EXPERIMENTAL ARTICLES

Algo—Bacterial Communities of the Kulunda Steppe (Altai Region, Russia) Soda Lakes

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Received February 24, 2014

Abstract—The composition and macroscopic structure of the floating oxygenic phototrophic communities from Kulunda steppe soda lakes (Cock Soda Lake, Tanatar VI, and Bitter Lake 3) was described based on the data of the 2011 and 2012 expeditions (Winogradsky Institute of Microbiology). The algo—bacterial community with a green alga *Ctenocladus circinnatus* as an edificator was the typical one. Filamentous *Geitlerinema* sp. and *Nodosilinea* sp. were the dominant cyanobacteria. Apart from *C. circinnatus*, the algological component of the community contained unicellular green algae *Dunaliella viridis* and cf. *Chlorella minutissima*, as well as diatoms (*Anomoeoneis sphaerophora*, *Brachysira brebissonii*, *Brachysira zellensis*, *Mastogloia pusilla* var. *subcapitata*, *Nitzschia amphibia*, *Nitzschia communis*, and *Nitzschia* sp.1). The latter have not been previously identified in the lakes under study. In all lakes, a considerable increase in salinity was found to result in changes in the composition and macroscopic structure of algo—bacterial communities.

Keywords: soda lakes, Kulunda Steppe, algo-bacterial communities, Ctenocladus circinnatus, cyanobacteria, diatoms

DOI: 10.1134/S0026261714060162

Soda lakes are environments of athalassophilic origin with stable extremely high pH ~10. The Kulunda Steppe lakes located in the zone of cold dry climate are the only occurrence of hypersaline soda lakes in Russia. They are characterized by a variable hydrological regime with cyclic high-amplitude fluctuations of temperature and brine concentration. According to our observations, they always remain soda solutions, although the ratio of carbonate/bicarbonate ions varies at insignificant pH fluctuations (from 9.7 to 10.5).

Extremely haloalkaliphilic phototrophic communities develop in these lakes. Cyanobacteria and eukaryotic algae are the oxygenic producers in these ecosystems and provide for high productivity of the lakes of this type, supporting a developed trophic chain of organisms up to birds. A description of Lake Tanatar by B.L. Isachenko is a good illustration: "Abundance of organic life in Tanatars is astounding. It literally swarms in the brine and leaves its traces all over the shore: innumerable Ephydra larvae swim in the brine, while its pupae cover everything above the water and seed the bottom. Immense amounts of Artemia flourish in the brine. On the shore, Ephydra larvae and pupae washed off the lake, together with bodies of various insects (to a large extent winged ants), form deposits several centimeters thick. Fattened water birds, from gulls to sandpipers and similar avians, sit at the shore and devour numerous ants and orthoptera" [1].

Russian soda lakes have been investigated since the early 20th century. Isachenko [1] and Voronikhin et al. [2–5] published classical works on the algal and microbial diversity of the Kulunda Steppe lakes. This was the period when the dominant green and bluegreen algae were described. While the presence of diatoms was reported, their species composition remained unstudied.

The goal of the present work was to describe the composition of oxygenic phototrophic communities of the Kulunda Steppe soda lakes using the material of the 2011–2012 expeditions, as well as the changes in these communities resulting from increased salinity.

MATERIALS AND METHODS

The subjects of research were the samples of floating phototrophic communities collected during the expeditions in early July 2011 and late June 2012 (Winogradsky Institute of Microbiology, Russian Academy of Sciences) at the following Kulunda Steppe lakes (Altai Region, Russia): Cock Soda Lake (Klyuchevskoi district), Bitter Lake 3, and Tanatar VI (Mikhailovskii district) (Table 1).

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Lake	Year	рН	S, g/L	Alkalinity, M		Phototrophic communities	
				Na ₂ CO ₃	total	(predominant organisms)	
Cock Soda Lake	2011	10.18	100	0.96	1.11	Accumulation of <i>C. circinnatus</i> and cyanobacteria (<i>Geitlerinema</i> , <i>Nodosilinea</i>)	
(52°6′16.48″ N 79°9′54.06″ E)	2012	9.80	200	2.4	2.7		
Bitter Lake 3 (51°40'3.38" N 79°54'36.14" E)	2011	10.30	90	0.80	1.00	Accumulation of <i>C. circinnatus</i> and cyanobacteria (<i>Geitlerinema</i> , <i>Nodosilinea</i>)	
	2012	9.90	200	2.6	3.0		
Tanatar VI (51°36′55.69″ N 79°49′17.55″ E)	2011	10.04	160	1.30	1.70	Biofilms predominated by <i>Geitlerinema</i> and <i>Nodosilinea</i> , with the presence of <i>C. circinnatus</i>	
/9 4 9 17.33 E)	2012	9.80	250	3.2	3.4	Bloom of cf. Chlorella minutissima in plankton	

Table 1. Investigated lakes and their phototrophic communities (S stands for salinity)

Salinity and pH of the brines were measured using a WTW field potentiometer—conductometer (Germany). For pH, the average between the values in the native brine and in its 1 : 5 dilution was used. Salinity was determined gravimetrically in the laboratory, and the average of the values obtained by two methods was used. Soluble carbonate alkalinity was determined by titration with 1 N HCl. The CO_3^{2-} was titrated with phenolphthalein to HCO_3^{-} (X) and the total HCO_3^{-} was then titrated with methyl orange (X + Y). The total carbonate alkalinity was calculated as 2X + (Y - X) = X + Y.

Total absorption spectra of the phototrophic community from the Cock Soda Lake were obtained using a Carry 100 Bio spectrophotometer (Varian, United States). The pigments were extracted from the phytobiomass with 96% ethanol.

Algologically pure cultures of diatoms and cyanobacteria were isolated from enrichments obtained on carbonate media simulating the composition of soda lake water.

Cyanobacterial morphology was examined in wet mounts under a Jenaval microscope equipped with a Zeiss Bundle Canon PS G9 digital camera (Germany). The images were obtained using the AxioVision Rel. 4.7 software package.

The dominant cyanobacteria were identified according to their morphology using the identification guide [6].

Phylogenetic position of the dominant cyanobacterial morphotypes was determined for cyanobacterial monocultures P-1104 and P-1105 from the Cock Soda Lake. To determine partial sequences of the 16S rRNA genes, DNA was extracted by the CTAB method [7], amplified with cyanobacteria-specific primers CYA359f and CYA781r [8], and sequenced in the Centre "Bioengineering," Russian Academy of Sciences (Moscow). Closely related organisms in the GenBank

database (NCBI, http://www.ncbi.nlm.nih.gov) were determined using the BLAST software package. The sequences were aligned using BioEdit. Phylogenetic trees of the 16S rRNA gene sequences (~400 bp) were constructed using the neighbor-joining algorithm and analysis of 1000 alternative trees with the TREECON software package [9].

Identification of diatoms, dinoflagellates, and euglenophytes was carried out by microscopy of live and ethanol-fixed material under Leica DMLS and Leica DM 2500 microscopes (×1000). Diatom species were identified in raw material by morphology and ornamentation of their shells.

Phylogenetic position was determined for Nitzschia communis strain T3-Nc11. DNA was extracted using the Diatom DNAprep 100 kit (Izogen, Russia). The 18S rRNA fragment was amplified using the standard primers Q5 (GTATCTGGTTGATCCTGCCAGT) and Q39 (TAATGATCCWTCYGCAGGTTCAC-CTAC) and the Encyclo PCR kit (Evrogen, Russia). Amplification was carried out as follows: 95°C, 3 min; 38 cycles (93°C, 20 s; 56°C, 30 s; 72°C, 1.5 min); 72°C, 5 min. PCR products were purified by preparative electrophoresis in agarose gel and sequenced on a capillary sequencer in the Center "Genom" (Moscow). The contig was assembled using SeqMan. For construction of the phylogenetic tree, 13 database sequences were used, including 8 Nitzschia sequences identified to the species level and two members of other genera (Tryblionella and Bacillaria). The 18S rRNA sequences of various diatoms were aligned using MEGA 5.1 [10] with the Muscle algorithm [11]. Phylogenetic trees were constructed in MEGA 5.1 using the Neighbor-Joining and Maximum Likelyhood algorithms accounting for the gamma distribution of the evolutionary rates along the sites and the presence of invariant sites (GTR+I). Statistical reliability of the trees was assessed using 100 bootstrap replicas.

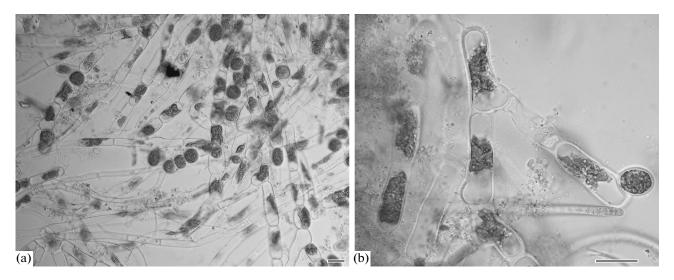


Fig. 1. Ctenocladus circinnatus Borzì from the Kulunda Steppe soda lakes Cock Soda Lake (a) and Tanatar VI (b). Scale bar, 20 µm.



Fig. 2. Dunaliella viridis from the Bitter Lake 3 (a) and Tanatar VI (b–f) in enrichment cultures (4 M Na⁺): morphological variability (a–d) and cell division (e–f). Scale bar, 5 μm.

RESULTS AND DISCUSSION

The phototrophic communities of the soda lakes studied comprised eukaryotic and prokaryotic forms.

Phototrophic communities predominated by *Ctenocladus circinnatus* Borzi developed in the Cock Soda Lake and Bitter Lake 3 (Fig. 1). This alga was described in the Kulunda Steppe lakes by Voronikhin and Popova [3, 12] as a new genus and species *Lochmiopsis sibirica* Woronich. et Popova. Identity of the genera *Lochmiopsis* and *Ctenocladus* was subsequently established with the preservation of the earlier genus and species names *Ctenocladus circinnatus* Borzì [13, 14]. This species was found in other saline lakes, including soda ones, in North America (United States and Canada), Peru, and Sicily [15, 16].

Unicellular eukaryotic algae *Dunaliella viridis* Teod. (Fig. 2) and cf. *Chlorella minutissima* were found in all lakes; their mass growth occurred under extreme salinization, similar to that of Tanatar VI in 2012. When bloom did not occur, both organisms were present in floating communities as minor components or were not revealed at all (Table 2), although they were invariably detected in enrichment cultures from all lakes. Occurrence of *D. viridis* in the Kulunda

Steppe soda lakes has been reported by Voronikhin [3]. *D. viridis* from the lakes under study was characterized by cell polymorphism (Fig. 2), both in environmental communities and in enrichments. Organisms similar to *C. minutissima* in morphology and ultrastructure were reported in Central Asian soda lakes and in Lake Magadi, Kenya by Gerasimenko et al. [17]. Presently, however, the morphologically and ecologically similar organisms predominant in soda lakes are described as members of new genera: *Picocystis salinarum* [18] or *Chloroparva pannonica* [19]. Thus, the taxonomic position of the organisms from the lakes studied in the present work is currently unclear, requiring a prefix "cf." and further phylogenetic identification.

Cyanobacterial growth was revealed in all lakes studied (Table 2). Unlike the works of the 1920s—1930s [1–5], their high diversity was not, however, revealed in environmental samples. Moreover, while early investigators reported mass development mostly of heterocystous cyanobacteria, in 2011–2012 only two dominant morphotypes of filamentous non-heterocystous cyanobacteria were revealed in environmental samples. A significant increase in salinity of the studied lakes by 2011–2012 was the most probable reason for this shift. Thus, salinity of the Cock Soda

Table 2. List of species and occurrence of oxygenic phototrophs in the lakes Cock Soda Lake, Bitter Lake 3, and Tanatar VI in summer 2011–2012

	Presence in the samples					
Oversonia whatateanha	Cock Soda Lake	Bitter Lake 3	Tanatar VI			
Oxygenic phototrophs	Cock Soda Lake	Bitter Lake 3	lake	shore		
	2011/2012	2011/2012	2011/2012			
Cyanobacteria						
Geitlerinema sp.	+++1	++	+++/-	+++/		
Nodosilinea sp.	++	+++	++/-	+++/		
Chlorophyta						
Ctenocladus circinnatus	+++	+++	+/-	_		
cf. Chlorella minutissima	_	_	±/+++	_		
Dunaliella viridis	±	_	_	_		
Diatoms (Ochrophyta, class Bacillariophyceae)						
Anomoeoneis sphaerophora	+++/-	+++/±	+++/-	_		
Brachysira brebissonii	_	++/+++	_	+++/-		
Brachysira zellensis	+++/±	+++	+++/-	+++/±		
Mastogloia pusilla var. subcapitata	±/-	_	±/-	_		
Nitzschia sp. 1	_	±/-	_	_		
Nitzschia amphibia	_	-/±	_	_		
Nitzschia cf. communis	_	±	_	±/+++		
Euglenozoa						
Euglena sp.	±/-	_	_	_		
Dinophyta						
Phytodinium simplex	_	_	_	±/-		
Chrysophyta cysts	_	_	+++	+++		

[&]quot;+++", "++", "±", and "-" stand for massive occurrence, frequent occurrence, infrequent occurrence, single cells, and not detected, respectively. 1 Occurrence was the same in both years.

Lake changed from 2.5–3°B in 1927 [3] (which approximately corresponds to 25–30 g/L NaCl) to 100 g/L in 2011 and 200 g/L in 2012.

The first phylotype was a filamentous cyanobacterium with straight motile trichomes and square cells 3.3–4.7 µm in diameter (Fig. 3a). This morphotype has been described by Voronikhin as *Oscillatoria tambi* Woronich. [3]. According to the presently accepted taxonomy [6], cyanobacteria of this morphotype could be identified as *Phormidium tambii* (Voronichin) Anagnostidis et Komárek 1988.

According to the phylogenetic tree of the 16S rRNA gene sequences, cyanobacteria of this morphotype, exemplified by strain P-1104 from the Cock Soda Lake) were most closely related to *Geitlerinema* spp. (Fig. 4). The botanical genus *Gei*-

tlerinema (Anagnostidis et Komárek) Anagnostidis 1989 is widespread in various habitats. Phylogenetically, however, it consists of at least three clusters [20]. While *Geitlerinema* sp. P-1104 falls into the cluster comprising the sequences of strains isolated from marine habitats and identified as *Geitlerinema*, it can not be assigned to *Geitlerinema* sensu stricto [20]. Thus, *Geitlerinema* sp. P-1104 may represent a new genus-level taxon.

A filamentous cyanobacterium with thinner trichomes (1–1.4 µm in diameter) and barrel-shaped square cells was a subdominant morphotype (Fig. 3b). In Kulunda Steppe soda lakes, this morphotype was described by Voronikhin [3] as *Phormidium tenue* (Menegh) Gom, during its mass growth in Cock Soda Lake. According to [6], cyanobacteria of this morpho-

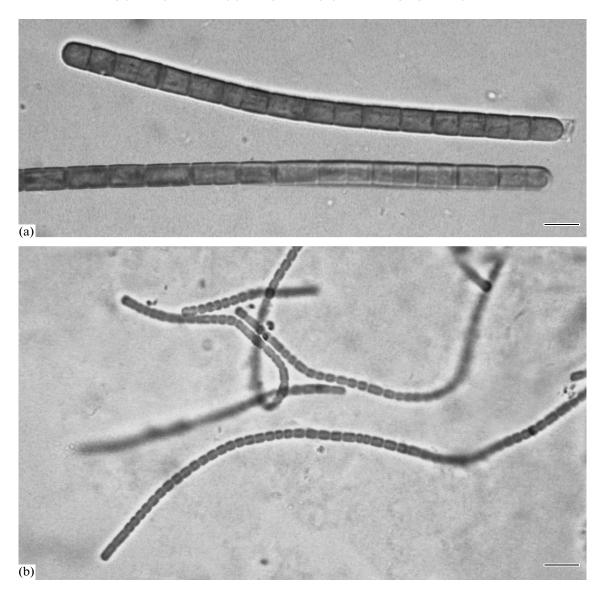


Fig. 3. Predominant cyanobacterial morphotypes: Geitlerinema sp. P-1104 (a) and Nodosilinea sp. P-1105 (b). Scale bar, 5 μm.

type may be identified as *Leptolyngbya halophila* (Hansgirg ex Gomont) Anagnostidis et Komárek 1988. On the phylogenetic tree (Fig. 4), members of this morphotype (exemplified by strain P-1105 from the Cock Soda Lake) fall within the genus *Nodosilinea* Perkerson & Casamatta, 2011, which was isolated from the genus *Leptolyngbya* Anagnostidis et Komárek based on its morphological and molecular genetic characteristics [21]. Only the sequences of uncultured cyanobacteria from saline and alkaline environments were among the GenBank sequences closely related to strain P-1105 (Fig. 4). Thus, the morphotype *Nodosilinea* sp. P-1105 may be subsequently described as a new species.

Predominance of two morphotypes of filamentous cyanobacteria in alkaliphilic phototrophic communities has been reported for a cyanobacterial mat from the alkaline lake Khilganta (Buryat Republic, Russia) [22], where *Microcoleus chthonoplastes* and *Phormidium molle* (according to Gollerbakh et al. [23]) developed. The differences in the ecological and physiological characteristics of these organisms provided for adaptive dynamics of community development during the salinization—desalination periods.

Dinoflagellates and euglenophytes each contained one species: *Phytodinium simplex* and *Euglena* sp. Reliable morphological identification of chrysomonad (*Chrysophyta*) cysts formed at high salinity and possessing considerable differences from the diagnosis [24] is difficult due to their possible significant phenotypic variability.

Analysis of the diatom composition in the lakes under study has not been carried out before. In the collections of two years, the combined diatom flora of

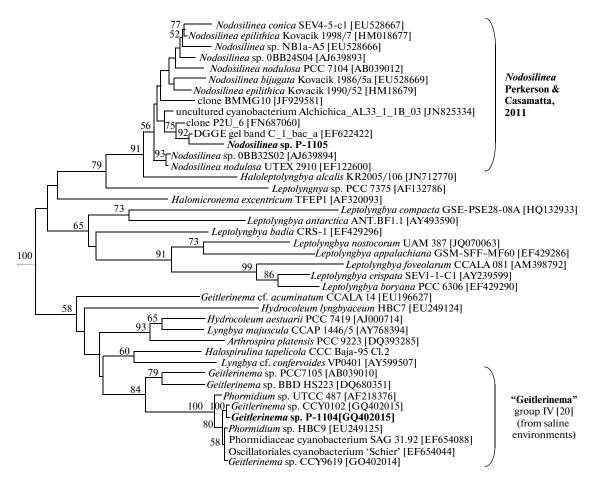


Fig. 4. Phylogenetic position of the dominant cyanobacterial morphotypes P-1104 and P-1105 isolated from the Cock Soda Lake. The tree is rooted at *Gloeobacter violaceus* PCC8105 [AF132791]. The numerals indicate bootstrap values.

three soda lakes with mineralization over 100 g/L consisted of seven species: *Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer, *Brachysira brebissonii* R. Ross in Hartley, *Brachysira zellensis* (Grunow) Round & D.G. Mann, *Mastogloia pusilla* Grunow var. *subcapitata* Hustedt, *Nitzschia amphibia* Grun., *Nitzschia communis* Rabh., and *Nitzschia* sp. 1 (Table 2). We have previously reported all these genera in continental water bodies with high chloride—sulfate mineralization [24, 25]. All the species found were motile and did not produce attached forms in the samples. Thus, no "brushlike" diatomic microepiphyton of *Brachysira* spp. cells was found on *C. circinnatus* trichomes, unlike some other hypersaline environments (western Aral Sea and adjacent areas [24].

Relatively few works dealt with diatom diversity in soda lakes. In Lake Mono (United States), where benthic algal communities with *C. circinnatus* develop, the number of diatom species varied from 30 to 12, decreasing drastically at salinity exceeding 50 g/L [26]. Thus, the relatively low number of diatom species revealed in hypersaline soda lakes of the Kulunda Steppe was probably due to extremely high water salinity during the sampling period.

Among the species revealed in Lake Mono at salinity up to 150 g/L were *Anomoeoneis sphaerophora* and *Nitzschia communis*, which were found in the Kulunda Steppe lakes at salinities of 90–160 and 250 g/L, respectively. According to our data, mass development of *Brachysira brebissonii* and *Brachysira zellensis* in the Kulunda Steppe lakes was probably typical of soda lakes. During 2002–2013 expeditions to the Aral Sea, Sapozhnikov observed this phenomenon in a number of small soda lakes in western Kazakhstan (unpublished data).

Importantly, the cells of *Anomoeoneis* and *Brachysira* contained abundant lipid inclusions, while the area of their chloroplasts was relatively small (Fig. 5). We observed a similar phenomenon in the stony and macrophyte biotopes of subtropical and tropical seas under conditions of high mineralization, intense insolation, and elevated temperatures. It is not anomalous and results from the low-area chromatophore being sufficient to satisfy the cellular energy requirements under high insolation. Large lipid vacuoles, apart from being storage compartments, act also as masking lenses scattering excessive light.

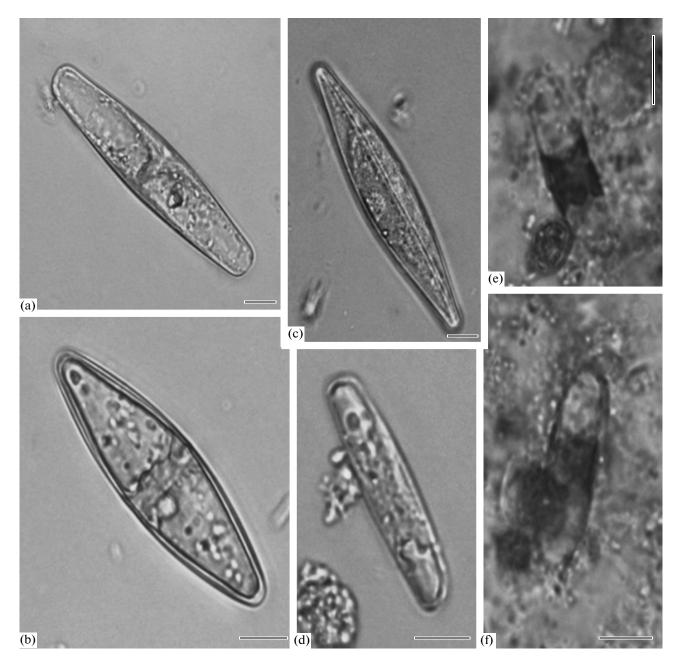


Fig. 5. Cells of the diatoms *Anomoeoneis sphaerophora* (a–c), *Brachysira brebissonii* (d), and *Brachysira zellensis* (e, f) from the Kulunda Steppe lakes. Relatively small chromatophores and abundant lipid inclusions may be seen. Scale bar, $10 \mu m$.

The cells of *Nitzschia communis*, which is normally motile, occurred not only singly, but also as short, band-shaped colonies (up to eight in a row), which is generally not characteristic of this species. The outlines and proportions of *Nitzschia communis* cells were often different from the typical ones, being more elongated and thin, especially in colonies. However, since transitional forms were also present, all specimens found belonged to the same species. To confirm the taxonomic position of this diatom, it was isolated in an algologically pure culture and identified by molecular genetic techniques. A-1677-bp fragment of

the 18S rRNA gene from *Nitzschia communis* T3-Nc11 was amplified. It exhibited quite high homology (97–99%) to the sequences of other *Nitzschia* species. The trees constructed using the neighbor-joining and maximum likelyhood algorithms had identical topology. Statistical support for most nodes exceeded 50%, indicating high reliability of the phylogenetic trees. The 18S rRNA gene sequence of the closest relative to *Nitzschia communis* T3-Nc11, strain *Nitzschia* sp. MD1, was deposited to GenBank by Chinese authors who did not provide species identification or any information concerning the sampling site.

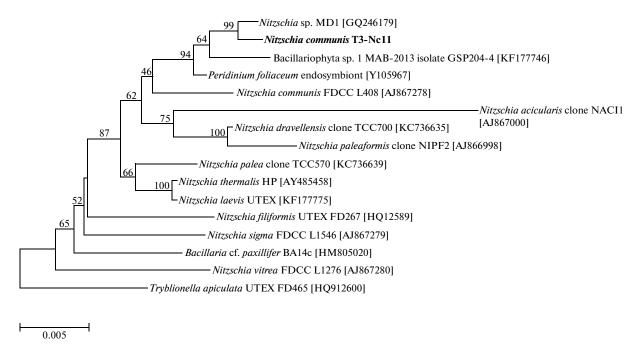


Fig. 6. Phylogenetic position of *Nitzschia communis* strain T3-Nc11 from the Tanatar soda lakes. The numerals indicate bootstrap values. The tree was constructed using the neighbor-joining algorithm.

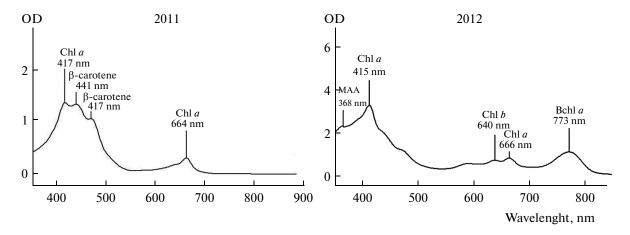


Fig. 7. Total absorption spectra from the Cock Soda Lake in 2011 and 2012. MAA indicates mycosporin-like amino acids, probably similar to Euhalothece-362 [25].

Another similar sequence belonged to a morphologically unidentified diatom from American hypersaline lakes. *Nitzschia communis* FDCC L408 from cave lakes in Luxemburg was the morphologically identified strain closest to our sample (Fig. 6).

Thus, several types of phototrophic communities were revealed in the lakes under study (Table 1):

(1) Cyano-bacterial communities, with mostly filamentous cyanobacteria (*Geitlerinema* sp. and *Nodosilinea* sp.) acting as edificators. Cyano-bacterial biofilms revealed in the lake Tanatar VI in 2011 belonged to this type. Perennial laminated cyano-bacterial

mats similar to those developing in Lake Khilganta [22] were not found.

- (2) Algo-bacterial communities with the filamentous eukaryotic alga *Ctenocladus circinnatus* Borzì as an edificator, with other phototrophs, such as cyanobacteria, diatoms, etc., developing between its filaments (Cock Soda Lake, Bitter Lake 3, 2011 and 2012).
- (3) Planktonic communities with mass development of a unicellular eukaryotic alga cf. *Chlorella minutissima* (Tanatar VI, 2012).

In one year (from 2011 to 2012), a significant increase in salinity, accompanied by an increase in

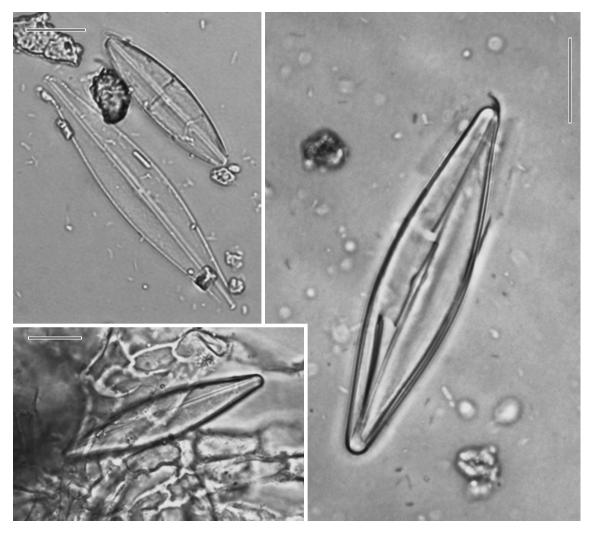


Fig. 8. Shells of the diatoms Anomoeoneis sphaerophora from the Kulunda Steppe hypersaline soda lakes. Scale bar, 20 µm.

total alkalinity, occurred in all studied lakes (Table 1). Salt concentration in the Cock Soda Lake, Bitter Lake 3, and Tanatar VI increased from 100 to 200, from 90 to 200, and from 160 to 250 g/L, respectively. Thus, the average salinity doubled in a year. The macrostructure and composition of phototrophic communities also changed. An increase in biomass of eukaryotic green algae (primarily D. viridis and cf. C. minutissima) and of anoxygenic phototrophic bacteria was a general pattern, as may be seen from emergence of the peaks of chlorophyll b and bacteriochlorophyll a in the Bitter Lake 3 and Cock Soda Lake (Fig. 7 and Table 1). The composition of the flora and the dominant diatom species varied not only from lake to lake, but also for the same lake in mid-summer at different years, probably due to the changes in total mineralization and the brine factor (ratio of dissolved salts) [24, 28] at specific periods.

The phototrophic communities with *C. circinnatus* as edificator were algo—bacterial aggregates floating throughout the brine column and often forming accu-

mulations at the windward side of the lake, where plant biomass became concentrated and compressed. This picture was observed in 2011 in the Cock Soda Lake and Bitter Lake 3 at salinity 90–100 g/L. Among the branching trichomes of *C. circinnatus*, cyanobacteria, diatoms, *Chrysophyta* cysts, coccoid forms of dinoflagellates, and motile, chlorophyll-containing *Euglenophyta* were found. In 2012, when salinity of the brine was 200 g/L and higher, *C. circinnatus* was found at the water edge and looked partially decomposed. Few isolated green areas (up to several m²) remained on the surface of the purple prokaryotic mat.

Drying of the lakes and associated evaporite concentration of the brine changed the shoreline, so that the *C. circinnatus* became completely exposed to air. It dried gradually, turning into felt, cracked crust. Subsequent watering may result in the germination of new *C. circinnatus* colonies. Most of the as-yet not decomposed biomass is buried in the silt and thus is accumulated in layers.

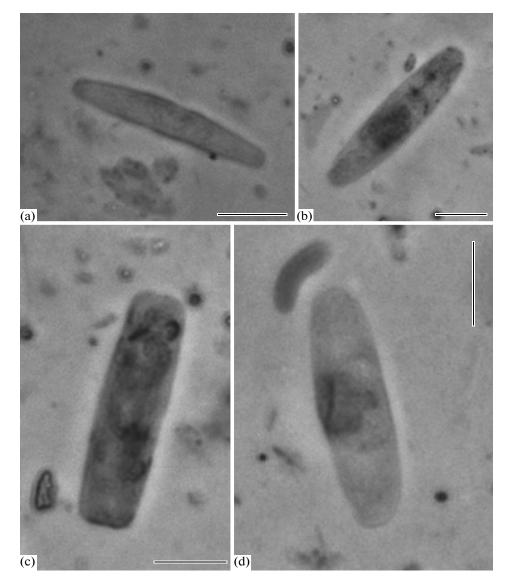


Fig. 9. Frequently occurring species of the *Brachysira* spp. diatoms form Bitter Lake 3, summer 2012: *B. brebissonii* (a, b) and *B. zellensis* (c, d). Scale bar, 10 μm.

In June 2011, A. sphaerophora was the predominant diatom in the Cock Soda Lake and B. zellensis was numerous, while only few M. pusilla var. subcapitata were found. The first two species often had very thin, locally amorphous, and even folded cell envelopes. Few cells of Euglena sp. were present. Next year, only B. zellensis was revealed in the same biotopes as an extremely rare species with an amorphous envelope.

In the Bitter Lake 3 at salinity 90 g/L (2011), *A. sphaerophora* and *B. zellensis* were the most abundant, while *Brachysira brebissonii* occurred somewhat less often, and few cells of *Nitzschia* sp. 1 were detected. The shells of all four species were relatively thin, although with clear outlines and ornamentation (Fig. 8). Next year, *A. sphaerophora* was not found in the cenoses, while both *Brachysira* were widespread

and almost equally abundant (Fig. 9), *N. amphibia* was revealed infrequently.

The most radical changes occurred in the Tanatar VI Lake. Salinity increased from 160 to 250 g/L and total alkalinity increased from 1.7 to 3.4 M. As a result, the phototrophic community changed from cyano—bacterial biofilms predominated by *Geitlerinema* sp. and *Nodosilinea* sp. (among diatoms, *A. sphaerophora* and *B. zellensis* were numerous, with infrequent cells of *M. pusilla* var. *subcapitata*) in 2011 to the monoalgological planktonic community with the green alga cf. *C. minutissima* in 2012 (Table 1). Development of cyanobacteria and diatoms was revealed only on the shore, on wet silt among the glasswort (*Salicornia altaica* Lomon) growth.

In 2011, *B. zellensis* predominated on the surface of the algal biomass drying at the shore, while *B. brebis*-

sonii occurred in high numbers. In 2012, cenoses of two types were observed. In less dry ones, mass growth of *N. communis* occurred. In drier habitats, only infrequent cells of *B. zellensis* occurred. Chrysomonad cysts were abundant in all biotopes of the lake in both years, while the cells of *Phytodinium simplex* were only seldom found at the dried bottom in 2011.

Thus, investigation of algo—bacterial communities from several Kulunda Steppe soda lakes with salinity from 90 to 250 g/L and pH above 9.8 revealed that the most typical community contained the green filamentous alga Ctenocladus circinnatus as an edificator and Geitlerinema sp. and Nodosilinea sp. as the dominant filamentous cyanobacteria; these may probably be described as new species. Application of molecular genetic techniques revealed the urgency of revision of the taxonomic diversity of cyanobacteria in soda lakes in accordance with the modern criteria of polyphasic approach. Apart from C. circinnatus, the algological component of the community was represented by unicellular green algae Dunaliella viridis and cf. Chlorella minutissima, as well as by diatoms. Phylogenetic confirmation of the taxonomic position of eukaryotic algae is also required for the purpose of reorganization of a number of taxa. The species composition of diatoms in the lakes under study has not been reported previously. Brachysira zellensis was the most persistent component of diatom cenoses of the lakes in both sampling periods. In the biotope of *Ctenocladus* accumulations developed in June 2011, it was often accompanied by Anomoeoneis sphaerophora and Brachysira brebissonii. While Anomoeoneis sphaerophora was not found in the cenoses in July 2012, mass development of *Nitzschia communis* occurred in some biotopes. including growth in colonies not reported previously for this species.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, projects nos. 12-04-31404, 12-04-00049, Program no. 28 of the Presidium of the Russian Academy of Sciences "Problems of Life Origin and Biosphere Development," and the Biodiversity program of the Presidium of the Russian Academy of Sciences. The expedition was supported by the Russian Foundation for Basic Research, project nos. 11-04-10011 and 12-04-10036.

REFERENCES

 Isachenko, B.L., Chloride, sulfate, and soda lakes of Kulunda Steppe, in *Kulundinskaya ekspeditsiya Akademii nauk SSSR 1931–1933 gg.* (Kulunda Expedition of the USSR Academy of Sciences, 1931–1933), 1934, part. 1, no. 8; also in *Izbrannye trudy* (Selected Works), vol. 2, Moscow: Izd-vo Akademii Nauk SSSR, 1951, pp. 143–162.

- 2. Voronikhin, N.N. and Khakhina, A.G., On biology of Kulunda Steppe salt lakes, *Izv. Bot. Sada*, 1919, vol. 28, pp. 1–2.
- 3. Voronikhin, N.N., Materials for research on the algological vegetation of Kulunda Steppe, *Izv. Glavn. Bot. Sada SSSR*, 1929, vol. 28, nos. 1–2, pp. 12–40.
- 4. Voronikhin, N.N., Comparative characteristics of algological vegetation of the Kulunda Steppe freshwater and saline lakes, in *Yubileinyi sbornik B.A. Kellera* (B.A. Keller Anniversary Collection), Voronezh, 1931.
- 5. Popova, T.G., On algal flora of West Siberian freshwater basins, *Izv. Glavn. Bot. Sada SSSR*, 1930, vol. 29, nos. 3–4, pp. 237–264.
- Komárek, J. and Anagnostidis, K., Susswasserflora von Mitteleuropa. 19/2. Cyanoprokaryota. 2. Teil: Oscillatoriales, Büdel, B., Gärtner, Krienitz, L., and Schagerl, M., Eds., Heidelberg: Elsevier, 2005.
- 7. Doyle, J.J. and Doyle, J.L., A rapid DNA isolation prosedure for small quantities of fresh leaf tissue, *Phytochem. Bull. Bot. Soc. Amer.*, 1987, vol. 19, pp. 11–15.
- 8. Nübel, U., Garcia-Pichel, F. and Muyzer, G., PCR primers to amplify 16S rRNA genes from cyanobacteria, *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 8, pp. 3327–3332.
- 9. Van de Peer, Y. and De Wachter, R., TREECON for windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment, *Comput. Appl. Biosci.*, 1994, vol. 10, pp. 569–570.
- 10. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S., MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol. Biol. Evol.*, 2011, vol. 28, pp. 2731–2739.
- 11. Edgar, R.C., MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Res.*, 2004, vol. 32, no. 5, pp. 1792–1797.
- 12. Voronikhin, N.N. and Popova, T.G., *Lochmiopsis*, a new genus of algae of the family *Leptosireae*, *Izv. Tomsk. Otd. Rus. Bot. Ob.*, 1931, vol. 3, nos. 1–2, pp. 17–27.
- 13. Smith, G.M., Freshwater Algae of the United States, 2nd ed., New York: McGraw-Hill, 1950.
- 14. Bourrelly, P., Les Algues D'eau Douce: Les Algues Vertes, Paris: N. Boubé et Cie, 1956, vol. 1.
- 15. Blinn, D.W. and Stein, J.R., Distribution and taxonomic reappraisal of *Ctenocladus* (Chlorophyceae: Chaetophorales), *J. Phycol.*, 1970, vol. 6, pp. 101–105.
- 16. Blinn, D.W., Autecology of *Ctenocladus circinnatus* Borzi (Chlorophyceae) in saline environments, *Canad. J. Bot.*, 1971, vol. 49, pp. 735–743.
- 17. Gerasimenko, L.M., Dubinin, A.V., Mityushina, L.L., and Zavarzin, G.A., A microscopic green alga from soda lakes, *Microbiology* (Moscow), 1999, vol. 68, no. 5, pp. 610–614.
- 18. Krienitz, L., Bock, C., Kotut, K., and Luo, W., *Picocystis salinarum* (Chlorophyta) in saline lakes and hot springs of East Africa, *Phycologia*, 2012, vol. 51, no. 1, pp. 22–32.
- Somogyi, B., Feleföldi, T., Solymosi, K., Makk, J., Homonnay, Z.G., Horváth, G., Turcsi, E., Böddi, B., Márialigeti, K., and Vörös, L., *Chloroparva pannonica*

- gen. et sp. nov. (Trebouxiophyceae, Chlorophyta)—a new picoplanktonic green alga from a turbid, shallow soda pan, *Phycologia*, 2011, vol. 50, no. 1, pp. 1–10.
- Perkerson, R.B.III, Perkerson, E.A., and Casamatta, D.A., Phylogenetic examination of the cyanobacterial genera *Geitlerinema* and *Limnotrix* (Pseudanabaenaceae) using 16S rDNA sequence data, *Algol. Stud.*, 2010, vol. 134, pp. 1–16.
- Perkerson, R.B.III, Johansen, J.R., Kovácik, L., Brand, J., Kastovsky, J., and Casamatta, D.A., A unique pseudanabaenalean (cyanobacteria) genus Nodosilinea gen. nov. based on morphological and molecular data, J. Phycol., 2011, vol. 47, no. 6, pp. 1397–1412.
- 22. Gerasimenko, L.M., Mityushina, L.L., and Namsaraev, B.B., *Microcoleus* mats from alkaliphilic and halophilic communities, *Microbiology* (Moscow), 2003, vol. 72, no. 1, pp. 84–92.
- 23. Gollerbakh, M.M., Kosinskaya, E.K., and Polyanskii, V.I., *Opredelitel' presnovodnykh vodoroslei SSSR* (Identification Guide fro Freshwater Algae of the USSR), Moscow: Sov. Nauka, 1953.
- Zav'yalov, P.O., Arashkevich, E.G., Bastida, I., Ginzburg, A.I., Dikarev, S.N., Zhitina, L.S., Izhitskii, A.S., Ishniyazov, D.P., Kostyanoi, A.G., Kravtsova, V.I., Kudyshkin, T.V., Kurbaniyazov, A.K.,

- Ni, A.A., Nikitina, A.B., Petrov, M.A., Sazhin, A.F., Sapozhnikov, F.V., Solov'ev, D.M., Khan, V.M., and Sheremet, N.A., *Bol'shoe Aral'skoe more v nachale XXI veka: fizika, biologiya, khimiya* (South Aral Sea in the Early 21st Century: Physics, Biology, Chemistry), Moscow: Nauka, 2012.
- 25. Sapozhnikov, F.V., Arashkevich, E.G., and Ivanishcheva, P.S., Biodiversity, in *The Handbook of Environmental Chemistry, vol. 7: The Aral Sea Environment, 1st ed.*, Kostianoy, A.G. and Kosarev, A.N., Eds., 2010, Berlin: Springer, pp. 235–282.
- 26. Herbst, D.B. and Blinn, D.W., Experimental mesocosm studies of salinity effects on the benthic algal community of a saline lake, *J. Phycol.*, 1998, vol. 34, pp. 772–778.
- 27. Kedar, L., Kashman, Y., and Oren, A., Mycosporine-2-glycine is the major mycosporine-like amino acid in a unicellular cyanobacterium (*Euhalothece* sp.) isolated from a gypsum crust in a hypersaline saltern pond, *FEMS Microbiol. Lett.*, 2002, vol. 208, pp. 233–237.
- 28. Khlebovich, V.V., Composition of aquatic fauna depending on ambient salinity, *Zh. Obshch. Biol.*, 1962, vol. 23, no. 2, pp. 90–97.

Translated by P. Sigalevich