

DIATOM SPECIES COMPOSITION AND THEIR SEASONAL DYNAMICS IN THE TIMOK RIVER BASIN

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ABSTRACT

The aim of this paper was to present the composition and seasonal dynamics of epilithic diatoms in the Timok River basin. The diatom samples were collected along the Timok River basin in March, May, August and November 2017. Permanent diatom slides were prepared after oxidizing the organic material by the hydrogen peroxide (H₂O₂). The cleaned diatom materials were mounted on permanent slides using ZRAX glue. A total of 85 taxa were identified in the Timok River Basin. The benthic diatom taxa belong to 31 genera with the highest diversity observed within *Navicula* Bory (15), *Nitzschia* Hassall (12) and *Gomphonema* Ehrenberg (8). Quantitative analysis showed that in all seasons, *Achnantheidium minutissimum* and *Amphora pediculus* were dominant taxa. In May, they were joined by the *Achnantheidium pyrenaicum*, *Gomphonema olivaceum* and *Ulnaria ulna*; in August by the *Cocconeis placentula*, *Denticula kuetzingii*, *Melosira varians*, *Navicula metareichardtiana*, *Nitzschia capitellata*, *N. fonticola* and *N. palea*; in November by the *D. kuetzingii*, *G. olivaceum*, *G. pumilum*, *N. metareichardtiana*, *N. veneta*, *Nitzschia capitellata* and *N. palea* and in March by *A. pyrenaicum*, *Diatoma moniliformis*, *G. olivaceum*, *N. palea* and *U. ulna*. Our research is the floristical and ecological study of benthic diatoms in this basin and can form the groundwork for further research work.

Keywords: Benthic diatoms, Species composition, Seasonal dynamics, Ecology, Timok River.

INTRODUCTION

Diatoms are a species rich group of eukaryotic unicellular microalgae and one of the most common organic matters in aquatic ecosystem (Wojtal, 2009). They play an important role the primary producers in freshwater and marine environments (Sarhou et al., 2005; Ishii et al., 2011). Diatoms are found in all aquatic habitats and in moist terrestrial habitats (Mann & Vanormelingen, 2013). They are cosmopolitan, with a wide geographical distribution and well-known autecology of most species (Wu et al., 2011; Van Dam et al., 1994). The taxonomic structure of diatom communities in rivers and streams is conditioned by water temperature, nutrient concentration, water flow, amount of light, grazing effect, substrate type and size (Wu et al., 2016). The impact of topography (altitude, longitude and latitude), geographically climatic characteristics (Tison et al., 2005), as well as glaciation (Stevenson et al., 1996), geomorphological characteristics and land use (Leland & Porter, 2000) must certainly not be neglected. The distribution, quality and quantity of diatom taxa on a temporal scale also depend on the characteristics of the species (growth rate, size) and the ecological niche of the species, since species of a broad ecological spectrum are more able to tolerate fluctuation of ecological factors in habitats than species with narrow ecological valences. The main study objectives were to present the floristic richness and seasonal dynamics of diatoms in the Timok River basin.

MATERIAL AND METHODS

The Timok is a across-border river. Most of the basin is located in eastern Serbia (98 %), while the smaller part is in the territory of Bulgaria (Paunović et al., 2008). It belongs to the Black Sea basin. The Timok River is formed by the joining of the Beli Timok and Crni Timok rivers downstream of the town Zaječar. The Beli Timok is formed by the joining of the Svrliški Timok and Trgoviški Timok rivers on the northern outskirts of the town Knjaževac. Timok is the largest river of eastern Serbia, the right and last tributary of the Danube in Serbia, which flows into the Danube at 845.65 river km and not far from the mouth of the Timok river is the border of Serbia and Bulgaria. In the area of the Timok River basin, the so-called Timok river system is being developed for water supply for hydroelectric power plants, supply for settlements and water industry, irrigation, and tourist valorization of watercourses (Paunović et al., 2008).

Data on previous studies of diatoms in the Timok River basin are scarce. The first data on algological investigations date from 1996 and relate to the Trgoviški Timok River, when 143 taxa from 7 phyla were identified: *Cyanophyta* (24), *Rhodophyta* (3), *Xanthophyta* (2), *Chrysochyta* (1), *Bacillariophyta* (100), *Euglenophyta* (1) and *Chlorophyta* (12) (Simić, 1996). With regard to exploration of other rivers in the Basin, data are rare and relate to the diatoms of the tributaries of the Beli Timok (Ćirić et al., 2018) and the Crni Timok (Nikitović & Laušević, 1995), as well as other groups of *Chlorophyta* and *Rhodophyta* algae in the Svrliški, Trgoviški and Beli Timok rivers (Simić et al., 2002; Simić & Ranković, 1998a, 1998b; Simić, 1995).

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The sampling was conducted during four seasons (March, May, August and November 2017) from 8 localities (Figure 1, Table 1) covering evenly the whole length of of the watercourse. Benthic diatoms were collected from a depth of 20-30 cm by

brushing the stones with a toothbrush following Taylor et al. (2005). The samples were immediately fixed in 4% formaldehyde (HCHO).

Table 1. Characteristics of the 8 sampling stations along the Timok River basin.

No.	River	Sampling site	Sampling site code	Latitude (°)	Longitude (°)	Altitude(m)
1	Trgoviški Timok	Štrbac	ST5	43.50306	22.31861	359
2	Beli Timok	Drenovac	BT2	43.68944	22.26944	177
3	Zlotska reka	Zlot	CT4	44.00583	21.98466	261
4	Lubnička reka	Lubnica	CT7	43.8585	22.19266	225
5	Crni Timok	Zvezdan	CT8	43.89166	22.22166	147
6	Borska reka	Kriveljska_Reka	BR5	44.03333	22.21667	231
7	Borska reka	Rgotina	BR7	44.00688	22.26733	153
8	Timok	Vražognac	VT1	43.9555	22.32017	111

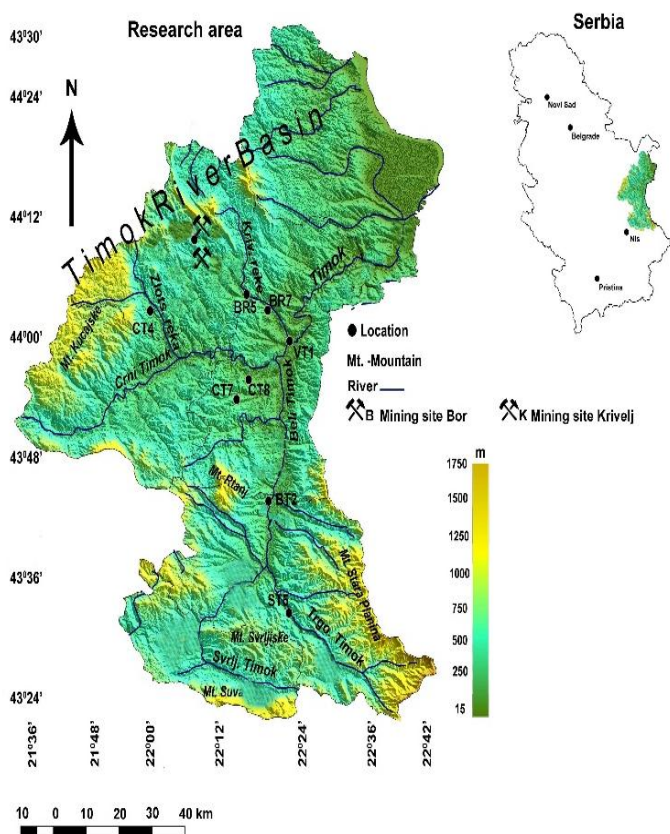


Figure 1. The Timok River basin and sampling stations.

At each site pH, conductivity, temperature and salinity were measured using a multiparameter device (PCSTester 35K), which was calibrated beforehand.

Diatom frustules were cleaned using 10% cold hydrochloric acid (HCL) and 30% hydrogen peroxide (H₂O₂) to remove organic matter from the sample according to the standard method described by Taylor et al. (2005). All samples were washed several times with distilled water, then the material was mounted in ZRAX glue and permanent slides were made. The relative abundance (%) of the identified taxa was determined by counting

400 valves on each slide on the same microscope that used for qualitative analysis of diatoms, and at 1000 x magnification (EN 14407, 2014).

Diatom species were identified according to Lange Bertalot et al. (2017) and Levkov (2009).

In order to analyse the diversity of epilithic diatom assemblages at different sampling sites and in different seasons, Shannon's Diversity Index was computed along with Pielou's evenness index following the calculations described in Heip et al. (1998).

RESULTS AND DISCUSSION

Values of the physical and chemical water characteristics from 8 sampling sites are presented in Table 2 and Table 3. The water temperature during the sampling period varied from 5.4°C (CT7) in March to 23.2°C (VT1) in August. pH ranged from 5 (BR7) in March to 8.8 (VT1) in August. Electrical conductivity values varied from 232 µS cm⁻¹ (ST5) to 4310 µS cm⁻¹ (BR7) and salinity from 0,1‰ (ST4) to 2,32‰ (BR7).

A total of 85 taxa were identified in the Timok River basin. The benthic diatom taxa of the Timok River basin belong to 31 genera with the highest diversity observed in *Navicula* (15), *Nitzschia* (12) and *Gomphonema* (8). The largest number of taxa was identified at the CT8 site (44) in May and August; in November, all localities are distinguished by a similar number of taxa (27 -35) and in March, the largest number of taxa was identified at the BR7 and VT1 sites (both 37 each). The smallest number of taxa was present at the sampling site CT7 (18) in May; in August at sampling site BR5 (17) and in March at the sampling site ST5 (18). *Achnantheidium minutissimum* (Kützing) Czarnecki, *Achnantheidium pyrenaicum* (Hustedt) Kobayasi, *Amphora pediculus* (Kützing) Grunow, *Cocconeis placentula* Ehrenberg, *Diatoma moniliformis* (Kützing) D. M. Williams, *Gomphonema olivaceum* (Hornemann) Brébisson, *Gomphonema pumilum* (Grunow) E. Reichardt & Lange-Bertalot, *Navicula cryptonella* Lange-Bertalot, *Navicula metareichardtiana* Lange-

Bertalot & Kusber, *Navicula tripunctata* (Müller) Bory de Saint-Vincent, *Nitzschia dissipata* (Kützing) Rabenhorst, *Nitzschia fonticola* (Grunow) Grunow, and *Nitzschia palea* (Kütz.) W.

Smith were the most frequent taxa in the Timok basin, represented in over 75% of the samples. The same taxa are also dominant.

Table 2. Values of the physical and chemical water characteristics from 8 sampling sites of the Timok River basin in March and May.

Season and site	March 2017								May 2017							
	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8
Temperature (°C)	6,3	7,3	7,3	5,4	8,3	8,6	7,8	7,5	10,8	13,7	12,5	12,3	14,7	15,8	15,2	13,5
pH	8,2	8,2	8,2	8,5	8,6	5,3	5,0	8,2	8,4	8,3	8,4	8,4	8,3	6,7	5,4	8,4
Conductivity (µS/cm)	232,0	411,0	280,0	685,0	438,0	1168,0	1433,0	401,0	258,0	436,0	322,0	710,0	466,0	1630,0	1896,0	421,0
Salinity (‰)	0,1	0,2	0,1	0,3	0,2	0,6	0,7	0,2	0,1	0,2	0,2	0,3	0,2	0,8	1,0	0,2

Table 3. Values of the physical and chemical water characteristics from 8 sampling sites of the Timok River basin in August and November.

Season and site	August 2017								November 2017							
	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8
Temperature (°C)	17,2	21,4	20,3	16,0	23,2	19,7	21,5	22,7	6,6	8,0	9,8	8,0	8,8	8,9	8,9	7,8
pH	8,4	8,5	8,5	8,4	8,8	6,3	6,0	8,5	8,2	8,2	8,4	8,0	8,7	6,5	5,3	8,3
Conductivity (µS/cm)	338,0	530,0	433,0	1290,0	564,0	3000,0	4310,0	464,0	232,0	402,0	409,0	887,0	502,0	1865,0	1753,0	537,0
Salinity (‰)	0,2	0,3	0,2	0,6	0,3	1,6	2,3	0,2	0,1	0,2	0,2	0,4	0,2	0,9	0,8	0,2

Halumphora montana (Krasske) Levkov, *Pantocsekiella ocellata* (Pantocsek) K. T. Kiss & Ács, *Cymbella neolanceolata* W. Silva, *Odontidium mesodon* (Kützing) Kützing, *Diploneis oculata* (Brébisson) Cleve, *Eunotia pectinalis f. undulata* (J. Ralfs) Berg, *Fallacia subhamulata* (Grunow) D. G. Mann, *Gyrosigma acuminatum* (Kützing) Rabenhorst, *Karayevia ploenensis* (Hustedt) Bukhtiyarova, *Navicula amphiceropsis* Lange-Bertalot & Rumrich, *Navicula reichardtii* Grunow, *Navicula viridulacalcis* Lange-Bertalot, *Nitzschia linearis var. tenuis* (Smith) Gr. in Cleve & Gr., *Nitzschia vermicularis* (Kützing) Hantzsch in Rabenhorst, *Pinnularia obscuriformis* Krammer and *Tryblionella apiculata* W. Gregory were present at only one locality. Quantitative analysis showed that in all seasons, *A. minutissimum* and *A. pediculus* were present with a percentage with 10% or more at least at one locality (Table 4, Table 5). In May, they were joined by the *A. pyrenaicum*, *G. olivaceum* and *Ulnaria ulna* (Nitzsch) Compère; in August by the *C. placentula*, *Denticula kuetzingii* Grunow, *Melosira varians* C. Agardh, *N. metareichardtiana*, *Nitzschia capitellata* Hustedt in Schmidt et al., *N. fonticola* and *N. palea*; in November by the *D. kuetzingii*, *G. olivaceum*, *G. pumilum*, *N. metareichardtiana*, *Navicula veneta* Kützing, *N. capitellata* and *N. palea* and in March by *A. pyrenaicum*, *D. moniliformis*, *G. olivaceum*, *N. palea* and *U. ulna*.

The Timok River basin is dominated by taxa that often occur in samples, as well as a large number of taxa that occur sporadically, which is typical of many rivers (Szabó et al., 2005; Chatháin & Harrington, 2008; Makovinska & Hlubikova, 2015). *A. minutissimum* and *A. pediculus* were dominant taxa in all four seasons. These taxa are cosmopolitan and have widespread distribution in the epilithic communities of diatoms of many rivers (Potapova & Charles, 2003; Bere & Tundisi, 2011). *A. minutissimum* is a widespread taxon in acidic, base,

oligo- to hypereutrophic waters (Van Dam et al., 1994; Wojtal & Sobczyk, 2006). *A. pediculus* is a widespread freshwater species that occasionally inhabits eutrophic and moderately polluted waters (Levkov, 2009). It is an alkalophilic species, which often occurs in waters with moderate conductivity (Van Dam et al., 1994), and is absent from acidic habitats with low electrolyte concentration (Lange-Bertalot et al., 2017). The most abundant genera were: *Navicula*, *Gomphonema* and *Nitzschia*. This is to be expected, since researches in Serbia (Andrejić et al., 2012; Vidaković et al., 2015; Jakovljević et al., 2016a, 2016b; Vasiljević et al., 2017) and other countries (Hlubiková et al., 2009; Sevindik & Kucuk, 2016; Noga et al., 2016) have shown that these three genera are dominant in the epilithic community.

Changes in the physical and chemical characteristics of water over time affect the seasonal dynamics of the diatom communities. Taxon distribution from upper to lower parts of the stream, water ionic composition and pH and temperature values are the three most important environmental factors that affect the structure of diatom communities on a temporal and spatial scale (Potapova & Charles, 2002). Diatoms are known to respond to annual variations in water temperature (Di Pippo et al., 2012; Lambert et al., 2016). *C. placentula* may increase abundance in the summer months, as observed in studies by Ní Chatháin & Harrington (2008) who found a greater number of this taxon during the early summer, while the *Gomphonema olivaceum* is adapted to lower temperatures (Potapova & Charles, 2002), which is consistent with our findings (seen in Table 4 and Table 5). Also, Wojtal & Sobczyk (2006) point out that appearance of certain taxa with higher relative representation may be related to the seasonal shift of diatom communities. They found an increased abundance of species of the genus *Navicula* in November, while the research by Ní Chatháin & Harrington (2008) showed that the species of the genus *Gomphonema* are

more common in the autumn, which is consistent with our results indicating an increased relative representation of species of genus *Navicula* (*N. metareichardtiana* and *N. veneta*) and species of genus *Gomphonema* (*G. pumilum*) during the said period. *G. pumilum* is considered to be the most widespread species of the genus *Gomphonema*, occurring in rivers and

streams with limestone bedrock, inhabits oligosaprobic to β -mesosaprobic water and can be tolerant of medium to high trophic level pollution (Lange-Bertalot et al., 2017). Undernatural conditions, water temperature and its annual variation depend on other factors (DeNicola, 1996).

Table 4. Diatom taxa with a percentage with 10% or more at least at one locality in the Timok River basin in 2017, in March and May.

Season and site Taxa	March 2017								May 2017							
	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8
<i>Achnanthydiumpyrenaicum</i>	73,9	0,5	1,7	0,0	0,0	7,7	2,9	7,4	38,0	0,4	44,8	0,0	1,5	9,5	1,4	2,4
<i>Achnanthydiuminutissimum</i>	0,0	1,4	45,2	1,4	4,8	44,5	33,5	13,4	3,0	8,9	32,9	6,3	6,1	34,2	53,0	8,7
<i>Amphora pediculus</i>	4,8	20,5	1,9	12,7	2,5	0,0	1,3	12,4	18,8	49,1	6,2	74,6	61,3	2,0	0,4	7,2
<i>Diatoma moniliformis</i>	0,0	0,2	2,6	0,0	10,7	0,0	2,7	2,5	1,9	1,8	0,2	0,5	0,0	3,2	1,9	7,5
<i>Gomphonema olivaceum</i>	5,0	54,7	3,8	35,6	22,0	0,0	4,1	18,8	3,2	8,9	2,9	6,8	2,9	0,0	0,5	18,6
<i>Ulnaria ulna</i>	0,0	0,0	0,0	2,6	10,7	2,5	1,0	2,5	1,4	2,2	0,0	0,5	1,5	0,5	2,9	11,3

Table 5. Diatom taxa with a percentage with 10% or more at least at one locality in the Timok River basin in 2017, in August and November.

Season and site Taxa	August 2017								November 2017							
	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8	ST5	BT2	CT4	CT7	VT1	BR5	BR7	CT8
<i>Achnanthydium Minutissimum</i>	1,4	3,5	45,7	2,7	3,7	71,5	58,0	2,3	1,9	10,2	39,5	0,5	1,7	26,5	29,3	5,4
<i>Amphora Pediculus</i>	8,6	18,1	2,6	35,3	5,2	0,7	2,6	64,8	21,4	13,8	22,6	5,1	7,7	5,0	0,7	48,4
<i>Cocconeis Placentula</i>	52,2	45,3	0,0	0,0	1,2	0,5	0,9	2,7	5,6	2,2	0,5	1,5	1,2	1,5	1,9	1,3
<i>Denticula Kuetzingii</i>	0,5	1,7	0,0	42,0	0,5	0,0	1,9	0,0	0,9	12,1	0,0	0,0	1,0	0,5	3,7	0,0
<i>Gomphonema Olivaceum</i>	3,5	5,2	4,5	1,4	4,0	3,5	0,8	3,9	21,4	13,6	1,4	0,5	3,7	0,5	1,5	6,7
<i>Gomphonema Pumilum</i>	7,2	1,7	3,3	3,4	2,2	0,5	0,9	3,4	21,1	19,1	2,1	1,9	2,5	1,5	0,9	5,8
<i>Melosira Varians</i>	0,0	0,0	11,5	0,5	0,0	2,5	3,8	0,5	0,2	0,0	1,6	0,0	0,2	0,0	4,2	0,0
<i>Navicula metareichardtiana</i>	0,7	0,0	2,9	0,5	11,9	0,5	1,4	0,7	1,2	3,9	0,5	0,5	12,9	3,0	0,5	4,0
<i>Navicula Veneta</i>	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,9	21,6	1,5	0,0	0,0
<i>Nitzschia Capitellata</i>	1,6	0,0	0,5	0,5	10,7	0,0	0,0	0,2	0,0	0,0	0,9	26,5	8,2	2,0	0,8	0,9
<i>Nitzschia Fonticola</i>	0,5	0,7	6,2	0,5	20,4	0,0	1,4	0,2	0,5	1,7	5,1	2,4	0,7	4,0	0,0	1,8
<i>Nitzschia palea</i>	0,0	1,4	4,5	0,0	22,6	0,0	4,7	0,9	0,5	3,9	2,3	26,2	24,1	9,5	7,6	0,9

As mentioned, the lower species richness of diatom assemblage was identified at BR5, which also had the acid reaction of water and highest values of conductivity (seen in Table 2 and Table 3). This was expected due to the mining-industrial complex Bor located after the BR5. Also, Verb & Vis (2000) reported that the species richness are typically reduced at acid mine drainage (AMD) site. The highest values of the Shannon's Diversity Index (Shannon & Weaver, 1949) at almost all localities along the Timok River basin were recorded in November while the lowest were during the summer (Figure 2).

In November, the values of the diversity index at almost all localities were above 3, and it was observed that the diversity of codominant taxa in the community increased and their distribution was the most uniform. The Pielou's evenness index (Pielou, 1966) follow the upward trend, ie. decreases in the value of the diversity index per season (Figure 3). Research of diatoms of the Velika Morava in Serbia has also shown the highest number of diatom taxa in the autumn (Vasiljević, 2017). The diversity index, as well as the structure of dominant taxa,

suggests a very similar community along the Timok River Basin with noticeable seasonal changes.

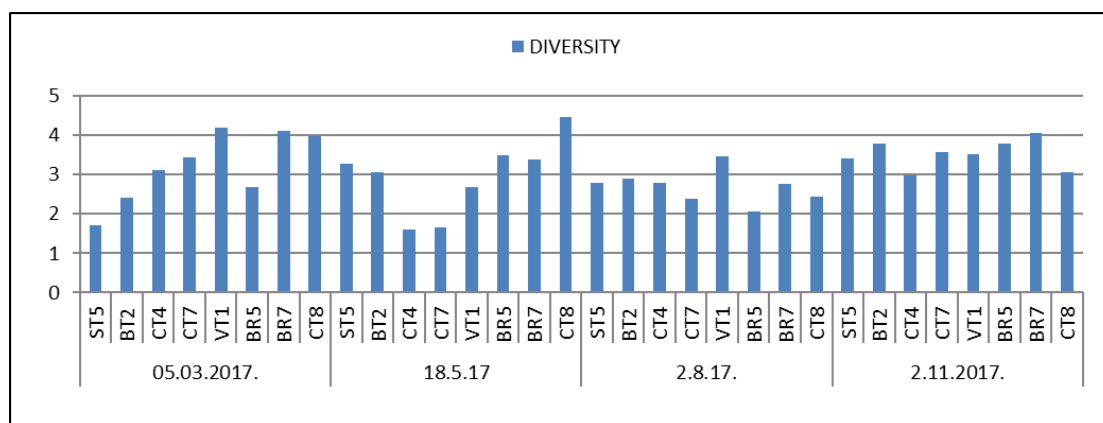


Figure 2. Shannon-Wiener Diversity Index during four seasons at the sampling sites (Figure 2) on the Timok Basin.

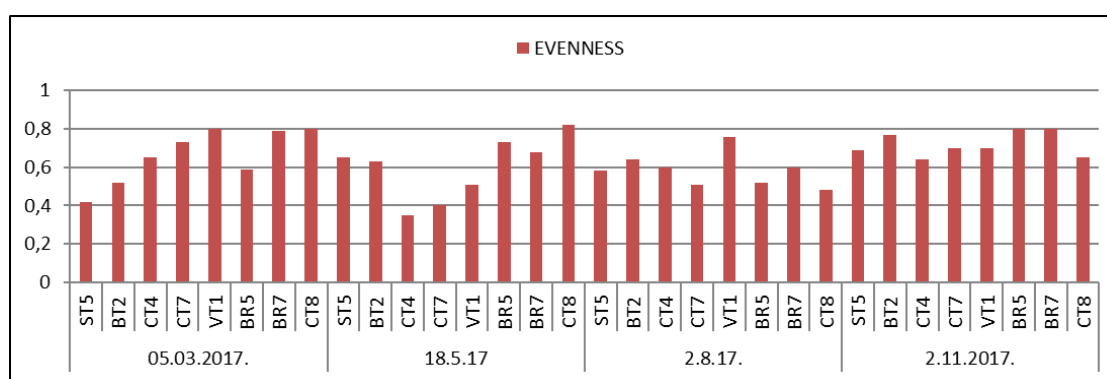


Figure 3. Pielou's evenness index during four seasons at the sampling sites (Figure 3) on the Timok Basin.

CONCLUSION

In this study, it was aimed to explore the composition and seasonal abundance of diatoms in the Timok River basin.

In terms of benthic diatom communities, previous data from the Timok River Basin is scattered. The presented data increase our knowledge of the river system, which is important for further prediction of diatoms as bioindicators; however, further fundamental investigations are necessary since the diatom microflora in this part of the Balkan Peninsula have been insufficiently studied regardless of a relatively long period of research.

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