

Epilithic diatoms in the river Spreča affected by urban and industrial pollution, Bosnia and Herzegovina

UNDER PEER REVIEW

ABSTRACT

Benthic diatoms have gained prominence as environmental bioindicators since the beginning of the twentieth century. Epilithic diatom taxa are frequently used for ecological water-quality assessments of rivers. Biodiversity of these dominant group of protists in the benthic communities can be indicative of changes in the freshwater ecosystem. The aim of this study was to analyze the diversity and ecology of diatoms of the Spreča River on the locations affected by urban and industrial pollution. Field sampling of the epilithic diatoms was carried out in the period of May 2021 at two locations, first before and second after the Lukavac city, which is known as industrial city in Bosnia and Herzegovina. Taxonomic analysis was carried out on light microscope on permanent slides and revealed in total 38 diatom taxa. The genera with the largest number of species were *Diatoma*, *Navicula* and *Nitzshia*. Identified taxa are widespread species that tolerate high levels of pollution. A slightly higher number of taxa was recorded at the first location, while at the second, a higher proportion of halophilic taxa was observed.

Key words: diversity, biological indicators, diatoms, Spreča River

1. INTRODUCTION

In the recent decades, a significant effort has been put forth all over the world to assess water quality attending to not only to chemical parameters (nutrients, metals, pesticides, etc.), which are obviously important, but also to biological indicators. In fact, one of the undesirable consequences of pollutants is their effect on biota.

Biotic parameters provide a better evaluation of environmental changes, because the community development integrates a period of time reflecting conditions that might not be present anymore at the time of sampling and analysis. Long-term ecological impacts are the important ones, since preservation of aquatic life is the ultimate goal. The limitations of chemical variables become even more serious when the object of study is a lotic system where current one promotes continuous renewal of the water at each site. Together they form the basis to a correct assessment of the quality of running waters (Lobo & Callegaro, 2000).

Benthic diatoms have gained prominence as environmental bioindicators since the beginning of the twentieth century (e.g., Kolkwitz & Marsson, 1908).

Today, silicate algae are widely accepted, both in scientific research and in the routine monitoring, as indicators of the state and quality of water in aquatic ecosystems (Lange-Bertalot, 1978, 1979; Whitton et al., 1991). They react quickly to changes in the environment and anthropogenic pressures, and are often investigated in monitoring the water quality of rivers, lakes, wetlands, oceans and estuaries (Smol and Stoermer, 2010), especially when monitoring changes caused by the influx of nutrients, i.e. trophic value (Rott et al. 1999) or the effects of anthropogenic influence (van Dam et al., 1994; Kelly and Whitton, 1998).

Agricultural intensification, urbanization, and industrialization as a result of human population growth and technological advancement, are the main factors responsible for water quality deterioration in many parts of the world (Nelson et al., 2009).

Agricultural runoff and wastes from urban areas that get their way into different aquatic systems could lead to changes in the physical and chemical characteristics of the receiving

water bodies (Peters, 2000). For instance, it can cause alteration of physicochemical parameters of water quality which in turn affects the assemblage of biological communities residing in the aquatic ecosystem (Karr JR, 1981).

The river Spreča is the largest recipient of the most wastewaters from Tuzla Canton in northeastern Bosnia and Herzegovina. It is a right tributary of the river Bosna and belongs to the catchment area of the river Sava. It originates below Velja Glava and flows into the Bosna River near Doboš with a total length of 127 km. The area of its basin is 1945 km² and it is one of the largest rivers in Bosnia and Herzegovina (Kulenović, 1994). The river system is influenced by two main types of water pollution: non-point sources from agricultural and local domestic sewage input, and point sources from untreated industrial waste water. The Spreča river basin includes 12 municipalities and is home to around half a million inhabitants. In its middle course, it passes through Sprečko polje, and on that gentle longitudinal profile, it has the characteristics of a plain stream. Its waters are used for the industry of Tuzla Canton (Đozić, 2019). It also flows through the City of Lukavac, which is known as an industrial city in Bosnia and Herzegovina.

The first investigations in Bosnia and Herzegovina in various aquatic ecosystems indicate a high species diversity of diatoms (Van Heurck, H. 1880-1883, Gutwinski, 1897; Protic, G. 1897; Protic, 1901; Matonickin, 1963). In the middle of the 20th and the beginning of the 21st century, research became somewhat more frequent. Algal studies in northeastern Bosnia and Herzegovina was carried out at the springs and streams of the Konjuh mountain, (Kamberovic et al., 2016; 2019a), the Modrac reservoir (Kamberovic et al., 2019b); the Šerićka Marsh (Kamberovic et al., 2017), pit lakes (Kamberovic et al., 2012), the Oskova River (Lukić et al., 2019) and only as additional research on the Spreča river at several locations (Skenderović, 2003, Habdija et al. 1983).

Skenderović (2003), show that composition of phytobenthos of the river Spreča indicates the characteristics of β - mesosaprobic water. The river Oskova, a tributary of the river Spreča, was investigated by Lukić et al. (2019), which determined a total of 106 species of diatoms, and poor water quality at the mouth of this tributary.

The main objective of this work is to analyze biodiversity and ecology of benthic diatoms on two locations of the river Spreča, first before and the second after inflow of urban and industrial waste water of the city Lukavac.

2. MATERIAL AND METHODS

Phytobenthos samples were collected at two locations on the Spreča River in 2021.

Location 1 (L1) is the river Spreča after the Modrac lake and before the inflow of urban and industrial waste water in the area of city Lukavac (GPS coordinates 44° 31' 06.0" N; 18° 30' 54.2" E). Location (L2) is the river Spreča downstream from the mouth of the Lukavac river, which carries municipal wastewater from the city into the Spreča and downstream from local industry (GPS coordinates 44° 32' 51.6" N 18° 29' 26.8" E).

Phytobenthos samples were collected according to the standard for Water quality - a guide for routine sampling and preparation of benthic diatoms from rivers and lakes (Institute for Standardization of Bosnia and Herzegovina, BAS EN 13946:2015, 2015), by scraping stones with a scalpel and a brush. Samples were immediately fixed with formaldehyde to a final concentration of 4%. Phytobenthic samples were analyzed according to the standard "Guidelines for routine sampling and pretreatment of benthic diatoms from rivers and lakes" (Institute for Standardization, BAS EN 13946:2004). Permanent preparations were prepared by the cleaning method using cold oxidation with concentrated sulfuric acid (Krammer and Lange-Bertalot 1986). Clean diatom frustules were mounted in a synthetic resin with high refraction index (Naphrax ©) and up to 400 valves were counted and identified to species or variety level in each sample using a light microscope with 1000x magnification. Identification

of diatom taxa was done using literature keys: Hofmann et al., 2011, Krammer and Lange-Bertalot 1985, 1986, 1988, 1991, Krammer, 1997, 1997a, 2000, 2002, 2003. The nomenclature of identified taxa is aligned with Guiry and Guiry, 2020 (algaebase.org).

Between 300 and 400 diatom frustules were counted from permanent preparations for each sample. In this way, the sample size was satisfied and statistical certainty was achieved according to the recommendations of the standard "Water quality - Standard instructions for monitoring, sampling and laboratory analysis of phytobenthos" (Institute for Standardization, BAS EN 14407:2015). The relative number of taxa in the examined samples is expressed by the percentage of frustules of each taxon in the ratio of the counted frustules. Shannon-Wiener Diversity Index (H') was calculated for biodiversity assessment.

3. RESULTS AND DISCUSSION

In total, 38 taxa of diatoms were identified by light microscopy, of which 30 were at site 1 and 23 at site 2.

The most dominant species is *Diatoma vulgare*Bory. In addition, the most common species, found in both localities, are: *Diatoma vulgare*Bory, *Gomphonema olivaceum* (Hornemann) Brébisson, *Naviculatripunctata* (O.F. Müller) Bory, *Cyclotella ocellata*Pantocsek.

The Spreča River is affected by many anthropogenic influences, which results in a change in the qualitative and quantitative composition of the diatom communities in the samples. The list of species is shown in Table 1.

Table 1. List of the epilithic diatoms, found in river Spreča

Taxon	1	2
1. <i>Cocconeis lineata</i> Ehrenberg	+	-
2. <i>Cocconeis pediculus</i> Ehrenberg	+	-
3. <i>Cyclotella meneghiniana</i> Kützing	+	-
4. <i>Cyclotella ocellata</i> Pantocsek	+	+
5. <i>Cymbellaneocistula</i> Krammer	+	-
6. <i>Cymbelladorsenotata</i> C.Agardh	+	-
7. <i>Cymbellaneolaceolata</i> W.Silva	+	-
8. <i>Diatoma ehrenbergii</i> Kützing	+	+
9. <i>Diatoma moniliformis</i> Kützing	+	-
10. <i>Diatoma tenuis</i> C.Agardh	-	+
11. <i>Diatoma vulgare</i> Bory	+	+

12. <i>Encyonemacaespitosum</i> Kützing	+	-
13. <i>Encyonemalange-bertalotii</i> Krammer	+	+
14. <i>Encyonemaventricosum</i> (C.Agardh) Grunow	+	+
15. <i>Fragilaria vaucheriae</i> (Kützing) J.B.Petersen	+	+
16. <i>Fragilariformabikapitata</i> (A.Mayer) D.M.Williams& Round	+	-
17. <i>Gomphonemacalcareum</i> Cleve	+	+
18. <i>Gomphonemaolivaceum</i> (Hornemann) Brébisson	+	+
19. <i>Merdionconstrictum</i>	+	-
20. <i>Naviculaantoniilange-Bertalot</i>	+	+
21. <i>Naviculacapitatoradiata</i> H.Germain ex Gasse	-	+
22. <i>Naviculacryptotenella</i> Lange-Bertalot	+	+
23. <i>Naviculaerifuga</i> Lange – Bertalot	+	-
24. <i>Naviculagermanii</i> J.H.Wallace	-	+
25. <i>Navicula lanceolata</i> Ehrenberg	+	+
26. <i>Naviculasalinarum var. rostrata</i> (Hustedt) Lange-Bertalot	-	+
27. <i>Naviculatripunctata</i> (O.F.Müller) Bory	+	+
28. <i>Naviculaveneta</i> Kützing	+	-
29. <i>Nitzschiacapitellata</i> Hustedt	-	+
30. <i>Nitzschiapaleavar. palea</i> (Kützing) W.Smith	+	+
31. <i>Nitzschia recta</i> Hantzsch ex Rabenhorst	+	+
32. <i>Odontidiummesodon</i> (Ehrenberg) Kützing	-	+
33. <i>Planothidiumlanceolatum</i> (Brébisson ex Kützing) Lange-Bertalot	+	-
34. <i>Reimeriauniseriata</i> Sala, Guerrero &Ferrario	+	-
35. <i>Rhoicospheniaabbreviata</i> (C.Agardh) Lange- Bertalot	+	-
36. <i>Surirellabrebissonii var. brebissonii</i>	-	+
37. <i>Surirellabrebissonii var. kuetzingii</i> Krammer etLange-Bertalot	-	+
38. <i>Ulnaria ulna</i> (Nitzsch) Compère in Jahn et al.	+	+

The largest number of species belong to genera *Navicula* (10), *Nitzschia* (5) and *Diatoma*(4).

Shannon Wiener's Diversity Index values are 2.59 for location 1 and 2.67 for location 2, which indicates a fairly high species diversity.

Species such as *Nitzschiacapitellata*Hustedt, *Nitzschiaincospicua*Grunow, *Naviculagermanii*J.H.Wallace, *Naviculasalinarum var. rostrata* (Hustedt) Lange-Bertalot which were found only at site 2, are species which prefer salt-rich, nutrient-rich waters, tolerate a high trophic and saprobic levels, prefer electrolyte-rich freshwater habitats.

Of the anthropogenic factors, who may be responsible for these conditions, several major ones can be singled out: organic load from the Jala river, communal water of individual households and nearby towns, and discharge water from local industry. Water temperature

and pH at site 2 are higher (T 15 °C, pH 8) compared to site 1 (T 11°C, pH 6), even though it is a short kilometer away.

Analysis of the phytobenthos of the Spreča River is reported in two studies by Habdija et al. (1983) and Skenderović (2003). According to Skenderović (2003), the phytobenthos composition of the Spreča River indicates a β - mesosaprobic water status. However, not a single study provides a list of taxa on the basis of which the results can be compared, and the communities and ecology of the species present can be better studied, and thus the water quality. Although Lake Modrac and the tributaries of the river Spreča (Jala, Oskova, Gostelja) were the subjects of earlier hydrobiological research, for the river Spreča as a separate water body, there are no published reference data on the composition of diatom communities. A large number of species found in the river Spreča coincides with the species found in its tributaries (Table 2).

Table 2. Comparative representation of the species of silicate algae

Taxon	Modrac (Kamberović et al., 2019)	Šerićka Marsh (Kamberović et al., 2017)	Oskova (Lukić et al., 2019)	Jala (Haračić et al., 2016)
<i>Achnanthydium eutrophilum</i> (Lange-Bertalot) Lange-Bertalot			+	
<i>Achnanthydium pyrenaicum</i> (Hustedt) H. Kobayasi			+	
<i>Achnanthydium minutissimum</i> (Kützing) Czarnecki	+		+	
<i>Achnanthydium</i> sp. Kützing		+		
<i>Amphipleura pellucida</i> (Kützing) Kützing	+			
<i>Amphora copulata</i> (Kützing) Schoeman et R.E.M. Archibald	+			
<i>Amphora inariensis</i> Krammer	+		+	
<i>Amphora ovalis</i> (Kützing) Kützing	+	+		
<i>Amphora pediculus</i> (Kützing) Grunow ex A. Schmidt	+	+	+	
<i>Anomoeoneis sphaerophora</i> Pfitzer	+	+		
<i>Aulacoseria</i> sp.	+			
<i>Breissonialanceolata</i> (C. Agardh) R.K. Mahoney et Reimer	+		+	
<i>Caloneisaerophila</i> W. Bock	+			
<i>Caloneis</i> sp. Cleve		+		
<i>Cocconeis lineata</i> Ehrenberg			+	
<i>Cocconeis pediculus</i> Ehrenberg		+	+	
<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurck	+			
<i>Cocconeis placentula</i> Ehrenberg	+			
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehrenberg) Grunow	+	+		
<i>Cocconeis placentula</i> var. <i>placentula</i> Ehrenberg		+	+	
<i>Cocconeis pseudolineata</i> (Geitler) Lange-Bertalot			+	
<i>Cocconeis</i> sp.				+

<i>Craticulacuspida</i> (Kützing) D.G.Mann	+	+		
<i>Craticulasubminuscula</i> (Manguin) Wetzel & Ector				+
<i>Cyclotella meneghiniana</i> Kützing		+		+
<i>Cymatopleura elliptica</i> (Brébisson) W.Smith	+	+		
<i>Cymatopleurasolea</i> (Brébisson) W.Smith	+	+		
<i>Cymbellaaspera</i> (Ehrenberg) Cleve		+		
<i>Cymbellacymbiformis</i> C.Agardh	+			
<i>Cymbellaexcisa</i> Kützing	+			+
<i>Cymbellaneocistula</i> Krammer var. <i>neocistula</i>	+	+		
<i>Cymbella</i> sp.				+
<i>Cymbellatumida</i> (Brébisson) Van Heurck	+			
<i>Diademesiparacontenta</i> Lange-Bertalot&Werum		+		
<i>Diatoma vulgare</i> Bory	+	+		
<i>Diploneisoculata</i> (Brébisson) Cleve	+			
<i>Discostellastelligera</i> (Cleve & Grunow) Houk et Klee	+			
<i>Encyonemaauerswaldii</i> Rabenhorst	+			
<i>Encyonemaminutum</i> (Hilse) D. G. Mann		+		+
<i>Encyonemaprostratum</i> (Berkeley) Kützing		+		+
<i>Encyonemasilesiacum</i> (Bleisch) D.G.Mann				+
<i>Encyonemaventricosum</i> (C.Agardh) Grunow	+			
<i>Encyonemavulgare</i> Krammer		+		
<i>Encyonopsismicrocephala</i> (Grunow) Krammer	+			
<i>Epithemiaadnata</i> (Kützing) Brébisson		+		
<i>Eunotia</i> sp. Ehrenberg		+		
<i>Fallaciapygmaea</i> (Kützing) Stickle et D.G.Mann in Round, R.M.Crawford et D.G.Mann	+	+		
<i>Fragilaria acus</i> (Kützing) Lange-Bertalot	+	+		
<i>Fragilaria gracilis</i> Østrup	+			
<i>Fragilaria radians</i> (Kützing) D.M.Williams et Round	+			
<i>Fragilaria</i> sp.				+
<i>Fragilaria vaucheriae</i> (Kützing) J.B.Petersen	+			
<i>Frustuliavulgaris</i> (Thwaites) De Toni				+
<i>Gomphonemaacuminatum</i> Ehrenberg	+	+		
<i>Gomphonemaangustum</i> C.Agardh		+		
<i>Gomphonemaaugur</i> Ehrenberg	+			
<i>Gomphonemaauritum</i> A.Braun ex Kützing	+			
<i>Gomphonemaclavatum</i> Ehrenberg	+			
<i>Gomphonemaextentum</i> E.Reichardt et Lange-Bertalot	+			
<i>Gomphonemainnocens</i> E.Reichardt	+			
<i>Gomphonemaitalicum</i> Kützing	+	+		
<i>Gomphonemamicropus</i> Kützing	+	+		
<i>Gomphonemaminutum</i> (C.Agardh) C.Agardh				+

<i>Gomphonemaolivaceum</i> (Hornemann) Brébisson	+	+	
<i>Gomphonemapala</i> E.Reichardt		+	
<i>Gomphonemaparvulum</i> (Kützing) Kützing	+		+
<i>Gomphonemapseudoaugur</i> Lange-Bertalot		+	
<i>Gomphonemapumilum</i> (Grunow) E.Reichardt & Lange-Bertalot		+	+
<i>Gomphonemapumilum</i> var. <i>elegans</i> E.Reichardt & Lange-Bertalot			+
<i>Gomphonemapumilum</i> var. <i>rigidum</i> E.Reichardt & Lange-Bertalot			+
<i>Gomphonemarhombicum</i> Fricke	+		
<i>Gomphonema</i> sp.			+
<i>Gomphonemasubclavatum</i> (Grunow) Grunow	+		+
<i>Gomphonematergestinum</i> (Grunow) Fricke			+
<i>Gomphonematruncatum</i> var. <i>turgidum</i> (Ehrenberg) R.M. Patrick in Patrick et Reimer	+		
<i>Grunowiasolgensis</i> (A.Cleve) Aboal	+		
<i>Grunowiatabellaria</i> (Grunow) Rabenhorst	+		
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	+	+	
<i>Gyrosigmaaenuatum</i> (Kützing) Rabenhorst			+
<i>Gyrosigmakuetzingii</i> (Grunow) Cleve	+		
<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowsk	+	+	
<i>Lemnicolahungarica</i> (Grunow) F.E.Round & P.W.Basson		+	
<i>Lindaviacomta</i> (Kützing) Nakov, Gullory, Julius, Theriot et Alverson	+		
<i>Melosiravarians</i> C.Agardh	+	+	+
<i>Meridioncirculare</i> (Greville) C.Agardh	+		
<i>Merdion</i> sp.			+
<i>Naviculaantonii</i> Lange-Bertalot	+		
<i>Naviculacapitato radiata</i> H.Germain	+		+
<i>Naviculacryptocephala</i> Kützing		+	+
<i>Naviculacryptotenella</i> Lange-Bertalot	+		+
<i>Naviculagermainii</i> J.H.Wallace	+		
<i>Naviculahofmanniae</i> Lange-Bertalot			+
<i>Navicula lanceolata</i> Ehrenberg	+		+
<i>Navicula oblonga</i> (Kützing) Kützing	+		
<i>Navicula</i> sp.			+
<i>Navicularadiosa</i> Kützing		+	
<i>Naviculatripunctata</i> (O.F.Müller) Bory	+		+
<i>Naviculatrivialis</i> Lange-Bertalot	+		
<i>Naviculaveneta</i> Kützing	+		
<i>Naviculawildii</i> Lange-Bertalot	+		
<i>Neidium affine</i> (Ehrenberg) Pfitzer	+	+	

<i>Nitzschia acicularis</i> (Kützing) W. Smith	+		
<i>Nitzschia amphibian</i> Grunow			+
<i>Nitzschia dissipata</i> (Kützing) Rabenhorst			+
<i>Nitzschia dissipata</i> var. <i>media</i> (Hantzsch) Grunow	+		
<i>Nitzschia fonticola</i> (Grunow) Grunow			+
<i>Nitzschia frustulum</i> (Kützing) Grunow		+	+
<i>Nitzschia gracilis</i> Hantzsch	+		
<i>Nitzschia heufleriana</i> Grunow		+	
<i>Nitzschia inconspicua</i> Grunow	+		+
<i>Nitzschia intermedia</i> Hantzsch	+		
<i>Nitzschia littoralis</i> var. <i>slesvicensis</i> Grunow	+		
<i>Nitzschia palea</i> (Kützing) W. Smith	+	+	+
<i>Nitzschia recta</i> Hantzsch ex Rabenhorst			+
<i>Nitzschia sigmoidea</i> (Nitzsch) W. Smith		+	+
<i>Nitzschia</i> sp.			+
<i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot		+	
<i>Nitzschia vermicularis</i> (Kützing) Hantzsch in Rabenhorst	+		
<i>Pantocsekiella ocellata</i> (Pantocsek) K.T. Kiss et E. Ács	+		
<i>Placoneis</i> sp.	+		
<i>Pinnularia acutobreissonii</i> Kulikovskiy, Lange-Bertalot & Metzeltin		+	
<i>Pinnularia</i> sp.			+
<i>Pinnularia subcommutata</i> var. <i>nonfasciata</i> Krammer	+		
<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg		+	
<i>Planothidium lanceolatum</i> (Brébisson ex Kützing) Lange-Bertalot	+	+	+
<i>Planothidium minutissimum</i> (Krasske) E.A. Morales		+	
<i>Reimeria uniseriata</i> S.E. Sala, J.M. Guerrero & M.E. Ferrario			+
<i>Reimeria sinuata</i> (W. Gregory) Kociolek & Stoermer			+
<i>Rhoicosphenia abbreviata</i> (C. Agardh) Lange-Bertalot	+	+	+
<i>Rhopalodia gibba</i> (Ehrenberg) Otto Müller	+	+	
<i>Sellaphora pupula</i> (Kützing) Mereschkovsky	+	+	
<i>Surirella amphioxys</i> W. Smith		+	
<i>Surirella angusta</i> Kützing	+		
<i>Surirella brebissonii</i> var. <i>kuetzingii</i> Krammer & Lange-Bertalot			+
<i>Surirella</i> sp.			+
<i>Surirella terricola</i> Lange-Bertalot & E. Alles		+	
<i>Synedra</i> sp.			+
<i>Tryblionella angustata</i> W. Smith	+		+
<i>Tryblionella apiculata</i> W. Gregory	+		
<i>Tryblionella hungarica</i> (Grunow) Frenguelli	+	+	

<i>Tryblionellalittoralis</i> (Grunow) D.G.Mann	+	+	
<i>Ulnaria capitata</i> (Ehrenberg) P.Compère		+	
<i>Ulnaria ulna</i> (Nitzsch) Compère in Jahn et al.	+	+	+

4. CONCLUSION

In the study of the impact of urban and industrial pollution on the communities of the Spreča River in the area of the Lukavac City, a slightly higher number of species was found before the wastewater discharge compared to the other location. The diversity index had similar values, but the presence of halophilic diatom taxa was more pronounced in the second location. It is indicative that wastewater changes the composition of epilithic diatoms in the context of adaptation of tolerant taxa to salinization pollution. The most dominant species are widespread and prefer nutrient rich waterbodies. Bearing in mind that this is an extremely polluted river, this pilot study provides initial results on the diversity of benthic algae, which will be investigated in more detail in the upcoming studies in connection with physico-chemical and hydromorphological changes.

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