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WATER QUALITY AND PROTECTION: ENVIRONMENTAL ASPECTS

Phytoplankton Structure and Microcystine Concentration in the Highly Eutrophic Nero Lake

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Abstract—The results of observations of some abiotic (water transparency, light intensity, depth, biogenic element concentrations) and algological (chlorophyll concentration, phytoplankton abundance and biomass, the concentration of microcystines—toxins of blue-green algae) characteristics of Lake Nero are given for study periods of 1999–2004 and 2005–2007. Variations of these characteristics in the latter period are shown to be significant. The lake phytoplankton is found to pass in a «catastrophic" manner to monodomination of planktotrichaetic complex of blue-green algae. High-performance liquid chromatography was used for the first time to determine the concentration of microcystines MC-LR and MC-RR in Lake Nero seston. The presence of these types of microcystines in samples was confirmed by mass-spectrometry. A statistically significant correlation was found to exist between the total concentrations of microcystines and the biomass of species of Microcystis genus, suggesting the possible toxicity of representatives of this type of algae in Lake Nero.

Keywords: planktotrichaetic complex of blue-green algae, highly eutrophic water body, microcystines.

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INTRODUCTION

In the studies of Lake Nero in 1999–2003, the use of functional classification of planktonic algae made it possible to suggest a hypothesis regarding the development of phytoplankton in the open part of the lake toward the stage of predominance of planktotrichaetic type (type S_1 according to [30]) as a final stage of phytoplankton development in shallow, highly eutrophic water bodies enriched with organic matter (OM) [18].

Thirty-one functional groups, characteristic of different types of water bodies, were identified in the ecological classification of algae [31]. Each group consists of a set of jointly developing species with similar morphology, physiological demands for light and biogenic substances, irrespective of taxonomic classes. The planktotrichaetic (oscillatorium) type includes phytoplankton with the predominance of fine filamentary heterocyst-free forms of blue-green algae Pseudanabaena limnetica (Lemm.) Kom, Limnothrix redekei (Van Goor) Meffert, Planktothrix agardhii Gom. This functional group shows tolerance to low light intensity, develops in shallow water bodies enriched with OM, and is sensitive to higher water flowage. In previous studies, the genotoxic effect of Lake Nero water was determined and the coincidence of its manifestation with seasonal increase in blue-green algae was demonstrated [7]. The established dynamics determined the scientific interest to studying the concentration of natural toxins of blue-green algae in Lake Nero. The appearance and development of blue-green algae in water bodies is generally accompanied by the release of various types of toxins: hepatotoxins, neurotoxins, and dermatotoxins. The best studied hepatotoxin—microcystine—can cause considerable damage to the health of both humans and animals [23]. World Health Organization in 1997 established a limit of 1 μg/l of microcystine-LR or equivalent of this toxin as the maximal allowable concentration in drinking water [35]. Microcystines are produced by representatives of Microcystis, Anabaena, Planktothrix, and Nostoc genera. In Europe, systematic studies of the qualitative and quantitative composition of the most widespread hepatotoxin—microcystine—have been carried out for more than 15 years.

Microcystines belong to the class of cyclic peptides and have the general form of $-_D$–Ala$^{(1)}$–$X^{(2)}$–$_D$–MeAsp$^{(3)}$–$Z^{(4)}$–AddA$^{(5)}$–$_D$–Glu$^{(6)}$–Mdha$^{(7)}$, where X and Z are different aminoacids, $_D$MeAsp: 3-methylasparaginic acid, AddA: 3-amino-9-methoxi-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid, Mdha: N-methyldehydroalanine [19]. More than 70 types of different microcystines have been described by now [19]. In periods of water blooming, blue-green algae can form both monodominant and polydominant phytoplanktocenoses with various pronounced toxic effect. The genotypic variability of blue-green alga species is very wide. Molecular–biological analysis shows the coexistence of toxic and nontoxic populations of blue-green algae [25, 26]. The hazard of water blooming with blue-green algae has been known in Russia since the mid-20th century; however, almost no studies of natural toxins are known now, as well as there are no studies in biology and ecology of toxic alga complexes. The objective of this study is to carry out comparative analysis of some abiotic characteristics and the structure of phytoplankton over the ten-year observation period and to assess the presence of microcystines in the highly eutrophic Lake Nero.

MATERIALS AND METHODS

Lake Nero is situated in Russia, in the Upper Volga region between 57°06′–57°12′N and 39°21′–39°30′E. This is the largest lake in Yaroslavl province (S ~ 58 km^2). The lake is shallow (the mean depth is 1.6, and the maximal depth is 4.7 m). It formed in late Middle Pleistocene, ~150000 years ago, in the period of degradation of moskovskii glacier. About 5000 years since the late boreal–early atlantic period, the lake features a complex of eutrophic organisms [3]. Lake Nero is a flow-through water body: it receives >20 tributaries, the largest among which is the Sara River; the Veksa Rostovskaya River flows out of the lake. Water level in the lake is regulated by a dam at the outlet of the Veksa River—a tributary of the Kotorosl River, whose water serve as a source of drinking water for several populated localities, including (up to the early 2009) Yaroslavl Town. The lake's drainage area is 1256.2 km^2, the coefficient of conventional water exchange is 1.9 [2].

By its water chemistry, Lake Nero differs from most lakes in the Yaroslavl area by higher concentration of dissolved mineral components with TDS of 277–1310 mg/l [2, 3]. The bed of the lake is covered by a thick layer of sapropels, which has a mean thickness of 4.9 m and serves as a powerful accumulator of biogenic elements. The northern and central parts of the lake water area refer to "phytoplankton" type, while its southern part, to "macrophyte" type [6]. By chlorophyll concentration [12], biomass, [6], and phytoplankton primary production [10], the lake in the late 1980s was classified as a water body of highly eutrophic type [16]. The studies of the early 21st century the trophic status of the lake was estimated as transitional between eutrophic and hypereutrophic by the majority çà parameters (total phosphorus P_{tot}, chlorophyll "a" concentration in seston and bottom sediments, phytoplankton biomass, photosynthesis rate, zooplankton structure characteristics) [14].

Lake water samples were taken on a monthly basis (March, May–October) on a conventional network of stations 3–5, 7, 8 (Fig. 1) from the surface water layer in the northern part of Lake Nero adjacent to Rostov Velikii Town [1, 6]. Averaged results for June–September of 1999–2004, 2005–2007 for abiotic and algological characteristics were analyzed. In 2008, for the first time, microcystine concentration in seston was determined on a monthly basis at station 5 and an integrated sample was taken at stations 3, 4, 7, and 8 from June to September. The spatial distribution of toxins was studied in September at five stations; a net plankton sample was also analyzed in this period. The study period corresponds to the highest abundance of phytoplankton in Lake Nero with the predominance of planktotrichaetic alga complex.

Dissolved reactive P was determined by colorimetric Denigs–Atkins method, P_{tot} was found after the transformation of organic components into inorganic forms by heating with concentrated sulfuric acid and subsequent application of the method mentioned above. Ammonium and nitrate N was determined with the use of Nessler reagent and sulfonated phenol, respectively, by colorimetry [15]. The light intensity was determined with the use of Secchi disk and by the ratio Z_{eu}/Z_{mix} of the depth of the euphotic zone, calculated as tripled transparency Z_{eu} to the depth of the mixed layer Z_{mix}; the latter, because of the shallow depth of the lake, was take equal to the depth of the sampling station [29]. Phytoplankton was concentrated by settling method from a volume of 0.5 l. The counting and determination of algae was carried out in Nageotte chamber, with the shape of cells assumed coinciding with known geometrical figures [21]. Alga biomass was determined by counting–volumetric method [4]. Standard spectrometric method was used to determine chlorophyll "a" [11, 32]. Chlorophyll "a" concentration was calculated by Jeffrey–Humphrey equation [22].

Analyses of microcystine concentrations in seston were carried out by high-performance liquid chromatography in the Institute of Limnology, Austrian Academy of Sciences, following certified procedures and standards ISO 20179:2005. Seston samples were concentrated on fibrous glass filters GF/A immediately after water sampling. Filters were dried at 50°C and stored frozen in a refrigerator at –18°C. For the analysis of a net sample, ~20 l of water from Lake Nero were filtered (st. 3) and concentrated to 250 ml using a planktonic net from capron screen no. 77. Microcystine extraction was carried out in 75% methanol [20]. Microcystine analysis was based on high-performance liquid chromatography HPLC with the use of a photodiode detector HPLC-DAD [24, 27]. Ultraviolet spectra were obtained from 200 to 300 nm, the spectra and the retention time were used to identify microcystines [27]. Microcystine standards were obtained from Cyanobiotech Center, GmbH (Germany). Additionally, a pure fraction of HPLC was sampled and analyzed by positive ion-mass-spectrometry (MALDI-TOF MS) [25]. To detect the difference between the mean values for two samples Student's t-test was used, assuming the normal distribution and statistically insignificant difference between the variances of the characteristics compared. When this condition was not met, rank Mann-Whitney U-test was used. To reveal linear

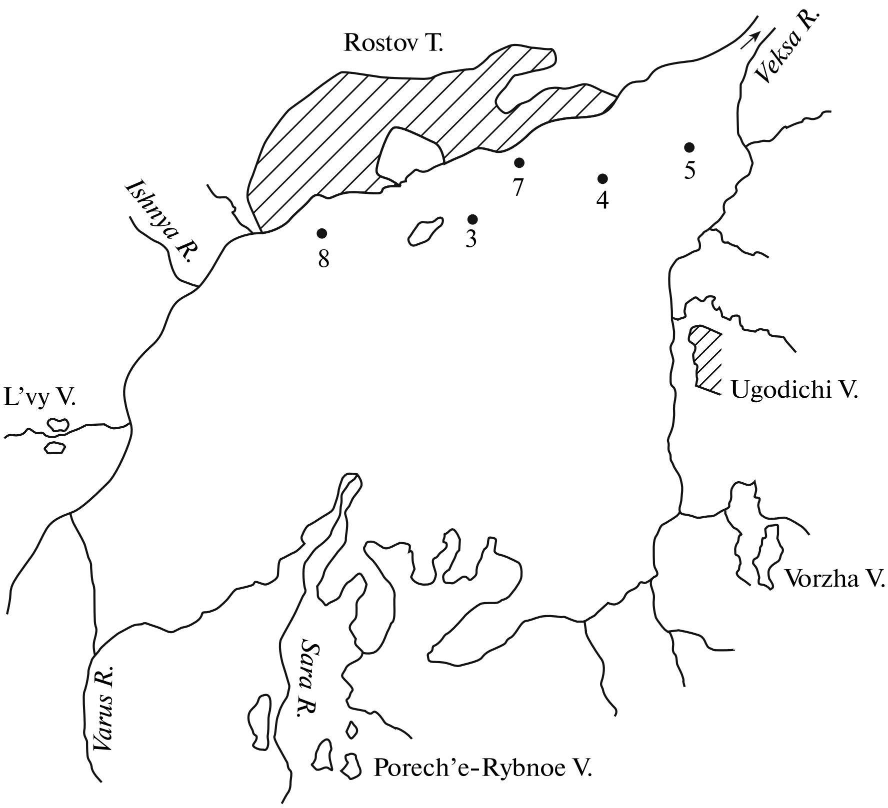

Fig. 1. Layout of stations in Lake Nero.

relationships between some parameters, correlation analysis with Pearson correlation coefficient was used [5].

RESULTS OF STUDIES

Changes in the abiotic and algological characteristics of Lake Nero over the ten-year study period were analyzed. A statistically significant decrease in the mean values of ammonium an and an increase in nitrate N, P_{min}, and P_{tot} were recorded in the periods of 1999–2004 and 2005–2007 (Table 1). A significant decrease was recorded in water transparency in the latter period against a rise in the lake level. The coefficients of variation decreased for all characteristics. Data on ammonium N concentrations for 2008 are close to those of 1999–2004; P_{min} concentration increased even greater relative to 2005–2007, while other characteristics fall within the range of values typical of 2005–2007 (Table 1).

Changes in the characteristics of phytoplankton development in the study periods were also statistically significant and correlated with change in abiotic characteristics (Table 2). The drop in chlorophyll "a" concentration observed in 1999–2004 [11, 12] changed by a new rise. In the recent 3–4 years, both the abundance and biomass of phytoplankton showed a reliable increase (Table 2). The reliable increase in the share of blue-green algae was mostly due to an increase in the biomass of algae of the type S_1 from 35 to 62%. The change had the form of a jump-like increase in the share of planktotrichaetic complex in the total phytoplankton biomass since 2005.

After describing long-term changes, we will consider in more detail the dynamics of characteristics in 2008. Phytoplankton biomass in the summer of 2008 showed high mean values (36.5 mg/l) with a gradual increase from June to August and an abrupt rise in September (Fig. 2). Cyanophyta dominated in the community (32–86% of total phytoplankton biomass), the major portion was algae of planktotrichaetic complex, accounting for 29.1–64.4% of the total biomass (Limnothrix redekei 22.2–30.2%, Pseudanabaena limnetica 3.7–19.2, Planktothrix agardhii 2–17.3, and Lyngbya limnetica Lemm. 0.4–1.3%). Limnothrix redekei dominated in the summer plankton of 2008; the abundance of this form could be higher. The difficulty of accurate identification of fine filamentous blue-green algae by light microscopy is well-known, and the stability of phenotypic characteristics and their use in taxonomy are the subject of considerable discussion [17, 33]. The authors of this paper faced problems with the identification of a subdominant of community Pseudanabaena limnetica. In addition to the form corresponding to the diagnosis, referred to

Table 1. Concentration of biogenic substances, mg/l; Secchi disk transparency S, m; depth H, m; the ratio of the euphotic zone depth Z_{eu} to mixing depth Z_{mix} in Lake Nero in year-to-year dynamics (here and in Table 2, the top numbers are the mean value ± the error of the mean; the bottom numbers are variation limits; the number in parentheses is variation coefficient, %)

Period, years (June–September)	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	$P\text{-}PO_4^{3-}$	P_{tot}	S	H	Z_{eu}/Z_{mix}
1999–2004	0.166 ± 0.02 0.02–0.29 (77)	0.056 ± 0.006 0.0003–0.17 (63)	0.015 ± 0.003 0.0001–0.07 (127)	0.089 ± 0.02 0.017–0.176 (65)	0.400 ± 0.01 0.2–0.6 (23)	1.37 ± 0.4 1.08–1.88 (15)	0.87 ± 0.05 0.55–1.64 (26)
2005–2007	0.090 ± 0.01 0.03–0.21 (52)	0.130 ± 0.016 0.045–0.24 (39)	0.030–0.009 0.004–0.085 (95)	0.130 ± 0.012 0.06–0.19 (31)	0.6330 ± 0.02 0.28–0.5 (17)	1.48 ± 0.3 1.30–1.68 (7)	0.67 ± 0.04 0.6–1.1 (24)
2008	0.155 ± 0.04 0.04–0.36 (72)	0.103 ± 0.023 0.04–0.18 (54)	0.060 ± 0.02 0.01–0.15 (82)	0.122 ± 0.024 0.08–0.24 (49)	0.350 ± 0.02 0.3–0.4 (14)	1.51 ± 0.2 1.43–1.56 (3)	0.67 ± 0.05 0.6–0.9 (14)

Table 2. Chlorophyll "a" concentration, μg/l; abundance N, million cell/l; total phytoplankton biomass B_{tot}, mg/l; share in the total blue-green biomass, B_{bg}, %, in planktotrichaetic phytoplankton B_{S1}, %, in Lake Nero in year-to-year dynamics

Period, years (June–September)	Chlorophyll "a"	N	B_{tot}	B_{bg}	B_{S1}
1999–2004	63.5 ± 4.8 9.7–181.8 (55)	584 ± 61.2 33.04–1697.9 (70)	18.6 ± 1.5 1.68–38.6 (54)	57.0 ± 3.6 5–93.5 (42)	34.8 ± 2.8 0.8–62.5 (52)
2005–2007	109.7 ± 10 50.2–170.5 (38)	832.9 ± 140.2 282.1–1636.6 (58)	30.7 ± 5.3 7.2–63 (60)	76.0 ± 4.0 54–93 (18)	62.3 ± 4.6 41.4–89 (26)
2008	96.7 ± 11 50–174.4 (40)	1024.3 ± 109.5 696.3–1339.8 (26)	36.5 ± 6.1 23.7–61.4 (36)	72.5 ± 6.9 32.6–95.6 (29)	60.8 ± 7.3 28.6–89 (36)

this species was also the form with weakly pronounced constrictions between cells and opalescent granulations within cells, which is very similar to Limnothrix redekei, but without gas vacuoles. The question arises of whether these forms belong to the same species. A surprising phenomenon was observed in the study where the quality of water supplied to Southern Water Treatment Station of Yaroslavl from the Kotorosl River was determined inlet to the . In the fixed samples taken at the conduit pipe, the majority of phytoplankton was represented by typical forms of Limnothrix redekei, while the form at the outlet was referred by

Table 3. Total concentration of microcystins RR and LR in seston and algal biomass $B_{microcystis}$ is the Microcystis genus biomass, $B_{planktothrix\text{-}agardhii}$ is the biomass of Planktothrix-agardhii

Date	Station	Microcystines (RR + LR), μg/l	B_{tot}	B_{bg}	$B_{microcystis}$	$B_{planktothrix\text{-}agardhii}$
			μg/l			
Sep. 13, 2008	3	12.91*	1393.00*	819.00*	16.93*	27.71*
June 19, 2008	5	1.13	30.90	10.10	0.34	0.83
Sep. 13, 2008	3	1.70	56.30	33.20	1.56	4.90
Sep. 13, 2008	4	0.62	44.64	28.92	0.71	5.09
Sep. 13, 2008	5	0.70	61.42	37.85	0.81	5.90
July 28, 2008	5	1.92	37.98	32.70	2.01	6.53
Aug. 20, 2008	5	1.70	48.28	33.38	1.77	3.29
June 19, 2008	3, 4, 7, 8	0.63	23.68	7.71	0.14	0.64
July 28, 2008	3, 4, 7, 8	1.07	29.05	25.00	0.79	5.02
Aug. 20, 2008	3, 4, 7, 8	0.55	36.91	25.53	0.85	2.53

* the values of characteristics in a net sample.

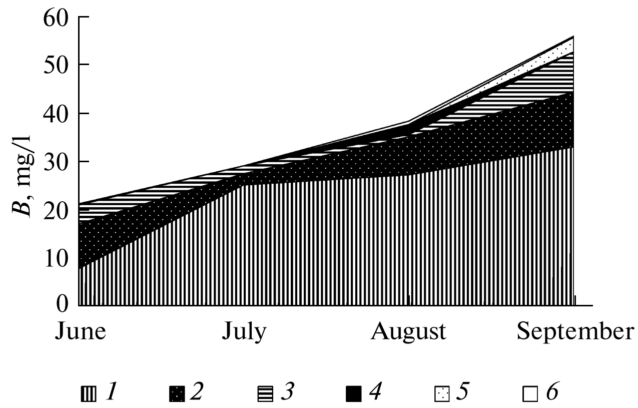

Fig. 2. Phytoplankton development dynamics in Lake Nero in summer 2008. (*1*) Blue-green, (*2*) diatoms, (*3*) green, (*4*) cryptophytes, (*5*) dinophytes, (*6*) euglenic algae.

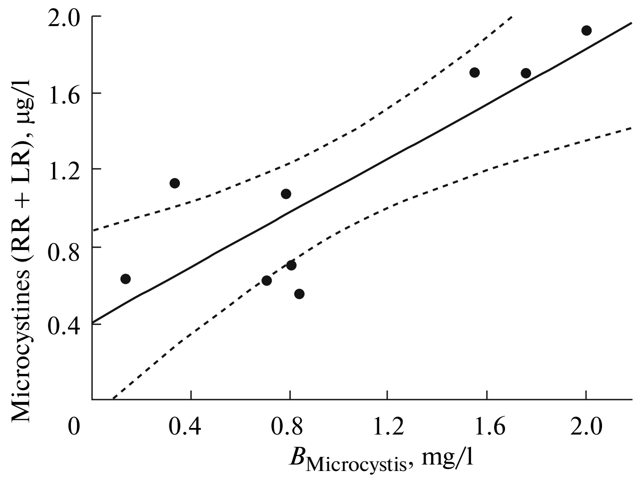

Fig. 3. Correlation between total concentration of microcystines LR + RR in seston and $B_{\text{Microcystis}}$ in Lake Nero.

the authors to Pseudanabaena limnetica with granulations. The conduit pipe is 2.5 km in length, and water residence time in it is ~1 h. It seems likely that changes in the physical conditions, including light intensity, caused changes in trichome morphology. O.A. Lyashenko, who was working at Lake Nero in the 1980s, also suggested that this is the same one species. At the same time, it is still possible that these are several species with similar morphology and life strategies; the only fact beyond question is their belonging to the planktotrichaetic complex S_1, according to functional classification [30]. Planktothrix agardhii (type S_1) developed at the level of subdominant (2.7–17.3%); from June to September its biomass was 0.84–6.5, that in the net sample was 27.7 mg/l (Table 3). Nitrogen-fixing blue-green alga Aphanizomenon flos-aquae f. gracile (Lemm.) Elenk. (4–13.5%) subdominated in the summer of 2008; the role of species from Lyngbya genus was insignificant.

The share of species of Microcystis genus in the total phytoplankton biomass was relatively small (0.5–5.7% of total phytoplankton biomass), varying in the seasonal dynamics from 0.14 to 3.45 mg/l; the largest amount was recorded in June at station 4. In September (a period of more thorough study of microcystine concentrations), the biomass at different stations varied from 0.71 to 1.56 mg/l. Dominating among the representatives of this genus in this period, on the average over three stations, were Microcystis wesenbergii (Komarek) (31.2%), Microcystis smithii Komarek et Anagnostidis (19.2), Microcystis viridis (A. Braun in Rabenhorst) Lemmermann (19.3), Microcystis aeruginosa Kutz. (16), and Microcystis flos-aquae (Wittrock) Kirchner (14%). The latter two alga forms dominated in the summer.

Among the representatives of other alga divisions, the share of Bacillariophyta in biomass was considerable (with a maximum of 39.3% in June and a minimum of 8% in July) (Fig. 3). Alga Aulacoseira ambigua (Grun.) Sim. (7.5–21.4%) dominated in biomass; benthic alga Fragilaria constuens var. binodis (Ehr.) Grun. formed an appreciable amount ins some samples (up to 5.3%). The contribution of Chlorophyta was low (2.6–16%) with higher values in spring and autumn (Fig. 1). The most abundant was the alga Scenedesmus communis Hegewald.—up to 10.3% in September; in June, Pediastrum duplex Meyen var. duplex developed to 6.4% of total phytoplankton biomass. The contribution of other alga divisions was insignificant; a rise in Cryptophyta in August and Dinophyta in September can be mentioned. Overall, the dynamics of phytoplankton development in the study period of 2008 corresponded to the trend of the most recent years of observations—an increase in quantitative characteristics and the predominance of planktotrichaetic type in phytoplankton structure.

The amount of microcystines in the analyzed samples was small (Table 3). The supposed microcystines were identified at the retention time of 14.3 and 19.7 min and showed high agreement in the comparison with standards MC-RR (14.3 min) and MC-LR (19.7 min). Relative to the standard curves constructed for MC-RR and MC-LR, the concentrations of these cyanotoxins in the analyzed extract were 198.33 and 124.38 ng, respectively. Converted to 1 l of water, this yielded in total 12.9 µg/l of net sample of phytoplankton. MALDI-TOF MS analysis of the identified peaks of HPLC fractions by the retention time 14.3 and 19.7 min showed the presence of M + H 1038 (MC-RR) and M + H 995 (MC-LR), respectively, thus confirming the results of high-performance liquid chromatography.

Dependences between the observed toxin concentrations and the biomasses of algae of different taxonomic levels (Table 3). In the calculation of correlation coefficient, the values for concentrated net samples were excluded, since, as outliers, they disturbed the homogeneity of the sample and unreasonably increased the correlation. The correlations with total alga biomass, the biomass of blue-green algae, and the biomass of Planktothrix agardhii was insignificant. A statistically significant correlation ($r = 0.84$, $p < 0.05$) was found to exist between the total concentrations of microcystines and the biomass of Microcystis genus (Fig. 2).

DISCUSSION OF RESULTS

A characteristic feature of phytoplanktonocenosis of Lake Nero from the late 1980s to 2004 was the stability of mean quantitative characteristics [1, 6, 18]. In terms of quantitative development and the occurrence of forms within the year, both in 1987–1989 and in 1999–2004, the community was determined by Limnothrix redekei, Pseudanabaena limnetica, Aphanizomenon flos-aquae f. gracile, Aulacoseira ambigua, Scenedesmus communis, and the species of Microcystis and Anabaena genera. In the observations of 1999–2004, the authors revealed a simplification of phytoplankton structure through the replacement form the dominating positions of green algae of genera Pediastrum, Coelastrum, Golenkinia, Scenedesmus, which were typical of the lake in 1987–1989 [18]. The studies of 2005–2007 showed a decrease in the relative role of species from general Microcystis, Aphanizomenon, Anabaena as compared with the late 1980s and the period of 1999–2004 [9].

A statistically significant increase in the concentration of biogenic substances (except for ammonium N) and a decrease in the size of euphotic zone in 2005–2007 suggest changes in abiotic characteristics of phytoplankton development in the lake (Table 1). The increase in phytoplankton abundance and biomass and chlorophyll "a" concentration in seston in this period was accompanied by an abrupt increase in the contribution of planktotrichaetic forms Limnothrix redekei, Pseudanabaena limnetica, which were typical of the lake, and Planktothrix agardhii, which has been classified as an associated form.

The year of 2005 can be regarded as a turning point in the year-to-year dynamics of abiotic characteristics and phytoplankton structure in Lake Nero. The trigger factor for this turn could be jams of higher aquatic plants driven by wind into the near-dam part of the Veksa River in the spring and summer of 2005 and 2006. The dam at the outlet from the lake was closed to prevent the mass of higher aquatic plants from entering the Veksa River and next the Kotorosl River. A sanitary gate alone was in operation, and water level in the lake was maintained at the level 10–20 cm above the ordinary level.

The same measures were taken in the subsequent years of observations (2007–2008). The flowage through the lake decreased, and the retaining capacity of the lake with respect to biogenic elements increased, as could be seen from an abrupt increase in N and P concentrations (Table 1). It is known from the literature that their high concentrations and a decrease in flowage are factors facilitating the development of planktotrichaetic complex of blue-green algae [30]. Accordingly, the decrease in water exchange and an increase in biogenic substance concentrations in the latest observation period could contribute to an abrupt increase in the share of algae of type S_1 in the total phytoplankton biomass. The exact factor that has caused the restructuring of the community is difficult to establish in this study. However, it is safe to say that the double increase in the share of planktotrichaetic complex in total algae biomass can be regarded as a transformation of phytoplankton structure in a "catastrophic" manner.

In the self-organization theory, a catastrophe is a jump-like change caused by changes in the ambient conditions in an open nonequilibrium system [34]. Characteristic features of such "catastrophic" change are considered for oscillatoria (planktotrichaetic) lakes in [31]. Data on 55 lakes in Germany (not deeper than 3 m) were analyzed with the aim to determine relationships between biogenic elements, light conditions, and the domination of Oscillatoriaceae. The data were collected in July–August, and several years of studies were considered for some lakes, so 118 observation series were analyzed. The relative abundance of Oscillatoriaceae was evaluated as the percentage in the total phytoplankton biomass. The authors of [31] used observational data to construct a model, which showed that the mechanism of competitive advantage of planktotrichaetic complex relative to other algal groups realizes itself through the creation of unfavorable light conditions in the process of mass development of representatives of the complex. Other photophilous alga groups, such as green chlorococci and blue-green from genera Aphanizomenon, Microcystis, Anabaena, lose the competition for light resource. At the same time, the complex itself is well adapted to low light intensity because of both the filamentous structure of the body (a high cell area-to-volume ratio) and the presence of an effective mechanism of "interception" of solar energy because of chromatic adaptation [16]. The result is that, the concentrations of P being the same and the biomasses being similar, the shadowing effect per unit P or biomass in lakes with the predominance of phytoplankton of planktotrichaetic type is much higher than in lakes with the predominance of other algal groups [31].

A correlation between a decrease in light intensity and the predominance of planktotrichaetic complex in Lake Nero has been established in [18]. Observations in the well-studied Veluwemeer Lake (Netherlands) also showed that low light intensity is the main factor determining the competitive advantage of filamentous blue-green algae in the structure of its phytoplankton [28]. An abrupt decrease in the abundance of blue-green algae in the lake was achieved by reducing the input of P. In the shallow Veluwemeer Lake, the share of Planktothrix genus in the total alga biomass dropped below 50% at the values of $P_{tot} \sim 0.12$ mg/l and $Z_{eu}/Z_{mix} = 0.5$. In their studies, the authors observed a reverse tendency toward an increase in the share of planktotrichaetic alga type in phytoplankton structure at P_{tot} concentration equal to 0.13 mg/l and $Z_{eu}/Z_{mix} = 0.67$. The similarity of the values of both P_{tot} and the light intensity in observations at Veluwemeer Lake and in the authors' studies may demonstrate the significance of these characteristics as the factors of jump-like change in phytoplankton structure of shallow, highly eutrophic water bodies.

The summer dynamics of phytoplankton in 2008 corresponded to the character of seasonal succession of the most recent years of observations with the predominance of planktotrichaetic phytoplankton (Table 2) and the development of typical subdominants of summer phytoplankton: Aphanizomenon flos-aquae f. gracile and Aulacoseira ambigua [9, 18].

Observations of 2006–2007 demonstrated a genotoxic effect of Lake Nero water and a coincidence of its manifestation with the seasonal increase in blue-green algae [7]. The authors first associated the manifestation of the genotoxic effect of Lake Nero water with the increase in the development of Planktothrix agardhii, whose potential toxicity owing to microcystine production is well known [25]. Correlation analysis showed significant correlation between microcyston concentrations and the biomass of species of the Microcystis genus, and the absence of such correlation with the biomasses of Planktothrix agardhii, blue-green algae, and total phytoplankton biomass.

Accordingly, we can suppose that the species of Microcystis genus in Lake Nero are toxic. The studies published earlier mentioned a considerable drop in the contribution of representatives of this genus to the total phytoplankton biomass [18]. In the recent 5 years, their share in summer phytoplankton decreased further from 7–15.3 (1999–2004) to 2.4–4.6% (2005–2008), estimated by maximal annual values. Nevertheless, in the presence of surging, hazardous amounts of algae of Microcystis genus can accumulate in Lake Nero, which is connected with the hydrographic river network serving as a source of drinking water supply to settlements and towns in Yaroslavl province. Considering the wide occurrence of these algae and their mass development up to blooming in rivers, lakes, and reservoirs used for recreation and drinking water supply, the presence of toxins of blue-green algae and the genotypes responsible for their production should become a subject of deeper analysis in both biological studies and the practice of water supply services.

CONCLUSIONS

Considerable changes are found to take place since 2005 in Lake Nero ecosystem in terms of some abiotic and algological characteristics. These included an increase in N and P concentrations, deterioration of subsurface light regime, and the associated increase in the quantitative characteristics of lake phytoplankton.

The structure of planktonic algacenosis was found to radically change. A significant increase was recorded in the share of blue-green algae due to an increase in the biomass of planktotrichaetic complex in a "catastrophic" manner. The results of analysis of summer phytoplankton in 2008 confirm the structural changes in phytoplanktonocenosis in 2005–2007.

The concentrations of natural toxins of blue-green algae—microcystines LR and RR—were determined for Lake Nero for the first time. A statistically significant correlation was found to exist between the total concentrations of microcystines and the biomass of species of Microcystis genus, suggesting the possible toxicity of representatives of this species in Lake Nero.

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