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Freshwater cyanobacteria in waters intended for human consumption in Serbia: two decades of changes in diversity

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Abstract: Herein we provide an assessment of cyanobacterial diversity and habitat preferences of potentially toxic and alien taxa, which could be an important tool for human health risk assessment regarding recreational and water-supply waterbodies. The diversity changes of cyanobacteria in waters intended for human consumption in Serbia were analyzed two decades after the first floristic study was published. The examination included phytoplankton and phytobenthic sample analysis from 35 localities in the period between 2012 and 2017, together with published literature records. The results indicate that the number of identified taxa doubled since the first Serbian Flora of Cyanobacteria was released two decades ago. The changes most likely occurred due to environmental factors, including hydrological transformations of habitats, cultural eutrophication and global warming. Many frequently recorded taxa are potentially toxic and bloom-forming. The spread of alien species with potentially invasive characteristics has also been noted. Canonical correspondence analysis (CCA) indicates that shallow waterbodies are the most vulnerable regarding the occurrence and expansion of bloom-forming, potentially toxic and invasive taxa. This shows the urgent need for a more detailed investigation. Additionally, although most of the research was focused on planktonic forms, benthic cyanobacteria represent an important component for public health risk assessment and therefore should be more frequently investigated.

Keywords: cyanobacteria; diversity; distribution; potentially toxic; alien/invasive

Abbreviations: deep reservoirs, over 20 m in depth (DR); shallow urban reservoirs (SUR); shallow lake (SL); lowland rivers (LR); highland river with a low basin (HR); mountain spring (MS); river/spring in an urban environment (UR/S); potentially toxic (T); bloom-forming (B-F); alien and/or invasive (A/I); very rare species (VR); rare species (R), common species (C); frequently occurring species (F)

INTRODUCTION

Cyanobacteria are often considered the oldest phototrophic organisms on Earth. The oxygen they released into the atmosphere in the Precambrian era was probably a precursor of the ozone layer [1], and it allowed the further development of plants and animals [2]. Therefore, they have played a key role in shaping the biosphere [3,4]. A long evolutionary history (~3.5 billion years) has allowed these organisms to develop diverse and very effective ecophysiological adaptations and strategies [2]. These strategies have enabled them to withstand various geochemical and climatic changes, as well as anthropogenically induced modifications of aquatic ecosystems, including increased nutrient concentrations (eutrophication), water level variation, desiccation and salinization [3], which is why they have a wide geographical distribution [2,5,6]. Also, when observing morphological characteristics, cyanobacteria represent a diverse group that includes single-celled and colonial forms, as well as multicellular filamentous forms [7].

Bearing in mind that cyanobacteria inhabit various environments, there are a number of characteristics that contribute to their competitive success, such as the

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ability to fix atmospheric nitrogen within special cells called heterocysts [8] or to form resting cells called akinetes [8,9]. Also, cyanobacteria produce a wide range of secondary metabolites, including substances that are used commercially as food additives, pharmaceuticals or have other industrial applications [10]. However, some of those compounds, such as cyanotoxins, have a negative effect on water quality and can be harmful to live organisms [3,4,6,10]. Due to their possible negative effect on human and environmental health, taxonomic identification and abundance assessments of cyanobacteria may provide an early warning for the potential presence of these harmful substances in waterbodies used by humans [11].

Over the past few years, the taxonomy of cyanobacteria has changed significantly [12-14] as new molecular methods and a genetic approach were used as the basic criteria. Nevertheless, these have also been combined with elements used in the traditional classification system (based primarily on morphological and ecological features), which is now called the 'polyphasic approach' [14]. Some recent studies suggest that there are over 300 cyanobacterial genera that are taxonomically accepted, and that over 50 of them have been described after the year 2000 and up to the present [15]. This means that the species lists must be continuously updated in accordance with the new findings and identification methods [16], and new discoveries are to be expected. Many countries in the region have been updating their checklists of cyanobacteria, such as the Czech Republic [17] and Greece [18]. The last study concerning cyanobacterial flora in the Republic of Serbia was published more than two decades ago [19]. Since then, studies on cyanobacteria were mostly related to individual waterbodies [20-27] or specifically focused on bloom-forming and potentially toxic taxa [28,29].

The present study includes the analysis of samples from 35 waterbodies used in water supply and/or human recreational activities in the Republic of Serbia, as well as literature data analysis aiming to determine the diversity of cyanobacteria in waterbodies related to water supply and recreation in Serbia, to create a database on their geographical distribution, to use findings obtained in this research (from 2012 to 2017) and those previously published, to compare them and observe potential changes across time and space, and to analyze potential preferences of some taxa regarding waterbody type (lake, reservoir, river, stream; assorted by size, maximum depth and altitude), with special reference to total diversity, potentially toxic taxa and invasive taxa.

MATERIALS AND METHODS

Sampling sites and procedure

Samples for the estimation of cyanobacterial diversity from the selected waterbodies intended for human use were collected in the period between 2012 and 2017. A total of 503 samples from 35 waterbodies in the Republic of Serbia were processed (Supplementary Fig. S1), of which 453 samples belong to the phytoplankton community (limnoplankton and potamoplankton), and 50 samples belong to the benthic community (phytobenthos). Phytoplankton samples were taken from 28 waterbodies, which included 3 lakes, 21 reservoirs and 4 larger rivers. The largest number of samples were related to limnoplankton. Samples of the benthic community were collected from 3 reservoirs intended for recreation and 9 rivers, among which 7 smaller rivers/streams and tributaries of waterbodies that are used for water supply and/or recreation. The research was conducted at different time intervals, according to the sampling dynamics of the Institute of Public Health of Serbia.

Phytoplankton sampling was performed in accordance with the standard method EN 16698:2016 [31]. Qualitative samples were taken by pulling the plankton net (mesh diameter 22-23 μm) along the water column of a waterbody, while quantitative samples were taken using Ruttner's bottle from various depths. The samples of benthic communities were mostly collected from rocky or stone substrates according to the following standard EN 15708:2010 [30]. Transparent 100-500 mL plastic bottles were used for sample storage. The bottles with samples were transported to the laboratory in the dark, in a hand refrigerator maintained at a temperature of +4-+10°C. In accordance with standard EN 16698:2016 [31], Lugol's solution was used to fix the samples.

Cyanobacteria analysis

Microscopic analysis was performed using an Axio Observer Z1 inverted microscope connected to the Zeiss Axiocam 506 color camera and Zen 2 software. Identification of taxa was carried out using standard identification keys for cyanobacteria according to Komárek and Anagnostidis [32,33] and Komárek [34]. The recorded taxa were reclassified according to Guiry and Guiry [35].

Data collection

Along with the data obtained from analysis of the samples from the selected waterbodies, literature data summarized in previously published studies [19,36] were used to create a list of cyanobacteria in waterbodies that are of interest for water supply and/or recreation. The research related to the distribution of cyanobacteria included only freshwater ecosystems linked to water supply and/or recreational activities (lakes, rivers, reservoirs, wetlands, etc.). Additionally, some waterbodies with a direct impact on water supply and recreation were also included, such as streams and smaller rivers that fit into reservoirs or larger rivers, but also wetlands that are connected to the large rivers. In some cases, due to the scarcity of published research in certain parts of our country, some rivers that are not direct tributaries of water supply and/or recreation waterbodies but are part of the upstream basin were included. This list of recorded taxa is given in Supplementary Table S1, which represents a revised checklist [36]. Previous studies [37-41] were used to analyze the occurrence of potentially toxic species and to assess the presence of alien and/or invasive species [42-44].

Statistical analysis

Multivariate analyses in software Canoco for Windows [45] were used for data analysis. Thereby canonical correspondence analysis (CCA) was performed on two sets of data. The first CCA observed the relationship between waterbody type (explanatory variable) and cyanobacterial planktic taxa (response variables) while for the second CCA, the same explanatory variable was used and was related to cyanobacterial benthic taxa. Planktic taxa included those from limnoplankton and potamoplankton. CCA was used since the gradient length was higher than 3.5 standard deviation units (SD units) in both cases. Thirty of the best fitted taxa are shown on both ordination diagrams.

RESULTS

During the analysis of the samples collected in this study, a total of 148 taxa of freshwater cyanobacteria were identified, including new findings for the examined group of waterbodies in Serbia. These include newly detected species for waterbodies used for water supply and recreation, such as *Anathece smithii*, *Aphanocapsa planctonica*, *Coelosphaerium aerugineum*, *Microcystis ichthyoblabe*, *Leptolyngbya notata*, *Leptolyngbya perforans*, *Limnothrix obliqueacuminata*, *Pseudanabaena contorta*, *Planktolyngbya contorta*, *Snowella atomus*, etc., and even several genera (*Cyanodictyon*, *Cyanosarcina*, *Cyanostylon*, *Cronbergia*, *Eucapsis* and *Lemmermanniella*). One-third of these newly detected taxa could be characterized as picocyanobacteria. Records from phytoplankton dominate. When observing samples of the benthic community, a total of 44 taxa of freshwater cyanobacteria were found, but it should be emphasized that planktonic forms were noted among them, as well. Based on the summarized data obtained from the analysis of samples from the selected localities, as well as the records from the published research, a total of 328 cyanobacterial taxa were recorded in waters related to water supply and recreation (Supplementary Table S1). According to the new taxonomic classification [12], they were classified into 85 genera, 28 families and 8 orders. In the flora of cyanobacteria of Serbia published in 1996 [19], the summarized data indicate that 154 taxa have been recorded in waterbodies related to this research, while regarding the findings of later scientific studies, 120 more taxa could be added to that list. Thus, this number has almost doubled through later scientific research.

Among the listed taxa (Supplementary Table S1; summarized data from literature and analyzed samples), 77 could be characterized as potentially toxic, such as representatives of bloom forming genera *Aphanizomenon*, *Microcystis*, *Dolichospermum* and *Planktothrix*, but also *Leptolyngbya*, *Anagnostidinema*, *Phormidium*, *Oscillatoria*, *Pseudanabaena* and *Woronichinia*. Among the recorded cyanobacterial taxa in Serbia, the following could be characterized as alien and/or invasive: *Anabaenopsis cunningtonii*, *Chrysosporum bergii*, *Cuspidothrix issatschenkoi*, *Raphidiopsis raciborskii*, *Dolichospermum lemmermannii*, *Nodularia spumigena*, *Planktothrix rubescens*, *Raphidiopsis mediterranea* and *Sphaerospermopsis aphanizomenoides*. During the sample analysis from two reservoirs, individuals that resemble *Anabaena minderi* Huber-Pestalozzi were noted. Among the detected invasive species, three were known from older literary findings – *D. lemmermannii*, *P. rubescens* and *R. mediterranea* [19], while the remaining 5 were recorded only in the past two decades. Regarding previously detected species, *P. rubescens* and *R. mediterranea* are still regularly found in many localities [22-24, 26], while *D. lemmermannii* has not been detected in recent literature.

The largest share of taxa (Fig. 1) belongs to the order Synechococcales (~35%), while slightly fewer of the recorded taxa belong to the order Oscillatoriales (~30%). When it comes to morphological characteristics, they are dominated by the non-heterocystous form of filamentous cyanobacteria (~47%).

The most frequent genera observed after qualitative analysis of collected samples in this study were: *Phormidium* Kützing ex Gomont (33 localities), *Leptolyngbya* Anagnostidis & Komárek (23 localities), *Oscillatoria* Vaucher ex Gomont (22 localities), *Chroococcus* Nägeli (22 localities), *Limnothrix* M.-E. Meffert (20 localities), *Pseudanabaena* Lauterborn (19 localities), *Aphanocapsa* Nägeli (17 localities) and *Anagnostidinema* Strunecký (15 localities), and the most diverse genera were *Phormidium* (10 taxa), *Leptolyngbya* (10 taxa), *Aphanocapsa* (9 taxa) and *Chroococcus* (8 taxa). When observing total diversity, it is important to note that *Dolichospermum*, *Microcystis*, *Oscillatoria* and *Pseudanabaena* had high numbers of recorded species as well.

Cyanobacterial taxa from the plankton community were related to waterbody type using CCA (Fig. 2), which described 22.57% variability in the data (F=1.7; P=0.002). A total of 144 taxa were included in the analysis, but only 30 of the best fitting are shown on the ordination diagram. Two large groups of planktonic cyanobacteria are distinguished: one in the upper part of the ordination diagram related to shallow lakes (SL) and the other in the right part of the ordination diagram related to highland river with a low basin (HR). Considering 30 of the best fitted taxa, coccoid and heterocystous representatives characterized the first group (i.e. *Aphanocapsa planctonica*, *Anabaenopsis circularis, A. cuningtonii*), while predominantly simple trichal taxa were connected to HR (*Jaaginema* cf. *quadripunctulatum, Leptolyngbya subtilis*, *Limnothrix lauterbornii*, *Microcoleus amoenus*, *Oscillatoria ornata*, *Pleurocapsa minor*, *Phormidium interruptum*, *Ph. favosum*, *Tapinothrix janthina*, *T.*

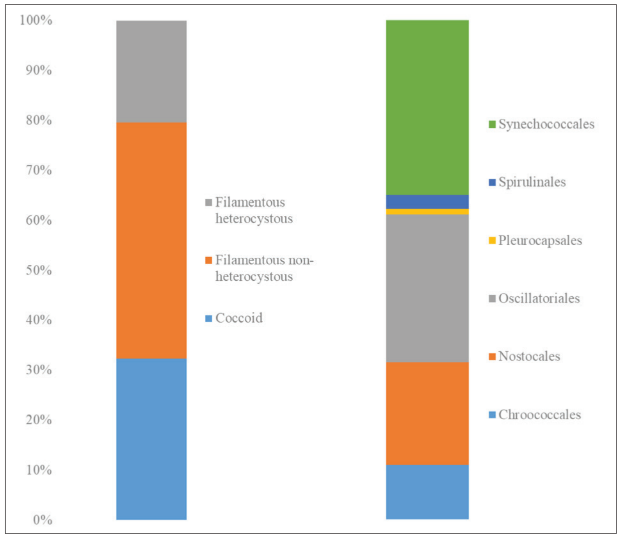

Fig. 1. Percentage of different groups of cyanobacteria based on the morphological forms (left) and taxonomic criteria (right).

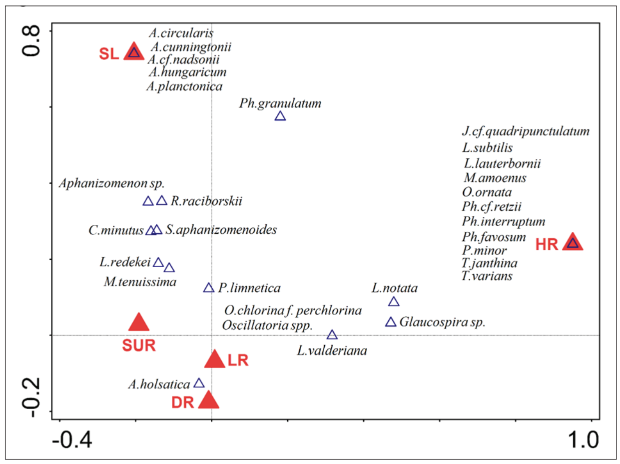

Fig 2. CCA showing the relationship between cyanobacterial planktonic taxa and waterbody type. DR – deep reservoirs, over 20 m in depth; SUR – shallow urban reservoirs; SL – shallow lake; LR – lowland rivers; HR – highland river – lower basin.

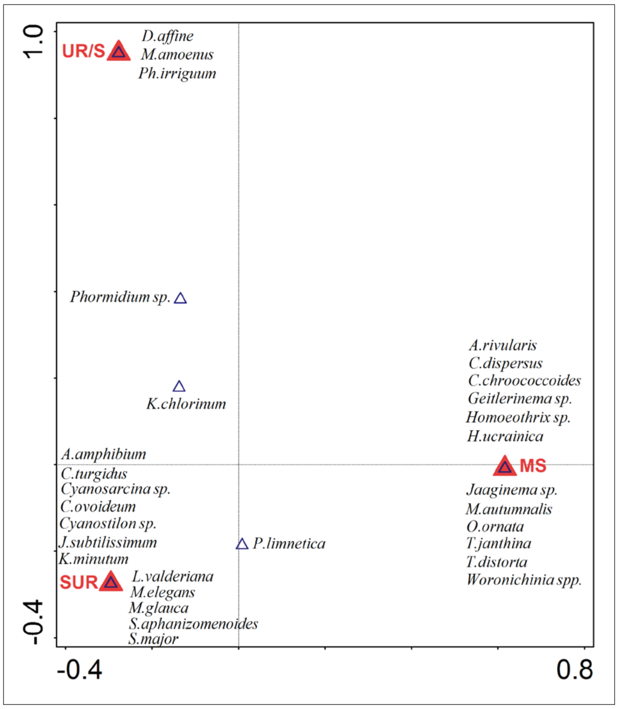

Fig. 3. CCA showing the relationship between cyanobacterial benthic taxa and waterbody type. SUR – shallow urban reservoirs; MS – mountain spring; UR/S – river/spring in an urban environment.

varians). Others were placed either between SL and three waterbody types – deep reservoirs (DR), lowland rivers (LR), shallow urban reservoirs (SUR), or between these three waterbody types and HR. The former are predominantly coccoid and heterocystous taxa (*Aphanizomenon* sp., *Chroococcus minutus, Raphidiopsis raciborskii, Limnothrix redekei, Merismopedia tenuissima, Sphaerospermopsis aphanizomenoides*), while the latter include simple trichal representatives (*Glaucospira* sp., *Leptolyngbya notata, L. valderiana, Oscillatoria chlorina* f. *perchlorina, Oscillatoria* sp.). *Aphanocapsa holsatica* was related to DR, LR and SUR.

The CCA plot (Fig. 3) where a relationship between cyanobacterial benthic taxa and waterbody type was observed, described 24.94% variability in data (F=1.3; P=0.004). Of 44 taxa that were included in the analysis, 30 of the best fitted are shown on the ordination diagram. Three groups of cyanobacterial taxa are clearly separated. The first group that included *Microcoleus amoenus* and *Phormidium irriguum* was connected to rivers/springs in an urban environment (UR/S). The second group related to SUR is composed of a large number of cyanobacterial benthic taxa of different morphology (*Anagnostidinema amphibium*, *Chroococcus turgidus*, *Cyanosarcina* sp., *Cyanostylon ovoideum*, *Cyanostylon* sp., *Jaaginema subtilissimum*, *Komvophoron minutum*, *Leptolyngbya valderiana*, *Merismopedia elegans*, *M. glauca*, *Spirulina major*). The third group, also composed of benthic cyanobacteria of different morphology, but with a slightly higher number of simple trichal representatives, is related to mountain springs (MS) (*Aphanocapsa rivularis*, *Chroococcus dispersus*, *Cyanosarcina chroococcoides*, *Geitlerinema* sp., *Heteroleibleinia ucrainica*, *Homeothrix* sp., *Jaaginema* spp., *Microcoleus autumnalis*, *Oscillatoria ornata*, *Tapinothrix janthina*, *Tolypothrix distorta*). *Phormidium chlorinum* can be connected to both UR/S and SUR, while *Pseudanabaena limnetica* is related to SUR and MS.

DISCUSSION

The number of cyanobacterial taxa recorded in waterbodies related to water supply and recreation in Serbia has increased substantially over the past two decades. It should be emphasized that 154 taxa had been recorded in previously published Serbian flora of cyanobacteria [19], and that this number has almost doubled after subsequent scientific research, including this study. Nevertheless, although an increase in the total number of species (presumably due to more advanced microscopy techniques and higher research frequency) was observed, there is also a possibility that some of the taxa recorded a few decades ago have been lost. In this regard, when observing aquatic habitats, changes in floristic composition inevitably occur, and several potential causes could be considered. The first among them is the change of habitat, which refers to the fact that some waterbodies have been significantly changed. A good example is the former moorland called Vlasinska Tresava, which was a research locality in a study performed at the beginning of the last century, and its data were later integrated into the Serbian Flora of Algae [19]. After the construction of a dam and the formation of the reservoir called Vlasina Lake [20], the habitat changed, and this undoubtedly had an impact on different communities within its ecosystem. In this regard, some cyanobacteria observed in the time before the formation of the reservoir, such as *Scytonema hofmannii* and *Tolypothrix tenuis*, may have disappeared, as they were not detected in later research. Organisms specific to different habitat types also differ in ecological characteristics [42], and changes in community composition are naturally expected with the change of waterbody type.

In addition to physical and hydrological changes, a deterioration in water quality can be a reason for species shifts. This primarily refers to nutrients, as different types of cyanobacteria react differently to changes in total phosphorus concentration, as well as to the form of nitrogen components that are dominant in the water [46]. Cultural eutrophication is a phenomenon that is considered to significantly favor the development of bloom-forming cyanobacteria [47], which can be clearly seen in the results of this research. For example, records of genera such as *Aphanizomenon*, *Dolichospermum*, *Microcystis* and *Planktothrix* in Serbia, as well as the number of found species belonging to them, have increased in recent years [36]. Representatives of these genera are considered to form blooms [3,5,48]. In addition, it should be emphasized that temperature has a strong influence on the physical, chemical and biological processes in lakes and reservoirs [2,5,9]. It is thought that temperature has a synergistic effect in increasing nutrient concentrations and therefore affects the trophic status of the waterbody itself [3,5]. In this regard, the temperature rise on a global level also favors the proliferation of bloom-forming cyanobacteria [2,9]. Furthermore, both cultural eutrophication and climate change increase the risk of the spreading of invasive species [5,49]. In Serbia, in the last two decades, the number of detected alien and/or invasive species has increased by more than 50% [36]. Nevertheless, it should be noted that advances in microscopy techniques, as well as the increase in research of cyanobacteria and new identification keys, have also contributed to such species list extensions [36].

When observing the presented floristic list, there are 77 taxa that could be characterized as potential producers of cyanotoxins [37-41]. The phenomenon known as harmful algal blooms (HABs) has already been a topic of scientific and social interest. It is defined as blooming that has a negative environmental and socio-economic impact and poses a threat to public health [50]. The findings for some common cyanobacterial taxa in waterbodies related to water supply and recreation that tend to cause HAB under favorable conditions are highlighted in the list. This is primarily significant because a toxic cyanobacterial bloom can accumulate near the water intake pipelines of drinking-water treatment plants and cause a collapse of the water supply system [5,49]. Having this in mind, it is extremely important for waterbody managers to have information on the taxa that have the ability to form toxic blooms and that are present in their water supply sources.

The number of reports of harmful cyanobacterial blooms in drinking and recreational waters has increased significantly worldwide in the last few decades [51]. This trend can be observed in Serbia as well [22,27,28]. Previous studies have shown that blooms are mostly caused by excessive development of representatives of the genera *Anabaena*/*Dolichospermum*, *Aphanizomenon*, *Microcystis* and *Planktothrix* [27]. Regarding the reservoirs used for water supply, cyanobacterial blooms were recorded in the following reservoirs: Bovan, Bresnica, Ćelije, Garaši, Grošnica, Grlište, Gruža, Krajkovac, Pridvorica and Vrutci [28]. CCA analysis in this study indicates that some potentially toxic bloom-forming taxa (*Anabaenopsis* spp., *Aphanizomenon* sp., *Raphidiopsis raciborskii*, *Limnothrix redekei*) have the tendency to occur more often in shallow waterbodies (shallow urban reservoirs and shallow lakes) in comparison to other investigated types of waterbodies. This suggests that shallow lentic aquatic ecosystems are at greater risk of HABs, which was also confirmed by other researchers [52]. Furthermore, urban reservoirs and lakes are generally disturbed dynamic systems due to land-use anthropogenic activity, which increases the discharge of nutrients, primarily nitrogen and phosphorous [53], and these conditions usually favor bloom-forming cyanobacteria [49].

CCA analysis indicates that some other cyanobacterial taxa, such as members of the genus *Aphanocapsa*, occur frequently in both deep and shallow, lotic and lentic ecosystems. *Aphanocapsa* is the most diverse genus that could be included in the group of picocyanobacteria. This is a specific group of cyanobacteria characterized by small cells (less than about 2-3 μm in diameter), which can appear as individual cells or under certain conditions form smaller or bigger colonies in both marine and freshwater ecosystems [54]. It is generally considered that single-celled picocyanobacteria prevail in deep, large oligo-mesotrophic lakes, while colonial forms prefer shallow and more nutrient-rich waterbodies [55]. However, colonial *Aphanocapsa* (especially the species *Aphanocapsa holsatica*) are frequently found in diverse types of waterbodies. Despite being often neglected in earlier studies, picocyanobacteria are increasingly investigated in recent years [56]. Records indicate that picocyanobacteria represent an important component in aquatic communities in Serbia. In this regard, with the use of advanced technologies, there is great potential for more extensive research that could provide additional information regarding their diversity and distribution, as well as environmental preferences. Although this group of photoautotrophs is still insufficiently studied, picocyanobacteria have a very important role in primary production, as well as a significant contribution to the phytoplankton biomass in both oligotrophic and eutrophic waters. It is expected that their role will be even more relevant due to the influence of climate change [57].

When benthic cyanobacteria were observed, CCA analysis indicated that a clear disjunction exists between three of the investigated types of waterbodies (SUR, MS and UR/S), especially between urban and non-urban environments. In this regard, mountain springs are suitable habitats for morphologically variable taxa (coccoid, simple trichal, heterocystous forms). However, those characterized by a simple trichal form stand out and include representatives of genera *Geitlerinema*, *Heteroleibleinia*, *Homeothrix* and *Jaaginema*, as well as the potentially toxic species *Microcoleus autumnalis* [40]. *Microcoleus autumnalis* is known to be a producer of anatoxins, potent neurotoxins commonly attributed to *Microcoleus* or *Phormidium* genera and considered the most widely reported toxins related to benthic mats [58]. On the other hand, two simple filamentous species show a strong preference for urban waterbodies – *Jaaginema subtilissimum* (for SUR) and *Kamptonema chlorinum* (for both UR/S and SUR). This is in accordance with the described ecological characteristics, as both species are most often found in benthic habitats that include organic mud, decaying substrates, along with sulfur bacteria that are tolerant to increased salt concentrations [33]. Nevertheless, although *Kamptonema* as former *Phormidium* [33,35] could be regarded a potential cyanotoxin producer [38,58], there are no data concerning toxin production for the species *K. chlorinum* and *Jaaginema subtilissimum*. When considering heterocystous cyanobacteria with potential toxicity, *Tolypothrix distorta* stands out as a potential microcystin producer [38]. According to the results of this study, this species is related to MS, which coincides with a previous study where it was detected for the first time in a similar habitat on the Stara Planina Mountain [21].

Planktonic cyanobacteria are generally more investigated than benthic, as mostly planktonic forms cause blooms in recreational and water supply waterbodies [47], which is the case in Serbia as well [36]. However, cyanobacteria have the potential to produce potent cyanotoxins that are harmful for human health [38]. It is necessary to organize and carry out further studies to assess the distribution of both benthic and planktonic cyanobacteria. Detection of the most important genera and species by microscopy provides some basic information for assessing the risk of potentially harmful events [7].

Regarding alien species, among the recorded cyanobacterial taxa in Serbia, *Raphidiopsis raciborskii* stands out as the most successful invasive allochthonous species and according to the number of findings it is followed by *Sphaerospermopsis aphanizomenoides* [36]. Bearing in mind that these can produce cyanotoxins and competitively suppress native species, invasive and allochthonous cyanobacteria are considered a serious threat to aquatic ecosystems and inhabiting organisms [44,59]. Although invasive species are an increasing research topic, microscopic organisms are still insufficiently investigated [42]. Among cyanobacteria, representatives of the order Nostocales are most often regarded as the group with the most successful competitive strategies for spreading to new habitats [59]. The same was noticed when it comes to allochthonous cyanobacteria that were observed in the investigated waters in Serbia, bearing in mind that, of all identified species, only one does not belong to the mentioned order. CCA analysis indicates that shallow lentic waterbodies represent suitable corridors for the spread of alien species such as *R. raciborskii* and *S. aphanizomenoides*, which is in accordance with previous observations [60].

CONCLUSION

During the last two decades, the number of recorded species of cyanobacteria in Serbia has increased substantially. The changes in cyanobacterial diversity most likely occurred due to environmental changes, including hydrological transformations of different habitats, cultural eutrophication, climate changes, and also due to the use of more advanced identification technology and methods, as well as increased research. Many of the frequently recorded taxa are potentially toxic and can also be bloom-forming. The spread of alien species with potentially invasive characteristics has been noted. This especially refers to *Raphidiopsis raciborskii* and *Sphaerospermopsis aphanizomenoides*, which show high expansive potential. Results indicate that shallow waterbodies (shallow urban reservoirs and shallow lakes) were the most vulnerable as regards the occurrence and expansion of potentially toxic and invasive taxa. However, more detailed research on the expansion of alien cyanobacteria in Serbia is necessary. Most scientific research concerning cyanobacterial occurrence and distribution has been focused on planktonic forms because bloom-forming taxa are mainly observed in the phytoplankton community. Nevertheless, benthic cyanobacteria represent an important component in terms of public health risk assessment in recreational and water supply waterbodies and should be investigated more.

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Supplementary Data

The Supplementary Material is available at: https://www.serbiosoc.org.rs/NewUploads/Uploads/Jovanovic%20et%20al_7790_Supplementary%20Material.pdf