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## Phytoplankton of the transboundary River Viliya (Neris): community structure and toxic cyanobacterial blooms

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**Abstract.** The 2011–2012 data on the hydrochemical mode of the transboundary River Viliya (Neris) and two of its tributaries in the territory of the Republic of Belarus and on structure of the phytoplankton community therein are presented. High phytoplankton biomass was determined in the summer period. For the first time, the presence of microcystin synthase genes (*mcyE*) was detected in the River Viliya (Neris), and four variants of microcystins were determined employing the MALDI-TOF method. Similarities in the species composition (including toxic species) of cyanobacteria from the River Viliya (Neris) and from the Curonian Lagoon of the Baltic Sea were noted. It can be assumed that the phytoplankton community structure of rivers can influence algal community formation in the downstream areas as well as in the coastal estuarine lagoons that eventually receive water from these rivers.

**Keywords:** biomass; cyanobacteria species composition; toxic species; microcystins

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## INTRODUCTION

The Baltic Sea, the only inland sea that is entirely in Europe, is one of the largest brackish-water basins in the world. The combination of a large catchment area, a small volume of water with limited exchange and high human population density on the shores makes the Baltic Sea extremely sensitive to nutrient enrichment (HELCOM 2009). As a result, eutrophication is the major problem in the Baltic Sea. Since the 1900s, the oligotrophic clear-water environment in the Baltic Sea has turned into the eutrophic marine environment. About 75% of the nitrogen load and at least 95% of the phosphorus load enter the Bal-

tic Sea via rivers or as direct waterborne discharges (HELCOM 2007). However, rivers bring macronutrients not only from the drainage basin, but also from the biological communities along their entire length, which, in accordance with the principles of the river continuum (Vannote *et al.* 1980; Lampert, Sommer 2007), can contribute to the species diversity of the downstream reaches, including the Baltic Sea itself. The influence of river flow is especially evident in semi-enclosed coastal water bodies with slow water-exchange such as the Curonian Lagoon (Vybernaite-Lubiene *et al.* 2018). It is not surprising that recurrent harmful cyanobacterial blooms are observed regularly in the Lagoon, including those of the

potentially toxic cyanobacteria (Schmidt-Ries 1940; Krevs *et al.* 2007; Paldavičienė *et al.* 2009; Belykh *et al.* 2013; Bresciani *et al.* 2012, 2014; Šulčius *et al.* 2015; Bartoli *et al.* 2018). However, toxic blooms can be formed not only in the Lagoon itself, but also in the rivers carrying their water into the Lagoon. The river basin approach to the ecological state monitoring and reduction of nutrient load in river drainage basins (EC – European Community 2000) also highlights the relevance of studying transboundary rivers.

The River Viliya (Neris) belongs to the Baltic Sea basin and is the largest tributary of the Neman River, which, in turn, is one of the sources of the Baltic Sea pollution (HELCOM 2013). In addition, the fact that the Viliya (Neris) River is the only waterway in the territory of Belarus used by *Salmonidae* for spawning (Pliuta *et al.* 2007) heightens the need for its water quality monitoring. Finally, the River Viliya (Neris) is the main source of water for the cooling system and a receiver of technical water from the Belarusian Nuclear Power Plant (Validation of investments... 2010), which is being built in close vicinity of the Lithuanian border and its capital Vilnius. The potential influx of significant amounts of major nutrients (especially, of mineral phosphorus) with wastewater from the Belarusian Nuclear Power Plant together with thermal pollution may further aggravate the trophic state of the river (Validation of investments... 2010).

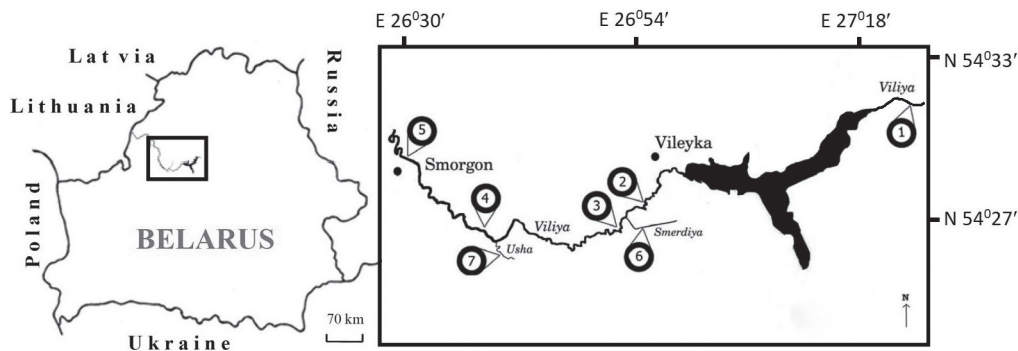
In this paper we present data on the phytoplankton community of the River Viliya (Neris), which were collected throughout the 2011–2012 vegetation seasons, well before the start of significantly increased industrialization and excessive urbanization of the river.

## MATERIALS AND METHODS

### Study area

The study was conducted in the River Viliya (Neris) and in two tributaries: the Smerdiya and Usha Rivers. Both of these tributaries have fish farms located on their watershed (Fig. 1). To study the ecological state of the River Viliya (Neris), five stations were selected (Table 1) in the River Viliya (Neris), and two additional stations (6 and 7) in tributaries of the River Viliya, the Smerdiya and Usha Rivers.

The River Viliya (Lithuanian name Neris) is a right-bank tributary of the River Neman in Belarus and Lithuania. Its length is 498 km, including 264 km in Belarus. The river is characterized by intense floods (which accounts for 45% of the annual flow) in spring, by mostly standing water during the summer low water period, which is annually disrupted by rain floods, and by stable water during the winter low water period. The Vileyski Reservoir was built on the



**Fig. 1** Location of sampling stations within the Republic of Belarus

**Table 1** The selected stations in the River Viliya (Neris)

| Stations | Latitude       | Longitude      | Sampling location   | Annual river discharge ( $m^3s^{-1}$ ) |
|----------|----------------|----------------|---|--|
| 1        | 54°33'50.31" N | 27°23'44.11" E | River Viliya (Neris) near village Steshitsa                           | 7.9*                                   |
| 2        | 54°27'15.01" N | 26°54'3.49" E  | River Viliya (Neris) near village Glinnoe                             | 28.1*                                  |
| 3        | 54°25'42.49" N | 26°50'54.57" E | River Viliya (Neris) downstream from the inflow of the River Smerdiya | 28.1*                                  |
| 4        | 54°25'23.11" N | 26°35'21.11" E | River Viliya (Neris) downstream from the inflow of the River Usha     | 28.1*                                  |
| 5        | 54°31'54.48" N | 26°23'53.82" E | River Viliya (Neris) downstream from Smorgon city                     | 79.6*                                  |
| 6        | 54°25'59.89" N | 26°51'3.98" E  | River Smerdiya upstream from the inflow to the River Viliya (Neris)   | 0.5**                                  |
| 7        | 54°24'55.27" N | 26°36'24.72" E | River Usha upstream from the inflow to the River Viliya (Neris)       | 6.01*                                  |

\*According to Pashkou *et al.* (2007)

\*\*According to the data from the Fish Industry Institute of Belarus.

River 70 km downstream of Minsk in 1973–1975. After the Reservoir had been put into operation, the level and flow regime below the dam in Belarus became dependent on the operation of the Reservoir. The catchment area of the River Viliya is 25.1 thousand km<sup>2</sup> (including 11 thousand km<sup>2</sup> in Belarus); 32% of which is covered with forest, and there are many lakes on the right bank of the River. The Viliya River is the power source of the Vileyka–Minsk water system (Pashkou *et al.* 2007). The River is to become the main source and receiver of technical water from the Belarusian Nuclear Power Plant (Validation of investments... 2010).

The River Smerdiya (length 14 km, catchment area 31 km<sup>2</sup>) and the River Usha (length 75 km, catchment area 780 km<sup>2</sup>) are left tributaries of the River Viliya (Neris). In the drainage basin of the River Smerdiya, there is a fish farm Vileyka with ponds (193 ha area). The fish farm Gritsevo, with ponds covering an area of 149 ha, is located in the River Usha drainage basin.

### Field sampling and methods

Samples for phytoplankton and water chemistry analysis were collected in April, July and September 2011 and in May, July and November 2012 monthly from the surface water layer (0–1 m) at each station using a tube sampler (volume 3 L). A total of 42 samples were collected for each parameter (phytoplankton and water chemistry).

Water quality parameters were analysed following the methods described in (Semenov 1977; Wetzel, Likens 2003). Mineral nitrogen was determined as the sum of ammonium, nitrate and nitrite nitrogen. Mineral forms of nitrogen and phosphate phosphorus were analysed employing the photo-colorimetric method. The content of organic matter was assessed by potassium permanganate oxidation. Dissolved oxygen was measured using the standard Winkler method. Water temperature was measured using a mercury deep-water thermometer with a scale resolution of 0.1°C.

Water samples (0.5 L) for phytoplankton analysis were fixed with Utermöhl's solution (Mikheyeva 1989). Utermöhl's technique is widely accepted as the best method for phytoplankton studies (Taylor *et al.* 1986). The samples were sedimented in total darkness at least for one week. Then the samples were concentrated up to 100–150 ml by pouring off all excess water using a thin silicon or rubber siphon covered with a two-layer 77 µm-size mesh plankton net. The resulting concentrate was poured into plankton bottles and allowed no less than 2–3 days to settle. Then the extra water was drawn out using a medical syringe with a thin vinyl tubule at its end. Finally, the actual sample volume was 15–30 ml depending

on the sediment thickness. The light Zeiss Axiolab microscope was used to analyse phytoplankton samples. A Fuchs-Rosenthal chamber with a volume of 3.2 mm<sup>3</sup> was used to count small (< 100 µm) phytoplankton organisms. The larger species such as *Ceratium*, *Asterionella*, *Melosira*, *Aulacoseira*, *Fragilaria*, *Microcystis*, *Coelosphaerium*, *Anabaena* and some other were counted using a 1 ml chamber while large colonial organisms were counted using the Bogorov one. Phytoplankton biomass was calculated using Kiselev's method of true volumes (Kiselev 1969). According to this technique, volumes were calculated for each species by applying a solid geometric shape (ellipsoid, sphere, rod, cone, etc.) most closely matching the shape of the cells or organisms (Hillebrand *et al.* 1999; Mikheyeva 1999). The total biomass of phytoplankton was calculated by summing up the calculated weights of each organism.

Samples for molecular analysis were collected in May and July 2012 using an Apstein net and were fixed with ethyl alcohol (80% final concentration). The samples were used for determination of *mcvE* gene responsible for the synthesis of microcystins (MCs). DNA from the samples was extracted using "Genomic DNA Purification Kit" (Thermo Scientific, USA). Amplification was done using primers hepF and hepR (Jungblut, Neilan 2006). The use of AMT domain as the target genetic marker showed its effectiveness in identifying toxigenic cyanobacteria (Belykh *et al.* 2011; Mikheyeva *et al.* 2012). DNA of a MCs-producing cyanobacterium strain *Microcystis aeruginosa* CALU 972 was used as a control. DNA isolation was performed using the phenol-chloroform method (Belykh *et al.* 2011). Polymerase chain reaction (PCR), cloning and sequencing were performed as described in the article by Belykh *et al.* (2011). The unique nucleotide sequences were deposited in GenBank under numbers KC900837-KC900842, amino acid — under numbers AGO06701-AGO06706.

Sequences of *mcvE* gene were edited using the BioEdit (v. 7.0.9) (Hall 1999). Search for immediate neighbours was conducted using BLAST analysis (E-value threshold – 1e-6). The phylogenetic tree was built using the MEGA 7 software and applying the neighbour joining method (NJ) (Kumar *et al.* 2016). The tree of amino acid sequences was constructed using the maximum likelihood method and bootstrap analysis.

Samples for MCs analysis were collected in July 2012 and prepared for analysis as described in the article by Belykh *et al.* (2011) and Mikheyeva *et al.* (2012). The detection and identification of MCs was performed from field phytoplankton samples collected on GF/F filters by the MALDI-TOF method (matrix activated laser desorption/ionization);  $\alpha$ -Cyano-4-hydroxycinnamic acid was used as a matrix. MCs

were detected using the mode of the positive ions registration (the working wavelength of the laser is 355 nm). The mass range is 500–3500 Da.

## RESULTS

### Water chemistry

No clear trends were found in the spatial dynamics of nitrogen mineral forms. The observed changes (Table 2) were likely caused by local sources of pollution along the river gradient. Almost at all sites, nitrogen concentrations were found to be higher in spring than in summer and autumn, when biological communities of the river are most actively involved in self-purification processes. Comparison of total mineral nitrogen concentrations at the river station No 1 with its content at other stations indicated that the change in mineral nitrogen downstream was comparatively small and did not exceed 30%. At individual river stations (i.e. No 2), the seasonal mean values exceeded 0.8 mg L<sup>-1</sup>, and in spring exceeded 1.8 mg L<sup>-1</sup>.

The dynamics of seasonal mean values (Table 2) indicated a weak trend of mineral phosphorus accumulation downstream. Low concentrations of mineral phosphorus at the river station No 2 located below the Reservoir can be explained by the fact that it is intensively consumed by aquatic organisms and is affected by sedimentation in the Reservoir.

During the study period, the content of organic matter remained almost unchanged throughout the river section.

### Phytoplankton composition and biomass

A total of 106 taxa of the species or lower rank were identified in the phytoplankton of the River Viliya (Neris), and additional 59 taxa were found in its two tributaries (Table 3). The number of taxa at different cross-sections varied slightly from 46 to 52. In the River Viliya (Neris), the largest number of species (38 taxa) belonged to Chlorophyta. Second in numbers were diatoms (22 taxa), and third were cyanobacteria (22 taxa) (Table 3). The most common genera of diatoms found at all sampling stations were *Asterionella*, *Cocconeis*, *Cyclotella*, *Navicula*, *Nitzschia*, *Pinnularia*, *Stephanodiscus*, *Synedra*; Chlorophyta – *Ankistrodesmus*, *Chlorella*, *Didymocystis*, *Micractinium*, *Oocystis*, *Pediastrum*, *Scenedesmus*, *Chlamydomonas*, and *Phacotus*. The most common genera of Chrysophyceae were *Chromulina*, *Kephyrion*, *Ochromonas*, the most common of Cryptophyta were *Cryptomonas*, *Rhodomonas*, of Cyanobacteria – *Dolichospermum*, *Aphanizomenon*, *Gomphosphaeria*, *Lyngbya*, *Microcystis*, *Planktothrix*, *Pseudanabaena*; and the most common genus of Euglenophyceae was *Trachelomonas*.

The phytoplankton biomass of the River Viliya (Neris) varied among the river sections studied and

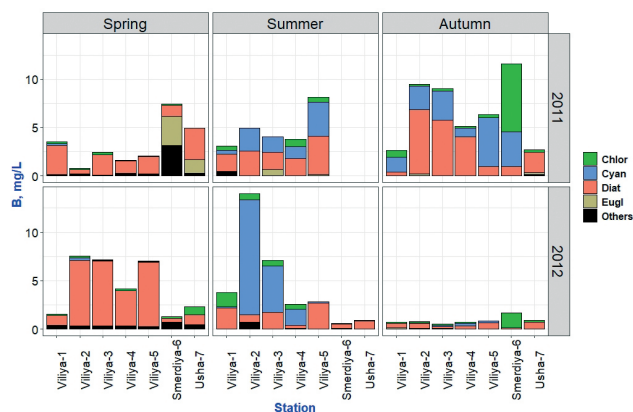
**Table 2** Environmental factors at different river stations of the River Viliya (Neris) and its tributaries (average (n = 6) ± SD for spring, summer and autumn 2011–2012)

|                                 | Units               | Stations    |             |             |             |             |             |             |
|---------------------------------|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                                 |                     | 1           | 2           | 3           | 4           | 5           | 6           | 7           |
| O <sub>2</sub>                  | mg L <sup>-1</sup>  | 8.9 ± 1.4   | 8.2 ± 1.9   | 8.3 ± 1     | 8.8 ± 1.1   | 9.3 ± 1.2   | 7.7 ± 1.1   | 8.0 ± 1.1   |
| Temperature                     | °C                  | 14.2 ± 5.8  | 14.8 ± 6.4  | 14.9 ± 5.8  | 15.1 ± 6.1  | 15.9 ± 6.4  | 15.2 ± 4.5  | 14.4 ± 4.9  |
| N-NH <sub>4</sub> <sup>+</sup>  | mgN L <sup>-1</sup> | 0.18 ± 0.1  | 0.39 ± 0.47 | 0.21 ± 0.06 | 0.34 ± 0.13 | 0.24 ± 0.16 | 0.30 ± 0.18 | 0.28 ± 0.11 |
| N-NO <sub>2</sub> <sup>-</sup>  | mgN L <sup>-1</sup> | 0.08 ± 0.09 | 0.01 ± 0.01 | 0.01 ± 0    | 0.07 ± 0.09 | 0.02 ± 0.04 | 0           | 0.11 ± 0.17 |
| N-NO <sub>3</sub> <sup>-</sup>  | mgN L <sup>-1</sup> | 0.43 ± 0.27 | 0.30 ± 0.23 | 0.29 ± 0.19 | 0.32 ± 0.24 | 0.48 ± 0.32 | 0.15 ± 0.11 | 0.54 ± 0.69 |
| mineral N                       | mg L <sup>-1</sup>  | 0.69 ± 0.3  | 0.69 ± 0.6  | 0.51 ± 0.23 | 0.73 ± 0.35 | 0.74 ± 0.32 | 0.46 ± 0.15 | 0.88 ± 0.64 |
| P-PO <sub>4</sub> <sup>3-</sup> | mgP L <sup>-1</sup> | 0.03 ± 0.02 | 0.01 ± 0.01 | 0.03 ± 0.03 | 0.04 ± 0.05 | 0.04 ± 0.03 | 0.03 ± 0.02 | 0.07 ± 0.04 |
| Organic matter                  | mgC L <sup>-1</sup> | 5.0 ± 1.9   | 4.8 ± 0.9   | 4.8 ± 0.6   | 5.1 ± 1.1   | 5.1 ± 0.9   | 5.4 ± 1.1   | 4.4 ± 1.1   |

**Table 3** The number of the phytoplankton taxa (Chlor – Chlorophyta, Chrys – Chrysophyceae, Crypt – Cryptophyta, Cyan – Cyanobacteria, Diat – diatom algae including Bacillariophyceae, Fragilariophyceae and Coscinodiscophyceae, Din – Dinophyta, Eugl – Euglenophyceae, Xant – Xanthophyceae) at the sampling stations

| Taxonomic groups | Stations |    |    |    |    |    |    | A total of taxa in River Viliya (Neris) | A total of taxa in tributaries |
|------------------|----------|----|----|----|----|----|----|---|--------------------------------|
|                  | 1        | 2  | 3  | 4  | 5  | 6  | 7  |   |                                |
| Diat             | 13       | 14 | 16 | 15 | 18 | 10 | 17 | 24                                      | 19                             |
| Chlor            | 22       | 14 | 15 | 17 | 12 | 13 | 8  | 38                                      | 17                             |
| Chrys            | 2        | 1  | 3  | 3  | 4  | 2  | 4  | 9                                       | 5                              |
| Crypt            | 4        | 3  | 2  | 4  | 2  | 3  | 2  | 7                                       | 3                              |
| Cyan             | 8        | 12 | 12 | 7  | 10 | 5  | 3  | 22                                      | 7                              |
| Din              | 1        | –  | –  | –  | –  | –  | –  | 1                                       | –                              |
| Eugl             | 2        | 2  | 2  | 2  | 1  | 5  | 4  | 4                                       | 8                              |
| Xant             | –        | –  | 1  | –  | –  | –  | –  | 1                                       | –                              |
| Total            | 52       | 46 | 51 | 48 | 47 | 38 | 38 | 106                                     | 59                             |

seasons (Fig. 2). The smallest seasonal variations in biomass, indicative of the relative phytoplankton community stability, were observed at the river station No 1 located upstream of the Reservoir. During both study years, a slight increase in biomass was noticeable at the station No 2 downstream of the Vileyka Reservoir. The biomass decreased slightly towards the river station No 4 and increased again downstream of the town of Smorgon at the station No 5, where the biomass was approximately twice higher than that at the river station No 1.



**Fig. 2** Changes in phytoplankton composition and biomass at sampling stations in the River Viliya (Neris) and its tributaries in 2011 and 2012 (Chlor – *Chlorophyta*, Cyan – *Cyanobacteria*, Diat – diatom algae including *Bacillariophyceae*, *Fragilariophyceae* and *Coscinodiscophyceae*, Eugl – *Euglenophyceae*, Others – algae including *Dinophyta*, *Chrysophyceae*, *Cryptophyta* and *Xanthophyceae*)

The two-year phytoplankton biomass is quite different, especially in spring and autumn seasons (Fig. 2), which can be explained by a considerable difference in temperature at the time of sampling in 2011 and 2012. The mean water temperatures at the time of sampling in spring and autumn in 2011 were 12.4 C° and 14.3 C° accordingly, while respective temperatures in 2012 were 16.3 C° and 5.6 C°. The total phytoplankton biomass significantly correlated

with water temperature (Spearman correlation coefficient = 0.60;  $p = 0.002$ ).

Diatoms that are typical inhabitants of lotic systems, were dominant at all river stations throughout the growth season and especially during spring (accounting for up to 94.3% of the phytoplankton biomass in spring 2012 at the station No 5) (Fig. 2). Cyanobacteria, which are typical indicators of a nutrient load in a watershed, also made a significant contribution to the phytoplankton biomass in summer and autumn (up to 84.4% and 80.4%, accordingly), indicating a high anthropogenic load in the River Viliya (Neris). In summer, the biomass of cyanobacteria at the station 2 downstream of the Vileyski Reservoir increased 3.7-fold in 2011, and 24.4-fold in 2012. Cyanobacteria became dominant downstream of the Vileyski Reservoir at the stations No 2 and No 3, and downstream of the town of Smorgon at the station No 5.

The biomass of phytoplankton fluctuated significantly in the tributaries among years (Fig. 2). However, the impact of tributaries on river plankton community was negligible due to the relatively small volume of water in the tributaries as compared to that in the River Viliya (Neris). The flow of the River Smediya to the River Viliya (Neris) actually stops in summer. During the study period, the biomass of phytoplankton in the River Usha was lower than in the River Viliya (Neris), indicating that this inflow water can only reduce the phytoplankton biomass in the main river bed.

### Potentially toxic species of Cyanobacteria

The high trophic state of the River Viliya (Neris) is indicated by the high phytoplankton biomass that reached 7.5 mg L<sup>-1</sup> as early as mid-May. However, no significant growth of cyanobacteria was recorded in that period and the cyanobacteria biomass reached up to 0.23 mg L<sup>-1</sup>. In July, the phytoplankton biomass

**Table 4** Composition (% in total biomass of phytoplankton) of cyanobacteria in the River Viliya (Neris) in May and July 2012

| Species  | Date        |              |
|--|-------------|--------------|
|  | 11 May 2012 | 06 July 2012 |
| <i>Aphanizomenon flos-aquae</i> (L.) Ralfs*  | –           | 69.88        |
| <i>Aphanothece clathrata</i> W. et G.S. West   | 0.31        | 4.33         |
| <i>Chroococcus minutus</i> (Kützing) Nägeli  | 0.5         | –            |
| <i>Cyanodictyon planctonicum</i> Meyer   | 0.12        | –            |
| <i>Dolichospermum</i> (Ralfs ex Bornet & Flahault) P. Wacklin, L.Hoffmann & J.Komárek sp.* | –           | 4.14         |
| <i>Gloeocapsa</i> (Kütz.) Hollerb. sp.   | –           | 0.69         |
| <i>Microcystis viridis</i> (A.Braun) Lemmermann*   | 1.07        | 4.39         |
| <i>Planktolyngbya limnetica</i> (Lemmermann) Komárková-Legnerová & Cronberg*               | 0.07        | 0.09         |
| <i>Planktothrix agardhii</i> (Gomont) Anagnostidis & Komárek*                              | 1.0         | –            |
| <i>Synechocystis</i> Näg. sp.  | –           | 1.55         |

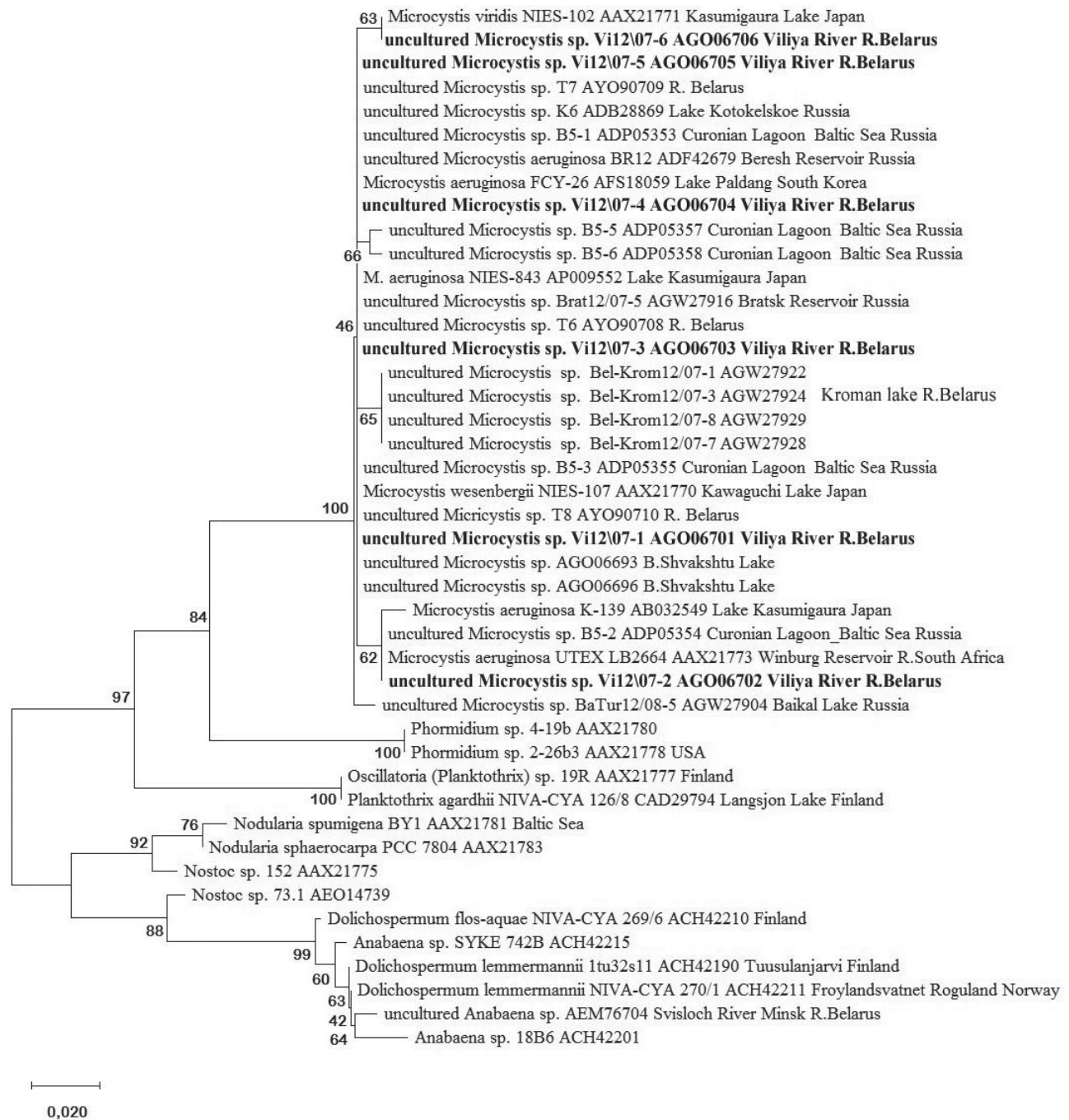
\*Species considered as potential toxin producers according to Bernard *et al.* (2017).

was still increasing (up to 13.98 mg L<sup>-1</sup>), and cyanobacteria became dominant (85% of phytoplankton biomass). The biomass of potentially toxic cyanobacteria was 10.97 mg L<sup>-1</sup> and comprised 78.5% of the total phytoplankton biomass. Changes in the biomass of cyanobacteria in the River Viliya (Neris) significantly correlated with water temperature (Spearman correlation coefficient = 0.43; *p* = 0.012).

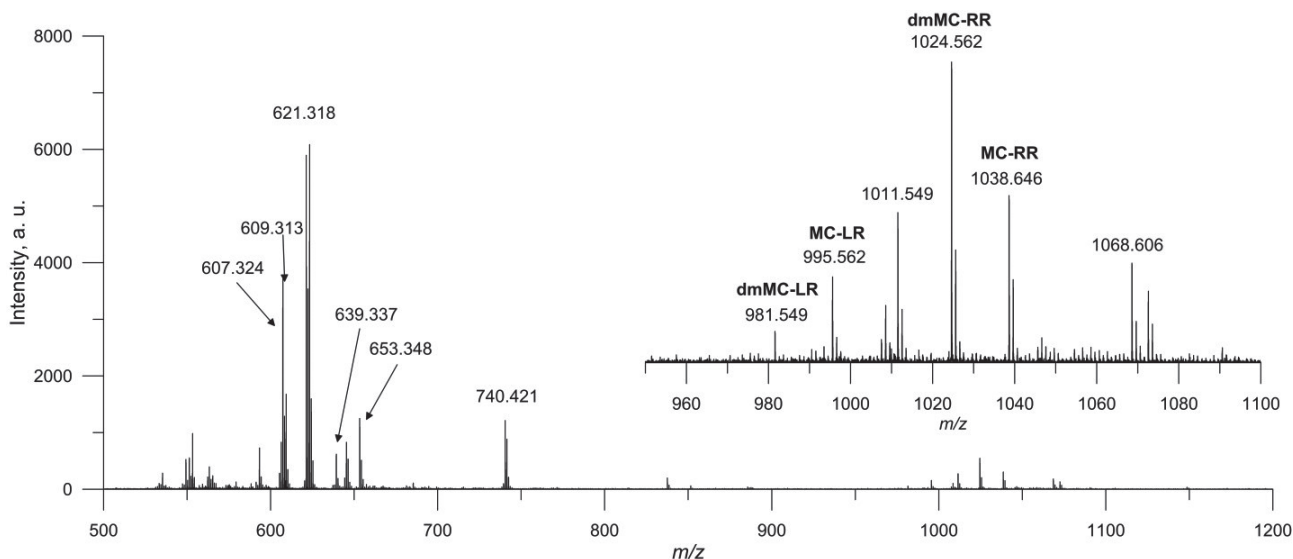
At that time, 10 representatives of cyanobacteria were recorded (Table 4). Five of the species, accord-

ing to Bernard et al. (2017), are considered to be potential toxin producers. *Aphanizomenon flos-aquae* dominated phytoplankton in summer accounting for up to 73.1 % of the total biomass (Table 4).

Thirty cloned sequences were obtained from the samples collected in July 2012 from the River Viliya (Neris) at the station No 2, six of which represented different genotypes bearing the 99 % similarity to one another (Fig. 3). The length of the nucleotide sequences was 470 bp.



**Fig. 3** The maximum-likelihood tree based on partial amino acid sequences of AMT domain. The sequences obtained in this study are marked in bold. Bootstrap values > 50 expressed as percentage of 1000 maximum-likelihood bootstrap replicates are given at the nodes. Scale bar corresponds to 2 substitutions per 100 amino acids



**Fig. 4** The MALDI-TOF mass spectrum of the samples collected from the River Viliya (Neris) on 6 July 2012

By performing MALDI-TOF analysis, we obtained peaks of  $[M+H]^+$  molecular ions (Fig. 4) and compared their characteristic ratios with literature data. Two most common microcystin variants, MC-LR and MC-RR, as well as the less common variant MC-WR, were detected in the mass spectrum. The molecular ion  $[M+H]^+$  with the mass value of  $m/z$  981.599 was found to correspond to the demethylated MC-LR microcystin variant (dmMC-LR).

## DISCUSSION

The River Viliya (Neris) is supposed to be exposed to a significant “enrichment” with mineral nitrogen throughout most of its length. Previously, nitrogen was shown to be one of the main nutrients contributing to water eutrophication (Rekolainen *et al.* 2005; Schindler 2006). The assessment of the River Viliya (Neris) water quality based on the concentration of mineral nitrogen showed that the station No 3 was least polluted. Taking into consideration the fact that the station No 5 was located downstream of the Smorgon town, which is not far from the border with the Republic of Lithuania, we assumed that the quality of the River Viliya (Neris) water flowing from the territory of the Republic of Belarus to the neighbouring country is similar to that of the water in the river stretch from the Vileyski Reservoir downstream. The analysis of phosphorus dynamics in water is complicated due to the high rate of mineral phosphorus turnover in the ecosystem. A small accumulation of mineral phosphorus at the station No 2 demonstrated an incomplete uptake of phosphorus by primary producers.

As environmental changes cause rapid structural and functional reorganization of aquatic communities, the biological assessment of water quality provides dynamic evaluation. Disruption of homeostasis in a water

body can lead to scenarios, where aquatic organisms themselves become additional contamination. And even if the input of chemical pollutants into the water body discontinues, the water body may already shift to a different trophic level, and it will take a long time and additional effort to restore the previous state of the water body. Phytoplankton community is one of the most important components in aquatic monitoring as it is mainly its abundance and structure that determine the trophic status of water bodies and water quality therein (Wetzel 2001; Spänhoff *et al.* 2012; Bilous *et al.* 2014).

The taxonomic composition of phytoplankton in the River Viliya (Neris) described in this study is typical of other rivers of Belarus. In recent decades, large river systems of Belarus experienced increased pressure from anthropogenic activities, as a result of which the species adapted to high levels of organic enrichment and nutrients became dominant in phytoplankton communities. According to the findings of several studies (Astapovich 1996; Prosyaniuk *et al.* 1996; Astapovich *et al.* 1998; Adamovich *et al.* 2009), the only large river of Belarus with the prevalence of diatoms in phytoplankton is the Zapadnaya Dvina, which is much less exposed to the anthropogenic load than other major rivers (Astapovich *et al.* 1998; Adamovich *et al.* 2012).

The effect of the phytoplankton input from two tributaries on the River Viliya (Neris) water quality was found to be weak, just like that of the phytoplankton input in autumn, when the discharge of water from fish farms is the largest. Based on quantitative indicators, and the dominance of cyanobacteria in phytoplankton determined in this study, the trophic status of the River Viliya (Neris) was higher than that of other major rivers of Belarus. The dominance of cyanobacteria and the presence of toxic species in phytoplankton are characteristic features of eutrophication (O’Neil *et al.* 2012; Pearl 2016). Our study is the first one to reveal the presence of

toxigenic cyanobacteria of the genus *Microcystis*, which possess microcystins synthesis genes, in water of the River Viliya (Neris). Four variants of microcystins (MC-LR, dmMC-LR, MC-RR and MC-WR) were identified using the MALDI-TOF method. MC-LR and MC-RR are the most common variants of them in blooms dominated by *Microcystis* spp. and *Planktothrix agardhii* in the Curonian Lagoon of the Baltic Sea (Paldavičienė *et al.* 2009; Ehzova *et al.* 2012; Šulčius *et al.* 2015). Cleavage of the methyl group is possible in three different amino acid residues of the microcystin molecule, providing three options: [D-Asp<sup>3</sup>]MC-LR, [Dha<sup>7</sup>] MC-LR or [DMAdda<sup>5</sup>] MC-LR (Sivonen, Jones 1999). Accurate identification of the demethylated version was impossible due to its low concentration in the sample. According to BLAST analysis, uncultivated immediate relatives (99% similarity) of nucleotide sequences from the River Viliya (Neris) are the sequences of the AMT domain of cyanobacteria from other water bodies, including those we identified in Lakes Baikal and Kotochelskoye in Eastern Siberia (Russia) (Gaevsky *et al.* 2011; Belykh *et al.* 2011), the Curonian Lagoon of the Baltic Sea (Belykh *et al.* 2013), in Lakes Bolshie Shvakshty and Kroman (Belarus) (Mikheyeva *et al.* 2018a, b), as well as in Lake Doirani (Greece) (Gkelis, Zaoutsos 2014). The cultivated species were found to show the greatest similarity (99%) with strains of *M. aeruginosa* K-139, *M. viridis* NIES-102, *M. aeruginosa* NIES-843, *M. aeruginosa* FCY-26, isolated during toxic cyanobacteria blooms in lakes of Japan (Nishizawa *et al.* 2000; Rantala *et al.* 2004; Kaneko *et al.* 2007) and Korea (Rhee *et al.* 2012).

On the tree, amino acid sequences of aminotransferase form stable clades corresponding to the species of cyanobacteria (*Microcystis*, *Dolichospermum*, *Planktothrix*, *Nostoc*, *Nodularia*, *Phormidium*) capable of producing MCs. All amino acid sequences of cyanobacteria from the River Viliya are included into the clade of the *Microcystis* species (boot-strep support of 100%) (Fig. 3). Inside the clade, the clone *Microcystis* sp. Vi12/07-2 is grouped with strains of *M. aeruginosa* K-139 from Lake Kasumigaura (Japan), *M. aeruginosa* UTEXLB 2664 from South Africa and the uncultivated sequence *Microcystis* sp. B5-2 from the Curonian Lagoon of the Baltic Sea. The sequence *Microcystis* sp. Vi12/07-6 forms a cluster with an isolate of *M. viridis* NIES-102 from Lake Kasumigaura. Four sequences from the River Viliya (Neris) form separate lines inside the clade as well as some sequences from the Curonian Lagoon and Lake Bolshie Shvakshty (Belarus). Separate clusters are formed by sequences from Lake Kroman (Belarus) and two sequences from the Curonian Lagoon.

Ecological studies of rivers are primarily based on several fundamental concepts such as river continuum and flood pulse concepts (Vannote *et al.* 1980; Dokulil

2014). The alternative point of view considers rivers as “neutral pipes” that transport organic matter to the sea (Cole *et al.* 2007). Based on their biogeochemical and microbiological data, Gladyshev *et al.* (2015) concluded that a river ecosystem can complexly combine features of a river mosaic, river continuum, and those of a “neutral pipe”. It can be assumed that the phytoplankton composition in rivers can be influenced by the algal community of their tributaries, and, in turn, can affect the phytoplankton community composition in the marine lagoons receiving water from these rivers (Lange, Makushenko 2018). The molecular genetic identification of the toxic representatives of *Microcystis* species in the River Viliya revealed their similarity with the *Microcystis* strains that occurred in the Curonian Lagoon (Belykh *et al.* 2013; Ehzova *et al.* 2012; Lange 2013). Moreover, the cyanobacterium *Aphanizomenon flos-aquae*, which dominates the River Viliya phytoplankton in summer, is also the main dominant species in the Curonian Lagoon of the Baltic Sea (Belykh *et al.* 2013), which receives waters from the River Neman and its major tributary, the River Viliya. Although *A. flos-aquae* is considered to be a toxin producer (Bernard *et al.* 2017), its strains from the Curonian Lagoon have been shown to be unable to produce toxins (Šučius *et al.* 2015). Thus, it is quite probable that the cyanobacterial strains causing blooms in the Curonian Lagoon historically originated in the large tributaries of the River Neman. In May–October 2013–2017, the mineral nitrogen content in the Curonian Lagoon was 78.3 mg L<sup>-1</sup> (Malfanov *et al.* 2017), which was very similar to our measurements of nitrogen at the station No 5 (Table 2). The content of mineral phosphorus in the Curonian Lagoon was somewhat lower and ranged from 4 to 9 µg L<sup>-1</sup> (Malfanov *et al.* 2017). In 2007–2016, the concentration of mineral phosphorus in the Curonian Lagoon ranged from 5 to 30 µg L<sup>-1</sup> (Aleksandrov *et al.* 2018) and was similar to the concentrations we found in the Belarusian part of the River Viliya (Neris) during our research. Therefore, at some historical stage, changes in phytoplankton community in the main tributaries of the River Neman could have affected phytoplankton including cyanobacteria in the Curonian Lagoon. It should be noted that changes in the biomass of cyanobacteria in the River Viliya (Neris) significantly correlated with water temperature, one of the main factors promoting cyanobacterial growth. The potential influx of major nutrient elements (especially mineral phosphorus) with wastewater from the Belarusian Nuclear Power Plant in combination with thermal pollution may further aggravate the trophic status of the River (Validation of investments... 2010), and contribute to an increase in cyanobacteria proliferation causing toxic blooms. This, in turn, will produce additional load on the River Neman, one of the main sources of the Baltic Sea pollution (HELCOM 2013).

Additional nutrient load can also directly affect the Curonian Lagoon of the Baltic Sea, which is already subjected to significant eutrophication (Belykh *et al.* 2013; Malfanov *et al.* 2017; Aleksandrov *et al.* 2018; Bartoli *et al.* 2018; Vybernaite-Lubiene *et al.* 2018). Phytoplankton monitoring, including toxic cyanobacteria, should be an essential part of biological monitoring in the River Neman and its major tributaries, such as the River Viliya (Neris).

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