

Original paper

Seasonal variability of the epilithic diatom community in the Radovanska River

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Summary. The present study provides detailed information on the seasonal variability of epilithic diatoms, based on analysis of their diversity and dominant taxa in the Radovanska River (Eastern Serbia) over 4 seasons at five sites. This river is the most important tributary of the Crni Timok River and has high biological diversity. This region of Eastern Serbia also has great tourism potential. Diatoms are widely recognized as excellent indicators of many environmental conditions. Spring and summer periods were characterized by the highest number of diatom taxa (143 and 145, respectively), and winter by the lowest (109 taxa). A total of 24 taxa were recorded as dominant (relative abundance higher than 5% for at least one locality). CCA ordination diagram clearly indicates that taxa recorded in more than two seasons are separated from those specific only for one sampling season (none were exclusively recorded only in winter). A large number of taxa were recorded in only two seasons.

Key words: diatoms, Radovanska River, seasonal dynamics.

INTRODUCTION

Diatoms are considered to be the most important component of benthic flora in rivers, streams, and lakes, comprising more than 80% of the total abundance and biovolume (Tan et al. 2013). They are widely recognized as excellent indicators of many environmental conditions, including the degree of pollution in aquatic ecosystems (Bilous et al. 2020). The high species and ecological diversity of benthic diatoms as well as their rapid response to many environmental pressures make them an irreplaceable component of the assessment of surface water quality. Moreover, they can provide unique and significant early warning signals of deteriorating conditions (Mao et al. 2018). Bearing in mind that freshwater ecosystems are continuously affected by anthropogenic pressure, their increased role in water quality management worldwide is justified (Chonova et al. 2019).

Natural and anthropogenic factors contribute to the seasonal and spatial variation of diatom assemblage compositions. The fact that the concentration and transport of many water pollutants (often human-induced) are season-dependent (Shibabaw et al. 2021) also contributes to the variability of diatom composition over time. Certainly, changes to physical and chemical parameters with respect to time must be taken into account during monitoring the seasonal shift of diatoms, but deviations must also be considered.

Different environmental conditions (e.g. nitrate, phosphorus, silica, temperature) in temperate regions lead to changes in biomass and the composition of dominant freshwater diatom species throughout the year (Wetzel 2001; Köster and Pienitz 2006). However, seasonal variability can be totally absent in some stable ecosystems such as springs in the Alps (Cantonati et al. 2006). Study of diatom community

structure and temporal and spatial distribution is indispensable for correct and appropriate monitoring of surface water bodies worldwide.

To date, there are only a few published reports focusing on diatom diversity, new recorded diatom taxa and ecological status assessment of the Radovanska River based on diatoms (Jakovljević 2017; Jakovljević et al. 2018, 2021; Vidaković et al. 2018, 2020).

The aim of this study, which is the first analysis of diatom seasonal dynamics in the research area, was to provide detailed information on the seasonal variability of epilithic diatoms by analyzing their diversity and dominant taxa in the Radovanska River (Eastern Serbia) over four seasons and at five different sites.

MATERIALS AND METHODS

Study area and collecting of diatom samples

The Radovanska River is an approximately 18 km long mountain river in Eastern Serbia (Fig. 1). This river is the most important tributary of the Crni Timok River and has high biological diversity. This region of Eastern Serbia also has great tourism potential (Petrović 1989). Sampling sites (RR1-RR5) along Radovanska River were selected with consideration of the trout pond's (FF) position. The first site (RR1) represented a control site and was located 100 m upstream of the trout pond. Four sites were located downstream of the trout pond at 20 m (RR2), 500 m (RR3), 1.6 km (RR4) and 3.1 km (RR5) away from the pond, respectively (Fig. 1). Sampling sites coordinates and altitude are given in Table 1.

Diatom samples for qualitative and quantitative analysis were collected over four seasons, from May 2011 to May 2012, from the five sampling sites mentioned above. During this period, a total of 30 samples were collected by scraping stones using a toothbrush and rinsing with distilled water (SRPS EN 13946:2015). A total of 30 samples were obtained by sampling six times (months) during four seasons (winter – March 2012, autumn – December 2011, summer – September and July 2011 and spring – May 2011 and 2012) at each of the five sites. Samples were fixed in 4% formaldehyde in the field and transported to the laboratory.

Laboratory and microscopic analyzes

In the laboratory of the Department of Algology, Mycology and Lichenology in the Institute of Botany and Botanical Garden "Jevremovac" in Belgrade, the cold acid method with H_2SO_4 , $KMnPO_4$ and $C_2H_2O_4$ (Krammer and Lange-Bertalot 1986) was used to remove the organic content of samples and diatom cells. Permanent slides of diatoms were made by mounting in Naphrax[®] mounting medium. A Carl Zeiss AxioImager M1 microscope equipped with a digital camera AxioCam MRc5 and AxioVision 4.8 software was used for detailed observation of diatom frustules. Identification of diatom taxa was made following standard taxonomic literature. In addition to qualitative analysis, quantitative observations were also conducted by counting 400 diatom valves on each slide (SRPS EN 14407:2015). Statistical analysis was performed using the CANOCO program for Windows, Version 5.0 (Ter Braak and Šmilauer 2012).

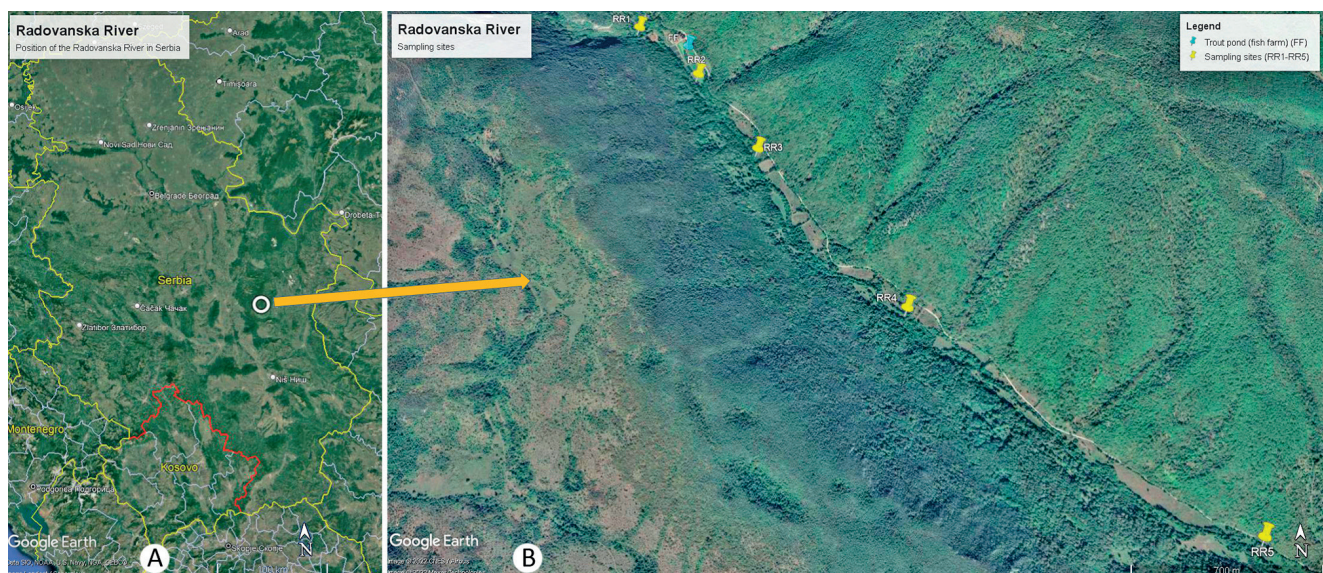


Fig. 1. Google Earth Image. ©2022. **A**, Position of the Radovanska River in Serbia; **B**, Sampling sites (RR1-RR5) and trout pond (fish farm) (FF).

Table 1. Altitude and coordinates of sampling sites (RR1-RR5) in the Radovanska River.

	RR1	RR2	RR3	RR4	RR5
Coordinates	43° 53.802 N	43° 53.666 N	43° 53.458 N	43° 53.066 N	43° 52.597 N
	21° 47.062 E	21° 47.274 E	21° 47.484 E	21° 47.946 E	21° 48.867 E
Altitude (m)	415	380	374	353	336

RESULTS AND DISCUSSION

During the spring period (May 2011 and 2012), a total of 143 diatom taxa were identified in Radovanska River. The largest number of taxa was recorded at the RR3 sampling site, 77 in May 2011 (Fig. 2). In this season, the lowest number of taxa (50) was observed at the RR1 site. Similar results were obtained in 2012, except that the highest number of taxa were detected at the RR2 site. Both RR2 and RR3 displayed the most diverse diatoms during the spring months, and are located immediately downstream from the trout pond. In fact, all downstream localities had a higher number of detected diatom taxa than the upstream RR1 site. Since spatial differences in the number of detected diatom taxa among the localities were higher than seasonal variability, our results support studies that highlight the relevance of location-specific factors (e.g., nutrients) over seasonal factors (e.g., temperature and solar irradiance) (Chonova et al. 2019). Hence, diversity and community structure of benthic diatoms more clearly react to local changes than seasonal ones. In Radovanska River, during the spring, 15 diatom taxa were identified with a relative abundance higher than 5% for at least one site (Table 2). Among them, 5 taxa were found at all 5 studied sites. The dominant taxa in the spring period were: *Nitzschia denticula* (73%), *Achnanthydium minutissimum* (36.5%) and *Meridion circulare* (33.3%). These species

have a wide geographic distribution and inhabit meso- and eutrophic waters (van Dam et al. 1994). Pinseel et al. (2017) pointed out that many *Nitzschia* species are successful colonizers of disturbed environments, whereas the high abundance of *Achnanthydium* spp. in almost all samples likely reflects its ability to rapidly colonize and adapt to a wide range of habitats.

In the summer period (July and September 2011), a total of 145 diatom taxa were recorded. The highest number of taxa was found at the RR4 (83), and the lowest number at the RR1 (43) (Fig. 2). Similar to the spring period, the locality upstream of the trout pond displayed the lowest diversity, while the downstream locality not far from the trout pond had the highest, indicating again the importance of local factors on the diversity of diatoms. The presence of 12 diatom taxa with a relative abundance higher than 5% was recorded over the summer period (Table 2). *Cocconeis lineata* was dominant taxon at RR1 with the highest relative abundance of 73.5% in September 2011. *Cocconeis* is a low-profile genus (Passy 2007) and its higher prevalence in summer can be explained by better adaptation of *Cocconeis* species to high current velocities and strong water turbulence present in the summer months (Chonova et al. 2019). At the RR2 and RR3 localities, the species *Denticula tenuis* was dominant (34.5% to 39.9%) while *Amphora pediculus* (9.22% to 70.8%) was the most abundant at RR4 and RR5 sites. Dominance of *D. tenuis*

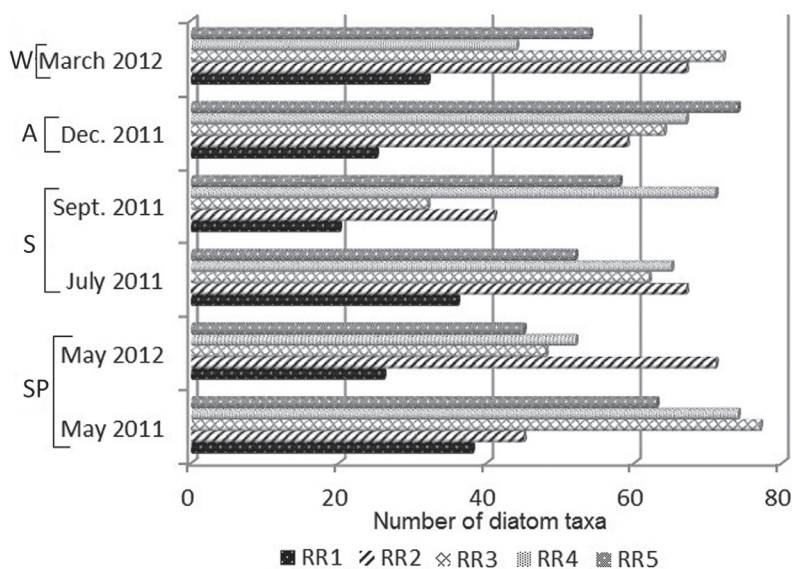


Fig. 2. Richness of detected diatom taxa in the Radovanska River at 5 sampling sites (RR1-RR5) in four seasons: winter (W), autumn (A), summer (S) and spring (SP).

at these sites is expected considering that it is well adapted to disturbed environments, such as stream-influenced water bodies, thanks to the strong connections of colonies with their substrate (Pinseel et al. 2017).

In the autumn period (December 2011), a total of 128 diatom taxa were identified. The highest number of taxa was found at RR5 site (74), and the lowest at RR1 (25) (Fig. 2). In the autumn period, 15 diatom taxa were present with a relative abundance higher than 5% for at least one locality (Table 2). *Cocconeis lineata* was the dominant taxon at the RR1 locality (69%), and *Denticula tenuis* at the RR2 and RR3 (46.8% and 28.4%, respectively). *Ulnaria ulna* was dominant at RR4 (27.5%) and *Amphora pediculus* at the RR5 (25.6%). Results for the autumn period were very similar to those obtained for summer, in terms of diversity, but also with respect to dominant species present. One exception is *U. ulna*, which was not dominant in summer. This taxon is considered to be indifferent to tolerant to the trophic state of the environment (van Dam et al. 1994). Unlike our findings, Dedić et al.

(2021) found *U. ulna* in all samples and seasons by studying diatom taxa of the Bunica, a small karstic river in Bosnia and Herzegovina.

In the winter period, a total of 109 diatom taxa were identified in the Radovanska River. This period is characterized by the lowest number of taxa. We must keep in mind that there were two samples in spring and summer, unlike in winter and autumn when only one sample was analyzed: which could be the reason for the lower detected richness of diatoms. Lu et al. (2020) suggested that low richness of diatoms can be attributed to limited light and low water temperature during winter. Similar results were also obtained in aquatic ecosystems in temperate regions of North America and Europe (Lotter and Bigler 2000; Frenette et al. 2008). RR3 site was characterized by the highest number of taxa (72), and RR1 by the lowest (33) (Fig. 2). In this period, 13 taxa had a relative abundance higher than 5% for at least one locality (Table 2). They occurred at all sites or were absent from one locality. *Meridion circulare* was the dominant taxon

Table 2. Dominant taxa and ranges of their relative abundances (%) throughout four seasons in the Radovanska River.

Taxa	Spring	Summer	Autumn	Winter
<i>Achnanthydium catenatum</i>	/	/	1.71-5.37	/
<i>Achnanthydium minutissimum</i>	5.95-36.5	0.49-22.8	1.47-15	8.98-35.1
<i>Achnanthydium pyrenaicum</i>	0.5-15.5	0.74-12	0.73-6.15	0.49-8.73
<i>Amphora pediculus</i>	0.99-22.7	0.74-70.8	0.48-25.6	1.72-29.4
<i>Cocconeis euglypta</i>	/	5.43-6.79	/	/
<i>Cocconeis lineata</i>	0.24-10.5	1.97-73.5	1.22-69	0.74-6.89
<i>Cocconeis pseudolineata</i>	/	0.41-10.4	0.73-7.88	/
<i>Denticula tenuis</i>	0.25-21.7	0.96-39.9	0.49-46.8	5.39-34.24
<i>Encyonema minutum</i>	0.24-5.17	0.49-5.91	/	0.72-10.1
<i>Encyonopsis minuta</i>	0.24-6.2	/	/	/
<i>Fragilaria gracilis</i>	/	/	0.49-8.8	/
<i>Gomphonema elengatissimum</i>	0.48-10.3	/	/	/
<i>Gomphonema micropus</i>	/	/	/	0.24-11.3
<i>Handmania glabriuscula</i>	/	/	0.24-6.11	/
<i>Meridion circulare</i>	1.23-60.9	0.72-6.89	/	0.24-39.9
<i>Navicula tripunctata</i>	0.73-9.6	0.24-10	0.24-20.4	0.49-7.59
<i>Nitzschia denticula</i>	73	/	/	/
<i>Nitzschia dissipata</i>	/	/	2.94-8.37	0.72-6.37
<i>Nitzschia fonticola</i>	/	/	1.23-17.8	1.45-27.2
<i>Planothidium dubium</i>	0.49-5.43	/	/	/
<i>Planothidium lanceolatum</i>	0.49-9.62	0.24-6.55	/	/
<i>Psammothidium grischunum</i>	6.38	/	/	7.63
<i>Staurosira mutabilis</i>	0.24-13.9	0.24-20.4	/	/
<i>Ulnaria ulna</i>	/	/	1.95-27.5	0.24-26

at RR1 (39.9%), *Achnanthydium minutissimum* at RR2 and RR3 (35.1% and 32.3%, respectively), *Amphora pediculus* at RR4 (29.4%), and *Ulnaria ulna* at RR5 site (26%).

A canonical correspondence analysis (CCA), which represents the relationship between recorded taxa in the Radovanska River and sampling seasons, is shown in Figure 3. In general, based on this ordination diagram we can distinguish taxa that were recorded in more than two seasons (the central part of the diagram), and taxa that were specific only for one sampling season. However, of the 45 taxa presented, none was exclusively recorded only in the winter. These analyses of the data suggest that changes in the epilithic diatom community structure can be explained by both seasonal and location-specific factors. The composition and structure of communities differ depending on the season at sites along small watercourses (Peszek et al. 2021). Although the similar characteristics of *Craticula* and *Sellaphora* species can lead to competition resulting in suppression of *Craticula* in warmer, and *Sellaphora* in colder months (Chonova et al. 2019), this was not indicated by our results. *Sellaphora pupula*, in addition to winter, was also recorded in summer

months (Fig. 3). As can be seen in the diagram, a large number of taxa were recorded only in autumn or spring, while two taxa, *Fragilaria capucina* and *Reimeria uniseriata*, stood out in summer. A large number of taxa were recorded for only two seasons, e.g. *Navicula viridula* in winter and summer, *Hantzschia amphioxys* in summer and spring (Fig. 3). *Hantzschia amphioxys* is more common in mesotrophic and eutrophic conditions than in waters poor in nutrients (Cuña-Rodríguez et al. 2018). Peszek et al. (2021) noted large differences between autumn and spring period also in agreement with the present study. The analysis presented in the present work demonstrates that the richness of diatoms and their seasonal dynamics are influenced by their location in relation to a trout pond, but also by temperature and other seasonally dependent factors.

CONCLUSIONS

In order to understand diatom community structure and better interpret results, one must consider complex interactions (the physiological characteristics of each species, anthropogenic factors, environmental conditions, resource availability, species competition etc.). Only after considering all of these factors, can conclusions be made about the structure of the diatom community over annual and inter-annual periods. In general, our study indicates that changes in the epilithic diatom community structure over a one-year period can be explained by location-specific and seasonal factors. Thus, position downstream from the trout pond had an effect on increased diatom richness, while environmental factors that are also seasonally variable had a significant impact on taxa shift. In order to protect the quality of rivers, continuous monitoring is necessary, which requires knowledge of the diatom community. Data about the structure and seasonal distribution of diatoms are essential in order to conduct water quality assessment and continuous monitoring on the basis of diatom indices.

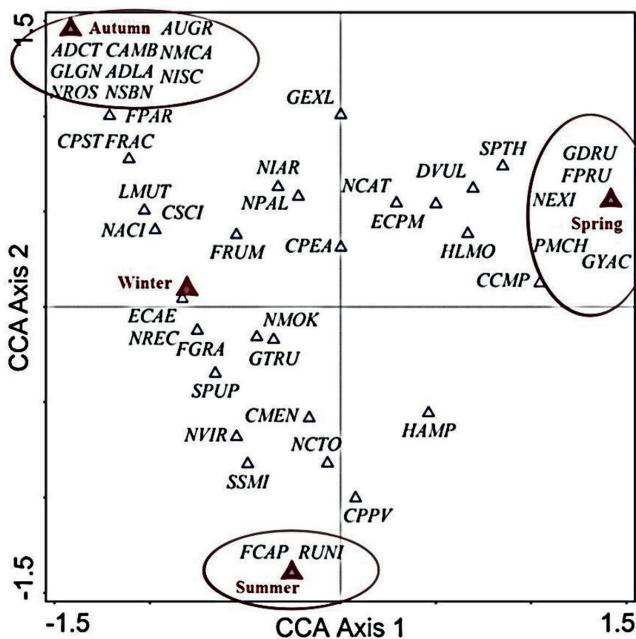


Fig. 3. Canonical correspondence analysis showing the relationship of diatom taxa identified in Radovanska River and sampling seasons. ADCT- *Achnanthydium catenatum*, GLGN- *Gomphonema lagenula*, NROS- *Navicula rostellata*, CAMB- *Craticula ambigua*, ADLA- *Achnanthydium latecephalum*, NSBN- *Navicula subalpina*, AUGR- *Aulacoseira granulata*, NMCA- *Navicula microcari*, NISC- *Nitzschia aff. scalpelliformis*, FPAR- *Fragilaria pararumpens*, CPST- *Cocconeis pseudolineata*, FRAC- *Ulnaria acus*, LMUT- *Luticola mutica*, CSCI- *Cymbella subcistula*, NACI- *Nitzschia acicularis*, FRUM- *Fragilaria rumpens*, CPEA- *Cocconeis euglypta*, NPAL- *Nitzschia palea*, NIAR- *Nitzschia archibaldii*, GEXL- *Gomphonema exilissimum*, NCAT- *Navicula catalanogermanica*, ECPM- *Encyonopsis minuta*, DVUL- *Diatoma vulgaris*, SPTH- *Stauroneis parathermica*, HLMO- *Halamphora montana*, CCMP- *Cymbella compacta*, GDRU- *Gomphonema drutelingense*, FPRU- *Fragilaria pararumpens*, NEXI- *Navicula exilis*, PMCH- *Pinnularia marchica*, GYAC- *Gyrosigma acuminatum*, ECAE- *Encyonema caespitosum*, NREC- *Nitzschia recta*, FGRA- *Fragilaria gracilis*, SPUP- *Sellaphora pupula*, NVIR- *Navicula viridula*, SSMI- *Stauroneis smithii*, NMOK- *Navicula moskalii*, GTRU- *Gomphonema truncatum*, CMEN- *Cyclotella meneghiniana*, NCTO- *Navicula cryptotenelloides*, CPPV- *Cymbella perparva*, HAMP- *Hantzschia amphioxys*, FCAP- *Fragilaria capucina*, RUNI- *Reimeria uniseriata*.

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