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Original scientific paper

SEASONAL VARIATIONS OF DIATOMS DIVERSITY AND COMPOSITION IN THE CRNICA RIVER

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The aim of this study was determining and comparing diatom diversity and dominant taxa in 4 seasons from the Crnica River. A total of 170 diatom taxa, 24 among them as dominant ones were recorded. The highest diversity expressed in number of taxa was observed in the spring (139) and the lowest in the autumn season (94). The most of 45 the best fitted taxa on the CCA ordination diagram are recorded in more than two seasons, often in all four seasons. One group of taxa (e.g. *Hanaea arcus*, *Meridion constrictum*, *Navicula gregaria*, *N. lanceolata*) was characteristic only for spring and other for winter season (e.g. *Gomphonema innocens*, *G. lagenula*, *Stauroneis phoenicenteron*, *Nitzschia oligotraphenta*). There were no taxa exclusively recorded only in summer and autumn.

Key words: diatom community, seasonality, Crnica River

INTRODUCTION

A high quality freshwaters are essential for healthy functioning of natural ecosystems, but also provides a variety of ecosystem services to humans (Várbíró *et al.* 2020, Lee *et al.* 2021). Rivers have been used for

centuries as a source of water used in domestic, agricultural and industrial activities, fisheries, etc. These activities have greatly contributed to changes in the river systems by affecting the communities of many organisms and water quality (Espinosa *et al.* 2020). Freshwater ecosystems are considered today the most endangered ecosystems worldwide (Várbíró *et al.* 2020, Lee *et al.* 2021). Their protection and management are not possible without regular studying and monitoring the communities of aquatic organisms (McCarthy *et al.* 2018), since these ecosystems are highly heterogeneous, with significant spatio-temporal variation in community structure (Wang *et al.* 2019).

Benthic diatoms are the key component of microalgal assemblages in freshwater ecosystems. They are the most abundant and diverse group of benthic algae, primary producers and therefore a crucial component in the nutrient cycle and energy flow in water ecosystems (Wang *et al.* 2018). Diatoms represent excellent, widely used bioindicators, because of their short generation time and fast response to environmental changes (Wu *et al.* 2017). Both biotic and abiotic factors, as well as chemical variables control diatom community composition. Benthic diatom communities usually are influenced by chemical conditions of the environment, but also biotic interactions, such as grazing and predation (Guo *et al.* 2020). Seasonal changes of controlling factors affect the functional composition and diversity of diatom communities. Virta *et al.* (2020) believe that high taxonomic diversity allows stability of ecosystem throughout the year, providing the resilience against environmental change. Some authors point out importance of ultimate factors (climate, geology, land use) in diatom assemblages variation (Pajunen *et al.* 2020).

Only a few studies on diatom communities of the Crnica River have been conducted (Ranković *et al.* 1995, Jakovljević *et al.* 2018, 2018a, 2021), without analysis of their seasonal dynamics. Study of seasonal analysis of epilithic diatom community from the Crnica River was conducted with the aim of determining and comparing diatom diversity and dominant taxa in 4 seasons, therefore examining the seasonal dynamics of the diatom community from the Crnica River.

MATERIAL AND METHODS

Study area and diatom sampling

The Crnica River is one of the most important right tributaries of the Morava River with the length of 32 km (Mustafić *et al.* 2012) (Fig. 1). The highest point (Debelo Brdo) of the basin (1005 m) is located in Srednji

Kučaj, while the lowest point (120 m) is at the mouth of the Velika Morava. Diverse geological structure caused the appearance of different types of soil. The average amount of precipitation in the lowest parts of the basin is 590 mm, while in the higher mountainous part an average of 880 mm of precipitation is excreted (Mustafić *et al.* 2012).

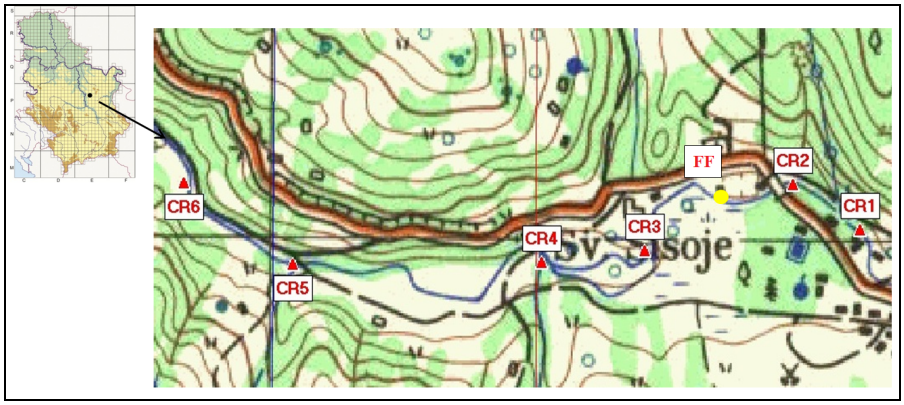

Fig. 1. – Location of the Crnica River with sampling sites (CR1-CR6) and position of the trout fish farm (FF).

Collection of epilithic diatoms was carried out at 6 sampling sites marked CR1-CR6 from April 2011 to May 2012, when a total of 36 samples were collected. CR1 and CR2 sampling sites represent the control localities above the fish farm (250 m and 20 m upstream from the fish farm) and 4 sites are located downstream at different distances from the farm (20 m, 400 m, 1.3 km and 1.7 km respectively) (Fig. 1). From each locality, five stones of medium size were collected and scraped with a toothbrush (SRPS EN 13946:2015). At the same time, the stones are washed with distilled water. The obtained material are transferred to plastic bottles and fixed with 4% formaldehyde.

Diatom analysis

In laboratory, the algological material was treated with cold strong acids (H_2SO_4 and $C_2H_2O_4$) and KM_nPO_4 (Krammer & Lange-Bertalot 1986). After laboratory treatment of diatoms, permanent slides were made using Naphrax® mounting medium. Qualitative analysis was done using Carl Zeiss AxioImager.M1 microscope, with AxioCam MRc5 camera and AxioVision4.8 software. Quantitative analysis was done taking into account the first 400 valves on each slide (SRPS EN 14407:2015). Standard literature was used for identification of diatom taxa: Krammer 2000, 2002, Lange-Bertalot 2001, Lange-Bertalot *et al.* 2017. For statistical analysis, we used CANOCO program for Windows, Version 5.0 (Ter Braak & Šmilauer 2012).

RESULTS

A total of 170 diatom taxa were recorded during this study and 24 were dominant among them (their percentage share in diatom community was higher than 5% at least at one sampling site in one season). By analyzing alpha diversity of diatoms during all four sampling seasons from the Crnica River, the highest diversity was observed in the spring and the lowest in the autumn season.

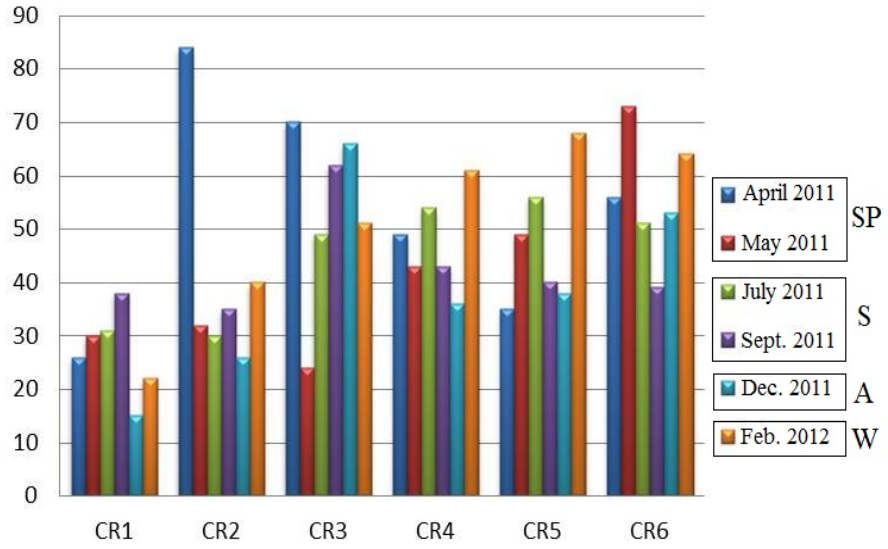

Fig. 2. – Diversity of diatom assemblages in the Crnica River at 6 sampling sites (CR1-CR6) in four seasons: spring (SP) – April and May 2011, summer (S) – July and September 2011, autumn (A) – December 2011 and winter (W) – February 2012.

A total of 139 diatom taxa were identified during the spring period (April 2011 and May 2012) in the Crnica River. It can be noticed a big difference in diversity concerning sampling sites. The highest number of taxa was recorded at CR2 (84 taxa in April 2011) and the lowest at CR3 (24 taxa in May 2012) (Fig. 2). 15 diatom taxa with a relative abundance higher than 5% at least at one site were identified in this season (Tab. 1). *Achnanthidium minutissimum*, *Cocconeis lineata*, *Gomphonema elegantissimum* and *Gomphonema micropus* were dominant taxa (Tab. 1). *A. minutissimum* was dominant taxon at three sampling sites (CR2, CR3 and CR6) with the highest abundance (57%) at CR2 in April 2011. *C. lineata* was dominant taxon at CR5 (26.5%) in April 2011, while *G. elegantissimum* and *G. micropus* were both dominant in May 2012, but at different sites. The first one at CR4 (36.5%) and the second at CR1 (55.7%).

Table 1. – Ranges of relative abundances (%) of dominant taxa throughout each season in the Crnica River.

Taxa	Spring		Summer		Autumn	Winter
	April 2011	May 2012	July 2011	Sep. 2011	Dec. 2011	Feb. 2012
Achnanthidium catenatum (Bily & Marvan) Lange-Bertalot 1999	/	/	/	/	9.9-10.1	/
Achnanthidium minutissimum (Kützing) Czarnecki 1994	11.2-56.9	3.4-31.7	8.3-35.2	19.4-45.6	4.6-25.9	8.1-29.8
Achnanthidium pyrenaicum (Hustedt) H.Kobayasi 1997	2.2-28.8	0.2-7.1	1.9-36	2.8-38.4	0.4-7.1	2.2-16.4
Amphora pediculus (Kützing) Grunow 1875	2.4-15.8	0.9-17.9	6.3-15.5	6.1-23.7	0.2-21.4	3.2-13.3
Cocconeis lineata Ehrenberg 1849	0.2-26.4	1.2-3.4	0.7-20.7	1.2-25	0.7-8.4	0.9-35.3
Cocconeis pseudolineata (Geitler) Lange-Bertalot 2004	0.2-1.6	0.2-1.2	0.2-6	0.4-8	/	/
Denticula tenuis Kützing 1844	0.4-4.1	0.2-3.4	2.2-5.4	0.2-5.4	0.7-18.6	0.7-31.6
Encyonema lange-bertalotii Krammer 1997	0.2	/	/	0.4	/	21.3
Encyonema silesiacum (Bleisch) D.G.Mann 1990	0.2	0.4-2.9	0.2-0.4	0.2-1.9	0.2-6.2	0.2-3.9
Encyonema ventricosum (C.Agardh) Grunow 1875	0.2	0.2-2.4	0.2-0.7	0.2-0.7	0.2-40.9	0.2-11.5
Encyonopsis minuta Krammer & E.Reichardt 1997	0.9-6.4	/	/	/	/	/
Fragilaria vaucheriae (Kützing) J.B.Petersen 1938	0.2	0.4-6.8	0.2-1.4	0.9-2.9	/	/
Gomphonema elegantissimum E.Reichardt & Lange-Bertalot 2011	0.2-19.6	1.7-36.4	2.2-47.1	1.9-5.4	0.9-6.9	0.9-4.1
Gomphonema micropus Kützing 1844	0.2-4.1	3.6-55.6	0.2	0.2-0.4	/	/
*Gomphonella olivacea (*Hornemann) Rabenhorst 1853	0.4-2.4	0.4-13.7	0.2	/	0.7-46.6	6.1-11.1
Meridion circulare (Greville) C.Agardh 1831	0.4-20.5	0.7-27.8	0.2-14	0.2-0.9	0.2-0.4	0.2-5.9
Navicula tripunctata (O.F.Müller) Bory 1822	1.2-3.2	2.4-5.1	0.2-3.6	0.2-4.6	0.4-13.8	0.4-12
Nitzschia fonticola (Grunow) Grunow 1881	2.6	1.4-4.4	0.2-0.4	0.4-0.7	2.2-32.6	2.2-47.2
Planothidium dubium (Grunow) Round & Bukhtiyarova 1996	1.2-1.9	0.4-1.9	0.7-5.4	0.4-3.9	/	/
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot 1999	0.8-8.8	0.7-1.9	0.2-5.1	0.7-4.7	/	/
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot 1999	0.4-4.4	0.4-15.5	0.7-1.7	0.4-1.1	0.4	0.2-7.4
Reimeria sinuata (W.Gregory) Kociolek & Stoermer 1987	0.4-5.8	0.4-15.2	1.2-4.1	0.7-8.2	0.4-7.4	0.4-1.7
Reimeria uniseriata S.E.Sala. J.M.Guerrero & M.E.Ferrario 1993	3.3	0.4	3.6	0.9-6.5	/	/
Staurosirella pinnata (Ehrenberg) D.M.Williams & Round 1988	0.4-6.1	0.9-3.4	2.7-3.9	2.2-9.6	0.9-32.2	0.2-2.6

In the summer period (July and September 2011), the presence of 109 diatom taxa was determined. The highest number of taxa was recorded at site CR3 (62 taxa in September 2011), while the CR2 is characterized by the lowest number of taxa (35 in the same sampling month) (Fig. 2). Downstream from the CR3, the number of taxa gradually decreases. In the summer period, 13 dominant taxa were recorded (Tab. 1). *A. minutissimum* was dominant taxon in this season at CR3, CR4 and CR6 sampling sites with relative abundance from 24.1% to 45.6%. At CR1, dominant species was *G. elegantissimum* with the highest abundance of 47.2%. *A. pyrenaicum* was dominant taxon at CR2 (38.4%) while *C. lineata* at CR5 (25.1%).

During the autumn season, a total of 94 diatom taxa were identified in the Crnica River. CR3 stood out as the sampling site with the highest number of taxa (67), and CR1 with the lowest (15) (Fig. 2). By analyzing diversity of taxa with relative abundance higher than 5% at least at one sampling site in the autumn, their number was 14 (Tab. 1). *Staurosira mutabilis* was dominant taxon at CR1 (32.2%), *Encyonema ventricosum* at CR2 (40.94%), *Nitzschia fonticola* at CR3 (32.67%), *A. minutissimum* at CR4 and CR5 (25.49% and 19.05%) and *Gomphonella olivacea* at CR6 (46.66%).

Concerning the winter period (February 2012), number of recorded diatom taxa in the Crnica River was 105. The highest number of taxa was recorded at CR5 (68 taxa), while the lowest at CR1 (22) (Fig. 2). 12 diatom taxa were found to be present during the winter with community share higher than 5% at least at one site (Tab. 1). Dominant taxa in this season were *A. minutissimum* (24.32%), *A. pyrenaicum* (48.02%), *C. lineata* (35.30%), *Denticula tenuis* (31.60%) and *N. fonticola* (47.26%).

The relationship between sampling seasons used as explanatory variable and taxa recorded in the Crnica River is shown in CCA (Fig. 3). A total of 45 the best fitted taxa are shown on the ordination diagram. Many of them are located in the central part of the diagram, meaning that they are recorded in more than two seasons, often in all four. However, one group of taxa in the lower left part of the ordination diagram was characteristic only for spring, and other in the lower right part for winter season. There were no taxa exclusively recorded only in summer and autumn. There were some representatives, however, found in two seasons only, e.g. *Encyonema minutum* and *Staurosirella martyi* were identified in spring and summer, *Gyrosigma sciotoense* in summer and autumn, *Cocconeis placentula* and *Navicula cryptotenelloides* in autumn and winter, *Nitzschia pusilla* in winter and spring.

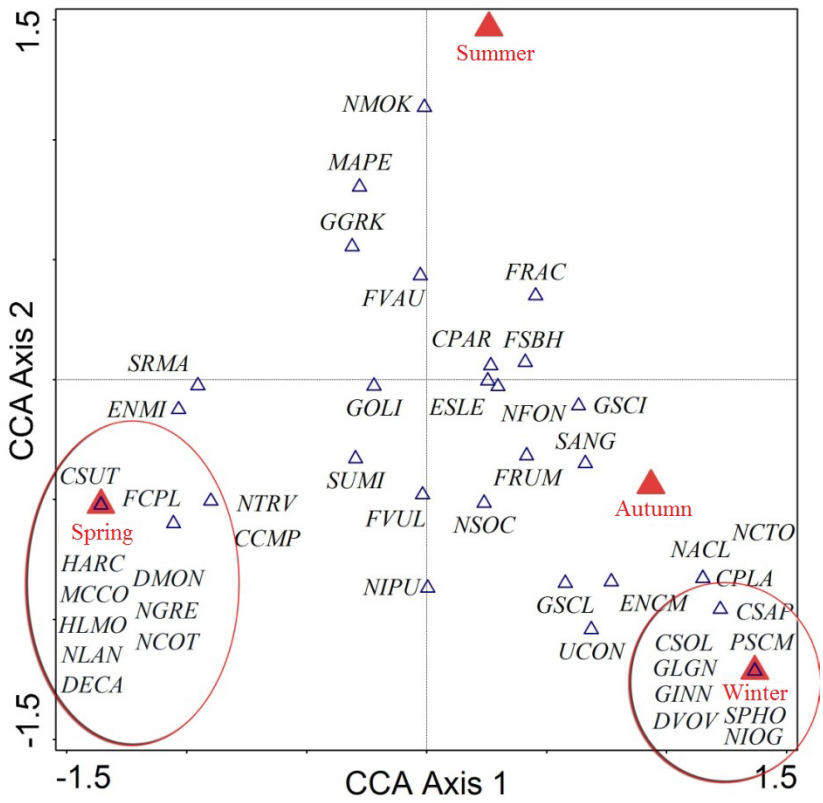

Fig. 3. CCA showing the relationship of diatom taxa identified in the Crnica River and sampling seasons. *Navicula moskalii* - NMOK, *Mayamaea permitis* - MAPE, *Geissleria gereckeii* - GGRK, *Fragilaria vaucheriae* - FVAU, *Staurosirella martyi* - SRMA, *Cymbella parva* - CPAR, *Ulnaria acus* - FRAC, *Fallacia subhamulata* - FSBH, *Encyonema minutum* - ENMI, *Gomphonella olivacea* - GOLI, *Encyonema silesiacum* - ELSE, *Surirella minuta* - SUMI, *Navicula tripunctata* - NTPT, *Frustulia vulgaris* - FVUL, *Cymbella compacta* - CCMP, *Nitzschia pusilla* - NIPU, *Cymbella subtruncata* - CSUT, *Fragilaria recapitellata* - FCPL, *Diatoma moniliformis* - DMON, *Hannaea arcus* - HARC, *Meridion constrictum* - MCCO, *Navicula gregaria* - NGRE, *Navicula lanceolata* - NLAN, *Diatoma ehrenbergii* var. *capitulata* - DECA, *Halamphora montana* - HLMO, *Nitzschia constricta* - NCOT, *Nitzschia fonticola* - NFON, *Gyrosigma sciotoense* - GSCI, *Surirella angusta* - SANG, *Fragilaria rumpens* - FRUM, *Nitzschia sociabilis* - NSOC, *Nitzschia acicularis* - NACI, *Gomphonema subclavatum* - GSCL, *Encyonopsis microcephala* - ENCM, *Ulnaria contracta* - UCON, *Cocconeis placentula* - CPLA, *Navicula cryptotenelloides* - NCTO, *Cymatopleura solea* - CSOL, *Gomphonema lagenula* - GLGN, *Stauroneis phoenicenteron* - SPHO, *Gomphonema innocens* - GINN, *Diatoma vulgaris* var. *ovalis* - DVOV, *Cymatopleura solea* var. *apiculata* - CSAP, *Nitzschia oligotraphenta* - NIOG, *Pinnularia subcommutata* - PSCM.

DISCUSSION

Observations from our study are supported by findings where the highest diversity of epilithic diatom taxa, in temperate zone, is recorded in spring period (Fig. 2) (Vidaković *et al.* 2020). According to Cardinale *et al.* (2002), efficiency of resource use, persistence under stressful conditions and high diversity are positively correlated. We found the small differences between taxa richness in summer and winter seasons (only 4 taxa). Seasonal changes in taxa richness were not significant, suggesting that epilithic diatom community in the Crnica River is best adapted to moderate disturbance. Considering sampling sites, influence of fish farm on diatom taxa diversity can be noticed in two seasons (taxa number was significantly higher at site after discharge (CR3). This can be explained by the increased concentration of nutrients after the farm. Virta *et al.* (2020) wrote about the importance of nutrients to diversity of diatoms. Temporal changes of diatom taxa diversity also reflect the intermediate disturbance hypothesis in the Crnica River.

Considering shift of dominant taxa throughout the seasons, *Achnanthidium minutissimum* and *Cocconeis euglypta* showed resistance in three and two seasons respectively (Tab. 1). *A. minutissimum* is regarded as indicator species with a broad ecological tolerance but recent studies (Pinseel *et al.* 2017) indicate that molecular data of *Achnanthidium* genus will help and give a new point to taxonomy and ecology of this group (Marquardt *et al.* 2017). *C. euglypta* is typical taxon of flowing waters with medium-high mineralization. However, habitat and ecology of this species is not yet well known due to inconsistencies in the identification of this species over time (Lai *et al.* 2019, Lange-Bertalot *et al.* 2017). In spring samples, in addition to the two mentioned taxa, *Gomphonema elegantissimum* and *G. micropus* were dominant (Tab. 1). *G. micropus* belongs to the group of alkaliphilous taxa, occurs in fresh and brackish waters with high oxygen level, and tolerate elevated concentrations of organically bound nitrogen (Wojtal 2003). Vidaković *et al.* (2020) recorded also species of the genus *Gomphonema* with high relative abundance in the spring period in the Studenica River. The same taxa were dominant in summer as in the spring period, but also *Achnanthidium pyrenaicum* (Tab. 1). This taxon often occurs together with *A. minutissimum*, but it never dominant in eu- and hypertrophic waters (Lange-Bertalot *et al.* 2017). A change of dominant species is observed in the autumn period. *Gomphonella olivacea* and *Encyonema ventricosum* recorded as dominant taxa (Tab. 1). They are frequent in eutrophic habitats with saprobic tolerance ranges from oligo-saprobic to α-mesosaprobic level (Lange-Bertalot *et al.* 2017). The winter period had the largest number of the same dominant taxa as the summer, but also *N. fonticola* and *Denticula tenuis* (dominant only in the winter)

(Tab. 1). Pinseel *et al.* (2017) indicated tolerance of *D. tenuis* for turbulent environments thanks to strong connections of cells in colonies and with their substrate.

Observing the relationship between sampling seasons and all taxa recorded in the Crnica River, its interesting that many taxa were characteristic only for spring and winter (Fig. 3), two periods often with the highest and lowest diatom diversity (Vidaković *et al.* 2020). Although many factors affect the structure of the diatom community (stochastic processes, biotic interactions, environmental filtering), it seems that the seasonal change of some environmental factors such as temperature, light and nutrients, affects the occurrence of a certain number of diatom species. These environmental factors change seasonally for most rivers (Fitzgerald 2017).

CONCLUSIONS

There are still unexplored habitats in Serbia, or habitats with insufficient data when it comes to diatoms. Studies of seasonal changes of diatom diversity and composition should be conducted on as many rivers as possible, since such research will improve monitoring of rivers in Serbia, in which diatoms have an irreplaceable role in water quality assessment in all European countries. Therefore, it is clear that such studies also can provide data for good conservation strategy of these valuable and endangered water ecosystems.

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СЕЗОНСКЕ ВАРИЈАЦИЈЕ ДИВЕРЗИТЕТА И САСТАВА СИЛИКАТНИХ АЛГИ РЕКЕ ЦРНИЦЕ

ОЛГА ЈАКОВЉЕВИЋ, СЛАЂАНА ПОПОВИЋ, ИВАНА ЖИВИЋ,
КАТАРИНА СТОЈАНОВИЋ, ЈЕЛЕНА КРИЗМАНИЋ

Р Е З И М Е

Циљ ове студије био је одређивање и упоређивање диверзитета и доминантних таксона силикатних алги реке Црнице током 4 сезоне. Забележено је укупно 170 таксона силикатних алги, од чега 24 доминантних. Највећи диверзитет је утврђен у пролеће (139), а најмањи у јесен (94). Велики број таксона је забележен у више од две сезоне, често у све четири. Једна група таксона била је карактеристична само за пролеће, а друга за зимску сезону. Није било таксона забележених искључиво у лето и јесен.