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The first report on periphytic diatoms on artificial and natural substrate in the karstic spring Bunica, Bosnia and Herzegovina

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Abstract – This study presents investigations of the periphytic diatoms on artificial (glass slides) and natural substrates in the karstic, limnocrene spring of Bunica situated in the south of Bosnia and Herzegovina. Investigations were performed in summer 2010. Samples were collected every seven days for eight weeks. Physical and chemical characteristics of water, temperature, oxygen saturation, dissolved oxygen, electric conductivity and nutrients as well as flow velocity at sample site, were measured simultaneously with each sampling. Physical and chemical characteristics showed low temperature oscillations, good aeration and oligotrophic conditions. In general, greater diatom diversity was noted on natural substrate. A total of 104 diatom species were found on natural substrate and 82 on glass slides. The best represented genera on both types of substrate were *Gomphonema* and *Navicula* (each with eight species), *Nitzschia* (with six species), and *Cocconeis* (with five species). *Achnanthidium exiguum*, *Achnanthidium minutissimum*, *Amphora pediculus, Cymbopleura amphicephala* and *Surirella minuta* were recorded in all samples of natural substrate and *Gomphonema minutum* in artificial substrate samples.

Key words: Bosnia and Herzegovina, Bunica Spring, glass slides, karst spring, periphytic diatoms

Introduction

Karst is a very specific geomorphological phenomenon, characterized by a rapid percolation of surface water into the underground as well as by the paucity of surface water flows. Most flows occur below the surface. Karst springs are a common occurrence in karst, as a special type of landscape, and they are places where groundwater comes to the surface

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creating a visible flow. Limnocrene springs are the most widespread type of springs in calcareous substrates, such as karst (DI SABATINO et al. 1997). According to their hydrological, physical and chemical characteristics, karstic springs are attractive and interesting ecological sites. They are characterized by physico-chemical stability. Almost all springs have a low water temperatures (8–12 °C) with small annual fluctuations, high concentrations of oxygen (8–12 mg L^{-1}) and high concentrations of carbon dioxide (up to 60 mg L^{-1}) (BLAGOJEVIĆ 1974, CANTONATI et al. 2007). Karst springs have high alkalinity, which is mainly due to carbonates, and high carbonate hardness (BLAGOJEVIĆ 1974, ŠTAMBUK-GILJANOVIĆ 1998). The concentration of nitrite is about 0.01 mg L^{-1} (CANTONATI et al. 2007) and concentrations of nitrate 0.4–8.0 mg L^{-1}. Sulphates are usually present with concentrations of a few tenths of mg L^{-1} while hundreds of mg L^{-1} can be found in springs fed by aquifers that also include evaporites or geological formations which contain gypsum (CANTONATI et al. 2007). Phosphorus is also present in low concentrations (0.005–0.050 mg L^{-1}) (BLAGOJEVIĆ 1974, WERUM 2001). Silicates are very important for the growth of diatoms. Underground waters in siliceous aquifers usually hold 3–10 mg L^{-1} and in carbonate aquifers about 1 mg L^{-1} SiO_2 (CANTONATI et al. 2007).

A common characteristic of karstic springs, whether permanent or temporary, is the strong dependence of discharge on precipitation. As a consequence, the ratio between minimum and maximum discharge is great (1:60, or more). The minimum and maximum discharges registered for Bunica Spring were 0.72 and 207 m^3 s^{-1}, respectively (MILANOVIĆ 2006). All values of physico-chemical parameters during major rainfall may be increased.

The periphytic communities of karstic springs in Bosnia and Herzegovina have not been sufficiently studied; only few authors have addressed the issue (BLAGOJEVIĆ 1974, 1976, 1979, HAFNER 2009, HAFNER et al. 2010, DEDIĆ et al. 2012). Algae, especially diatoms, in springs have received little attention in Bosnia and Herzegovina, despite the fact that springs provide specific conditions that cannot be found in any other freshwater ecosystem and notwithstanding their great importance in terms of general environmental changes.

The aim of this study was to determine the qualitative and quantitative composition of periphytic diatoms on artificial (glass slides) and natural substrates in fast and slow water flow in the Bunica Spring.

The results of this study give us preliminary results from the investigations of periphytic diatoms in springs and are a first step towards a detailed elaboration of diatom distribution and taxonomy in this very poorly investigated area.

Materials and methods

Study area

Bunica Spring is a deep siphonal limnocrene spring of the eponymous river Bunica. It is located in the southeastern part of the Mostar valley, near the towns of Blagaj and Mostar (Fig. 1) in the south of Bosnia and Herzegovina. The Bunica River is 6 km long and is the main left tributary of the river Buna, a tributary of the river Neretva. There are no direct hydrogeological connections between the Buna and the Bunica (MILANOVIĆ 2006). The outlet channel was researched to the depth of 73 m and over a length of 160 m (MILANOVIĆ 2006). Bunica Spring is the surface outlet of the underground part of the Zalomka River,

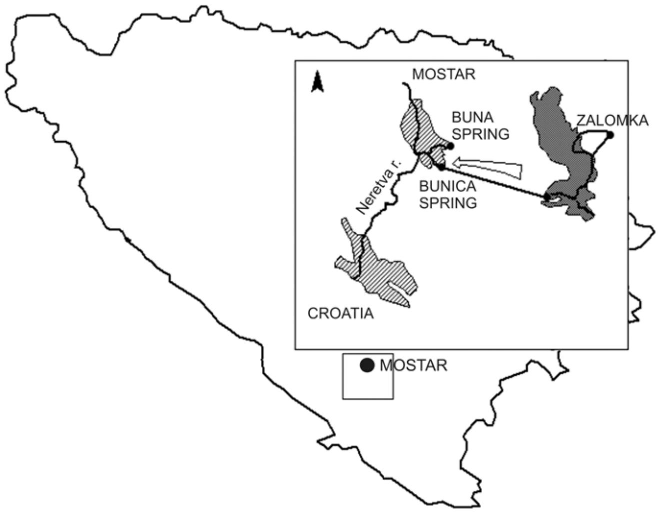

Fig. 1. Study area site of Bunica Spring located in the southeastern part of the Mostar valley, near the towns of Blagaj and Mostar in the south of Bosnia and Herzegovina.

subsequent to its sinking into the Biograd Ponor (Fig. 1). The catchment area of Bunica Spring is estimated at approximately 160 km^2 (not including the Zalomka River) (Milanović 2006). Biograd Ponor and Bunica Spring are directly connected with the karst channel which is one of the most direct underground connections in this part of Dinaric karst (Milanović 2006).

Bunica Spring is located at the contact between karstified carbonate rocks and Miocene deposits. It is located in an area influenced by the Mediterranean climate, which is characterized by long, hot summers and rainy winters with rare occurences of snow (Galić 2011). The average annual air temperature is 14.6 °C. The highest daily average temperature is 23.2 °C in July and the lowest below 5.8 °C in January (data for the city of Mostar, for the period 1971–2000, recorded by Meteorological and Hydrological Service of Bosnia and Herzegovina). The total annual precipitation is about 1,515 mm. This area has approximately 2,291 hours of sunshine per year. Average relative humidity is 69. Water vegetation in Bunica Spring consists of algae and mosses, leaves, branches and roots of plants.

Sampling

Samples from artificial and natural substrate and water for physical and chemical analyses were collected in summer 2010 in Bunica Spring. Summer minimum riverine water discharge and higher temperature conditions provide favorable and stable conditions for the development of periphytic diatoms (Hillebrand and Sommer 2000).

Diatom colonization was monitored after 7, 14, 21, 28, 35, 42, 49 and 56 days of exposure of artificial substrates (glass slides) that were horizontally placed and oriented parallel to the current velocity. Two different microhabitats were defined with regard to the current velocity. Current velocities of water were measured using a DOSTMANN P 670 probe.The microhabitats were made of seven glass slides that were fixed on the upper side of a brick.

Glass slides were washed with distilled water before being placed into the spring and one or two slides were taken every seven days. Samples from the artificial substrate were stored in a labeled bottle in the field and upon return to the laboratory they were scraped with a razor blade and scalpel.

Simultaneously, samples from the natural substrate were collected applying the standard procedure of scraping sludgy material from the rock's surface making sure that the total surface of all rocks is about 500 cm^2 (EUROPEAN STANDARD EN 13946, 2003). Rocks were chosen randomly according to standard procedures.

Identification and morphological examination of diatoms

All the samples were fixed with 4% formaldehyde. Permanent preparations of diatoms were prepared using the HUSTEDT (1930) method. The species were identified using the microscopes Olympus BX53 and Carl Zeiss Jena. Different keys and guides were used for taxa determination: HUSTEDT (1930), KRAMMER and LANGE-BERTALOT (1986, 1988a, 1988b), KRAMMER (1988, 2000, 2004, 2010), LANGE-BERTALOT (2001, 2002). The nomenclature of taxa is determined by the nomenclature set out in algae base (GUIRY and GUIRY 2014).

Measurement of physico-chemical parameters of spring water

The physical and chemical parameters considered in this study were: water temperature, oxygen saturation, dissolved oxygen, electric conductivity and nutrients (nitrate, nitrite, silicate and orthophosphate) as well as flow velocity. Water temperature, dissolved oxygen, oxygen saturation and conductivity were measured using the WTW probe (Wissenschaftlich-TechnischeWersätten GmbH & Co. KG-Weilheim) whereas nutrient concentrations (mg L^{-1}) were determined using the standard spectrophotometrical method (APHA, 1995) in Public Utility Company in Ljubuški.

To test the differences between the flow regimes and different exposure periods, one-way ANOVA with post hoc Bonferoni test was employed.

Results

Physico-chemical parameters of Bunica Spring

Results of physico-chemical measurements of Bunica Spring water (temperature, conductivity, dissolved oxygen concentrations, oxygen saturation, nutrients and current velocities) are shown in table 1. Data show low temperature oscillations, a low concentration of nutrients, good aeration and generally, oligotrophic conditions. The slow current velocity at the site had an average value of about 0.01 m s^{-1} and fast current velocity of about 0.9 m s^{-1}.

Diatom community on artificial substrate

In periphyton from artificial substrates a total of 82 diatom taxa were determined (Tab. 2). The genus *Gomphonema* dominates in terms of number of taxa (with 8 taxa), *Navicula* is next (with 7 taxa), followed by *Cocconeis* and *Nitzschia* (each with 5 taxa). The most taxa, 77 of them, were recorded after 56 days of exposure of the artificial substrate in the slow flow, and the fewest, with only 2, after 21 days in the fast flow (Fig. 2).

Tab. 1. Physical and chemical parameters of water at Bunica Spring. Min. – minimum value, Max. – maximum value, AVG – average value, SD – standard deviation, F – fast flow, S – slow flow.

Parameter	Min.	Max.	AVG	SD
Temperature (°C)	11.3	12.3	12.05	0.31
Dissolved oxygen (mg L^{-1})	9.73	12.4	10.73	0.951
Saturation (%)	91	115.6	99.828	9.805
Conductivity (µS cm^{-1})	339	413	399.6	24.86
Nitrite (mg L^{-1})	0.0002	0.0015	0.0009	0.0004
Nitrate (mg L^{-1})	1.91	2.85	2.408	0.333
Orthophosphate (mg L^{-1})	0.0093	0.0494	0.0288	0.0132
Silicate (mg L^{-1})	2.06	2.53	2.475	0.136
Current velocity F (m s^{-1})	0.58	1.44	0.85375	0.268
Current velocity S (m s^{-1})	0	0.02	0.0175	0.007

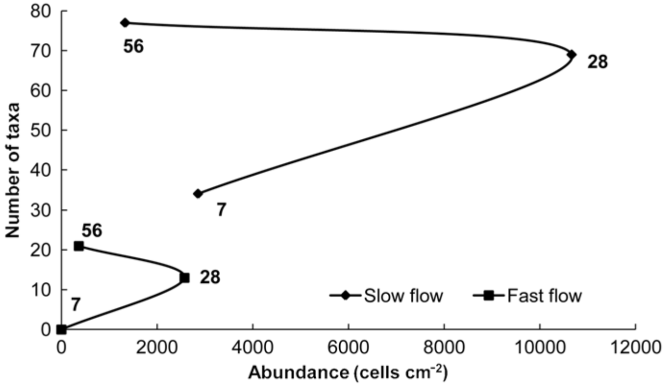

Fig. 2. Abundance and diversity of periphytic diatoms on the artificial substrate on the 7th, 28th and 56th days of the exposure time in fast and slow flow in Bunica Spring.

Generally, abundance as well as diversity of diatom species was higher in the slow than in the fast flow throughout the monitored exposure period although the values were very similar in both types of sites (no statistically significant differences, $p > 0.05$). The first colonizers on the artificial biofouling substrate, regardless of the water current velocity were *Achnanthidium*, *Amphora* and *Cocconeis*. *Gomphonema minutum*, *Cocconeis placentula*, *Cocconeis placentula* var *lineata* and *Achnanthidium minutissimum* were the most common taxa in biofouling from the artificial substrate. The aforementioned taxa were present in more than 68% of samples but with low abundance (Fig. 3). Taxa started to adhere to the artificial biofouling after 7 days of exposure in the slow flow (total number of cells per cm^2 was 2854), and in the fast flow after 21 days (total number of cells per cm^2 was 10) (Fig. 2). In 28 to 56 days of exposure, more massive colonization of *Cymatopleura*, *Epithemia*, *Encyonema*, *Gomphonema*, *Meridion* and *Nitzschia* was recorded and they were more dominant in the slow flow.

Tab. 2. List of diatom taxa determined in the Bunica Spring samples.

Taxa	Frequency of occurrence (%)	
	Natural substrate	Artificial substrate
Achnanthes inflata (Kützing) Grunow	5.9	0
Achnanthidium exiguum (Grunow) D. B. Czarnecki	100	12.5
Achnanthidium helveticum (Hustedt) Monnier, Lange-Bertalot & Ector	5.9	6.3
Achnanthidium minutissimum (Kützing) Czarnecki	100	68.8
Achnanthidium pyrenaicum (Hustedt) H. Kobayasi	76.5	37.5
Amphora ovalis (Kützing) Kützing	64.7	37.5
Amphora pediculus (Kützing) Grunow ex A. Schmidt	100	56.2
Brebissonia boeckii (Ehrenberg) E. O. Meara	5.9	6.3
Caloneis alpestris (Grunow) Cleve	23.5	6.3
Caloneis bacillum (Grunow) Cleve	70.6	43.8
Caloneis ventricosa(Ehrenberg) F. Meister	35.3	6.3
Cocconeis neodiminuta Krammer	11.8	25
Cocconeis pediculus Ehrenberg	52.9	50
Cocconeis placentula var. *lineata* (Ehrenberg) van Heurck	64.7	68.8
Coconeis placentula Ehrenberg	82.4	75
Coconeis placentula var. *euglypta* (Ehrenberg) Grunow	29.4	25
Cyclotella meneghiniana Kützing	29.4	6.3
Cyclotella ocelatta Pantoscek	41.2	12.5
Cyclotella sp.	23.5	18.8
Cymatopleura elliptica (Brébissoni) W. Smith	41.2	31.3
Cymatopleura solea (Brébissoni) W. Smith	23.5	31.3
Cymbella affinis Kützing	29.4	18.8
Cymbella cymbiformis C. Agardh	17.6	12.5
Cymbella excise Kützing	11.8	0
Cymbella helvetica Kützing	5.9	0
Cymbella tumida (Brébisson) van Heurck	11.8	0
Cymbopleura amphicephala (Nägeli) Krammer	100	12.5
Cymbopleura naviculiformis (Auerswald ex Heiberg) K. Krammer	5.9	12.5
Denticula tenuis Kützing	47.1	31.3
Diatoma ehrenbergii f. *capitulate* (Grunow) Lange-Bertalot	17.6	18.8
Diatoma hiemalis (Lyngbye) Heiberg	17.6	18.8
Diatoma mesodon (Ehrenberg) Kützing	35.3	25
Diatoma vulgaris Bory de Saint-Vincent	64.7	31.3
Diploneis marginestriata Hustedt	11.8	0

Taxa	Frequency of occurrence (%)	
	Natural substrate	Artificial substrate
Diploneis oblongella (Nägeli ex Kützing) Cleve-Euler	29.4	25
Diploneis ovalis (Hilse) Cleve	11.8	18.8
Ellerbeckia arenaria (Moore ex Ralfs) R. M. Crawford	17.6	18.8
Encyonema gracile Rabenhorst	82.4	0
Encyonema minutum (Hilse) D. G. Mann	58.8	50
Encyonema prostratum (Berkeley) Kützing	5.9	0
Encyonema silesiacum (Bleisch) D. G. Mann	82.4	50
Encyonopsis microcephala (Grunow) Krammer	23.5	0
Epithemia argus (Ehrenberg) Kützing	52.9	37.5
Eunotia arcus Ehrenberg	5.9	12.5
Eunotia bilunaris (Ehrenberg) Schaarschmidt	23.5	6.3
Eunotia pectinalis (Kützing) Rabenhorst	11.8	6.3
Eunotia praerupta Ehrenberg	11.8	31.3
Fragilaria capucina Desmazières	41.2	12.5
Fragilaria capucina var. g*racilis* (Oestrup) Hustedt-Unchecked	47.1	18.8
Fragilaria capucina var. v*aucheriae* (Kützing) Lange-Bertalot	29.4	6.3
Fragilaria constricta Ehrenberg	5.9	12.5
Fragilaria construens (Ehrenberg) Grunow	64.7	0
Frustulia vulgaris (Thwaites) De Toni	11.8	0
Gomphonema acuminatum Ehrenberg	23.5	6.3
Gomphonema gracile Ehrenberg	11.8	18.8
Gomphonema intricatum Kützing	82.4	50
Gomphonema micropus Kützing	52.9	6.3
Gomphonema minutum (C. Agardh) C. Agardh	76.5	93.8
Gomphonema olivaceum (Hornemann) Brébisson	41.2	18.8
Gomphonema parvulum (Kützing) Kützing	94.1	31.3
Gomphonema truncatum Ehrenberg	23.5	12.5
Gomphosphenia lingulatiformis (Lange-Bertalot & E. Reichardt) Lange Bertalot	17.6	25
Gyrosigma acuminatum (Kützing) Rabenhorst	52.9	31.3
Gyrosigma attenuatum(Kützing) Rabenhorst	17.6	0
Halamphora veneta (Kützing) Levkov	5.9	0
Melosira sp.	11.8	0
Melosira varians C. Agardh	58.8	6.3
Meridion circulare (Greville) C. Agardh	52.9	37.5

Taxa	Frequency of occurrence (%)	
	Natural substrate	Artificial substrate
Navicula capitatoradiata Germain	4.2	6.3
Navicula cari Ehrenberg	17.6	25
Navicula cryptocephala Kützing	58.8	31.3
Navicula cryptotenella Lange-Bertalot	17.6	0
Navicula exilis Kützing	47.1	37.5
Navicula radiosa Kützing	35.3	6.3
Navicula tripunctata (O. F. Müller) Bory de Saint-Vincent	88.2	37.5
Neidium dubium (Ehrenberg) Cleve	5.9	0
Neidium productum (W. Smith) Cleve	5.9	0
Nitzschia amphibia Grunow	41.2	0
Nitzschia dissipata (Kützing) Rabenhorst	64.7	50
Nitzschia linearis West	23.5	37.5
Nitzschia palea (Kützing) W. Smith	76.5	62.5
Nitzschia sigmoidea (Nitzsch) W. Smith	52.9	6.3
Nitzschia sublinearis Hustedt	82.4	6.3
Placoneis clementioides (Hustedt) Cox	100	25
Planothidium delicatulum (Kützing) Round&Bukhtiyarova	29.4	6.3
Planothidium ellipticum(Cleve) Round&Bukhtiyarova	17.6	6.3
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	11.8	18.8
Planothidium rostratum (Oestrup) Lange-Bertalot	5.9	31.3
Psammothidium oblongellum (Østrup) Van de Vijver	11.8	6.3
Reimeria sinuata (Gregory) Kociolek&Stoermer	5.9	0
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	70.6	12.5
Rossithidium linearis(W. Smith) Round & Bukhtiyarova	58.8	25
Sellaphora pupula (Kützing) Mereschkovsky	17.6	6.3
Stauroneis anceps Ehrenberg	5.9	0
Stauroneis smithii Grunow	11.8	6.3
Staurosirella pinnata (Ehrenberg) D. M. Williams & Round	52.9	31.3
Stephanodiscus hantzschii Grunow	76.5	31.3
Stephanodiscus sp.	41.2	6.3
Surirella angusta Kützing	11.8	6.3
Surirella brebissonii Krammer& Lange-Bertalot	5.9	0
Surirella linearis W. Smith	17.6	15.8
Surirella minuta Brébisson	100	0
Ulnaria ulna (Nitzsch) P. Compère	58.8	43.8

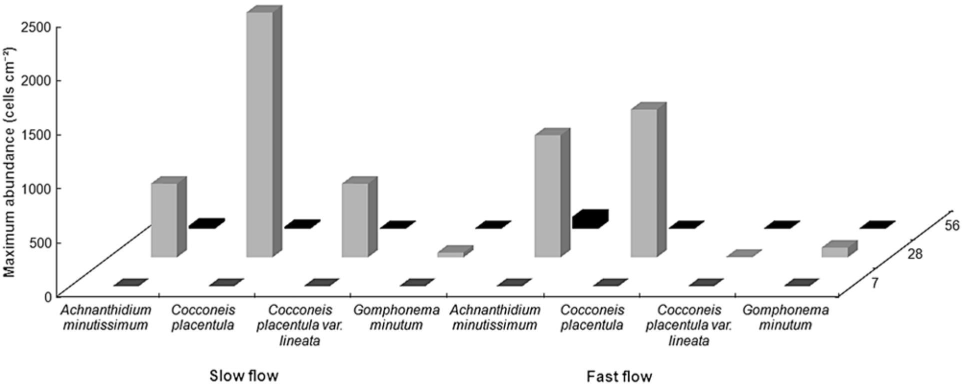

Fig. 3. Maximum abundance of the most common taxa on the artificial substrate on the 7th, 28th and 56th days of the exposure time in fast and slow flow in Bunica Spring.

Massive accumulation of taxa as well as number of cells was recorded from the 7th to the 35th day of periphyton colonization on the artificial substrate in both fast and slow flow. From 35th to 56th day the number of taxa was relatively constant but the number of cells was on the decrease (Fig. 2).

Diatom community on natural substrate

A total of 104 diatom taxa were determined in periphyton from natural substrates (Tab. 2). In periphyton from natural substrates *Gomphonema* and *Navicula* were the most numerous genera (each with 8 taxa), followed by *Nitzschia* (with 6 taxa) and *Cocconeis*, *Cymbella* and *Fragilaria* (each with 5 taxa). *Achnanthidium exiguum* (Grunow) Czarnecki, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Amphora pediculus* (Kützing) Grunow ex A. Schmidt, *Cymbopleura amphicephala* (Nägeli) Krammer and *Surirella minuta* Brébisson were present in all samples during the research period. The highest number of taxa, 87 of them, were registered on the 2nd sampling in the slow flow, and the lowest, 55, on the 9th sampling in the fast flow (Fig. 4).

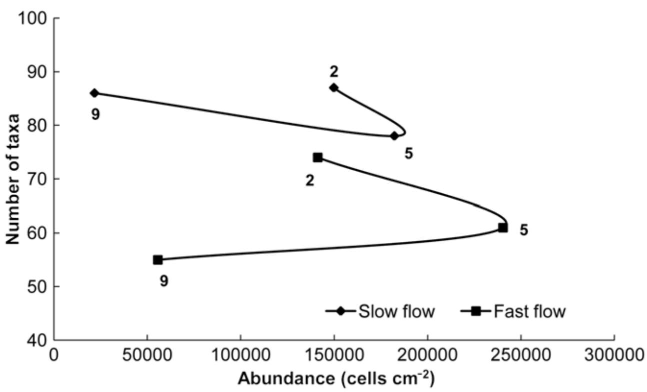

Fig. 4. Abundance and diversity of periphytic diatoms on the natural substrate at the 2th, 5th and 9th samplings.

All the taxa recorded in the biofouling from the artificial substrate were present in the samples from the natural substrate as well. In the samples from the natural substrate there were 22 taxa, which were not found in the biofouling from the artificial substrate and these are *Achnanthes inflata* (Kützing) Grunow, *Cyclotella* sp., *Cymbella excisa* Kützing, *Cymbella helvetica* Kützing, *Cymbella tumida* (Brébisson) van Heurck, *Diploneis marginestriata* Hustedt, *Encyonema gracile* Rabenhorst, *Encyonema prostratum* (Berkeley) Kützing, *Encynopsis microcephala* (Grunow) Krammer, *Fragilaria construens* (Ehrenberg) Grunow, *Frustulia vulgaris* (Thwaites) De Toni, *Gyrosigma attenuatum* (Kützing) Rabenhorst, *Halamphora veneta* (Kützing) Levkov, *Melosira* sp., *Navicula cryptotenella* Lange-Bertalot, *Neidium dubium* (Ehrenberg) Cleve, *Neidium productum*(W. Smith) Cleve, *Nitzschia amphibia* Grunow, *Reimeria sinuate* (Gregory) Kociolek & Stoermer, *Stauroneis anceps* Ehrenberg, *Surirella brebissonii* Krammer & Lange-Bertalot and *Surirella minuta* Brébisson. The aforementioned taxa were present with low appearance frequency in samples, with the exception of *Encyonema gracile* Rabenhorst, *Fragilaria construens* (Ehrenberg) Grunow and *Surirella minuta* Brébisson that were present in more than 50% of samples but with low abundance.

Total number of cells per cm^2 was very similar in both fast and slow flow from 1^{st} to 4^{th} sampling, whereas on the 5^{th} sampling there was a larger number of cells recorded in the fast flow than in the slow part of the flow, on the 9^{th} sampling the number of cells sharply decreased in both flows (Fig. 4). Taxa and their frequency of appearance were similar in the fast as well as in the slow flow.

Discussion

This paper presents research of periphytic diatoms sampled from natural substrate and artificial substrate in Bunica Spring. Bunica Spring showed low temperature oscillations, low concentration of nutrients, good aeration and oligotrophic conditions, which are characteristic of karstic springs (BLAGOJEVIĆ 1974) and the measured values correspond to values recorded in other springs (BLAGOJEVIĆ 1974, WERUM 2001).

Use of artificial substrates (glass slides) for quantification of periphyton was recommended by WAHL and MARK (1999), ALBAY and AKCAALAN (2003), LIBORIUSSEN (2005) who consider them more favorable for experimental growth than natural substrates. Glass slides are the most frequently used artificial substrates because of their uniform size and inert surface (ÀCS and KISS 1993, ÀCS et al. 2000, BARBIERO 2000). Total surface area is known and the problems associated with the measurement of irregular natural substrates are eliminated (LAMBERTI and RESH 1985). In the early days of fouling, increased diversity can be related to the colonization of new taxa (HILLEBRAND and SOMMER 2000). Through development of fouling on artificial substrate, stages of growth and loss take turns. The growth stage is characterized by colonization and growth of algae whereas the loss stage can be related to emigration, dying, peeling off and grazing (BIGGS 1996). At the end of the exposure period, the recorded decrease of the taxa number was influenced by the weather conditions (42^{nd} day) accompanied by the high current velocity, which caused flushing. Slow flow showed a greater diversity and abundance of taxa possibly because it gives more favorable habitat for vegetation growth, which leads to greater colonization of epilithic taxa (FALASCO et al. 2012).

The relatively small number of taxa recorded in Bunica Spring, 104 on natural substrates and 82 on artifical substrates, matches the results in the reviewed papers (BLAGOJEVIĆ 1974, SABATER and ROCA 1990, 1992, CANTONATI 1998, TAXBÖCK and PREISIG 2007, HAFNER et al. 2008, CANTONATI and SPITALE 2009, ANGELI et al. 2010, DEDIĆ et al. 2012, WOJTAL and SOBEZYK 2012). Bunica Spring also showed great similarity to the others with regard to the composition of diatom taxa. TOWNSEND and GELL (2005) as well as CANTONATI and SPITALE (2009) argue that similar taxa occur on a large number of substrates. Genera with the highest number of taxa on both types of substrate are *Gomphonema*, *Navicula*, *Nitzschia*, *Achnanthidium*, *Cocconeis*, *Fragilaria*, *Planothidium* and *Cymbella*, which is very similar to results of ANGELI et al. (2010).

BARBIERO (2000) compared epilithic diatoms on natural and artificial substrates and concluded that if there was any difference between the substrates, the difference was insignificant. In this study samples from natural substrates showed greater species diversity. That was probably due to the fact that fouling on the natural substrate lasted for an unknown but probably longer time than on the artificial substrate, so the equilibrium was reached and competitive preferences of early colonizers were not limiting for the attainment of maximum richness under those conditions. *Achnanthidium minutissimum* and *Cocconeis placentula* were the most abundant taxa in this research, on both natural and artificial substrate. They were recorded as the first colonizers of the artificial substrate in springs as well as of other habitats. The reason for their high abundance is their ability to respond well to disturbance and relatively high growth rates, which enable them to populate the surface before their competitors (BARBIERO 2000). WOJTAL and SOBEZYK (2012) carried out research into springs, finding that *Achnanthidium minutissimum* was the most represented taxon. In both of the systems (fast and slow) and on the natural and the artificial substrate the same taxa were recorded. The most numerous taxa were similar as well; they had similar appearance frequency, which implies they are characteristic spring species. Although numerous studies (MUNTEANU and MALY 1981, PLENKOVIĆ et al. 2008) showed that flow velocity greatly influences diatom community, this study did not confirm such findings. That is probably caused by the small difference between the velocities (about 1 m s^{-1}) or unrecorded changes in the flow regime between two sampling occasions. KRALJ BOROJEVIĆ (2011) also recorded similar dominant and subdominant taxa in lothic and lenthic biotopes of the Krka River. This study has shown that diatoms on an artificial substrate adequately represent diatoms on natural substrates, with respect both to taxa composition and abundance.

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