

Composition and structure of the epilithic diatom communities from Izbucl Cernei, Cernișoara and the confluence area (Gorj County)

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Abstract. The paper presents the results obtained after determining and processing some relatively quantitative samples used to characterize the epilithic diatom communities installed in the biggest carstic spring from Romania, Izbucl Cernei, into Cernișoara brook, as well as in the upstream of Cerna river, formed from the two mentioned streams. Recent articles (Wynn et al 2010; Onac et al 2011) approach unique hydro-geological and geo-chemical aspects present in the upstream area of Cerna Valley, but a diatom community characterization, an especially important algal group (Vanormelingen et al 2008) for this upstream area has not been done yet. Samples were harvested seasonally, from several sampling points; 121 specific and intra-specific taxa were identified as part of the epilithic diatom communities, dominating, both quantitatively and qualitatively, *Achnanthes minutissima* Kützing. Some structural parameters of the epilithic diatom communities were determined (floristic similarity through Sørensen index and specific diversity using Shannon index). Comparing the obtained results we conclude that in these streams, where the anthropic impact is minimal and the chemistry of water does not differ significantly, the determinant factors of the composition and structure of the epilithic diatom communities are those related to the morphology of the streams and the physical parameters of water.

Key Words: diatoms, epilithic communities, Izbucl Cernei, Cerna river, *Achnanthes minutissima* Kützing.

Rezumat. Lucrarea prezintă rezultatele obținute în urma determinării și prelucrării unor probe relativ cantitative care și-au propus caracterizarea comunităților de diatomee epilactice instalate în cel mai mare izvor carstic din România, Izbucl Cernei, în pârâul Cernișoara, precum și în zona de amonte a râului Cerna, format din cele două cursuri anterior enunțate. Articole recente (Wynn et al 2010; Onac et al 2011) abordează aspecte hidrogeologice și geochimice unice prezente în zona de aval a văii Cernei, dar o caracterizare a comunităților de diatomee, grup algal deosebit de important (Vanormelingen et al 2008) pentru zona de amonte încă nu a fost făcută. Probele au fost recoltate cu frecvență sezonieră, din mai multe puncte de colectare, iar în urma determinărilor în compoziția comunităților epilactice au fost identificați 121 taxoni specifici și intraspecifici, dominant atât calitativ, cât și cantitativ fiind *Achnanthes minutissima* Kützing. Au fost determinați unii parametri structurali ai comunităților de diatomee epilactice (similaritatea floristică, prin indicele Sørensen și diversitatea specifică, cu ajutorul indicelui Shannon). Compararea rezultatelor obținute duce la concluzia că, în aceste cursuri de apă, unde impactul antropic este minim și chimismul apei nu diferă semnificativ, factorii determinanți ai compoziției și structurii comunităților epilactice de diatomee sunt cei legați de morfologia cursurilor și de parametri fizici ai apei.

Cuvinte cheie: diatomee, comunități epilactice, Izbucl Cernei, râul Cerna, *Achnanthes minutissima* Kützing.

Introduction. The streams that samples were harvested from are the ones forming Cerna River, with a complex hydrographic basin (Povară 1997). The first one is Izbucl Cernei (Cerna's Spring), considered the most important carstic spring from Romania, and formed out of complex carstic drainage, from several different neighbouring streams (Badea 1981; Povară 1997). Water temperature in Izbucl Cernei is relatively constant, within 6.9 – 7.2°C, but the flow (within approx. 1.0 m³·s⁻¹ and 7.0 m³·s⁻¹, with a multiannual average of 1.5 – 1.6 m³·s⁻¹), and salt content vary (Povară 1997; Badea 1981). Water speed is relatively high (1.228 m·s⁻¹). The spring is at an altitude of 709 m

and a length of approx. 150 m up to the confluence with the second stream that samples were harvested from, Cernișoara brook (together forming Cerna river). Other samples were harvested from Cerna River, at approx. 400 m after the confluence of the two streams, from both riverbanks.

The vegetation is dominated by beech wood, the sample harvesting area being within the inferior mountain level (Cristea 1993). Sample harvesting area is located in the north part of the Domogled – Cerna Valley National Park, in the Ciucevele Cernei Scientific Reserve, in the Natura 2000 ROSCI0069 and ROSPA0035 Domogled – Cerna Valley sites, respectively (***) - 2012a).

The epilithic samples were harvested seasonally, brushing the rocks, treating them afterwards with strong and concentrated mineral acids (HCl, HNO₃), incinerated and included in colophony. Taxonomic determination of the samples led to the identification of 121 specific and intra-specific taxa that form the epilithic diatom communities. Most of these taxa are cosmopolitan, characteristic to clean waters, specific to the mountainous part of the streams. The dominant species, both quantitatively and qualitatively, is *Achnanthes minutissima*, alongside which few species show a constant presence. Sporadically was identified the invasive species *Dydimosphaenia geminata* M. Schmidt, but not in the Izbucul Cernei samples.

The assessment of some structural parameters of the epilithic diatom communities (floristic similarity and specific diversity) evidenced the existence of a high floristic similarity (generally over 50%), especially between samples from Izbucul Cernei, but also between these and some correspondent samples, found on the same bank, downstream of the confluence with Cernișoara brook and of a floristic diversity between 0.945 – 3.581, higher in the Cernișoara brook.

Materials and Methods. Sample harvesting involved the selection of some sampling points distributed so that they reflect as objectively as possible the scientific reality from the researched area by the number and their location in the field and to be in accordance with the specialty literature (Lowe & Pan 1996; Biggs & Kilroy 2000; *** 2009b). Five sampling points were selected, two from Izbucul Cernei (upstream and downstream), one on Cernișoara (upstream of the confluence with Izbucul Cernei) and two points downstream of the two streams' confluence, from both banks of newly formed Cerna river. Samples were harvested during 2000-2003, seasonally; alongside these samples a series of samples was harvested, for comparison, in the fall of 2008. From each sampling point were selected three to five rocks from the river bank, fully covered by water, and the sample detaching was done by energetic brushing in a small quantity of water, with a tooth brush. After brushing, sample was deposited in two containers, preserved (in formalin 4%) and labelled. Samples processing was done in order to remove the organic component of the diatoms and to obtain clean frustules with well visible ornamentations, suitable to taxonomic identification. A combined method was used treating samples with strong mineral acids then incinerating them. Specialty literature recommends the combined use of the two methods for satisfactory results (Patrick & Reimer 1966). Mineral acid (HCl and HNO₃) treating was done at low temperature; samples were initially treated with a small quantity of hydrochloric acid (HCl), to avoid effervescence (due to the presence of calcium carbonate, CaCO₃) which would lead to the loss of part of the sample, and after 2-3 days with concentrated nitric acid (HNO₃), in a volume approx. equal to that of the sample. During the use of acids for processing (14 days), samples were stored in a very well aerated place. Subsequently, through a series of consecutive operations (removal of the supernatant with a vacuum tube, distilled water addition, sample agitation for uniformity, and sample sedimentation for 24 hours) repeated three times, the used acid was removed. Incineration (≈600°C) was done using a gas plate, on metal plates for 6 hours. Previously, on the metal plates degreased microscope cover slips were disposed, on which a small quantity of sample was placed (usually one drop), as well as a drop of surfactant (Tween 20, 2%). For each sample, two cover slips were prepared, dried for 24 hours, and then incinerated until the material placed on the slides received a greyish colour.

Slides were mounted in colophony, after degreasing and heating them to 90°C on an electric plate with thermostat. On the slides, a small quantity of colophony is placed. When it liquefies, the cover slip with the incinerated sample is flipped and carefully placed, thus obtaining the mount. After mounting they are placed in a cool place (on a tile) for 24 hours, then colophony excess removed and labeled. Taxonomic identification of the diatom species was done using a trinocular microscope (OLYMPUS BX51), immersion objective (100x, with determined IM). For identification, main diatom taxonomic elements were looked for (valve form, its ornamentation, dimensions, number of striae and/or fibulae on the 10 µm unit, their disposal etc.). Taxonomic determination and establishment of ecological preferences of the identified species was done using identification books (Krammer 2000, 2002, 2003; Krammer & Lange-Bertalot 1997, 2000, 2004, 2008a, 2008b; Lange-Bertalot 2001). Some websites dedicated to diatoms were also used (** 2008a; ** 2008b; ** 2009a).

Through qualitatively processing of the epilithic samples (recording the presence or absence of species), diatom communities were characterized regarding the floristic composition and floristic affinity. To determine the floristic affinity Sørensen index was used, in binary version (Cristea et al 2004). Sørensen index calculation and dendrogram drawing were done using PAST statistic program (Hammer et al 2001). To analyze the structure of the epilithic diatom communities, samples were processed relatively quantitatively (counting approximately 600 frustules/mount). This allowed the determination of the specific relative abundance (number of frustules/species/sample) and made possible the assessment of the specific diversity (using Shannon index, adapted for diatoms (Werner 1977)). To assess the specific diversity was used a Microsoft Excel application, especially designed.

Results and Discussion. Taxonomic determination of samples led to the identification of 121 specific and intra-specific taxa, 113 species respectively and 8 varieties that form the epilithic diatom communities upstream Cerna river and the influents that form it (see Table 1). Best represented is *Navicula* genus, with 18 taxa, followed by, in decreasing order of number of taxa, *Cymbella* (15), *Nitzschia* (13), *Gomphonema* and *Cocconeis* (eight each). The rest of the genera are represented by a more reduced number of taxa, often times by only one taxon (only one species: *Caloneis*, *Cymbopleura*, *Diploneis*, *Dydimosphaenia*, *Hannaea*, *Meridion*, *Synedra* etc.). Such situations, in which the community structure is composed of different species as taxonomic belonging, and also with different ecological preferences (such as *Hannaea arcus*, *Meridion vernal* characteristic to mountain levels of streams, but also species tolerant for higher levels of trophicity - *Gomphonema parvulum*, *Navicula lanceolata*, *Nitzschia inconspicua*), were recorded in mountain streams from the Alps and Pyrenees (Gomà et al 2005).

The area of the diatom species forming the epilithic communities is predominantly cosmopolitan (e.g. species like: *Achnanthes minutissima*, *Cocconeis placentula*, *Cymbella affinis*, *C. minuta*, *Diatoma hyemalis*, *Gomphonema parvulum*, *Hannaea arcus*, *Navicula capitatoradiata*, *N. lanceolata*, *Nitzschia brevissima*, *N. palea*, *Pinnularia divergens*, *Rhoicosphaenia abbreviata*, and *Synedra ulna*), but there are also species with circum-temperate area (such as: *Cymbella caespitosa*, *C. compacta*), northern (*Amphora fogediana*), northern-alpine (*Cymbella parva*), or vague or incompletely known. Taxa with mountain distribution prevail, as it is normal, but taxa vegetating on plains or with alpine and sub-alpine distribution are also present.

Regarding water chemistry, diatoms that prefer waters with a medium electrolyte content (followed by those from waters with high electrolyte content and those from waters with low electrolyte content), with predominantly alkaline pH, oligotrophic (but there are also present taxa characteristic for eutrophic or mesotrophic waters) prevail. We thus conclude that in the analyzed streams there are epilithic diatom communities relatively sensitive to trophicity level, those from the eutrophic rivers being adapted to frequent fluctuations of the nutrient concentrations, whereas diatoms from mesotrophic and oligotrophic rivers are sensitive to these fluctuations (Lavoie et al 2009).

Table 1

Epilithic diatom communities composition* in the Cernișoara brook, Izbucl Cernei, and downstream of their confluence

Taxon	summer 2001					fall 2001					winter 2002					spring 2002					summer 2002					fall 2002					spring 2003					fall 2008				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<i>Achnanthes biasolettiana</i> Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>A. bioretii</i> Germain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>A. exigua</i> Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>A. impexiformis</i> Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>A. lanceolata</i> (Brébisson) Grunow	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	
<i>A. minutissima</i> Kützing	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>A. subatomoides</i> (Hustedt) Lange-Bertalot	-	+	-	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+	-		
<i>Amphora aequalis</i> Krammer	-	+	+	-	+	-	-	-	-	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
<i>A. fagediana</i> Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>A. libyca</i> Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>A. pediculus</i> (Kützing) Grunow	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Caloneis bacillum</i> (Grunow) Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cocconeis disculus</i> (Schumann) Cleve	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
<i>C. neodiminuta</i> Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. pediculus</i> Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. placentula</i> Ehrenberg	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>C. placentula</i> var. <i>euglypta</i> (Ehrenberg) Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. placentula</i> var. <i>lineata</i> (Ehrenberg) van Heurck	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. scutellum</i> Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. scutellum</i> var. <i>parva</i> (Grunow) Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cyclotella planctonica</i> Brunthaler	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. pseudostelligera</i> Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Cymbella affinis</i> Kützing	-	-	+	+	+	+	-	+	-	+	-	-	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. amphicephala</i> Naegelli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. caespitosa</i> (Kützing) Brun	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. cistula</i> (Ehrenberg) Kirchner	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. compacta</i> Østrup	-	-	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. excisa</i> Kützing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	
<i>C. gracilis</i> (Ehrenberg) Kützing	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. helvetica</i> Kützing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Table 1

Epilithic diatom communities composition* in the Cernișoara brook, Izbucl Cernei, and downstream of their confluence (continuation)

Taxon	summer 2001					fall 2001					winter 2002					spring 2002					summer 2002					fall 2002					spring 2003					fall 2008							
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5			
<i>G. angustum</i> Agardh	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	+	+	+	+	+	-	+	-	-	-	+	+	+	+			
<i>G. clavatum</i> Ehrenberg	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-			
<i>G. minutum</i> (Agardh) Agardh	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
<i>G. olivaceum</i> (Hornemann) Brébisson	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-			
<i>G. parvulum</i> (Kützing) Kützing	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
<i>G. tergestinum</i> Fricke	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>G. truncatum</i> Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Gyrosigma nodiferum</i> (Grunow) Reimer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>Hannaea arcus</i> (Cleve) Foged	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>Meridion vernale</i> Agardh	-	+	+	+	+	-	-	+	-	-	-	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
<i>Navicula arctotenelloides</i> Lange-Bertalot & Metzeltin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>N. capitatoradiata</i> Germain	+	+	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>N. cincta</i> (Ehrenberg) Ralfs	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. cryptocephala</i> Kützing	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. cryptotenella</i> Lange-Bertalot	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. erifuga</i> Lange-Bertalot	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. expecta</i> van Landingham	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. lanceolata</i> (Agardh) Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. menisculus</i> Schumann	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. moskalii</i> Metzeltin, Witkowski & Lange-Bertalot	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. pupula</i> Kützing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. radiosa</i> Kützing	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. reichardtiana</i> Lange-Bertalot	-	+	+	+	+	-	+	+	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. reichardtii</i> (Grunow) Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. tripunctata</i> (O.F. Müller) Bory	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. veneta</i> Kützing	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. viridula</i> (Kützing) Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. vitabunda</i> Hustedt	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Neidium alpinum</i> Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Nitzschia brevissima</i> Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>N. capitellata</i> Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1

Epilithic diatom communities composition* in the Cernișoara brook, Izbucl Cernei, and downstream of their confluence (continuation)

Taxon	summer 2001					fall 2001					winter 2002					spring 2002					summer 2002					fall 2002					spring 2003					fall 2008				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
<i>N. dissipata</i> (Kützing) Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. fonticola</i> Grunow	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-			
<i>N. heufleriana</i> Grunow	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. inconspicua</i> Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. linearis</i> (Agardh) W. Smith	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. littorea</i> Grunow	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. palea</i> (Kützing) W. Smith	-	-	-	-	+	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-	+	-			
<i>N. pellucida</i> Grunow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-			
<i>N. perminuta</i> (Grunow) M. Peragallo	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. recta</i> Hantzsch	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>N. sigmoidea</i> (Nitzsch) W. Smith	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Pinnularia borealis</i> Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>P. divergens</i> W. Smith var. <i>sublinearis</i> Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
<i>P. divergentissima</i> (Grunow) Cleve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>P. intermedia</i> (Lagerstedt) Cleve	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>P. isselana</i> Krammer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>P. marchica</i> Ilka Schönfelder	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>P. nodosa</i> (Ehrenberg) W. Smith	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Reimeria sinuata</i> (Gregory) Kociolek & Stoermer	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+		
<i>Rhoicosphaenia abbreviata</i> (Agardh) Lange-Bertalot	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+		
<i>Sellaphora bacillum</i> (Ehrenberg) D.G. Mann	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Staurosira venter</i> (Ehrenberg) Cleve & Möller	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>Surirella angusta</i> Kützing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
<i>S. brébissonii</i> Krammer & Lange-Bertalot	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>S. brébissonii</i> var. <i>kuetzingii</i> Krammer & Lange-Bertalot	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>S. minuta</i> Brébisson	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-			
<i>Synedra ulna</i> (Nitzsch) Ehrenberg	+	+	-	+	-	+	-	-	+	+	+	+	-	+	+	-	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	+			
<i>Tetracyclus rupestris</i> (Braun) Grunow	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

* +: presence; -: absence; 1 – Cernișoara brook, upstream of the confluence with Izbucl Cernei, 2 - Izbucl Cernei, upstream, 3 - Izbucl Cernei, downstream 4 – Cerna river, downstream of the confluence of Izbucl Cernei with Cernișoara, left bank 5 – Cerna river, downstream of the confluence of Izbucl Cernei with Cernișoara, right bank.

Regarding the organic pollution, the communities found in the studied streams are ranked within clean waters, prevailing the oligosaprobies and β - α -mesosaprobies (Krammer 2000, 2002, 2003; Krammer & Lange-Bertalot 1997, 2000, 2004, 2008a, 2008b; Lange-Bertalot 2001). Numerous studies show tight correlation between diatom communities, pH and water conductivity, as well as with the habitat structure in which they vegetate (Van de Vijver & Beyens 1996). Also, such a composition of the epilithic diatom communities can be related to the fact that the streams are of small orders, a correlation between stream order and trophicity being demonstrated; stream order increase involves the water trophicity level increase (Vilbaste & Truu 2003). It seems that a determinant factor in the diatom community composition is the geologic one, as well as the reduced level of water pollution (Rimet 2009).

Even though epilithic communities were sampled and determined, alongside strictly epilithic taxa were identified some vegetating on other types of substratum (epiphytic, epipellic, and epipsammic). The species that dominates both qualitatively and quantitatively the epilithic diatom communities from the analyzed samples is *Achnanthes minutissima*. It is one of the most frequently found diatoms from the benthos of fresh waters, globally (Potapova & Hamilton 2007), from oligotrophic to eutrophic waters, with acid to basic pH (Vilbaste & Truu 2003), often times opportunistic and with a great competition capacity (Stevenson & Bahls 1999). *Achnanthes minutissima* prefers waters with low nutrient level, its relative abundance decreases directly with the increase of nutrient level (Griffith et al 2005). In the studied area, the prevalence of *Achnanthes minutissima* can be explained probably by the existence of some perturbations induced by physical parameters (morphological) of the streams samples were harvested from, among which rapid water flow is a determinant factor, such situations, in which there is a stronger conditioning of distribution and composition of diatom communities related to the typological parameters of those streams were found in the case of some mountain streams from the French Alps and Pyrenees (Gomà et al 2005).

A constant presence, in most samples, is also recorded by the following species: *Cocconeis placentula*, *Cymbella minuta*, *Diatoma mesodon*, *Gomphonema minutum*, *Hannaea arcus*, and *Rhoicosphaenia abbreviata*. There were also found representative species for the epilithic flora of diatoms from springs and brooks (e.g.: *Cymbella laevis*, *Diatoma hyemalis*, *Eunotia minor*, *Meridion vernale*, *Neidium alpinum*, *Surirella minuta*). To be noted is the presence of the invasive species *Dydimosphaenia geminata* (Momeu 2009), sporadically recorded, in the Cernișoara brook and in the two samples downstream of the two streams' confluence, and never found in Izbucl Cernei.

Benthonic diatom communities are characterized by a more or less obvious dynamic, both in the unaltered streams, but especially in the ones under the influence of different anthropic activities. In the studied area, which is relatively a bit under the anthropic influence, a seasonal dynamic occurs, but with small differences, insignificant between the series of harvested samples. If we globally assess the species richness, samples in which it is the highest are those from Cernișoara brook, and those in which it is the lowest are from Izbucl Cernei. Downstream of the confluence of the two streams, the number of recorded species is intermediate (see Table 1). Seasonally, the richest samples in species are those from the summer and winter of 2002. The other samples have a more reduced number of species, though close to these.

Relative specific abundance assessment shows a structure of the epilithic diatom species with a relatively reduced number of species (between 2 -11) with over 1% percentage prevalence. Between the different seasons, no major changes are recorded in the community composition, but seldom, the dominant species being the same one (*Achnanthes minutissima*). In almost half of the samples, no other species reaches the 10% threshold, and in the samples in which other species occur with a more significant prevalence, most often *Gomphonema minutum* is recorded, then *Cymbella minuta*, *Reimeria sinuata*, rarely *Hannaea arcus* and *Diatoma mesodon* and very rarely *Cocconeis placentula*, *Cymbella tumida*, *Fragilaria capucina* and *Meridion vernale*. As seasonal presence, *Gomphonema minutum* occurs most of the times in the summer (with high prevalence especially in summer samples of 2001; being dominant in Cernișoara), then in the spring-time, fall and winter; *Cymbella minuta* in the fall, then spring and summer,

being absent during winter. Except communities in which is dominant, in five of the samples, other species are dominant: *Gomphonema minutum* in two samples (from Cernișoara point), a summer one (2001) and a winter one (2002), *Diatoma mesodon* in other two samples, a spring one (2002, downstream of the confluence point, left bank) and a summer one (2002 downstream Izbucul Cernei point) and *Cymbella minuta* in a spring sample (2003, downstream of the confluence point, left bank).

As a general tendency, a greater number of species (with prevalence $\geq 1\%$) occur in the vernal and autumnal communities, and the communities with the most species exceeding 10% prevalence are those from spring 2003; an interesting correspondence is recorded between the Cernișoara sample and that harvested downstream the confluence, on the same bank (left): *Cymbella minuta*, codominant upstream, becomes dominant downstream, whereas in the two samples from Izbucul Cernei it does not reach the 1% threshold. If we compare the two influents, Cernișoara (point 1) with Izbucul Cernei (points 2 and 3) and with those after the confluence (4 left bank, and 5 right bank respectively), we can say that in the structure of the communities the highest number of taxa (with more than 1% of the percentage abundance) is found in Cernișoara, followed by the point downstream the confluence, on the left bank (on the Cernișoara side), and in the points from Izbucul Cernei and that downstream the confluence, from the right bank, their number is smaller. We can ascribe this situation to the physical characteristics (and implicitly to the influence of the abiotic factors on the communities) of the two streams, Cernișoara flowing already on a 4-5 km distance to the confluence with the Izbucul Cernei, less speed and smaller flows and is affected by seasonal alteration, whereas Izbucul Cernei has only a flow of approx. 150 m, high speed and flow, seldom affected by periodical alterations. After the confluence, the stream that seems to "impose" in marking the epilithic diatom communities' characteristics is Izbucul Cernei.

The values of floristic similarities between the analyzed samples are high and very high, ranked almost entirely at over 50% (see Figure 1). The highest values (greater than 80%) correlate samples from Izbucul Cernei (sampling points 2 and 3) with samples from downstream of its confluence with Cernișoara, from the right bank, the side Izbucul Cernei flows its waters in (sampling point 5).

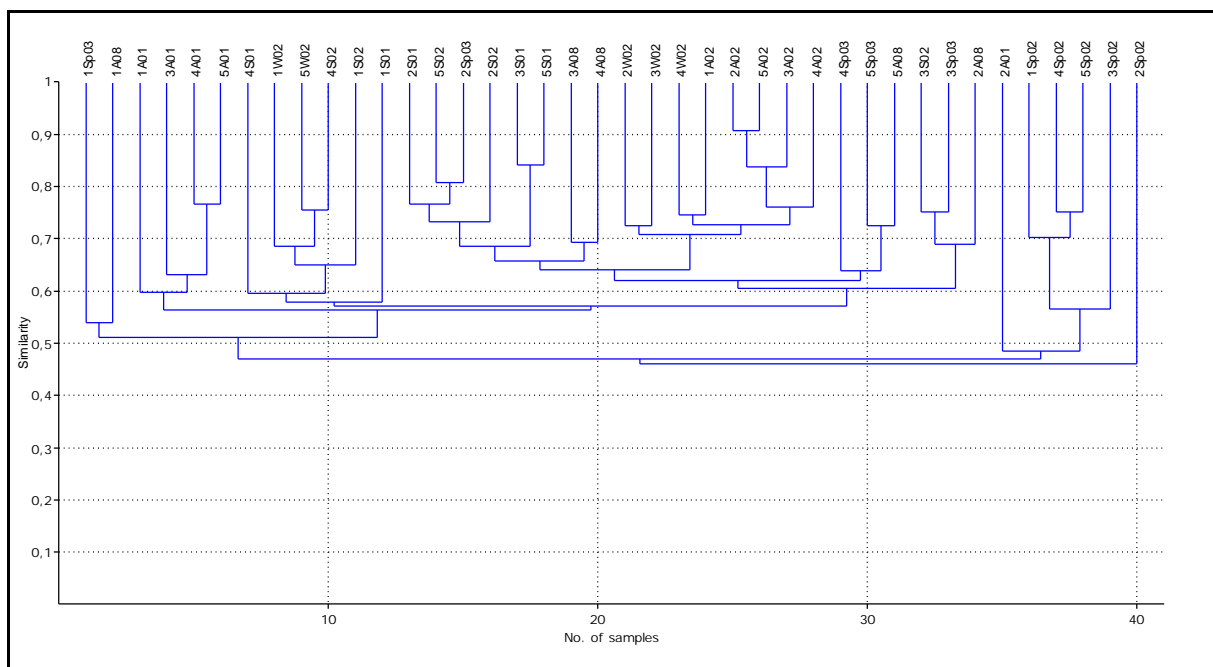


Figure 1. Floristic similarity dendrogram (Sørensen index) between the analyzed epilithic diatom communities: 1 – 5 – sampling points, A, S, Sp, W – seasons (A – autumn, S – summer, Sp – spring, W - winter), 01, 02, 03, 08 – sampling years (01 – 2001, 02 – 2002, 03 – 2003, 08 - 2008).

It seems that the waters of the two confluent streams (Cernișoara and Izbucl Cernei) do not homogenize after the 400 m of the Cerna river flow at which the downstream sampling points are (4 and 5), most probably because of the debits and increased speed Izbucl Cernei stream comes into the confluence, as well as the sharp angle of the confluence. Can also be noticed the generally smaller values to which correlate the samples harvested from Cernișoara brook; in two situations these are found within the 70-80% interval; in both situations the correlation is with samples from downstream of the confluence with Izbucl Cernei, from the correspondent bank (left bank, sampling point 4). Can also be noticed the correlation to high values of similarity between samples belonging to the same series (same season and same year), but not absolutely. In fact, benthonic diatom community analysis evidenced that these were affected by the morphology and type of the habitat of the stream in which they occur, suggesting the fact that benthonic diatoms can be used to assess the alteration level, from a physical point of view, of the rivers' habitats (Pan et al 2006). On the other hand, comparative analyses of different diatom communities led to the conclusion that between them and their environmental conditions there is a very strong relation (Gillett et al 2009).

Specific diversity is frequently used as structural indicator of the benthonic diatom communities (Lobo & Kobayasi 1990; Stevenson & Bahls 1999; Vilbaste & Truu 2003; Nagy & Péterfi 2008), including as indicator of organic pollution (Stevenson & Bahls 1999) or estimation of the impact resulted after mining activities (Ferreira da Silva et al 2009). In the case of the epilithic communities downstream Cerna river, floristic diversity values (see Figure 2) are within a broad interval (from 0.945 to 3.581, with a general average of 1.696). Even though the values reaching the superior threshold can be interpreted as being high, Shannon index is usually between 1.5 – 3.5, rarely greater than 4.5 (Cristea et al 2004). In a study of diatom communities from several streams from Estonia, Shannon index values are between the 2.09 – 4.63 interval (Vilbaste & Truu 2003). Between the different sampling points, the highest values are recorded in the Cernișoara brook point (sampling point 1), and the smallest in the Izbucl Cernei point, downstream (sampling point 2). For the floristic diversity, too, can be noticed a correspondence between the sampling point from Cernișoara brook with the corresponding one after the confluence, from the left bank (sampling point 4), between the two sampling points from Izbucl Cernei with that downstream of the confluence, de from the right bank (sampling point 5), respectively. Seasonally, values recorded high variations, the highest being in the spring of 2003, and the smallest in the fall of 2008.

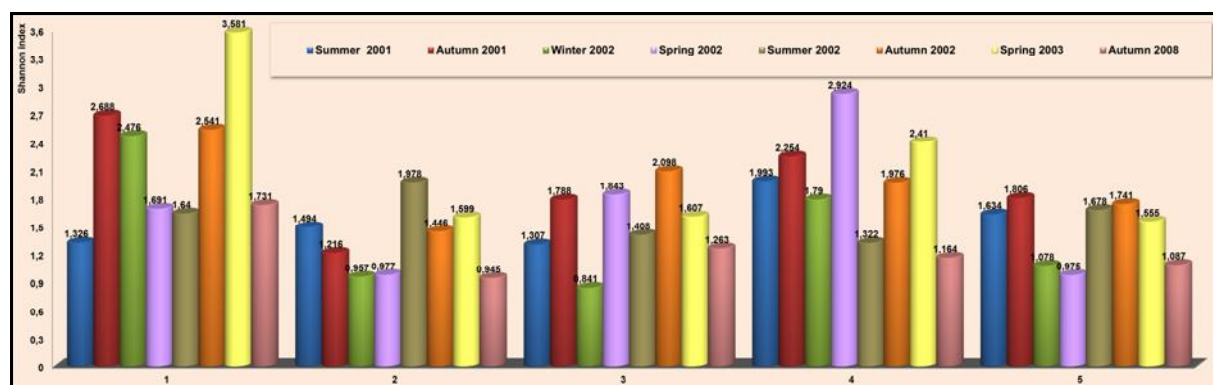


Figure 2. Seasonal dynamic of floristic diversity in the case of the analyzed epilithic diatom communities: 1 – 5 – sampling points.

In the two sampling points from Izbucl Cernei, Sørensen index values are higher in the upstream point in the winter and summer samples, and in the spring and fall they are higher in the downstream point.

Conclusions. The composition of the epilithic diatom communities habitating downstream of Cerna River (Izbucl Cernei, Cernișoara brook and the area in the

immediate proximity of their confluence) is given by a relatively great number of taxa (121). Especially in the Izbucl Cernei, rheophilic species, characteristic to the mountain sector of streams, vegetate, but with many sub-alpine and alpine interferences, which are well adapted rather especially for the morphologic characteristics of this stream than to the chemistry of the water. Even though many species were recorded, relatively few of these have a constant presence in the analyzed samples, even fewer with significant abundance, alongside the dominant species of *Achnanthes minutissima*. In the seasonal dynamic of the epilithic diatom communities, variations are reduced, the communities with the highest number of taxa with significant presence are in the Cernișoara brook. Floristic similarity records high values, especially between samples from Izbucl Cernei as well as between these and the corresponding sampling points (from the same bank, the right one) downstream of the confluence with Cernișoara. Floristic diversity shows relatively high values, higher in the Cernișoara brook and lower in Izbucl Cernei. In fact, after the confluence, the stream that determines the morphologic and chemical characteristics of the newly formed Cerna river, regarding the composition, dynamics, as well as some structural parameters of the epilithic diatom communities (similarity and floristic diversity), is Izbucl Cernei. This situation supports the statement that on the streams where there are no pollution sources (anthropic impact) or significant differences between the chemical characteristics of the chemistry of water, the morphological parameters of the streams and the physical ones of the water are determinant factors of the composition and structure of the epilithic diatom communities.

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