Original Research

Cyanobacterial Blooms in Oligosaline and Alkaline Microaccumulation Before and After Rehabilitation

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Abstract

This work describes the occurrence and blooming of some Cyanobacteria in an artificial, oligosaline, alkaline, microaccumulation in Serbia, which was formed on salines in 1963-64, and rehabilitated in 2009-10. The appearance and specific blooming of species common for tropical regions in Aleksandrovac Lake (N 42º29'22", E 21º53'54") before reconstruction (*Cylindrospermum stagnale* (Kützing) ex Bornet et Flahault, *Synechococcus lividus* Copeland, *Chrysosporum bergii* (Ostenfeld) Zapomělová et al., *Anabaenopsis elenkinii* Miller, *Lyngbya aestuarii* (Mertens) Liebman ex Gomont) and after reconstruction (*Cylindrospermopsis raciborskii* (Woloszińska) Seenayya et Subba Raju, *Chrysosporum bergii*, *Anabaenopsis elenkinii*, as well as *Glaucospira* sp. Lagerheim) was recorded for the first time in Serbia. The work also demonstrates the potential hazard to the environment (fishkill) due to the presence of specific blooming of Cyanobacteria. Alkaline salt ponds and lakes with extreme ecological conditions are places suitable for the development of alien and potentially invasive species.

Keywords: cyanobacterial bloom, microaccumulation, salines, *Cylindrospermopsis raciborskii*, *Glaucospira* sp.

Introduction

Cyanobacteria is an extensive group of photosynthetic prokaryotes that often plays a key role in the production of organic matter in soda, brackish, and saline lakes, as well as artificial reservoirs formed on salty ground. They are unique ecosystems with extremely high pH values and high mineralization levels (up to saturating concentrations) [1, 2]. Under extremely high pH values (>10.5). Cyanobacteria form up to 99% of overall phytoplankton [2].

During the last 90 years the presence and blooming of Cyanobacteria has been described in about a hundred different lakes, reservoirs, rivers, and canals of Serbia [3-9]. A total of 21 genera with 67 species of Cyanobacteria has been noted. Obvious blooms were in the form of water colour change, foam, and skim of different colors. The research so far has shown that the blooming most commonly occured due to the excessive multiplication of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Planktothrix* [5]. Recent work has indicated the appearance of certain tropical, potentially invasive species, especially in the salt ponds and lakes of Serbia [10-12]. The two invasive Cyanobacteria *Chrysosporum bergii* (Ostenfeld) Zapomělová et al. (as *Anabaena bergii* Ostenfeld) and *Cylindrospermopsis raciborskii* (Woloszińska) Seenayya et Subba Raju [10, 12] were first found in Serbia in the salty puddles and marshes near the river Tamiš. Also, in these salt ponds, was first found *Arthrospira fusiformis* (Voronichin)

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Komárek et Lund, up to now mainly known for salty alkaline lakes from tropical regions in Africa and Asia [11]. In Serbia at the salty alkaline lakes the appearance of the taxa *Anabaena*, *Cylindrospermopsis* and *Arthrospira* has not caused blooming up to now and there were no negative consequences for the wildlife, the only detected blooming of the *Cylindrospermopsis raciborskii* species in Serbia was in the Ponjavica River [8].

The aim of this work is to indicate the presence and specific blooming of Cyanobacteria in an oligosaline, alkaline microaccumulation in Serbia before and after its rehabilitation, as well as to demonstrate potential hazards to the environment (fishkill) due to the presence of specific blooming of Cyanobacteria. The formed water bloom, as well as the species that created it, are new to Serbia.

Material and Methods

Aleksandrovac Lake is situated in the southernmost part of Serbia (N 42º29′22″, E 21º53′54″), at an elevation of 412 m (Fig. 1). It is an artificial reservoir formed by creating an earth-fill dam 8 meters high on the Aleksandrovac River. The lake area is 12 ha, max. volume 250,000 m^3, length 580 m, width 300 m, maximum depth of 4 m, and average depth of 2 m. The water mass of the lake covers most of the so-called Aleksandrovac salines. The reservoir covers about 2/3 of the salines, while 1/3 is outside the reservoir. Soda ($NaHCO_3$) appears on the coast in the form of a white lime powder. This reservoir was originally used for irrigation of agricultural areas, and later for sports and recreational purposes – above all fishing. In the last few years several fish kills have occurred. Complete rehabilitation of the lake was performed from September 2009 to May 2010. The remaining fish were fished out, the water was released from the lake, and a complete rehabilitation [13] was performed. The salines were not considered within the rehabilitation project.

The samples of cyanobacteria were taken before the rehabilitation (June and September 2008) and after the rehabilitation of the lake (April, June, July, and September 2011). Before rehabilitation, the gelatinous mass was col-

Fig. 1. Study area map. Sampling and saline ground location at Aleksandrovac Lake.

lected from the surface (in June and September 2008), as well as from the bottom of the lake (in September 2008). Before and after the rehabilitation, phytoplankton samples were gathered by standard methods – phytoplankton net (net frame 25 cm, mesh net e.g. 22 µm) and Rutner bottle (2 l) in all aspects. All samples were preserved at once in a 4% solution of formaldehyde. Microscopic identification of cyanobacteria was performed, based on morphological characteristics on microscope Amplival-Jena with magnification up to 800 -1000x. Identification was carried out using the following literature [14-21]. Before rehabilitation only the relative abundance of the present taxa was estimated, due to the specificity in the structure of the mats. A 6-grade scale was used: 1 – occasional, 2 – rare, 3 – common, 4 – frequent, 5 – very frequent, and 6 – abundant. After rehabilitation, quantitative analysis of Cyanobacteria was made using the Utermöhl method [22] with a Carl Zeiss inverted microscope and it is expressed as the number of individuals per liter. The colonial or filamentous species were counted as one individual. The relative abundance was determined as well for the sake of comparing the results before and after the rehabilitation of the lake.

Simultaneously with gathering Cyanobacteria samples in the field, the following physical and chemical parameters were measured: water temperature (ºC), pH, oxygen concentration ($mg \cdot l^{-1}$), saturation (%), conductivity ($\mu s \cdot cm^{-1}$), nitrate, ammonia, and phosphate concentrations ($mg \cdot l^{-1}$), and water transparency (Secchi disc) (m) [23].

Table 1. Mean value physical and chemical parameters of Aleksandrovac Lake before and after rehabilitaton.

	Before rehabilitation		After rehabilitaton			
Month	6	9	4	6	7	9
Year	2008	2008	2011	2011	2011	2011
Water temperature (ºC)	34.4	15.9	12.9	21.0	26.2	21.6
pH	10.37	6.68	8.78	8.78	9.23	10.21
Oxygen ($mg \times l^{-1}$)	10.15	3.60	10.05	6.02	7.24	7.36
Saturation (%)	146.00	41.20	102.55	71.5	96.4	85.80
Nitrate ($mg \times l^{-1}$)	2.10	-	0.48	2.52	1.88	1.85
Phosphate ($mg \times l^{-1}$)	1.25	0.20	0.08	0.74	0.39	0.33
Ammonia ($mg \times l^{-1}$)	0.23	0.10	0.4	0.23	0.27	0.14
Conductivity ($\mu s \times cm^{-1}$)	1238	1234	362	367	377	423
Water transparency (m)	0.1	0.1	1.0	1.2	0.8	0.6

Results and Discussion

Before Rehabilitation

The results of physical and chemical analysis (Table 1), especially the conductivity values at 1,234 $\mu s \cdot cm^{-1}$ and pH at 8.7 to 10.3 of water of Aleksandrovac Lake, indicate that this is an oligosaline [24] and alkaline lake. The ground on which the lake was formed, morphological characteristics of the lake (average depth of 2 m), small flow of fresh water, physical and chemical characteristics of the water itself (increased level of biogenic salts, high temperature to 34ºC, pH, and conductivity), had an effect on the formation of a specific cyanobacteria community. This includes both the presence of certain species and the way of their joining and the formation of a compact gelatinous mat on the surface of the water.

Throughout 2008 a specific blooming in the form of gelatinous green-orange mats was formed on the surface of the water of Aleksandrovac Lake, up to 0.5 cm thick. By June the mats had spread, covering about 50% of the lake surface. A gelatinous green-orange mat was made out of densely intertwined and compact cyanobacteria, both benthic and plankton filamentous, unicellular and colonial ones, along with green filaments (*Cladophora* sp. and *Oedogonium* sp.) and plankton unicellular and colonial algae of the order Desmidiales and Chlorococcales. Twenty-seven Cyanobacteria were found in the biofilm (Table 2): Chroococcales 11, Oscillatoriales 8, and Nostocales 8. In relation to the relative abundance, *Cylindrospermum stagnale* (Kützing) ex Bornet et Flahault (Fig. 2A-C) and *Synechococcus lividus* Copeland (Fig. 2E), dominated, subdominated by *Chroococcus* spp. Nine Cyanobacteria were present in the plankton (Chroococcales 3, Oscillatoriales 3 and Nostocales 3) dominated by *Synechococcus lividus*. Such thick gelatinous green-orange mats formed by a combination of the above-mentioned species of Cyanobacteria was first recorded in Serbia [3-5]. Such a biofilm with similar cyanobacteria structure is mostly characteristic of alkaline waters, with conductivity values over 1,000 $\mu s \cdot cm^{-1}$. *Cylindrospermum stagnale* is a species tolerant of high pH and conductivity in water (and soil), especially in the tropical regions in Asia and Africa [25-27]. In foliose floating mats, *Cylindrospermum stagnale* always had gas bubbles trapped within the mucilaginous matrix, thus helping the buoyancy of the species [25].

In Aleksandrovac Lake, in hyper-saturated conditions (146% of oxygen), mats were elevated due to air bubbles formed within them and remained on the surface of the water. The formed mats obscured the light, which resulted in the elevation of all the other algae and their presence in the above-mentioned mats on the surface of the water. That explains the diversity and mass appearance of a number of primarily plankton forms in the biofilm and their absence or small number in the plankton. In the plankton, under the transparency of 0.1 m at 34ºC ae cells of termophilic species *Synechococcus lividus* dominate (Table 2).

Table 2. Species diversity, relative abundance (1 – occasional, 2 – rare, 3 – common, 4 – frequent, 5 – very frequent, and 6 – abundant) of Cyanobacteria in mats (M) and plankton (P) of Aleksandrovac Lake before and after rehabilitation.

Cyanobacteria	Before rehabilitation				After rehabilitation			
Year	2008				2011			
Month	6		9		4	6	7	9
	M	P	M	P	P	P	P	P
Chroococcalles								
Aphanocapsa grevillei (Berkeley) Rabenhorst	1		1				1	
Aphanocapsa holsatica (Lemmermann) Cronberg et Komárek	1							
Chroococcus minutus (Kützing) Nägeli	4	1	4					
Chroococcus bituminosus (Bory) Hansgirg	1							
Chroococcus dispersus (Keissler) Lemmermann	3		5					
Chroococcus minimus (Keissler) Lemmermann	3		4					
Chroococcus turgidus (Kützing) Nägeli			1				1	
Gloeocapsa montana Kützing	1							
Gloeocapsa minima f. smithii Hollerbach, Kosinskaja et Poljanskij	1							
Merismopedia glauca (Ehrenberg) Kützing							1	
Merismopedia punctata Meyen	1							
Microcystis aeruginosa (Kützing) Kützing			1	1				
Synechococcus aeruginosus Nägeli	1		1					
Synechococcus elongatus (Nägeli) Nägeli		1	1					
Synechococcus lividus Copeland	6	6	6	6		2	3	2
Oscillatoriales								
Glaucospira sp. Lagerheim								6
Limnothrix redekei (Van Goor) Meffert	2	1			1	1	1	1
Lyngbya aestuarii Liebman ex Gomont	1		6	6			1	1
Lyngbya limnetica Lemmermann	1	1	1	1				3
Oscillatoria sp. Vaucher ex Gomont						1		
Planktothrix sp. Anagnostidis et Komárek						1		
Porphyrosiphon martensianus (Meneghini ex Gomont) Anagnostidis et Komárek	1		1					
Pseudanabaena constricta (Szafer) Lauterborn	1							
Pseudanabaena limnetica (Lemmermann) Komárek	4	4	4	4	2	2	2	2
Spirulina sp. 1 Turpin ex Gomont	1							
Spirulina sp. 2 Turpin ex Gomont	1							
Nostocales								
Anabaena sp. 1 Bory de Saint-Vincent ex Bornet et Flahault	1		1	1	1	1		
Anabaena sp. 2 Bory de Saint-Vincent ex Bornet et Flahault				1	1	1		
Anabaenopsis elenkinii Miller	1	1	2	5				3
Aphanizomenon sp. Morren ex Bornet et Flahault					1	1		
Calothrix fusca (Kützing) Bornet et Flahault	2							
Calothrix pulvinata (Mertens) C. Agardh	1							
Chrysosporum bergii (Ostenfeld) Zapomelová et al.		1	2	2	1	1	1	2
Cylindrospermum stagnale (Kützing) ex Bornet et Flahault	6	2	3	1	1	1	1	1
Cylindrospermopsis raciborskii (Woloszińska) Seenayya et Subba Raju								4
Gloeotrichia echinulata (Smith) P. Richter	2							
Gloeotrichia sp. J. Agardh ex Bornet et Flahault	1							
Nostoc paludosum Kützing ex Bornet et Flahault	2		2					

Synechococcus spp. is common and numerous in shallow, turbid soda lakes [28].

In September, apart from existing gelatinous biofilm on the surface, a leathery-like dark-green scum on the sludge was observed. Eighteen Cyanobacteria were present (Table 2): Chroococcales 9, Oscillatoriales 4, and Nostocales 5. In this period dominant species in the mats at the bottom were *Lyngbya aestuarii* Liebman ex Gomont (Fig. 2D-E), subdominated by *Pseudanabaena limnetica* (Lemmermann) Komárek (Fig. 2F). Ten Cyanobacteria were present in the plankton (Chroococcales 2, Oscillatoriales 3, and Nostocales 5), dominated by *Lyngbya aestuarii*, *Synechococcus lividus*, subdominated by *Anabaenopsis elenkinii* Miller (Fig. 2G).

The conditions that prevailed in the lake toward the end of the summer (low light intensity and high conductivity) enabled the appearance of benthic *Lyngbya aestuarii* in the sludge in both coastal areas of the lake, where it formed thick green mats out of trichomes in sheath, and the plankton, where a large number of trichomes out from a sheath were present. The trichomes of *Lyngbya aestuarii* (along with the trichomes of *Chrysosporum bergii*, *Anabaenopsis elenkinii*, *Pseudanabaena limnetica,* and cells of *Synechococcus lividus*) colored the water dark green. The *Lyngbya aestuari*, *Chrysosporum bergii* (Fig. 2H), and *Anabaenopsis elenkinii* have rarely been found in Serbia so far [5, 10, 15]. All these algae are known to produce toxins that can lead to the endangerment of aquatic life forms (fishkill), or health problems with people who use such water for recreational purposes (swimming, fishing) [5]. The fish kill at Aleksandrovac Lake happened on September 22, 2008, when 65 specimens of white silver carp (*Hypophthalmichthys molithrix*), with a total weight of 1,600 kg, died in one day.

It was in the time of the domination of these algae that the death of white silver carp occurred, an allochthonous planktivore species of fish introduced to the lake. The death of these species occurred at a period of biomass decomposition in conditions of low temperature, measured at 15.6ºC in this period, a sudden decrease of oxygen concentration in the water (3.6 mg·l^{-1}), as well as the sudden decrease of the pH value to 6.8. Potential causes for the death of this species could be the toxins of these algae [29]. Silver carp have been introduced worldwide for both aquaculture fish production and algal control. Stocking of silver carp as a biomanipulation tool to reduce phytoplankton biomass in lakes remains controversial [30, 31]. The present study indicates that the phytoplanktivorous silver carp can be an efficient biomanipulation fish, to reduce nuisance blooms of cyanobacteria in eutrophic lakes where large herbivorous zooplankton are lacking [31]. Some experimental research indicate that the presence of this species in the same conditions resulted in the decrease of growth of certain species (*Anabaena* sp., *Aphanizomenon* sp.), but on the other hand it stimulated the growth of certain Cyanobacteria such as *Microcystis aeruginosa* [32]. Other studies indicate that Cyanobacteria blooms (*Aphanizomenon flos-aquae* and *Microcystis aeruginosa*) often result in white silver carp deaths [33].

After Rehabilitation

The results of the research of physical and chemical parameters in Aleksandrovac Lake after rehabilitation indicate that only some parameters changed. Conductivity of the water is significantly lower at 362 μs·cm^{-1} (before rehabilitation it was 1,234 μs·cm^{-1}). The pH value and especially phosphate concentration indicate that the conditions for a rapid natural eutrophication are present in the lake (Table 1).

After rehabilitation in April to September 2011, no visible cyanobacteria-induced water blooms was observed. From April to September 2011 the number of Cyanobacteria species present in the phytoplankton was small (7-10) (Table 2). One year after filling the lake the heterocite species of Nostocales (*Cylindrospermum stagnale*, *Chrysosporum bergii*, *Anabaenopsis elenkinii*) are still present. Their appearance right after rehabilitation was possible due to the akinetes, which helped them to remain in the sludge that had not been completely removed during rehabilitation [13]. The total abundance of Cyanobacteria was 635 ind·l^{-1} in April, 2,087 ind·l^{-1} in June, 6,726 ind·l^{-1} in July, and 1,033,427 ind·l^{-1} in September. The Cyanobacteria community in September was dominated by *Glaucospira* sp. Lagerheim (74%), and subdominated by *Cylindrospermopsis raciborskii* (23%) (Fig. 2J-K).

According to Komárek and Anagnostidis [20], *Glaucospira* is an insufficiently known genus, rarely found in the plankton of a lake. These cyanobacteria are very rare in Europe and the USA. One species known from Europe (Greece, Hungary, Caspian Sea – estuary of Volga River and one from a thermal spring in Yellowstone Park (USA). Few species are known from plankton of lakes and reservoirs and from metaphyton among water plants in swamps, usually in tropical regions (African lakes), also in Australia, India, Brazil, Cuba, Japan, Equador, Venezuela, Malaysia, Mexico, etc. [20]. Santos and Sent'Anna [1] provide data on the appearance of *Glaucospira* sp. with *Anabaenopsis elenkinii* in a Brazilian salt lake Salina de Meio, with extreme ecological conditions (pH>10, conductivity >3,800 μs·cm^{-1}, 28.2ºC). This is the first record of genus *Glaucospira* in the waters of Serbia [5]. The *Cylindrospermopsis raciborskii*, until recently known as a tropical species, is spreading its areal into moderate climate regions, and is defined as an invasive, harmful algae, that can produce toxins [34, 35]. Akinetes, the resting cells of nostocalen Cyanobacteria, help to withstand unfavorable conditions and play a key role in their invasion [36]. Cvijan and Fužinato [12] were the first to provide data on the findings of this species in 2006 in water of Slatina Pond, Serbia (about 960 trichomes per l), with high pH (8.4), conductivity (1900±100 μs·cm^{-1}), and temperature (28ºC). In Serbia we detected the first blooming of the *Cylindrospermopsis raciborskii* species in 2008 in the Ponjavica River [8]. The presence and blooming of *Cylindrospermopsis raciborskii* in Aleksandrovac Lake indicates the expansion of this species.

Alkaline salt ponds and lakes with extreme ecological conditions are places suitable for the development of non-native and potentially invasive species. This argument is

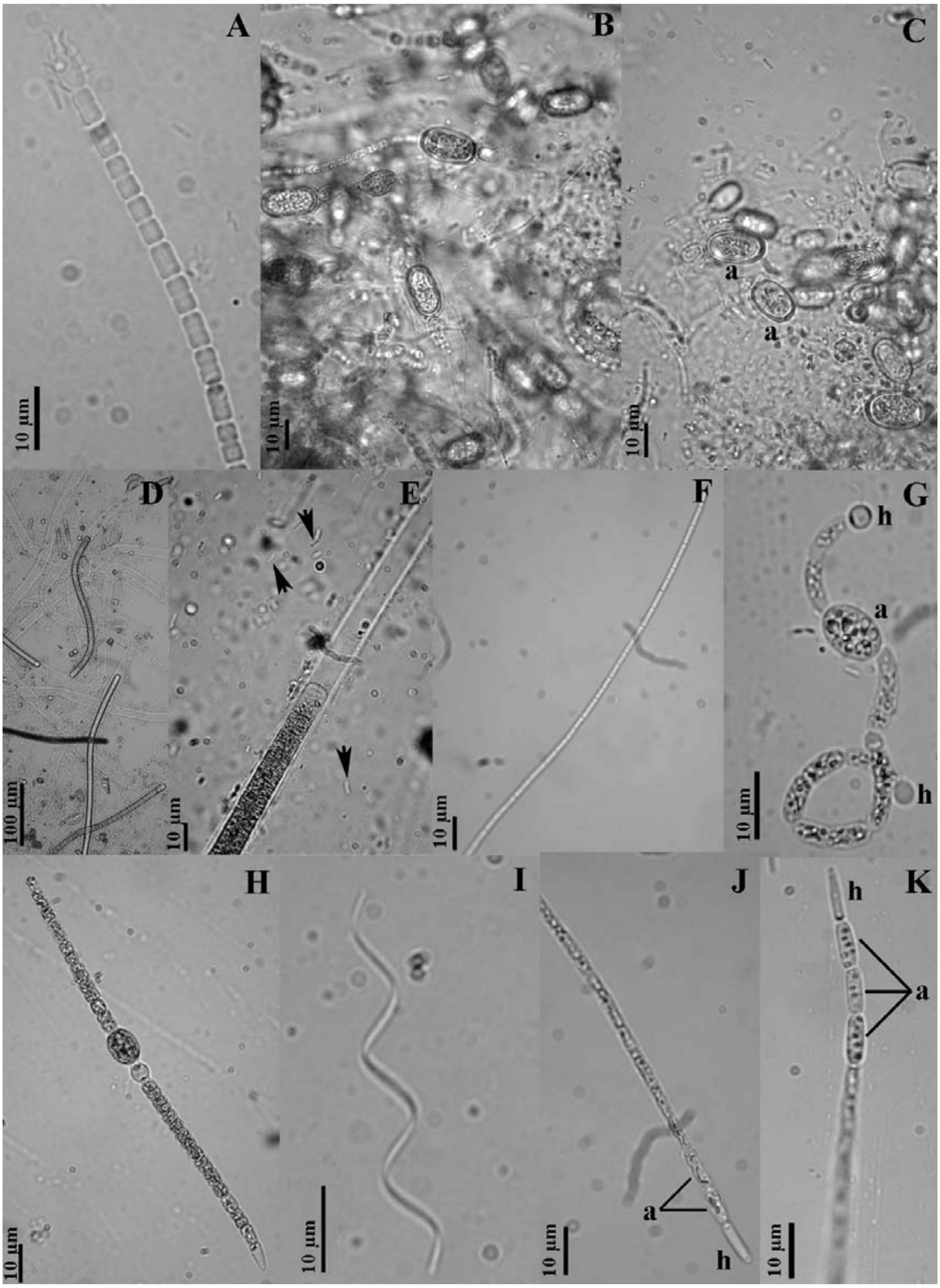

Fig. 2. Micrographs of different Cyanobacteria observed by optical microscope in Aleksandrovac Lake. Panels correspond to:
A) *Cylindrospermum stagnale* – simmetric trichom and terminal heterocytes,
B) development of akinetes of *Cylindrospermum stagnale*,
C) akinetes of *Cylindrospermum stagnale*,
D) *Lyngbya aestuarii* in mats with and without sheath,
E) *Lyngbya aestuarii* and *Synechococcus lividus* (arrows),
F) *Pseudanabena limnetica*,
G) *Anabaenopsis elenkinii*,
H) *Chrysosporum bergii,*
I) *Glaucospira* sp.,
J) *Cylindrospermopsis raciborskii* – solitary subsimetric trichom with terminal heterocytes and development two subapical akinetes;
K) *Cylindrospermopsis raciborskii* – trichom with terminal heterocytes and three subapical akinetes.
Legend: h – heterocyta, a – akinet.

justified by the fact that the cyanobacteria *Chrysosporum bergii* [10], *Arthrospira fusiformis* [11], and *Cylindrospermopsis raciborskii* [12], previously known as foreign species, have already been found in the waters of similar physical and chemical characteristics.

Conclusion

The occurence and blooming of *Cylindrospermum stagnale*, *Synechococcus lividus*, *Chrysosporum bergii*, *Anabaenopsis elenkinii*, and *Lyngbya aestuarii* before reconstruction and *Cylindrospermopsis raciborskii*, *Chrysosporum bergii*, *Anabaenopsis elenkinii*, as well as *Glaucospira* sp. after reconstruction in Aleksandrovac Lake, characteristic and common for tropical regions indicates that the development of these Cyanobacteria, as well as their blooming, are not conditioned merely by latitude, but, above all, by specific conditions ruling in an aquatic ecosystem. In the case of this lake, extreme ecological conditions (high pH values and high mineralization levels, as well as high temperature) can be singled out as important factors for the appearance and blooming of these cyanobacteria. The base on which the lake was formed as well as the salines on the left bank of the lake also affect the specificity of ecological conditions.

Taking into account all of the mentioned facts, it is clear that the process of rehabilitation has not given results. After the filling of the lake, blooming of potentially toxic cyanobacteria occurred. All this indicates the necessity for further monitoring of this lake and a controlled use of these waters provided for sport and recreational purposes (swimming and fishing). Alkaline salt ponds and lakes with extreme ecological conditions are places suitable for the development of alien and potentially invasive species.

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