.

Among them the largest number of new for the science taxa were found for Chlorophyta, Euglenophyta, Xanthophyta, Cyanoprocarvota and Charophyta;

– 60 species of algae from Xanthophyta (1), Rhodophyta (18), Phaeophyta (11), Chlorophyta (13) and Charophyta (17) included in the latest edition of the Red Data Book of Ukraine (2009), corresponding to the algosozological criteria;

– biotechnological specifics and the nature of the practical using of algal flora in Ukraine are determined by species-producers of biomass - profitable for economic needs (35 strains), prospective raw material strains for biodiesel production or use in subsequent energy and bioconversion studies (10 strains): (*Spirulina (Arthrospira)*, *Dunaliella*, *Haematococcus*, *Chlorella*, *Scenedesmus*, *Desmodesmus*, *Monoraphidium*, *Tetradesmus*), as well as being considered as potential material for further study the types of algae cultures within the IBASU-A collection (M.G. Kholodny Institute of Botany of NAS of Ukraine) and HPDP (Institute of Hydrobiology). The IBASU-A collection is the object of the National Heritage of Ukraine and the form of conservation and practical use of species of national flora containing more than 500 strains and 100 species of algae and constitutes a potential source of biotechnology objects.

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**MICROALGAE STRAINS OF THE IBASU-A COLLECTION AS A BASIS FOR BIOTECHNOLOGICAL STUDIES**

Microalgae Culture Collection (IBASU-A) is an object of national heritage of Ukraine. It was established in 1960s for using algal cultures in research on the morphology, systematics, ecology, biogeographic of the *Dunaliella* species and their biotechnological potential as a source of  $\beta$ -carotene, bioactive compounds, pharmaceuticals, microalgae mass culture, animal and aquaculture feeds (Massjuk, 1973, in Rus.). Since then an assortment of strains has been gradually developed according to actual topic of research projects. Now IBASU-A includes 500 strains of halophilic, freshwater and aerophytic mainly green microalgae. It consists of several

separate collections including sets of authentic strains, mutants, cultures of rare species, hyper producers of biomass, algae with valuable properties for biotechnology etc. This provides reliable material for use in education, scientific and applied studies (Borysova, Tsarenko, Konishchuk, 2017, in Ukr.).

The considerable number of strains of species belonging to genera *Chlorella* s.l. (27 strains), *Dunaliella* Teodor. (70), *Scenedesmus* s.l. (145) allowed realizing in 1980-90s such research projects as an application of microalgae cultures in bioassays/toxic testing (detergents, carcinogens), biodegradation of synthetic organic compounds and biological treatment of different type industrial effluence (Lenova, Stupina 1990; Lenova et al., 1991; Stupina, Borisova, 1998, in Rus.).

In 2009 the screening of IBASU-A was conducted out in regard to species hyperproducing biomass as a feedstock for biofuel (biodiesel). The main criteria for selection were capability of algae to accumulate a significant volume of lipids, high kinetic characteristics (specific growth rate ( $\mu$ ) and productivity (P), their resistance to stress factors and biological contaminations. Consequently, 33 strains of 9 species from genera *Acutodesmus* (E. Hegew.) Tsarenko, *Botryococcus* Kütz., *Chlorella* Beijer, *Chloroidium* Nadson, *Desmodesmus* (Chodat) An et al., *Enalax* Pascher, *Euglena* Ehrenb., *Monoraphidium* Komárk.-Legn., *Parachlorella* Krienitz et al. were chosen (Tsarenko, Borysova, Blume, 2011, 2016).

The further biotechnological studies on the basis of these selected strains IBASU-A have showed the high potential of many of them, especially some species from genera *Acutodesmus* and *Desmodesmus* as hyper producers of biomass for biofuel industrial needs. It was supported by two patents (Tsarenko et al., 2013, 2014, in Ukr.).

The presence in culture collections of algal strains with relevant properties and optimized to the local climatic conditions is of fundamental importance for their successful mass cultivation. Therefore, the collection IBASU-A is steadily supplemented by strains isolated from different parts of Ukraine. Recently, a number of new strains of representatives of family Selenastraceae (genera *Monoraphidium*, *Raphidocelis* Hindák, *Selenastrum* Reinsch) have been isolated from some water bodies in Donetsk, Kyiv and Volyn regions, while continuing to search the perspective high biomass producers. According to published data the species of *Monoraphidium* are considered as promising candidates for liquid biofuel production, which characterized by high biomass productivity and neutral lipids

with favorable fatty acids profile (Bogen, 2013; Zhao et al., 2014). Actually, our preliminary investigation of *M. minutum* (Nägeli) Komárk.-Legn IBASU-A 574, isolated from a small lake in Kyiv-city, revealed its active growth with specific growth rate and productivity equaled  $0,44 \text{ day}^{-1}$  and  $72,5 \cdot 10^6 \text{ cells ml}^{-1}$  per day, that is similar to growth characteristics of the known biomass producer *Chlorella vulgaris* IBASU-A 189 under the same cultivation conditions. There are more 11 strains of *Monoraphidium griffithii* (Berk.) Komárk.-Legn, *M. minutum*, *Raphidocelis subcupitata* (Korschikov) Nygaard et al. and *Selenastrum gracile* Reinsch in IBASU-A which we will research in near future.

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## **EFFECT OF HYDROXYCINNAMIC ACIDS ON GREEN ALGAE AND CLADOCERANS**

The photosynthesis intensity of green algae varies depending on the concentration and composition of dissolved physiologically active phenolcarboxylic acids (PhCAs), which are formed due to vital and postmortem secretion of hydrobionts. These peculiarities of algal response to biologically active substances may be one of the main factors in the formation of phytoplankton structure of continental waters owing to chemical interactions between higher water plants and algae (Sakevich, Usenko, 2008).

Species with a large cellular specific surface area are more sensitive to BASs (Usenko, Sakevich, Balanda, 2010). The promising area of application of these substances can be increasing of biomass yield under green algae cultivation in artificial conditions, and especially their application as a component of cultural media during green algae and cladocerans joint cultivation for production high-calorie agricultural feed. However, the literature on the effect of PhCAs on aquatic invertebrates is quite limited.

The aim of this investigation was to study the mode of action of some hydroxycinnamic acids on growth of green algae cultures *Selenastrum gracile* Reinsch. IBASU-317 and *Monoraphidium contortum* (Thur.) Kom-Legn. IBASU-A



364, as well as productivity of *Daphnia magna* Straus. For this purpose, chemically pure grade caffeic and *p*-coumaric acids were added in cultural media in the range of concentrations 0.05–3.2 mg/L. Exposures for green algae and *D. magna* were 14 and 21 days respectively.

The effect of hydroxycinnamic acids on the growth of green algae was diverse. Stimulation of growth by *p*-coumaric acid was observed for *S. gracile* at concentrations 0.05–0.4 mg/L with the highest increase at 0.1 mg/L (30 %), however stimulation of *M. contortum* growth was not demonstrated. Moreover, the inhibitory effect of *p*-coumaric acid was observed in the range of 0.6–1.6 mg/L for *S. gracile* and 0.2–1.6 mg/L for *M. contortum*.

Addition of caffeic acid to the cultural medium resulted in the substantial decrease of the relative growth rate. Stimulation was observed only for *M. contortum* at concentration 0.05 mg/L (14 %). As for *S. gracile*, the inhibitory effect was found throughout the exposition.

*p*-Coumaric acid caused an adverse effect on *D. magna* fertility rate in the all range of concentrations studied (0.1–3.2 mg/L), as confirmed by both reduction in the average number of offspring per female and increasing the mortality of organisms.

Caffeic acid in the concentration range 0.1–0.8 mg/L exerted a divergent effect on the productivity of *D. magna*. Increased mortality rate of juveniles during the first 7 days of exposure showing no dose-response relationship was found, which indicated the absence of toxic effect in its typical manifestation. It is confirmed by a significant increase in the productivity rate of survived females at concentrations of 0.1; 0.4 and 0.8 mg/L compared to control (2.1, 1.8 and 1.7 times respectively). Caffeic acid at concentration 3.2 mg/L caused typical toxic effect, as reflected by the increase in the mortality of *D. magna* juveniles and the reduction in the productivity of parthenogenetic females.

Hence, the effect of hydroxycinnamic acids on green algae and cladocerans is species-specific and multidirectional. Caffeic acid in concentrations 0.1–0.8 mg/L stimulates productivity rate in *D. magna*, but it is questionable to consider this effect as hermetic because of increased mortality of juveniles. Species-specific physiological and biochemical features of freshwater green algae determine their response to the changes in content of *p*-coumaric and caffeic acids. Our study has shown that *p*-coumaric acid in a range of concentrations 0.05–0.4 mg/L is promising cultural media component for increasing of *S. gracile* productivity.

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### **A TAXONOMIC STUDY OF STRAINS FROM THE GENUS *STICHOCOCCUS* NÄGELI BASED ON ECOPHYSIOLOGICAL, MORPHOLOGICAL, AND MOLECULAR DATA**

*Sitichococcus* Nägeli is a cosmopolitan terrestrial genus belonging to the Trebouxiophyceae. It is a key player in terrestrial soil crust productivity, where it is commonly found. Due to its simple rod-shaped morphology and lack of autapomorphic features, identification and taxonomic study for this genus has been difficult. Furthermore, the presence of Group I introns make it a challenge to sequence. Previous works (Sluiman et. al., 2008; Hodac et al., 2016) have suggested that the genus is closely related to other genera, such as *Gloeotila* Kützing, *Desmococcus* F. Brand, and *Diplosphaera* Bialousuknia. This study aims to resolve the genera's taxonomic position through an integrative taxonomic approach.

Monoalgal strains identified as *Stichococcus* were acquired from culture collections worldwide. Ecophysiological testing consisted of salt tolerance testing with a liquid medium gradient of up to 90 PSU as well as desiccation tolerance and recovery testing after Holzinger & Karsten (2013). Strains were observed in liquid and agar medium over a period of eight weeks, with most strains being photographed and analyzed weekly.

The 18S-ITS-28S locus was used to build phylogenetic trees. For many strains, it was not possible to completely sequence this locus using common primers from the literature due to intronic effects; the results are thus preliminary. Further primer development specific for this class will be developed.

First results indicate that there may be a higher-than-expected diversity within *Stichococcus* and that a generic revision recommended, particularly in relation to the genus *Gloeotila*.

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**NEW AND INTERESTING RECORDS OF CYANOBACTERIA IN  
BIOLOGICAL SOIL CRUSTS FROM CHALK OUTCROPS OF KHARKIV  
REGION (UKRAINE)**

Biological soil crusts are of great importance in ecosystems where water availability limits vascular plant cover: they decrease runoff and soil erosion and increase the silt, clay, carbon, and nitrogen in the soil (Belnap et al., 2016). On sites representing initial stages of soil formation, such as chalk outcrops, cyanobacteria play a leading role in the biocrust formation (Büdel, 2003). In such conditions, they expose to various abiotic stresses, including high temperatures, UV, salinity, pH, low moisture, etc. It would seem that this should limit their diversity. However, due to unique ecophysiological strategies of cyanobacteria, their diversity in xerophytic environments is amazing: more than 320 species in over 70 genera are known to occur in biocrusts worldwide (Büdel et al., 2016).

The study of cyanobacteria using a polyphasic approach allows more accurately assess their species and taxonomic diversity (Komárek, 2016). Recently, a number of taxa new for science and for the flora of Ukraine was discovered in seashore biocrusts using this approach (Mikhailyuk et al., 2016, 2018; Vinogradova et al., 2017). They include representatives of genera *Oculatella* Zammit, Billi et Albertano (Synechococcales) and *Roholtiella* M. Bohunická, Pietrasiak et Johansen (Nostocales).

Investigating cyanobacterial crusts commonly occurred on chalk outcrops of Dvurechansky National Nature Park (DNNP) and its environs (Kharkiv Region, Ukraine) we re-discovered some of these taxa and add new details to their morphology and ecology. The samples were studied by direct microscopy (Olympus BX51 and BX53 light microscopes with Nomarski DIC optics) and using cultures on Bold (1N BBM) and BG 11 agarized media. Cultures were grown under fluorescent lights ( $25 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) with a 12:12 light:dark photoperiod at  $+20 \pm 5 \text{ }^\circ\text{C}$ . Molecular study procedure see in Vinogradova et al., 2017.

Morphotype, later described as *Oculatella ucrainica* O.M. Vynogr. et Mikhailyuk first was isolated from biocrusts (both epigeic on conquina beach and

clay outcrops, and hypolithic) found on the coast of the Sea of Azov (Mikhailyuk et al., 2016). On chalk outcrops of the DNNP, thin filaments of *O. ucrainica* with reddish eyespot in mature apical cells (autapomorph of the genus) occur quite common: we found them in 71 % of the samples. Morphological and molecular (16S rRNA gene sequence concatenated with the 16S-23S ITS region) evaluation confirmed taxonomical designation of this species. Interestingly, the crusts in which *O. ucrainica* was part of the dominant complex occurred in sites with varying degrees of plant cover: from scree slopes, devoid of vegetation, to steppe communities on chalky soils. Heteropolar false branched cyanobacterial filaments possessing heterocytes and chains of arthrospores (*Roholtiella* morphotype) we have recorded in biocrusts from two locations: chalky outcrops near settlm. Dvirichna and hill slopes covered with steppe near vil. Protopopivka. Near Dvirichna, *Roholtiella*-like filaments occurred in the cyanobacterial crust dominated by *Scytonema ocellatum* Lyngbye ex Bornet et Flahault and *O. ucrainica*. In second locality, the soil crust dominated by *O. ucrainica* and *Nostoc punctiforme* Hariot was formed not only by cyanobacteria, but also by various eukaryotic algae, which is typical of steppe areas. Revealed specimens both pheno- and genotypically (16S-ITS) correspond to *Roholtiella edaphica* Bohunická et Lukešová known from temperate climate soils of the USA, Czech Republic and Russia (Bohunická et al., 2015; Gaysina et al., 2018). Details of filament and cell morphology, dimensional ranges and ecological peculiarities of investigated populations of *O. ucrainica* and *R. edaphica* are discussed.

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## **RESPONSE OF DIATOMS TO THE MINING OF THE Zn-Pb ORE (SOUTH POLAND)**

We studied the effects of over 40 years of Chechło River water pollution caused by discharge from a lead-zinc ore mine. The examined aquatic system (A.S.)

included the river and a group of subsidence ponds. Some water contamination occurred during the period of metal mining, and some appeared after cessation of mining. Currently the river is recovering to its natural conditions, but many channel and floodplain locations still preserve the sediments that accumulated before, during, and after the mining period. The aim of our investigations was to model the response of the A.S. to the reduction of mine water discharge into the river. The model predictions are based on reconstructed changes of diatom assemblages and on physical characteristics and chemistry of sediment stored in subsidence ponds. The diatom materials consist of present planktonic samples and eighteen subfossil cores. The CCA analysis and Monte Carlo permutation test were performed using multivariate statistics in CANOCO software. In the CCA analysis of the species-locality relationship, the first two principal components accounted for 44.5 % (axis 1) and 16.4 % (axis 2). The most important diatoms in the samples most polluted by copper were *Fragilaria* cf. *rumpens*, *Planothidium lanceolatum*, and *P. frequentissimum*. The sediments heavily polluted by zinc and cadmium were rich in *Aulacoseira ambigua*, *Craticula buderii*, and *Pinnularia frequentis* valves. The material collected from the deepest part of the cores (representing the pre-mining period) show many taxa which were lost during the time of mining, when diatoms resistant to heavy metals appeared. In the pre-mining time the waters were inhabited by oligosaprophilous and oligotraphentic diatoms – *Brachysira brebissoni*, *Chamaepinnularia mediocris*, *Encyonema neogratile*, *Eunotia incisa*, *E. tenella*, *E. tetradon* or *Frustulia saxonica*. Currently the majority of diatoms are recognised as eurytraphentic and the most abundant species indicate in the uppermost sample the water of  $\alpha$ -meso-polysaprobous or  $\beta$ -mesosaprobous status.

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**PHYCOCYANIN CONTENT ASSESSMENT BY THE SPECTRAL RESPONSE OF *ARTHROSPIRA PLATENSIS* GOMONT BIOMASS**

*Arthrospira platensis* Gomont is one of the most widely used microalgae culture which has a gainful potential as a source of valuable substances, such as

murein, phycocyanin,  $\beta$ -carotene, chlorophyll, carbohydrates, proteins, fats, vitamins, fatty acids, essential amino acids and bioavailable mineral forms (Khan et al., 2005). High biomass growth rates and relatively simple cultivation technologies provide the promising background to use this cyanobacteria as a source for a broad variety of industries and applications, such as pharmaceuticals, food, polymers and fuel production. In case of some applications it is vitally important to get predictable and stabilized amounts of targeted substances in a net biomass. Since *A. platensis* is a cyanobacteria, this culture could adapt quickly for cultivation environment changes (such as microelements balance, temperature, pH, light spectrum, nutrition components, etc.). This adaptation bears the changes in a net biomass content, growth rate and other strain features. For such stabilization it is obvious to use a permanently stabilized growth environment, however the real-life cultivation facility conditions could fluctuate widely as a result of human factor and system of external conditions changes. For this reason it is important to use control systems with feedback option based on real-time biomass features monitoring.

The main objective of the described research is to determine the sets of typical reflective spectral responses which appear as an *A. platensis* reaction for different impact-factors, in particular medium vitally important or vitally stressful additives. Another objective is to build the response-content correlations and to estimate how these spectral responses could be used to predict the content of valuable substances in a net biomass.

Typical experiment had following framework. The 48 samples (plastic cups, 6\*8 matrix) were used for a data collection. Each inoculated sample contained 100 ml of Zarrouck medium with a different additives concentration in each. 5 samples were used as a control (no additives). As an additives the different concentrations and proportions of Cobalt nitrate, Potassium iodide (Kotinsky et al., 2004), dextrose (Chojnacka, Saeid, World, 2012) and some other substances were used.

The data collection was performed with state-of-the-art computer vision module which collects data in RGB and IR bands for each separate sample simultaneously. After data collection the computer vision module uploads the calculated spectral stats, biomass density values and images into a cloud database. Information about phycocyanin content was calculated after biomass samples extraction and final dry weight measurement.

The working model for predictive analysis of phycocyanin content in a net biomass of *A. platensis* is proposed and discussed. Model was applied for feedback control to correct the medium additives and conditions to fit the required biomass parameters.

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## **THE INFLUENCE OF NITROGEN TO PHOSPHOROUS RATIO ON INTER-ANNUAL VARIABILITY OF PHYTOPLANKTON STRUCTURE OF THE ODESSA REGION (THE BLACK SEA, UKRAINE)**

Studies of the variability of specific surface ( $S/W$ ;  $m^2 \cdot kg^{-1}$ ) and relative abundance (density  $N$ , biomass  $B$ , surface area  $S$ ; %) of unicellular algae taxonomic groups revealed the relationship between the reorganization of the morphological and taxonomic structure of phytoplankton (Zotov, 2018). The deviations of the distribution of taxonomic groups  $S/W$  values in the phytoplankton community from the distribution that characterizes the overall variability of  $S/W$  of these taxa were accompanied by the redistribution of the relative abundance of taxonomic groups. Groups that dominated during the year lost their dominant status. And the maximum contribution to abundance of phytoplankton was formed by one of the accompanying groups. The revealed relationship demonstrates the mechanism of structural self-organization of phytoplankton in accordance with changes in environmental conditions. Therefore, the task of searching for environmental factors that determine the interrelated morphostructural rearrangements of phytoplankton is relevant.

In accordance with the "resource ratio" theory, which considers nutritional needs as a species-specific characteristic, the main factor regulating the taxonomic rearrangement of phytoplankton is the nutrient concentrations ratio, primarily nitrogen to phosphorus ratio (N/P) (Levich, 2000). However, to identify this relationship in natural communities is problematic. Uneven consumption of nitrogen and phosphorus leads to a change in the value of N/P during the implementation of the community structural adjustment. An additional process

that transforms the N/P ratio is the entry of nutrients into the aquatic environment. An analysis of the inter-annual variability of phytoplankton can smooth out the problem of factor transformation during the implementation of impact. Intra-annual changes in the phytoplankton structure can be considered as an integral response to intra-annual changes in environmental parameters.

In 2006–2009 and 2012–2013, up to 90 % of the phytoplankton biomass of the Odessa coast was formed by the dominant (Bacillariophyta) and subdominant (Dinophyta) groups of algae. The influence of the ratio of the mineral forms of nitrogen and phosphorus on the variability of the contribution to biomass of both the dominant (F-Ratio 5.03) and subdominant (F-Ratio 4.61) taxonomic groups of phytoplankton was statistically significant (P-Value 0.0271 and 0.0341). Similar values of the influence of the N/P on the variability of the contributions to the biomass of the dominant groups were also found for the ratio of mineral nitrogen to total phosphorus (F-Ratio 5.36 and 4.46 with P-Value 0.0226 and 0.0372). This is natural due to the high rate of turnover of organic forms of phosphorus. These relationships correspond to theoretical perceptions about the effect of the N/P ratio on the taxonomic structure of phytoplankton. The influence of N/P ratio on the morphological structure of phytoplankton can be analyzed on the basis of inter-annual trends of various indicators. The synchronous inter-annual dynamics of  $S/W$  and N/P was revealed during the study period. In the same period, the interannual variability of N/P was synchronous with the variability of the contributions to the biomass of the dominant group, and asynchronous with the same indicator of the subdominant group. The value of  $S/W$  Bacillariophyta ( $960 \text{ m}^2 \cdot \text{kg}^{-1}$ ) is significantly higher than the value of  $S/W$  Dinophyta ( $330 \text{ m}^2 \cdot \text{kg}^{-1}$ ) (Zotov, 2018). Thus, the interannual redistribution of relative abundance between Bacillariophyta and Dinophyta in response to a change in the N/P ratio can be considered as a redistribution of the contributions of morphological groups. An increase in the relative abundance of Bacillariophyta may be accompanied by an increase in the contribution of cells with higher  $S/W$ . And the increase in the relative abundance of Dinophyta, on the contrary, - may be accompanied by decrease in the contribution of cells with higher  $S/W$ . The coordinated changes in the morphological and taxonomic structure of phytoplankton make it possible to optimize the intensity of consumption of nutrients and, consequently, the production process.



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