Conservation of freshwater habitats on Vranica Mountain and establishment of long-term monitoring of biodiversity

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ABSTRACT

Freshwater oligotrophic habitats are considered to be threatened due to various anthropogenic influences. The significance of such habitats was recognized by EU authorities and they are listed as important habitats for protection (NATURA 2000). Due to the presence of these habitat types, Vranica mountain was taken for our study. Numerous springs, creeks, and small rivers determine the specific hydrological regime of this area. Prokoško lake at the 1.635 meters of altitude gives the subalpine belt a particular ecological value. In addition to numerous springs and mountain creeks, this area is characterized by the presence of peatlands. Water temperature, pH, dissolved oxygen and specific electrical conductivity were measured with a portable multimeter (Orion Star A329). Light microscope observation and micrographs were made using a Best Scope 2020 microscope, equipped with a digital camera (MD-130). Diatoms identification and nomenclature were based on specific diatom literature and identification keys. A total of 174 taxa belonging to 58 genera were determined in the analysed samples. The most species-rich genera were *Eunotia* (22), *Gomphonema* (14) and *Pinnularia* (12). The most frequent and abundant taxa were *Achnanthidium minutissimum*, *Cocconeis placentula*, *Meridion circulare*, *Pinnularia borealis*, *Pinnularia neomajor*, *Staurosirella pinnata* and *Ulnaria ulna*. This study has shown that the analysed sampled sites offered suitable habitats for taxa of conservation interest. The highest number of taxa included in the Red List (Lange-Bertalot & Steindorf, 1996) was found in peatlands (43%) and springs (19%), while in other habitats the number of rare and endangered species is slightly lower. The obtained results from this study could serve, in the future, as a good basis for restoration and conservation of this specific habitat types on a wide area of Vranica mountain.

Key words: *Vranica, diatoms, mountain, peatlands, springs, conservation.*

INTRODUCTION

Despite the effort to significantly reduce biodiversity loss by 2010, recent research has confirmed the extinction of several plant and animal species listed as endangered in the IUCN Red Lists, while allochthonous taxa are increasing globally (Butchart et al. 2010 in Falasco & Bona, 2011).

Therefore, fragile ecosystems such as Alpine streams should be carefully monitored, as they are exposed to extreme conditions due to their harsh nature (Cantonati & Spitale, 2009 in Falasco & Bona, 2011) and human exploitation (Fráncova et al. 2009 in Falasco & Bona, 2011). Over the last years, the increasing awareness of the importance of biodiversity has led governmental agencies and the scientific community to improve the knowledge and management of these ecosystems in order to preserve their integrity (Falasco et al. 2011). In high mountains, freshwater oligotrophic habitats represent usually shelter for high biodiversity due to low variability of environmental factors over the seasons. Because of their low nutrient content, moderate water flow and constant temperature, these types of habitat can be considered suitable for a wide range of species. In that sense, very important habitats are springs, creeks, streams, lakes and peatlands. Indeed, springs offer protection for endangered and rare species taxa in addition to the common oligosaprobous ones (Cantonati 2001; Cantonati & Spitale, 2009 in Falasco et al. 2011).

On the other side mountain creeks and streams are extremely oligotrophic ecosystems where the geology of the substrate represents an important environmental factor in shaping especially algal (diatoms) community. Siliceous substrates shelter the highest biodiversity, with indicator species characteristics of low Ca water content. At local scales, current velocity and pH are the most important variables influencing algal community composition since the geology is the same among habitats (Falasco et al. 2011). From the literature data, it can be concluded that freshwater oligotrophic habitats which are distributed in the high mountain are very fragile ecosystem types and that from that point of view they must be included in a different monitoring program. At local level, the main threats to this type of habitats include : habitat reduction due to the increase of pastures, the establishment of artificial snow basins, and construction of roads and infrastructure. From a global point of view, an increase in temperature, lengthening of the growing season, habitat fragmentation, and alteration of the snow free-period duration are the most important environmental factors affecting high-mountain ecosystems. As far as climatic warming and global changes are concerned, mountain regions are among the most potentially vulnerable areas, thus being strongly affected by species loss (Körner, 1995 in Falasco et al. 2011). The ecological status of freshwater oligotrophic habitats in recent years has usually been assessed using bioindicator organisms. Benthic diatoms are regularly used as biological indicators for the environmental assessment of river water quality (included in the Water Framework Directive 2000/06). The analysis of diatom communities is a tool to guarantee an ecological and sustainable use of the water resources and the correct elaboration of the guidelines for their preservation (Falasco & Bona, 2011). Vranica Mountain is characterized by an extremely high degree of oligotrophic freshwater habitats. Due to global climate change and intense anthropogenic activities, reduction and threatening of these types of habitats in the area of Vranica are increasing each day. In order to protect these habitat types and high diversity of species, it is necessary to assess their condition. In many cases, oligotrophic habitats are used as reference sites due to their high ecological status. A diversity of diatoms were taken as a tool for assessment of the state of oligotrophic freshwater habitats.

Our results show that the living world of oligotrophic freshwater habitats, despite their surface, is extremely distinctive and diverse in the wider area of Vranica. In addition to numerous springs and mountain creeks, this area is also characterized by the presence of peatland ecosystems. These habitat types are extremely valuable elements of biological and ecological diversity. The most prominent plant species with high conservation value are *Alchemilla xanthochlora*, *Eleocharis palustris*, *Eriophorum gracile*, *Parnassia palustris*, *Philonotis fontana*, *Pinguicola leptoceras* and *Saxifraga stellaris*. Bryophyte species which inhabit oligotrophic freshwater habitats are very rare, and they also have high conservation value and the most prominent are listed as follows: *Bryum schleicheri*, *Calliergon stramineum*, *Climacium dendroides*, *Ctenidium molluscum*, *Plagiomnium affine*, *Sphagnum russowii* and *Sphagnum subsecundum*. Preliminary research on diversity of diatoms in the peatland ecosystems has revealed a large number, which according to Lange-Bertalot & Steindorf, (1996), have a certain degree of vulnerability, as follows: *Adlafia bryophila*, *Caloneis tenuis*, *Cymbopleura amphicephala*, *Cymbella aspera*, *Diploneis krammeri*, *Diploneis petersenii*, *Encyonema neogracile*, *Eunotia arcubus*, *Eunotia arcus*, *Eunotia glacialis*, *Eunotia tetraodon*, *Frustulia crassinerivia*, *Gomphonema parvulius*, *Neidium affine*, *Neidium bisulcatum*, *Pinnularia subrupestris*, *Pinnularia microstauron*, *Placoneis ignorata* and *Stauroneis phoenicenteron (*Barudanović et al., 2019*)*. In mountain springs and small creeks, during fieldwork, one crenic species of macroalgae was discovered (*Hydrurus foetidus* (Villars) Trevisan). This species is an indicator of the good ecological state of freshwater oligotrophic habitats, especially mountain springs and creeks. It is indicative that oligotrophic freshwater habitat types on Vranica Mountain are a "*hot spot*" of biodiversity. However, due to global climate change and variation in hydrological regime and strong anthropogenic influences, these habitat types and the species contained therein are extremely vulnerable. In order to protect these habitat types, in the future, it is necessary to establish long-term monitoring of biodiversity, as well as their condition.

The aim of this monitoring is to create a plan for the future restoration and conservation activities of these very unique and sensitive habitat types and to protect the high degree of species diversity. According to preliminary data main aim of this study is to establish a database of abiotic and biotic parameters which will enable further action, especially towards their restoration, conservation and long-term monitoring of biodiversity, as well as their conditions. Results of these comprehensive projects might help in establishing reference conditions not only for Bosnia and Herzegovina but also for neighbouring countries.

Study area

Vranica mountain is located in the central part of Bosnia and Herzegovina (Redžić, 2007). Boundaries of the mountain range Vranica are determined by coordinates 43° 30' and 44° 00' N; 17° 30' and 18° 00, E and cover app. 288 km. Vranica has very heterogeneous geology and petrography. Various eruptive rocks and crystal shales, feldspars, muscovite and biotite play the dominant role here. According to authors (Drešković & Mirić, 2017), the area of Vranica Mountain belongs to the Central Dinarides parts, i.e. lies in the zone of Palaeozoic and Mesozoic limestones. The uniqueness of the geological structure of Vranica Mountain is also reflected in the same geological processes. Palaeozoic deposits are presented by the insufficiently established Silurian to Permian-Triassic. Lower Devon sediments are represented by light grey limestone and dolomites that build the highest part of Vranica Mountain. Middle Devon sediments are represented by massive, rarely layered limestone and dolomite, while deposits upper Devon less represented. The whole system is rich in numerous springs, brooks, and small rivers, which are active throughout the year, and they determine the specific hydrological regime of this area. Prokoško Lake (46.039 m^2) at 1.635 m of altitude gives the subalpine belt a particular ecological value. The most important river which springs on Vranica Mountain is the river Fojnica. This river appears in the area on the northern and eastern slopes of Vranica Mountain from numerous small creeks as follows: Jezernica, Borovnica, Razdobolja, Dobruška Vranica, Pogorelica and Bitovnja. The length of the river basin (river Fojnica) along with spring line is 45.74 km, and the total area is 727,4 km^2. When flowing through Fojnica town it is called the river Dragača, but when it reaches the river Željeznica which is located near the town, it iscalled the river Fojnica. Downstream from the river Željeznica, it acquires the characteristics of medium-sized river. It has numerous tributaries, and the river Čemernica, the Mlava, the Željeznica and the Lepenica are the most important. The river flows through the following towns: Fojnica, Kiseljak and Visoko as a left tributary of the river Bosna an altitude of 430 meters (Drešković & Mirić, 2017; Basler & Benac, 1979). The vegetation is characterized by the following vegetation belts of climatogenous forests: *Quercion roboris*, *Quercion petraeae*, *Aremonio-Fagion*, *Luzulo-Fagion*, *Piceion excelsae*. Upper timberline makes the presence of *Pinion mugi* and *Alnion viridis*. This area is a habitat for numerous steno-endemic plant species. Vranica Mountain is rich in numerous springs, brooks, and small rivers, which are active throughout the year, and they determine specific hydrological regime of this area. The glacier Lake Prokoško (46 039 m^2) at 1635 m of altitude gives the subalpine belt a particular ecological value (Spahić, 2001 in Redžić, 2007). According to Redžić, (2007) plant coverage of this area could be divided into 28 classes, 44 orders, 73 alliances and 165 communities of the level of association. Very sensitive vegetation from the classes *Scheuzerio-Caricetea fuscae* and *Montio-Cardaminetea* has been developed in this area, including peatland ecosystems. Despite their relatively small areas, the vegetation from these two classes is very diverse, which has been confirmed by comparative investigations carried out in the other parts of Europe (Figure 1).

MATERIAL AND METHODS

Physical and chemical analysis

Basic properties of water were measured directly on sampling sites. Water temperature, pH, dissolved oxygen and specific electric conductance were measured with portable multimeter Orion Star A329, while turbidity was measured with Portable turbidimeter AQ3010 and TDS with PCE-CM 41.

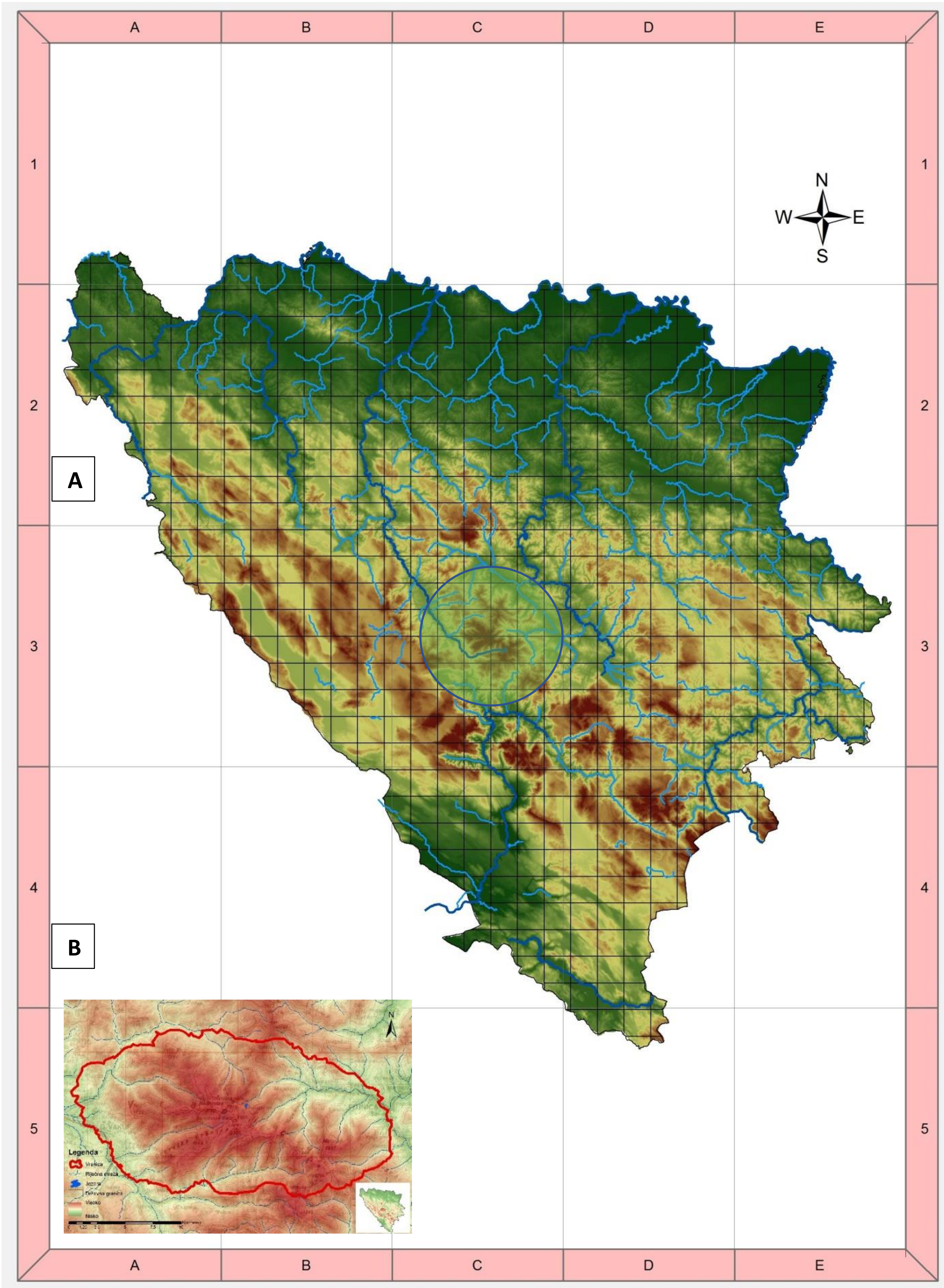


Figure 1. Position of Vranica Mountain in Central Dinarides [A.) Duraković, B. 2019 and B.) Boškailo, A. 2018]

Water samples collected on the field were analysed at the Department of Chemistry at Faculty of Science, University of Sarajevo. All samples were collected in plastic bottles. The plastic bottles were soaked in 10% (v/v) HNO_3 and then washed with double distilled water. The bottles were rinsed out three times with the sample water prior to taking the sample. The analysis of water samples was performed in the following 24 hours after the sampling. All reagents used for the preparation of calibration standard solutions were of analytical grade. Single standard stock solutions (CertPur, Merck, Darmstadt, Germany) containing 1000 mg/L of K, Na, Ca, Mg, Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn were used. Caesium chloride and potassium nitrate (Merck, Darmstadt, Germany) were used as ionization suppressor. The measurements were performed on an atomic absorption spectrometer model AA240FS, Varian, Australia. The concentration of Na and K in water samples was determined by flame atomic emission spectrometry (FAES). The concentration of Ca, Mg, Cr, Cu. Mn, Fe, Ni, Cd, Pb and Zn in water samples was determined by flame atomic absorption spectrometry (FAAS).

Diatom analysis

Samples of phytobenthos were collected from different types of substrates: epilithon, epiphyton, epipsamon and epipelon during summer, autumn and spring seasons in 2018 and 2019. Sample from submerged stones was collected by scraping with a scalpel blade or brushing the upper surface of submerged stones. Stones overgrown by filamentous algae or covered by mud were avoided due to possible contamination by nonepilithic diatoms. Portions of the samples were preserved with 4% formaldehyde solutions. Laboratory processing of diatoms was carried out applying methods used by Hustedt (1930). In order to obtain pure valves of diatoms, part of the obtained material was digested with sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid ($C_2H_2O_4$). The cleaned valves of diatoms were then mounted in a special mountant (Canada balsam) with a high refractive index in order to make it easier to see surface ornamentation such as striae and other characteristic structures (Mašić et al. 2018). Five permanent slides have been prepared for each sample and a total of 300 valves were counted to asses relative abundance. Species with content above 5% in a given assemblage were defined as abundant. All slides were scanned for taxa with low relative abundance. Light microscope observation was conducted using Best Scope 2020 microscope. Species composition and the quantitative relationship of diatoms were estimated from the permanent slides under 1000x magnification. Species abundance of diatoms were estimated on a five-degree scale as follows: 1-rare (single valve or frustule), 2-sparse (up to 10% of the sample), 3-frequent (11-15% of the sample), 4-very frequent (51-75% of the sample), 5-common (in more than 75% of the sample).

The identification of diatom was supported by the following references: Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot & Metzeltin (1996), Krammer (1997a,b., 2000, 2001, 2003), Lange-Bertalot, H. (1993, 2001), Reichardt (1999), John et al. (2003), Bey & Ector (2013a-f), Hofman et al. (2013), John (2015), Cantonati et al. (2017) and a number of specialized references listed with respective taxa: Wojtal (2003, 2006), Witkowski et al. (2004), Schmidt et al. (2004), Wojtal & Kwandras (2006), Wojtal (2009), Wojtal et al. (2011), Levkov et al. (2010), Levkov & Ector (2010), Cantonati et al. (2010)., Levkov & Wiliams (2012), Pavlov et al. (2013), Pavlov & Levkov (2013),Bucko et al. (2013), Lange-Bertalot & Wojtal (2014), Pavlov et al. (2016). The nomenclature of diatoms was adjusted according to the following Internet base: Guiry & Guiry, 2019. In order to further verify the name of the taxon they were used in the addition to the following database: Jahn, R. & Kusber, W.-H. (2019) (AlgaTerra Information System), Spaulding, S.A., Lubinski, D.J. and Potapova, M. 2019 (Diatoms of the United States) and Jüttner, I., Bennion, H., Carter, C., Cox, E.J., Ector, L., Flower, R., Jones, V., Kelly, M.G., Mann, D.G., Sayer, C., Turner, J.A., Williams, D.M. (2019) (Freshwater Diatoms Flora of Britain and Ireland). Research on diatoms in the wider area of Vranica Mountain has not been carried out so far. First data about the biodiversity of cyanobacteria and algae in some specific habitat types were described by Protić (1926), Kapetanović & Hafner (2007) and Barudanović et al. (2017).

It is very important to highlight that this comprehensive study is first in regards to inventorisation of cyanobacteria and algae in the wider area of Vranica Mountain with special emphasis on diatoms. All collected samples were stored in the Laboratory for Systematics of Algae and Fungi at the Faculty of Science, University of Sarajevo, and also aliquot with diatoms and permanent slides. It is very important to note that from each sample (aliquot), we prepared five permanent slides which are associated with unique field protocol.

Data analysis

Omnidia software (Lecointe et al. 1993) version 6.0.8, was used to calculate diatomaceous indices, including ecological and taxonomic data. The ecological status of freshwater oligotrophic habitats was assessed based on the following diatom indices: IPS – Specific, pollution sensitivity Index (Cemagref, 1982), SLAD - Sládeček's index (Sládeček, 1986), TID - ROTT trophic index (Rott, 1999) and SID - ROTT saprobic index (Rott et al., 1997). Range of diatom indices varied from 1 to 20 and corresponding to the ecological statuses as follows: bad (1-4), poor (5-8), moderate (9-12), good (13-16) and very good (17-20). In order to determine variables important for a number of species, i.e. determining the correlation between dependent (diversity index) and the independent variables (temperature, pH, dissolved oxygen, specific conductance, turbidity, TDS) Pearson's coefficient of simple linear correlation (r) was used. Pearson's coefficient of correlation between analysed variables was tested using Student t-test at a significance level of <0.05. A univariate statistical analysis was performed using the software package PAST v.3.24 (Hammer, 2019). Species diversity in diatom assemblages was determined using Shannon (H') index:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

where p_i is the proportion of characters belonging to the i_{th} type of letter in the string of interest. p_i is often the proportion of individuals belonging to the i_{th} species in the dataset of interest (Shannon & Weaver, 1949). Indicator values of diatoms were used (Van Dam et al. 1994) for the purpose of understanding the complexities of environmental conditions in investigated freshwater oligotrophic habitats. According to the Red List of diatoms (Lange-Bertalot & Steindorf, 1996), the threatened diatoms were attributed to the categories: Threatened with extinction [1], Highly endangered [2], Endangered [3], At risk [G], Very rare [R] and Declining [V]. In order to analyse the differentiation of individual samples collected at different peatlands ecosystem on Vranica mountain, the Principal Coordinate Analysis (PCoA) was used. The ordination was conducted on the Bray-Curtis similarity matrix of species data (Legendra & Legendre, 1998; Kamberović et al. 2016; Mašić et al. 2018). The data were transformed by $\log_{(x+1)}$ after standardising the matrices. Environmental variables (water temperature, pH, dissolved oxygen, specific conductance and turbidity) were presented as vectors after normalisation (Pearson's correlation). The statistical analysis with graphical interpretation was performed using the software package PRIMER v6 (Anderson et al. 2008).

RESULTS AND DISCUSSION

A. Overview of basic investigated physical, chemical and biological parameters

Results of physical, chemical and biological parameters of freshwater oligotrophic habitat types are shown in Table 1 and Figures 2 - 7. On studied sites the following physical parameters were measured: air temperature, relative humidity and light intensity. From physical and chemical parameters on studied sites the following were measured: water temperature, pH, concentration of dissolved oxygen, specific electroconductivity, turbidity and TDS. Biological parameters were determined with the presence of diatom taxa, but during the studied period other groups of algae were taken into account. Diatom diversity is presented through two indices as follows: Margalef and Shannon diversity indices, while the ecological state of studied sites was presented through four diatom indices as follows: IPS, SLA, TID and SID.

Average air temperature ranged from 13.26°C (H5) to 18.94°C (H1). The lowest average air temperature was measured in peatlands and the highest air temperature was measured in springs. Some lower temperature was measured in the lake (18.55°C), creeks (18.69°C) and stream (16.79°C). Average air humidity ranged from 60.78% (H4) to 83.51% (H3). The lowest average air humidity was measured in Prokoško Lake and the highest air humidity was measured in streams. Some lower humidity was measured in springs (69,43%), peatlands (68,01%) and creeks (65,65%). The highest average light intensity was measured in springs (9197,00 lux) and the lowest in streams (1596.89 lux). Water temperature ranged from 9.55°C (H1) to 17.43°C (H4). The lowest pH values was measured in peatlands (5.88), while the highest values were measured in streams (8,40). The values of dissolved oxygen ranged from 7.37 mgl^{-1} to 9.43 mgl^{-1}. The highest values were measured in streams, and the lowest values were measured in peatlands. The values of electric conductivity ranged from 140.53 μScm^{-1} (H5) to 223.00 μScm^{-1} (H3).

Table 1. Overview of physical, chemical and biotic parameters in studied habitats

Habitat types	**Springs**	**Creeks**	**Streams**	**Lake**	**Peatlands**
Physical site conditions / ID	***H1***	***H2***	***H3***	***H4***	***H5***
Air temperature (°C)	18.94	18.69	16.76	18.55	13.26
Air humidity (%)	69.43	65.65	83.51	60.78	68.01
Light intensity (lux)	9197.00	8344.46	1596.89	8802.00	7337.00
Chemical parameters / ID	***H1***	***H2***	***H3***	***H4***	***H5***
Water temperature (°C)	9.55	12.49	11.40	17.43	13.11
pH	7.59	8.15	8.40	8.32	5.88
Dissolved oxygen (mgL^{-1})	9.30	9.18	9.43	8.63	7.37
Specific conductance (μScm^{-1})	184.35	186.91	223.00	196.59	140.53
Turbidity (NTU)	1.18	1.42	0.80	1.70	314.30
TDS (ppm)	103.00	135.39	163.80	204.92	120.00
Biotic parameters	***H1***	***H2***	***H3***	***H4***	***H5***
Diatom taxa	**82**	**61**	**59**	**52**	**78**
d – Margalef diversity index	5.44	5.06	5.22	4.05	5.48
H' - Shannon diversity index	2.63	2.65	2.63	2.30	2.68
Bangiophyceae	0	1	0	0	0
Charophceae	0	0	0	1	0
Chlorophyceae	0	0	0	19	0
Chrysophyceae	1	1	0	2	0
Conjugatophyceae	3	9	0	22	14
Cyanophyceae	3	5	1	13	5
Dinophyceae	0	0	0	1	0
Euglenophyceae	0	0	0	7	0
Trebouxiophyceae	0	0	0	4	0
Xanthophyceae	1	1	1	0	1
Total without diatoms	8	17	2	69	20
Total with diatoms	90	78	61	121	98
Ecological state / ID	**H1**	**H2**	**H3**	**H4**	**H5**
IPS/20	18.29	17.11	16.43	17.22	18.79
Sla/20	16.41	16.51	14.85	13.59	16.29

Rott TI/20 (TID)	10.81	9.98	8.41	12.48	16.58
Rott TI/20 (SID)	16.83	16.65	14.54	17.46	19.48

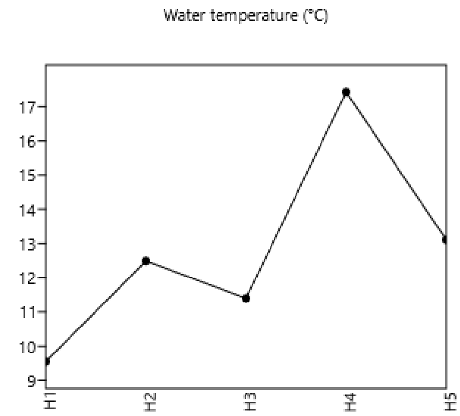


Fig. 2. Variation of water temperature

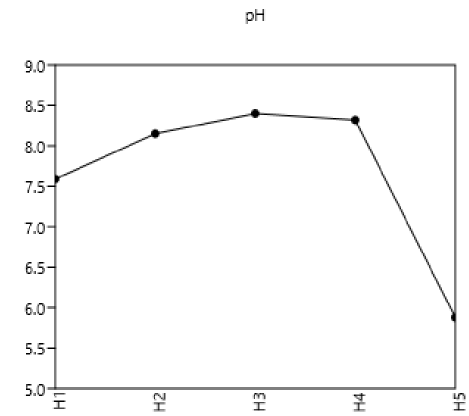


Fig. 3. Variation of pH values

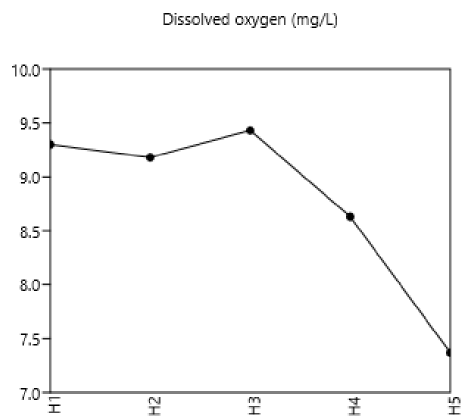


Fig. 4. Variation of dissolved oxygen

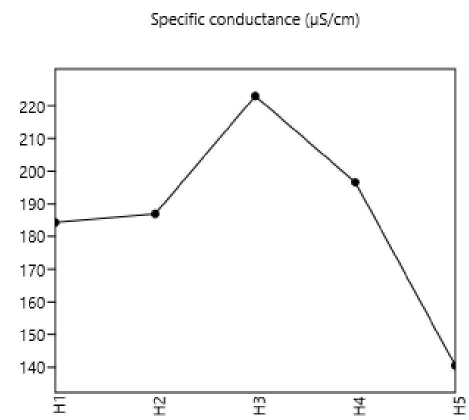


Fig. 5. Variation of specific conductance

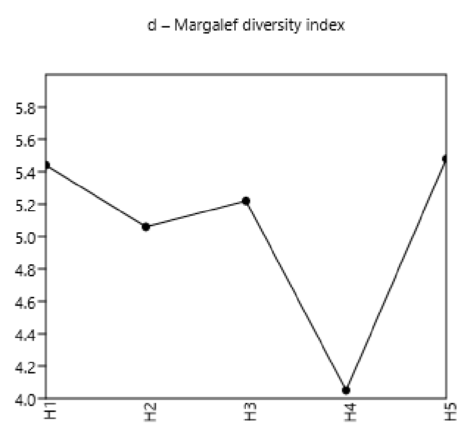


Fig. 6. Variation of Margalef diversity index

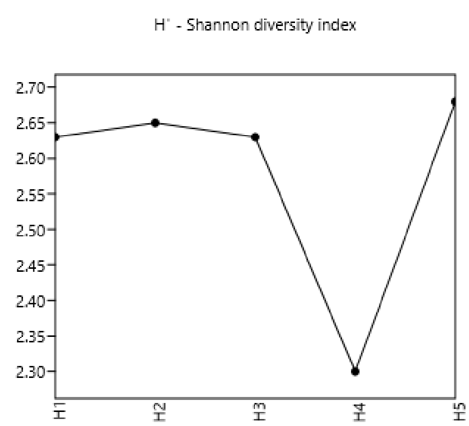


Fig. 7. Variation of Shannon diversity index

The highest value of turbidity was recorded in peatlands (314.30 NTU), while the lowest value was recorded in streams (0.80 NTU). TDS ranged from 103.00 ppm (H1) to 204.92 ppm (H4). The value of Margalef diversity index ranged from 4.95 (H4) to 2.68 (H5), while the value of the Shannon diversity index ranged from 2.30 (H4) to 2.68 (H5). The highest number of diatom species was recorded in peatlands (78), while the lowest number of diatom species was recorded in Prokoško Lake (59). Taking into account the other group of algae we can conclude that habitat type (H4) was the richest with the species (121), while the lowest number of species was recorded in streams (61).

B. Heavy metal analysis

In order to analyse the selected heavy metals, water sampling was carried out at six locations as follows: one sample was collected from the lake and peatlands, and two samples were collected from creeks and springs. Sampling from the streams was not performed. A total of 12 chemical parameters of water were analysed. Of the total of six samples collected, cadmium (Cd) was not detected at any site of research. Natrium (Na) and zinc (Zn) were determined at one site, while manganese (Mn) and iron (Fe) were determined at two sites. Chromium (Cr) and nickel (Ni) were determined at five sites, while potassium (K), calcium (Ca), magnesium (Mg), copper (Cu) and lead (Pb) were determined at all investigated sites. The results of the concentration of heavy metals from freshwater oligotrophic habitats collected on the Vranica Mountain are shown in Table 2.

Table 2. Concentration of metals (mgL^{-1}) in water samples collected at Vranica Mountain [CR – creek, LE – lake, SP – spring and PE – Peatland]

Samples	Concentration (mgL^{-1})											
	K	**Na**	**Ca**	**Mg**	**Cr**	**Cu**	**Mn**	**Fe**	**Ni**	**Cd**	**Pb**	**Zn**
CR26	0.077	n.d.	10.4	0.524	0.026	0.005	n.d.	n.d.	0.006	n.d.	0.039	n.d.
CR27	0.052	n.d.	25.6	1.97	n.d.	0.008	n.d.	n.d.	n.d.	n.d.	0.019	n.d.
17LE	0.185	n.d.	27.1	2.01	0.009	0.011	0.004	0.146	0.007	n.d.	0.003	n.d.
10SP	0.039	n.d.	26.9	1.97	0.008	0.009	n.d.	n.d.	0.017	n.d.	0.005	n.d.
11SP	0.117	n.d.	0.142	0.081	0.004	0.003	n.d.	n.d.	0.002	n.d.	0.029	n.d.
11PE	1.38	0.108	0.691	0.263	0.009	0.004	0.063	0.272	0.013	n.d.	0.022	0.012

n.d. – not detected by using the determination technique

The values of the potassium (K) range from 0.039 to 1.38. The highest values were measured in the peatlands, and the lowest values were measured in the mountain springs. The values of calcium (Ca) range from 0.142 to 10.40. The highest values were measured in the mountain streams, and the lowest values were measured in the mountain springs. The highest values of magnesium (Mg) were measured in the mountain lake (2.01), and the lowest values were measured in the mountain springs (0.081). Within the investigated habitats, there is an extremely low concentration of copper, nickel, lead, and also the remaining measured heavy metals. It is important to note that cadmium (Cd) was not detected by using the determination technique.

C. Ecological state of studied freshwater oligotrophic habitats on Vranica Mountain

In order to assess the ecological status of freshwater oligotrophic habitat types on Vranica Mountain, the results of four diatom indices (IPS, SLA, TID and SID) were taken. The IPS index ranged from 16.43 (H3) to 18.79 (H5). H1, H2, H4 and H5 habitats have very good ecological status, while H3 has good ecological status. The Sla index ranged from 13.59 (H4) to 16.51 (H2). According to the above index, all the analysed habitats have moderately good ecological status. The Rott TID index ranged from 8.41 (H3) to 16.58 (H5). According to the index, H4 and H5 habitats have a good ecological status, while habitats H1, H2 and H3 have a moderate ecological status. The Rott SID index ranged from 14.54 to 19.48. Habitats H1, H2 and H3 have good ecological status, while habitats H4 and H5 have very good ecological status. The results of variation of diatom indices are shown in the figures (Fig 8 – Fig 11).

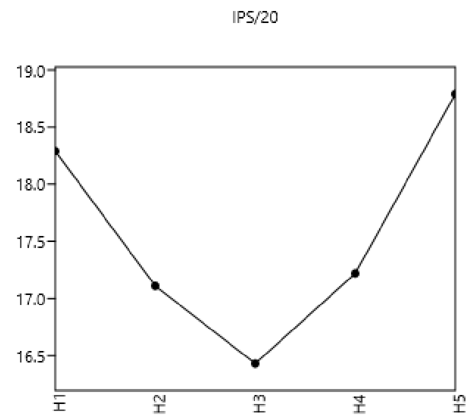


Fig. 8. Variation of IPS index

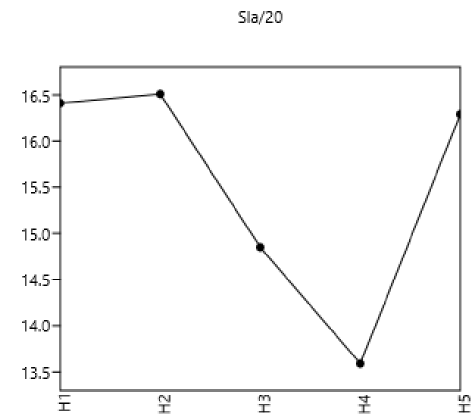


Fig. 9. Variation of Sla index

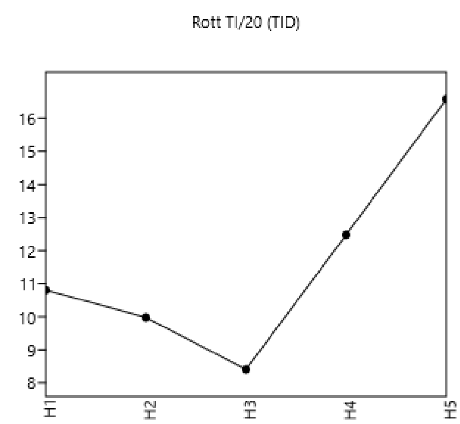


Fig. 10. Variation of TID index

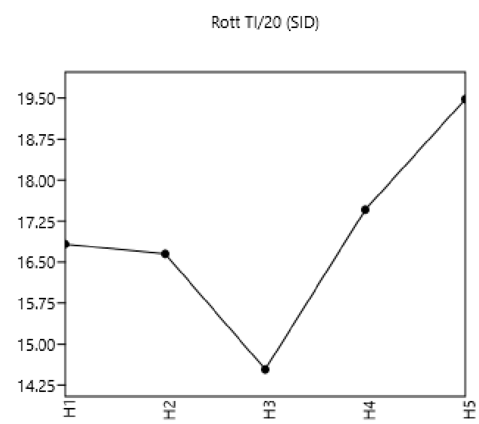


Fig. 11. Variation of SID diatom index

D. Correlation between diversity index and physical and chemical parameters

Diversity indices were in correlation with water temperature and TDS, and slightly with pH. High value of Pearson's correlation coefficient obtained between TDS and water temperature ($r=0.817$), between d and water temperature ($r=-0.879^*$; $p<0.05$), between H' and water temperature ($r=-0.829$), DO and pH ($r=0.860$), SC and pH ($r=0.927^*$; $p<0.05$), SC and DO ($r=0.854$), T2 and pH ($r=-0.953^*$; $p<0.05$), T2 and DO ($r=-0.934^{**}$; $p<0.05$), T2 and SC ($r=-0.859$), d and TDS ($r=-0.896^{**}$; $p<0.05$), H' and TDS ($r=-0.840$) and between H' and d ($r=0.960^*$; $p<0.05$). Statistically significant correlation was marked with an asterisk. Moderate correlation obtained between TDS and pH ($r=0.572$), TDS and SC ($r=0.529$), d and pH ($r=-0.539$). Week values of Pearson's correlation coefficient was obtained between pH and T1($r=0,126$), DO and T1($r=-0,390$), SC and T1($r=-0,034$), T2 and T1($r=0.062$), TDS and DO($r=0.094$), TDS and T2($r=-0.354$), d and DO($r=-0.070$), d and SC ($r=-0.352$), d and T2($r=0.410$), H' and DO ($r=-0.024$), H' and SC($r=-0.304$), H' and T2 ($r=0.362$). Moderate and week values of Pearson's correlation coefficient are not significant at the $p<0.05$ level. Correlation between diversity indices and measured physical and chemical parameters and level of significance are presented in Table 3 and Figure 12.

Table 3. Correlation between diversity indices and measured physical and chemical parameters and level of significance [H – diversity index, T1 – temperature, DO – dissolved oxygen, SC – specific conductivity, T2 – turbidity, TDS – total dissolved substance, d – Margalef diversity index and H' – Shannon diversity index].

	T1 (°C)	pH	DO (mgL^{-1})	SC (µScm^{-1})	T2 (NTU)	TDS (ppm)	d	H'
T1 (°C)	**1.00**	.	.	.	.	.	.	.
pH	0.126	**1.00**	.	.	.	.	.	.
DO (mgL^{-1})	-0.390	0.860	**1.00**	.	.	.	.	.
SC (µScm^{-1})	-0.034	0.927*	0.854	**1.00**	.	.	.	.
T2 (NTU)	0.062	-0.953*	-0.934*	-0.859	**1.00**	.	.	.
TDS (ppm)	0.817	0.572	0.094	0.529	-0.354	**1.00**	.	.
d	-0.879*	-0.539	-0.070	-0.352	0.410	-0.896*	**1.00**	.
H'	-0.829	-0.449	-0.024	-0.304	0.362	-0.840	0.960*	**1.00**

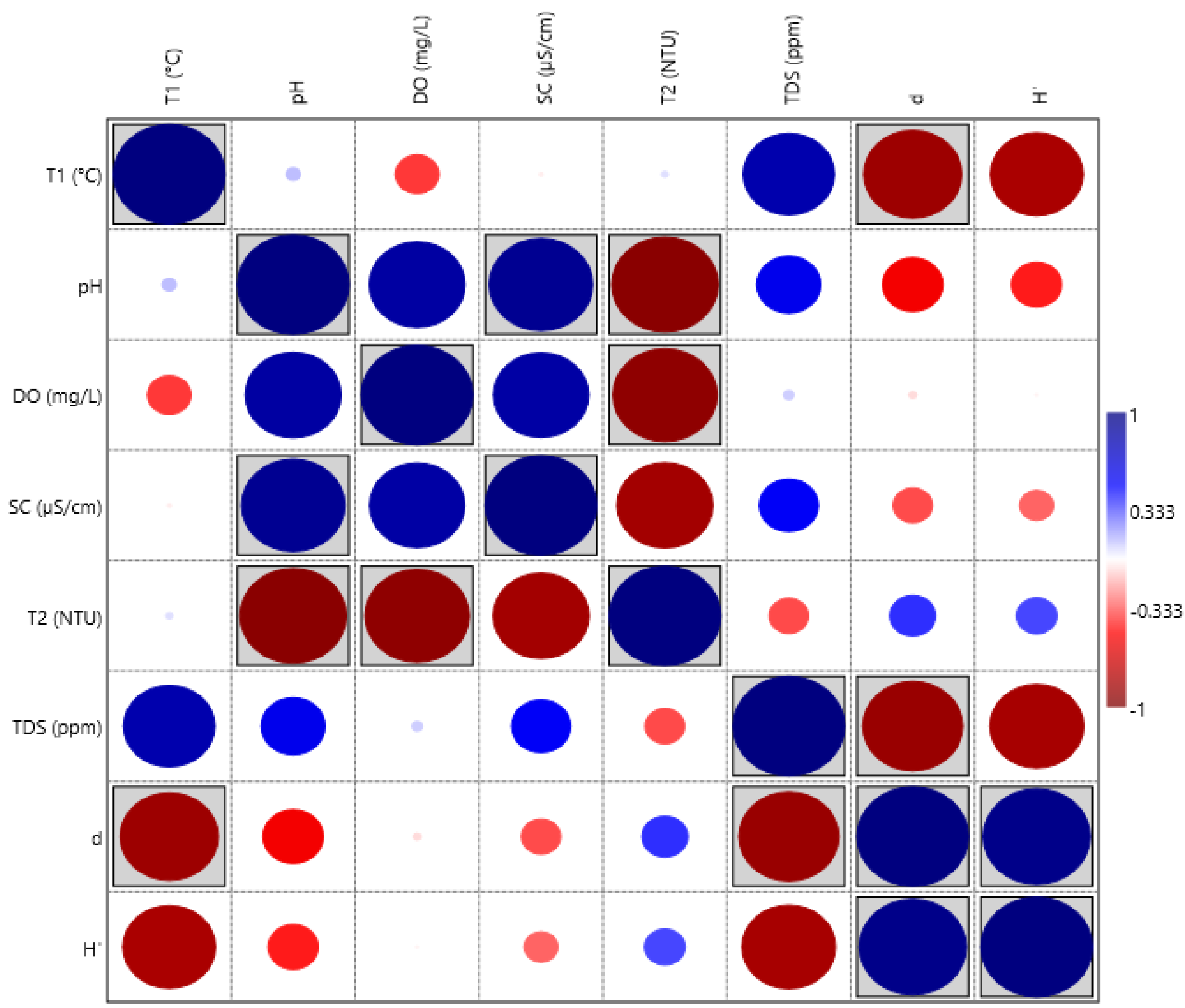


Figure 12. Correlation and level of significance between diversity indices and measured physical and chemical parameters [Statistically significant correlation is marked with a square]

E. Diversity of cyanobacteria and algae in freshwater oligotrophic habitats

Taking into account all groups of algae during the investigated period, a total of 272 taxa were recorded. The highest number of taxa was identified within the class Bacillariophyceae, Chlorophyceae, Conjugatophyceae, while taxa of other classes occurred with a smaller numerical value. The largest number of taxa was found in the lake (121 species or 27.10%), while the smallest number of taxa were recorded in the stream (61 species or 13.62%). In all studied habitat types, algae from class Bacillariophyceae were the most dominant. The largest number of species was found in springs (82 species or 24.70%), while the smallest number of species was found in Prokoško Lake (52 species or 15.66%). Summary of the recorded taxa in each investigated habitat type is shown in Table 4. Comparative overview of the recorded number of taxa (Bacillariophyceae vs other algal groups) in studied habitat types is shown in Figure 13.

Table 4. Summary of the recorded taxa in each investigated habitat type is shown in tables

Class	Springs	Creeks	Streams	Lake	Peatlands
Bacillariophyceae	82	61	59	52	78
Bangiophyceae	0	1	0	0	0
Charophceae	0	0	0	1	0
Chlorophyceae	0	0	0	19	0
Chrysophyceae	1	1	0	2	0
Conjugatophyceae	3	9	0	22	14
Cyanophyceae	3	5	1	13	5
Dinophyceae	0	0	0	1	0
Euglenophyceae	0	0	0	7	0
Trebouxiophyceae	0	0	0	4	0
Xanthophyceae	1	1	1	0	1
TOTAL	**90**	**78**	**61**	**121**	**98**

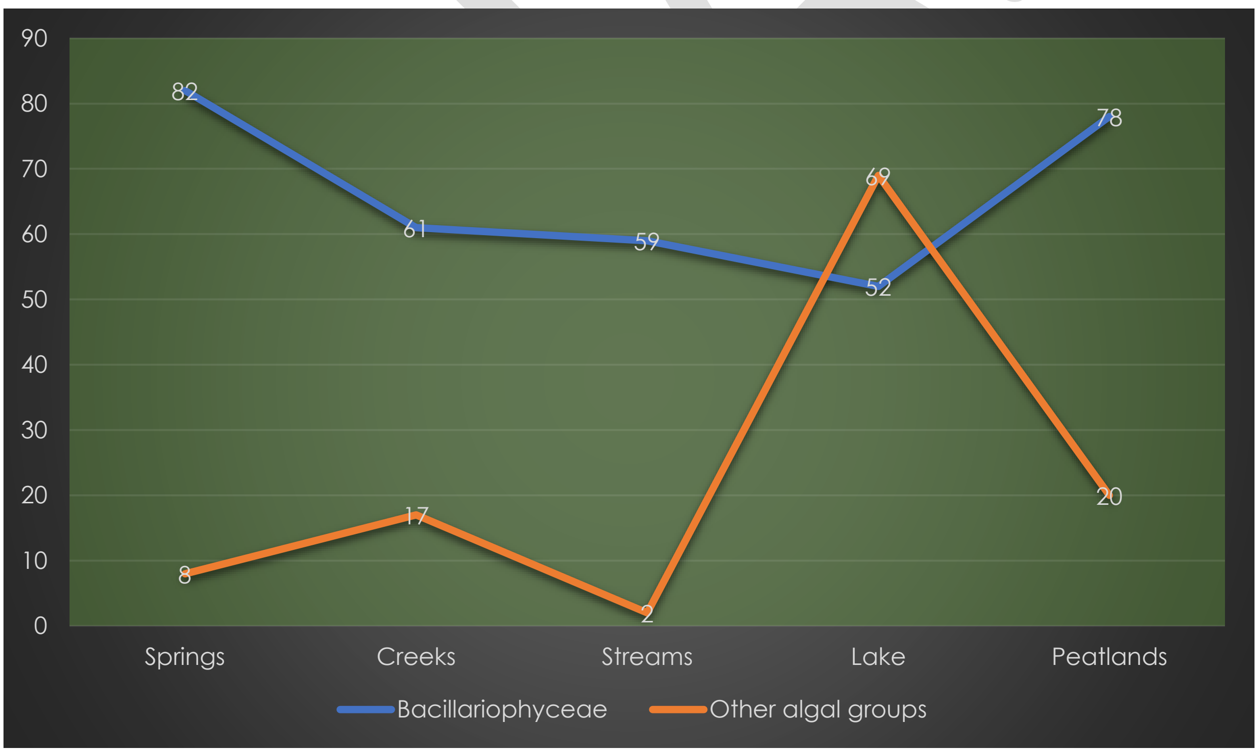


Figure 13. Total number of diatoms vs other algal group found in studied habitat types

F. Diversity of diatoms in freshwater oligotrophic habitats on Vranica Mountain

A total of 174 taxa belonging to 57 genera were determined in the material. Genera with the highest number of species were *Eunotia* (22), *Gomphonema* (14), *Pinnularia* (12), *Encyonema* (7), *Stauroneis* (6), *Cocconeis* (5), *Cymbopleura* (5), *Navicula* (5), *Neidium* (5) and *Nitzschia* (5). Altogether 169 diatom taxa mostly belonging to pennate diatoms were found and five centric diatoms. The most common pennate diatoms were *Cocconeis placentula* Ehrenberg 1838, *Achnanthidium minutissimum* (Kützing) Czarnecki 1994, *Staurosirella pinnata* (Ehrenberg) D.M.

Willams et Round 1988, *Meridion circulare* (Gréville) C. Agardh 1831, *Odontidium mesodon* (Ehrenberg) Kützing 1844, *Cocconeis lineata* Ehrenberg 1849, *Gomphonema minusculum* Krasske 1932, *Ulnaria ulna* (Nitzsch) Compère 2001, *Nitzschia fonticola* Grunow in Cleve & Möller 1879, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot 1999, *Navicula radiosa* Kützing 1844, *Diatoma ehrenbergii* Kützing 1844, *Encyonema ventricosum* (C.Agardh) Grunow in A. Schmidt et al. 1885, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot 2004, *Encyonema minutum* (Hilse) D.G. Mann 1990, *Pinnularia borealis* Ehrenberg 1843, *Tabellaria ventricosa* Kützing 1844, *Navicula tripunctata* (O.F. Müller) Bory 1822 and *Pinnularia neomajor* Krammer 1992, while centric diatoms were *Aulacoseira crenulata* (Ehrenberg) Thwaites 1848, *Orthoseira roeseana* (Rabenhorst) O'Meara 1876, *Aulacoseira alpigena* (Grunow) Krammer 1991, *Ellerbeckia arenaria* (Moore ex Ralfs) Crawford 1988 and *Melosira varians* C. Agardh 1827 (Table 5 and Plate I and II).

Within 57 recorded genera (174 taxa), three genus (Eunotia, Gomphonema and Pinnularia) consist of 49 taxa. Within the genus of Eunotia 22 taxa were identified as follows: *Eunotia arcubus, Eunotia arcus, Eunotia bilunaris, Eunotia boreoalpina, Eunotia curtagrunowii, Eunotia exigua, Eunotia glacialifalsa, Eunotia glacialis, Eunotia implicata, Eunotia incisa, Eunotia minor, Eunotia mucophila, Eunotia nymanniana, Eunotia paludosa, Eunotia praerupta, Eunotia rhomboidea, Eunotia soleirolii, Eunotia subherkiniensis, Eunotia tenella, Eunotia tetraodon, Eunotia triodon and Eunotia valida.*

Within the genus of Gomphonema 14 taxa were identified as follows: *Gomphonema acidoclinatum, Gomphonema acuminatum, Gomphonema angustum, Gomphonema exilissimum, Gomphonema gracile, Gomphonema hebridense, Gomphonema italicum, Gomphonema minusculum, Gomphonema olivaceum, Gomphonema parvulum, Gomphonema productum, Gomphonema rhombicum, Gomphonema subclavatum and Gomphonema truncatum.*

Within the genus of Pinnularia 12 taxa were identified as follows: *Pinnularia appendiculata*, *Pinnularia borealis, Pinnularia microstauron*, *Pinnularia neomajor, Pinnularia obscura, Pinnularia perirrorata, Pinnularia rupestris*, *Pinnularia schoenfelderi*, *Pinnularia stomatophora*, *Pinnularia subcapitata* var. *elongata*, *Pinnularia subrupestris* and *Pinnularia viridis.*

It is important to note that these species in the future can serve as significant bioindicators of the state of freshwater oligotrophic habitats in the wider area of Vranica Mountain. Some of these species indicate an oligotrophic state, while some species indicate processes of eutrophication or natural acidification within these freshwater habitats. Also, some of the identified species are extremely rare so that habitats have settled a major conservation significance.

During the investigated period in the studied samples of phytobenthos, a certain number of extremely rare diatoms were presented and some of them were presented with only one genus: *Amphipleura pellucida, Cavinula cocconeiformis, Chamaepinnularia mediocris, Craticula cuspidata, Cymatopleura solea, Decussiphycus hexagonus, Denticula tenuis, Ellerbeckia arenaria, Hannea arcus, Hippodonta coxiae, Karayevia laterostriata, Kobayasiella parasubtilissima, Luticola mutica, Melosira varians, Odontidium mesodon, Orthoseira roeseana, Paraplaconeis placentula, Planothidium lanceolatum, Pseudostaurosira parasitica, Reimeria sinuata, Rhoicosphaenia abbreviata, Rhoppalodia gibba, Staurosira construens, Tabellaria ventricosa* and *Tetracyclus rupestris.* Similar to the previously mentioned diatom taxa, even these rare species can serve as significant bioindicators of the state of freshwater oligotrophic habitat types and in monitoring of certain ecological phenomena. A comprehensive checklist of diatoms species found in freshwater oligotrophic habitat types is presented in Table 5.

Table 5. Comprehensive check list of diatom species in freshwater oligotrophic habitat types on Vranica Mountain

No.	OMNIDIA	Taxon name according to Cantonati et al. 2017.	RL	H1	H2	H3	H4	H5
1.	ADKR	*Achnanthidium kranzii* (Lange-Bertalot) Round et Bukhtiyarova 1996	G	0	0	1	0	0
2.	AMIN	*Achnanthidium minutissimum* (Kützing) Czarnecki 1994	?	1	1	1	1	0
3.	ABRY	*Adlafia bryophila* (Petersen) Lange-Bertalot in Mooser et al. 1998.	V	0	0	0	0	1
4.	APEL	*Amphipleura pellucida* (Kützing) Kützing 1844	*	0	0	1	0	0
5.	ACOP	*Amphora copulata* (Kützing) Schoemann et Archibald 1986	?	1	1	0	0	0
6.	AINA	*Amphora inariensis* Krammer 1980	3	1	0	0	0	0
7.	AMNU	*Amphora minutissima* W. Smith 1853	z	1	0	1	0	0
8.	AUAL	*Aulacoseira alpigena* (Grunow) Krammer 1991	G	0	0	0	0	1
9.	AUCR	*Aulacoseira crenulata* (Ehrenberg) Thwaites 1848	V	1	1	0	0	0
10.	BBRE	*Brachysira brebissonii* Ross in Hartley 1986	*	1	0	0	0	1
11.	BINT	*Brachysira intermedia* (Østrup) Lange-Bertalot in Lange-Bertalot & Moser 1994	z	0	0	0	1	1
12.	CAER	*Caloneis aerophila* Bock 1963	R	1	0	0	0	0
13.	CAPS	*Caloneis alpestris* (Grunow) Cleve 1894	G	1	1	0	0	1
14.	CSIL	*Caloneis silicula* (Ehrenberg) Cleve 1894	*	1	0	0	1	1
15.	CATE	*Caloneis tenuis* (Gregory) Krammer 1985	*	0	0	0	0	1
16.	CCOC	*Cavinula cocconeiformis* (Gregory) D.G. Mann et Stickle 1990	G	0	0	0	1	0
17.	CHME	*Chamaepinnularia mediocris* (Krasske) Lange-Bertalot 1996	V	0	0	0	0	1
18.	CLNT	*Cocconeis lineata* Ehrenberg 1849	?	1	1	1	1	0
19.	CPED	*Cocconeis pediculus* Ehrenberg 1838	?	1	0	1	0	0
20.	CPLA	*Cocconeis placentula* Ehrenberg 1838	?	1	1	1	1	1
21.	CPLK	*Cocconeis placentula* Ehrenberg 1838 var. klinorafis	*	0	0	1	0	0
22.	COPL	*Cocconeis pseudolineata* (Geitler) Lange-Bertalot 2004	D	1	1	1	0	0
23.	CCUS	*Craticula cuspidata* (Kützing) D.G. Mann in Round et al. 1990	V	0	0	0	1	0
24.	CSOL	*Cymatopleura solea* (Brébisson) W. Smith 1851	?	0	0	1	0	0
25.	CASP	*Cymbella aspera* (Ehrenberg) H. Peragallo 1889	V	1	1	1	0	1
26.	CNCI	*Cymbella neocistula* Krammer 2002	V	1	0	1	1	0
27.	CNLC	*Cymbella neolanceolata* W.Silva 2013	V	0	1	0	1	1
28.	CPRX	*Cymbella proxima* Reimer in Patrick & Reimer 1975	V	0	0	0	1	0
29.	CBAM	*Cymbopleura amphicephala* (Nägeli) Krammer 2003	V	0	1	0	1	1
30.	CBAU	*Cymbopleura austriaca (*Grunow) Krammer 2003	V	1	1	1	0	1
31.	CBCU	*Cymbopleura cuspidata* (Kützing) Krammer 2003	V	1	0	0	1	0
32.	CIQL	*Cymbopleura inaequalis* (Ehrenberg) Krammer 2003	V	0	0	0	1	0
33.	CBNA	*Cymbopleura naviculiformis* (Auerswald) Krammer 2003	*	1	0	1	1	1
34.	DHEX	*Decussiphycus hexagonus* (Torka) Guiry & Gandhi 2019	z	0	1	0	0	0
35.	DTEN	*Denticula tenuis* Kützing 1844	*	1	1	0	0	0
36.	DEHR	*Diatoma ehrenbergii* Kützing 1844	?	1	1	1	0	0
37.	DELO	*Diatoma elongatum* (Lyngbye) Agardh 1824	z	0	1	0	0	0
38.	DMON	*Diatoma moniliformis* (Kützing) Williams 2012	?	0	1	0	0	0
39.	DVUL	*Diatoma vulgaris* Bory 1824	D	0	1	1	0	0
40.	DKRA	*Diploneis krammeri* Lange-Bertalot et Reichardt 2004	V	1	1	0	0	1
41.	DOVA	*Diploneis ovalis* (Hilse) Cleve 1891	V	0	1	0	0	0
42.	EARE	*Ellerbeckia arenaria* (Moore ex Ralfs) Crawford 1988	?	0	0	1	0	0
43.	EAUE	*Encyonema auerswaldii* Rabenhorst 1853	z	0	0	1	0	0

44.	ECAE	*Encyonema caespitosum* Kützing 1849	?	0	0	0	1	0
45.	ENMI	*Encyonema minutum* (Hilse) D.G. Mann 1990	*	1	1	1	1	0
46.	ENPE	*Encyonema perpusillum* (A.Cleve) D.G. Mann 1990	G	0	0	0	0	1
47.	ENSL	*Encyonema silesiacum* (Bleisch) D.G. Mann 1990	z	0	1	0	1	1
48.	ENVE	*Encyonema ventricosum* (C.Agardh) Grunow in A. Schmidt et al. 1885	*	1	1	1	1	0
49.	EVUL	*Encyonema vulgare* Krammer 1997	3	0	0	0	0	1
50.	ECES	*Encyonopsis cesatii* (Rabenhorst) Krammer 1997	*	0	0	0	1	0
51.	ENCM	*Encyonopsis microcephala* (Grunow) Krammer 1997	*	0	0	0	1	0
52.	EADN	*Epithemia adnata* (Kützing) Brébisson 1838	?	0	1	0	0	0
53.	EGOE	*Epithemia goeppertiana* Hilse 1860	R	0	0	0	0	1
54.	ESOR	*Epithemia sorex* Kützing 1844	?	0	0	0	1	0
55.	ETUR	*Epithemia turgida* (Ehrenberg) Kützing 1844	*	1	1	0	1	0
56.	EARB	*Eunotia arcubus* Nörpel et Lange-Bertalot 1993	G	0	0	0	1	0
57.	EARC	*Eunotia arcus* Ehrenberg 1837	2	1	0	0	0	0
58.	EBIL	*Eunotia bilunaris* (Ehrenberg) Schaarschmidt 1881	?	1	0	1	0	1
59.	EBOA	*Eunotia boreoalpina* Lange-Bertalot et Nörpel-Schempp in Lange-Bertalot & Metzeltin 1998	z	0	0	0	0	1
60.	ECTG	*Eunotia curtagrunowii* Nörpel-Schempp & Lange-Bertalot in Lange-Bertalot & Metzeltin 1996	G	0	1	0	0	0
61.	EEXI	*Eunotia exigua* (Brébisson) Rabenhorst 1864	?	1	0	0	0	1
62.	empty	*Eunotia glacialifalsa* Lange-Bertalot in Krammer & Lange-Bertalot 2000	-	0	0	0	0	1
63.	EGLA	*Eunotia glacialis* Meister 1912	G	1	0	0	0	0
64.	EIMP	*Eunotia implicata* Nörpel-Schempp, Alles et Lange-Bertalot 1991	G	0	0	1	0	1
65.	EINC	*Eunotia incisa* Gregory 1854	*	0	0	0	0	1
66.	EMIN	*Eunotia minor* (Kützing) Grunow in Van Heurck 1881	*	0	0	0	1	0
67.	EMUC	*Eunotia mucophila* (Lange-Bertalot et Nörpel) Lange-Bertalot in Metzletin, Lange-Bertalot & Garcia-Rodríguez 2005)	G	0	0	0	0	1
68.	ENYM	*Eunotia nymanniana* Grunow in Van Heurck 1880	3	0	0	0	0	1
69.	EUPA	*Eunotia paludosa* Grunow 1862	V	1	0	0	1	1
70.	EPRA	*Eunotia praerupta* Ehrenberg 1843	3	0	0	1	0	0
71.	ERHO	*Eunotia rhomboidea* Hustedt 1950	V	1	0	1	0	1
72.	ESOL	*Eunotia soleirolii* (Kützing) Rabenhorst 1864	G	1	0	0	0	0
73.	ESHK	*Eunotia subherkiniensis* Lange-Bertalot in Lange-Bertalot, Bak & Witkowski 2011	2	0	1	0	0	0
74.	ETEN	*Eunotia tenella* (Grunow) Hustedt in A. Schmidt et al. 1913	V	0	0	1	0	1
75.	ETET	*Eunotia tetraodon* Ehrenberg 1838	2	1	0	0	0	1
76.	ETRD	*Eunotia triodon* Ehrenberg 1837	1	0	0	0	0	1
77.	EVAL	*Eunotia valida* Hustedt 1930	G	1	0	0	0	1
78.	FRCP	*Fragilaria recapitellata* Lange-Bertalot & Metzeltin in Metzeltin, Lange-Bertalot & Nergui 2009	z	0	1	0	0	0
79.	FRUM	*Fragilaria rumpens* (Kützing) Carlson 1913	*	0	0	1	0	0
80.	FVAU	*Fragilaria vaucheriae* (Kützing) Petersen 1938	?	0	0	1	1	0
81.	FVIR	*Fragilaria virescens* (Ralfs) D.M. Williams et Round 1988	V	0	0	0	0	1
82.	FCRS	*Frustulia crassinervia* (Brébisson) Lange-Bertalot et Krammer in Lange-Bertalot & Metzeltin 1996	V	1	0	0	0	1
83.	FSAX	*Frustulia saxonica* Rabenhorst 1853	V	0	0	0	0	1
84.	FVUL	*Frustulia vulgaris* (Thwaites) De Toni 1891	?	1	1	0	0	0
85.	GADC	*Gomphonema acidoclinatum* Lange-Bertalot et Reichardt 2004	D	0	0	1	0	1
86.	GACU	*Gomphonema acuminatum* Ehrenberg 1836	?	0	1	0	1	0
87.	GANT	*Gomphonema angustum* C. Agardh 1831	V	1	0	1	0	0
88.	GEXL	*Gomphonema exilissimum* (Grunow) Lange-Bertalot et Reichardt 1996	V	1	0	0	1	1

89.	GGRA	*Gomphonema gracile* Ehrenberg 1895	D	0	1	0	0	0
90.	GHEB	*Gomphonema hebridense* Gregory 1854	V	0	0	0	0	1
91.	GITA	*Gomphonema italicum* Kützing 1844	z	0	0	0	1	0
92.	GMIS	*Gomphonema minusculum* Krasske 1932	z	1	1	1	0	1
93.	GOLI	*Gomphonema olivaceum* (Hornemann) Brébisson 1838	?	1	1	1	0	0
94.	GPAR	*Gomphonema parvulum* (Kützing) Kützing 1849	?	0	1	0	0	0
95.	GPRO	*Gomphonema productum* (Grunow) Lange-Bertalot et Reichardt in Lange-Bertalot 1993	D	1	1	0	0	0
96.	GRHO	*Gomphonema rhombicum* M. Schmidt 1904	•	0	0	1	0	0
97.	GSCL	*Gomphonema subclavatum* (Grunow) Grunow 1884	*	1	1	0	0	1
98.	GTRU	*Gomphonema truncatum* Ehrenberg 1832	*	0	0	0	1	0
99.	GYAC	*Gyrosigma acuminatum* (Kützing) Rabenhorst 1853	V	0	0	1	0	0
100.	GYAT	*Gyrosigma attenuatum* (Kützing) Rabenhorst 1853	?	0	0	1	0	0
101.	HARC	*Hannea arcus* (Ehrenberg) R.M.Patrick in Patrick & Reimer 1966	?	0	1	1	0	1
102.	HAMP	*Hantzschia amphioxys* (Ehrenberg) Grunow 1880	?	1	0	0	1	0
103.	HCAL	*Hantzschia calcifuga* Reichardt et Lange-Bertalot 2004	D	1	0	0	0	0
104.	HCOX	*Hippodonta coxiae* Lange-Bertalot 2001	z	1	0	0	0	0
105.	HUCO	*Humidophila contenta* (Grunow) Lowe, Kociolek, Johnsen, Van den Vijver, Lange-Bertalot et Kopalová 2014	?	1	1	0	0	0
106.	HUPE	*Humidophila perpussila* (Grunow) Lowe, Kociolek, Johnsen, Van den Vijver, Lange-Bertalot et Kopalová 2014	-	1	0	0	0	0
107.	KLAT	*Karayevia laterostrata* (Hustedt) Bukhtiyarova 1999	3	1	0	0	0	0
108.	KOPA	*Kobayasiella parasubtilissima* (Kobayasi et Nagumo) Lange-Bertalot 1999	V	0	0	0	0	1
109.	LMUT	*Luticola mutica* (Kützing) D.G. Mann 1990	?	1	0	0	0	1
110.	MVAR	*Melosira varians* C. Agardh 1827	?	1	0	0	0	0
111.	MCIR	*Meridion circulare* (Gréville) C. Agardh 1831	?	1	1	1	1	1
112.	MCON	*Meridion constrictum* Ralfs 1843	z	1	1	0	0	0
113.	NCCT	*Navicula concentrica* Carter 1981	2	1	0	0	0	0
114.	NLAN	*Navicula lanceolata* (C.Agardh) Ehrenberg 1893	?	1	1	1	0	0
115.	NRAD	*Navicula radiosa* Kützing 1844	?	1	0	1	1	1
116.	NSPD	*Navicula splendicula* Van Landingham 1875	V	0	0	1	0	0
117.	NTPT	*Navicula tripunctata* (O.F. Müller) Bory 1822	?	0	1	1	0	0
118.	NEAF	*Neidium affine* (Ehrenberg) Pfitzer 1871	V	1	0	0	0	1
119.	NEAM	*Neidium ampliatum* (Ehrenberg) Krammer in the sense of Krammer et Lange-Bertalot 1985	V	1	0	0	0	1
120.	NBIS	*Neidium bisulcatum* (Lagerstedt) Cleve 1894	3	0	0	0	0	1
121.	NBSA	*Neidium bisulcatum* var. *subampliatum* Krammer 1985	3	0	0	0	0	1
122.	NLGI	*Neidium longiceps* (Gregory) Ross 1947	G	1	0	0	0	1
123.	NACD	*Nitzschia acidoclinata* Lange-Bertalot 1976	*	0	1	0	0	1
124.	NDEN	*Nitzschia denticulata* Grunow in Cleve & Grunow 1880	*	1	0	1	0	0
125.	NFON	*Nitzschia fonticola* Grunow in Cleve & Möller 1879	?	1	1	1	1	0
126.	NREC	*Nitzschia recta* Hantzsch in Rabenhost 1862	?	1	0	0	0	0
127.	NSIN	*Nitzschia sinuata* (W.Smith) Grunow 1880	V	0	0	0	0	1
128.	OMES	*Odontidium mesodon* (Ehrenberg) Kützing 1844	*	1	1	1	0	0
129.	OROE	*Orthoseira roeseana* (Rabenhorst) O'Meara 1876	V	1	0	1	0	0
130.	PPPL	*Paraplaconeis placentula* (Ehrenberg) M.S.Kulikovskiy & Lange-Bertalot in Kulikowskiy et al. 2012	z	0	1	0	0	0
131.	PAPP	*Pinnularia appendiculata* (C. Agardh) Cleve 1896	*	0	1	0	0	1
132.	PBOR	*Pinnularia borealis* Ehrenberg 1843	?	1	1	1	1	1
133.	PMIC	*Pinnularia microstauron* (Ehrenberg) Cleve 1891	V	1	0	0	1	1

134.	PNEO	*Pinnularia neomajor* Krammer 1992	G	1	1	1	1	1
135.	POBS	*Pinnularia obscura* Krasske 1932	?	0	0	0	1	0
136.	PPRI	*Pinnularia perirrorata* Krammer 2000	z	0	0	0	0	1
137.	PRUP	*Pinnularia rupestris* Hantzsch in Rabenhorst 1861	G	0	1	0	0	1
138.	PSHO	*Pinnularia schoenfelderi* Krammer 1992	G	0	0	0	0	1
139.	PSTO	*Pinnularia stomatophora* (Grunow) Cleve 1891	G	0	0	0	0	1
140.	PSEL	*Pinnularia subcapitata* var. *elongata* Krammer 1992	*	0	0	0	0	1
141.	PSRU	*Pinnularia subrupestris* Krammer 1992	G	0	1	0	0	1
142.	PVIR	*Pinnularia viridis* (Nitzsch) Ehrenberg 1843	*	1	0	1	1	1
143.	PELG	*Placoneis elginensis* (Gregory) Cox 1988	*	0	1	0	0	0
144.	PGAS	*Placoneis gastrum* (Ehrenberg) Mereschkowsky 1903	V	1	0	0	0	0
145.	PLIG	*Placoneis ignorata* (Schimanski) Lange-Bertalot in Rumrich et al. 2000	3	0	0	0	0	1
146.	PTLA	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot 1999	?	1	1	1	1	0
147.	PBIO	*Psammothidium bioretii* (Germanin) Bukhtiyarova et Round 1996	V	0	0	0	0	1
148.	PSFR	*Psammothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot 1999	-	0	0	1	0	0
149.	PHEL	*Psammothidium helveticum* (Hustedt) Bukhiyarova et Round 1996	*	0	0	0	0	1
150.	PSRE	*Psammothidium rechtense* (Leclercq) Lange-Bertalot 1999	R	1	0	0	0	0
151.	PSAT	*Psammothidium subatomoides* (Hustedt) Bukhtiyarova et Round 1996	V	0	1	0	1	1
152.	PPRS	*Pseudostaurosira parasitica* (W.Smith) Morales in Morales & Edlund 2003	?	0	0	0	1	0
153.	RSIN	*Reimeria sinuata* (Gregory) Kociolek & Stoermer 1987	?	0	1	0	0	0
154.	RABB	*Rhoicosphaenia abbreviata* (C.Agardh) Lange-Bertalot 1980	?	1	1	1	0	0
155.	RGIB	*Rhopalodia gibba* (Ehrenberg) O. Müller 1895	*	1	1	0	1	1
156.	SPPU	*Sellaphora pseudopupula* (Krasske) Lange-Bertalot in Lange-Bertalot et al. 1996	z	0	0	0	0	1
157.	SPUP	*Sellaphora pupula* (Kützing) Mereschkowsky 1902 (species group)	?	1	0	1	0	0
158.	SADC	*Stauroneis acidoclinata* Lange-Bertalot et Werum 2004	z	0	0	0	1	1
159.	STAN	*Stauroneis anceps* Ehrenberg 1843	V	1	0	0	0	0
160.	SGRC	*Stauroneis gracilis* Ehrenberg 1841	V	0	0	1	1	0
161.	SPHO	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843	V	1	0	0	0	0
162.	STSE	*Stauroneis separanda* Lange-Bertalot et Werum 2004	z	1	0	0	0	0
163.	SSVH	*Stauroneis silvahassiaca* Lange-Bertalot et Werum 2004	z	1	0	0	0	0
164.	SCON	*Staurosira construens* Ehrenberg 1843	?	0	0	0	1	1
165.	SLEP	*Staurosirella leptostauron* (Ehrenberg) D.M. Williams et Round 1988	?	0	1	0	0	1
166.	SPIN	*Staurosirella pinnata* (Ehrenberg) D.M. Willams et Round 1988	z	1	1	1	1	1
167.	SANG	*Surirella angusta* Kützing 1844	*	1	0	0	1	0
168.	SRBA	*Surirella roba* Leclercq 1983	*	1	0	0	0	1
169.	SSPI	*Surirella spiralis* Kützing 1844	V	1	1	1	0	1
170.	TVEN	*Tabellaria ventricosa* Kützing 1844	G	0	0	1	1	1
171.	TRUP	*Tetracyclus rupestris* (Braun) Grunow in Van Heurck 1881	G	1	1	1	0	0
172.	UACU	*Ulnaria acus* (Kützing) Aboal 2003	*	0	0	1	0	0
173.	UDEL	*Ulnaria delicatissima* (W.Smith) Aboal et P.C. Silva 2004	z	0	0	1	1	0
174.	UULN	*Ulnaria ulna* (Nitzsch) Compère 2001	*	1	1	1	1	1

G. Diversity of rare and endangered species

According to the Red List of freshwater diatoms (Lange-Bertalot & Steindorf, 1996) about 74 taxa or 42,52% are cited under various categories: 1 – threatened of extinction (1 taxon), 2 – highly endangered (4 taxa), 3 – endangered (8 taxa), G – at risk (20 taxa), R – very rare (3 taxa), V – declining (38 taxa). The largest number of rare and endangered diatom taxa was determined in mountain peatlands (44 taxa or 59,45%), while the smallest number was determined in mountain creeks (16 taxa) and mountain lake (16 taxa). In total 33 rare and endangered taxa were determined in mountain springs and 17 taxa in mountain streams. Comparative overview of rare and endangered taxa determined in freshwater oligotrophic habitats on Vranica Mountain is shown in Table 6 and 7 and Figure 14.

Table 6. Comparative overview of rare and endangered diatom taxa found in freshwater oligotrophic habitats on Vranica Mountain: H1 – mountain springs, H2 – mountain creeks, H3 – mountain stream, H4 – mountain lake, H5 – mountain peatlands. [RL – Red List (Lange-Bertalot & Steinforf, 2996): 0 – extinct, 1 – threatened of extinction, 2 – highly endangered, 3 – endangered, G – at risk, R – very rare, V – declining]

Habitat code	1	2	3	V	G	R	Sum
H1	.	3	2	19	7	2	33
H2	.	1	.	9	6	.	16
H3	.	.	1	11	5	.	17
H4	.	.	.	12	4	.	16
H5	1	1	5	23	13	1	44

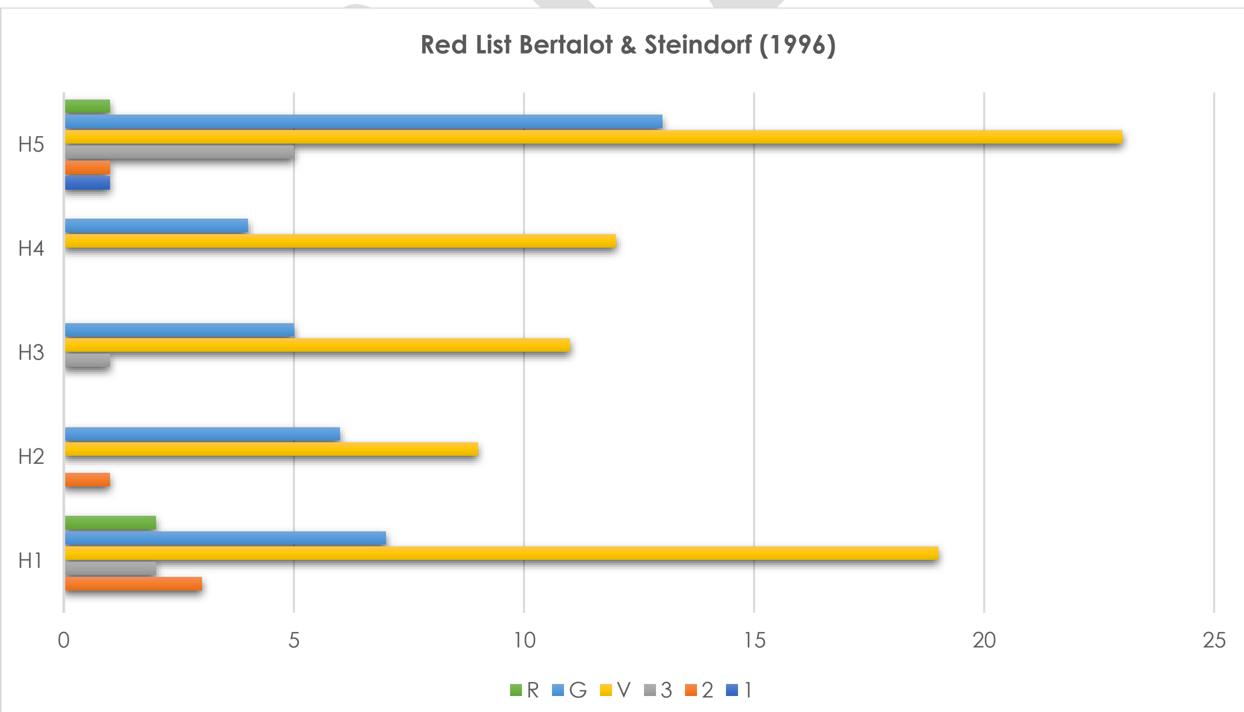


Figure 14. Comparative overview of rare and endangered diatom taxa found in freshwater oligotrophic habitats on Vranica Mountain

Table 7. Detailed overview of rare and endangered diatom taxa found in freshwater oligotrophic habitats on Vranica Mountain

No	Taxon name according to Cantonati et al. 2017.	RL	H1	H2	H3	H4	H5
1.	*Pinnularia neomajor*	G	1	1	1	1	1
2.	*Cymbella aspera*	V	1	1	1	0	1
3.	*Cymbopleura austriaca*	V	1	1	1	0	1
4.	*Surirella spiralis*	V	1	1	1	0	1
5.	*Tetracyclus rupestris*	G	1	1	1	0	0
6.	*Caloneis alpestris*	G	1	1	0	0	1
7.	*Diploneis krammeri*	V	1	1	0	0	1
8.	*Cymbella neocistula*	V	1	0	1	1	0
9.	*Eunotia rhomboidea*	V	1	0	1	0	1
10.	*Eunotia paludosa*	V	1	0	0	1	1
11.	*Gomphonema exilissimum*	V	1	0	0	1	1
12.	*Pinnularia microstauron*	V	1	0	0	1	1
13.	*Cymbella neolanceolata*	V	0	1	0	1	1
14.	*Cymbopleura amphicephala*	V	0	1	0	1	1
15.	*Psammothidium subatomoides*	V	0	1	0	1	1
16.	*Tabellaria ventricosa*	G	0	0	1	1	1
17.	*Aulacoseira crenulata*	V	1	1	0	0	0
18.	*Gomphonema angustum*	V	1	0	1	0	0
19.	*Orthoseira roeseana*	V	1	0	1	0	0
20.	*Stauroneis gracilis*	V	0	0	1	1	0
21.	*Cymbopleura cuspidata*	V	1	0	0	1	0
22.	*Eunotia tetraodon*	2	1	0	0	0	1
23.	*Eunotia valida*	G	1	0	0	0	1
24.	*Frustulia crassinervia*	V	1	0	0	0	1
25.	*Neidium affine*	V	1	0	0	0	1
26.	*Neidium ampliatum*	V	1	0	0	0	1
27.	*Neidium longiceps*	G	1	0	0	0	1
28.	*Pinnularia rupestris*	G	0	1	0	0	1
29.	*Pinnularia subrupestris*	G	0	1	0	0	1
30.	*Eunotia implicata*	G	0	0	1	0	1
31.	*Eunotia tenella*	V	0	0	1	0	1
32.	*Amphora inariensis*	3	1	0	0	0	0
33.	*Caloneis aerophila*	R	1	0	0	0	0
34.	*Eunotia arcus*	2	1	0	0	0	0
35.	*Eunotia glacialis*	G	1	0	0	0	0
36.	*Eunotia soleirolii*	G	1	0	0	0	0
37.	*Karayevia laterostrata*	3	1	0	0	0	0
38.	*Navicula concentrica*	2	1	0	0	0	0
39.	*Placoneis gastrum*	V	1	0	0	0	0
40.	*Psammothidium rechtense*	R	1	0	0	0	0
41.	*Stauroneis anceps*	V	1	0	0	0	0

42.	*Stauroneis phoenicenteron*	V	1	0	0	0	0
43.	*Diploneis ovalis*	V	0	1	0	0	0
44.	*Eunotia curtagrunowii*	G	0	1	0	0	0
45.	*Eunotia subherkiniensis*	2	0	1	0	0	0
46.	*Achnanthidium kranzii*	G	0	0	1	0	0
47.	*Eunotia praerupta*	3	0	0	1	0	0
48.	*Gyrosigma acuminatum*	V	0	0	1	0	0
49.	*Navicula splendicula*	V	0	0	1	0	0
50.	*Cavinula cocconeiformis*	G	0	0	0	1	0
51.	*Craticula cuspidata*	V	0	0	0	1	0
52.	*Cymbella proxima*	V	0	0	0	1	0
53.	*Cymbopleura inaequalis*	V	0	0	0	1	0
54.	*Eunotia arcubus*	G	0	0	0	1	0
55.	*Adlafia bryophila*	V	0	0	0	0	1
56.	*Aulacoseira alpigena*	G	0	0	0	0	1
57.	*Chamaepinnularia mediocris*	V	0	0	0	0	1
58.	*Encyonema perpusillum*	G	0	0	0	0	1
59.	*Encyonema vulgare*	3	0	0	0	0	1
60.	*Epithemia goeppertiana*	R	0	0	0	0	1
61.	*Eunotia mucophila*	G	0	0	0	0	1
62.	*Eunotia nymanniana*	3	0	0	0	0	1
63.	*Eunotia triodon*	1	0	0	0	0	1
64.	*Fragilaria virescens*	V	0	0	0	0	1
65.	*Frustulia saxonica*	V	0	0	0	0	1
66.	*Gomphonema hebridense*	V	0	0	0	0	1
67.	*Kobayasiella parasubtilissima*	V	0	0	0	0	1
68.	*Neidium bisulcatum*	3	0	0	0	0	1
69.	*Neidium bisulcatum var. subampliatum*	3	0	0	0	0	1
70.	*Nitzschia sinuata*	V	0	0	0	0	1
71.	*Pinnularia schoenfelderi*	G	0	0	0	0	1
72.	*Pinnularia stomatophora*	G	0	0	0	0	1
73.	*Placoneis ignorata*	3	0	0	0	0	1
74.	*Psammothidium bioretii*	V	0	0	0	0	1
	TOTAL NUMBER OF RARE AND ENDAGERED TAXA		**33**	**16**	**17**	**16**	**44**

Very rare diatom taxa which inhabit freshwater oligotrophic habitat types on Vranica Mountain are *Eunotia triodon* (1), *Eunotia tetraodon* (2), *Eunotia arcus* (2), *Navicula concentrica* (2), *Eunotia subherkiniensis* (2), *Amphora inariensis* (3), *Karayevia laterostrata* (3), *Eunotia praerupta* (3), *Encyonema vulgare* (3), *Eunotia nymanniana* (3), *Neidium bisulcatum* (3), *Neidium bisulcatum* var. *subampliatum* (3), *Placoneis ignorata* (3) and high number of diatom taxa from category G (at risk), R (very rare) and V (declining).

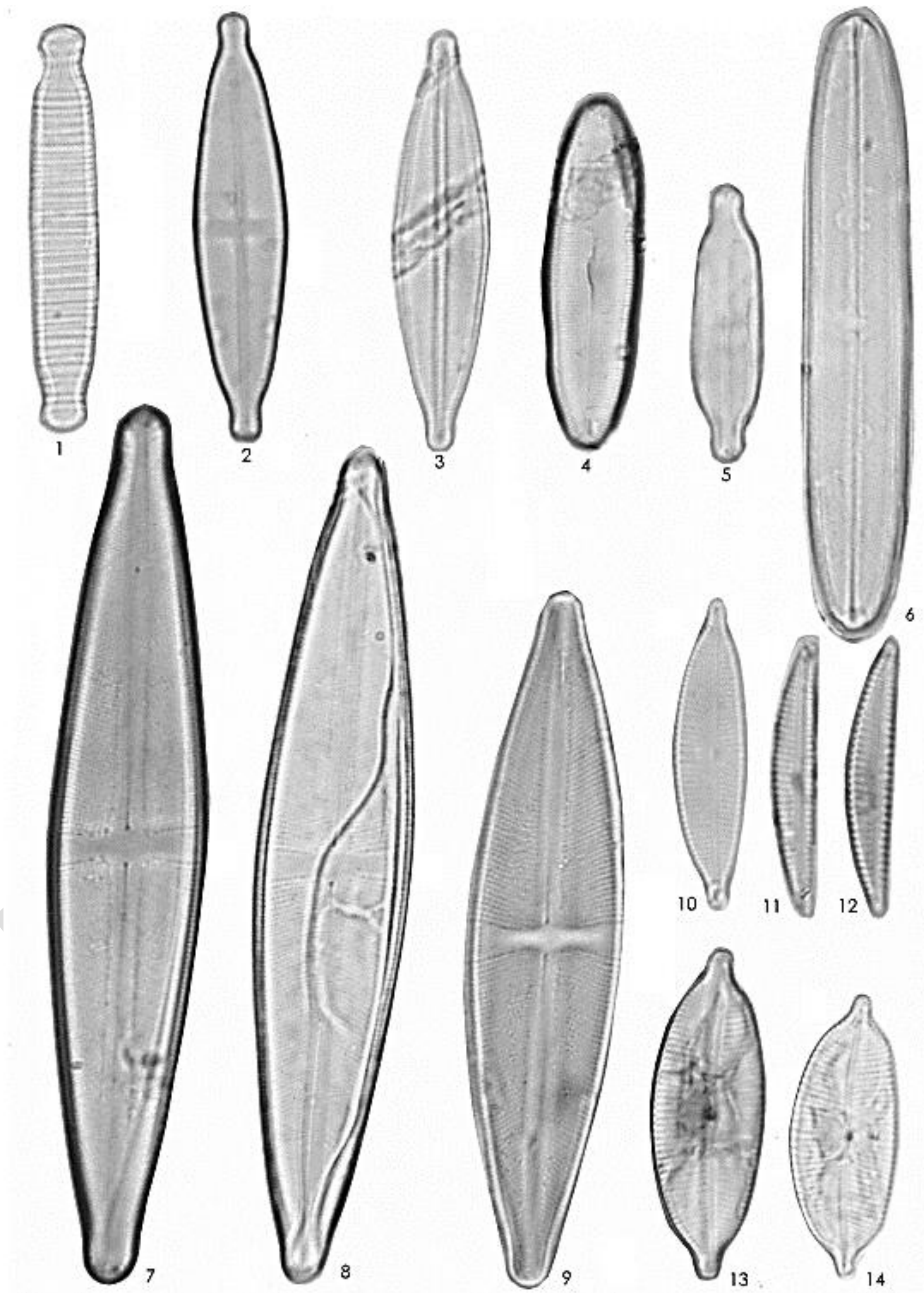
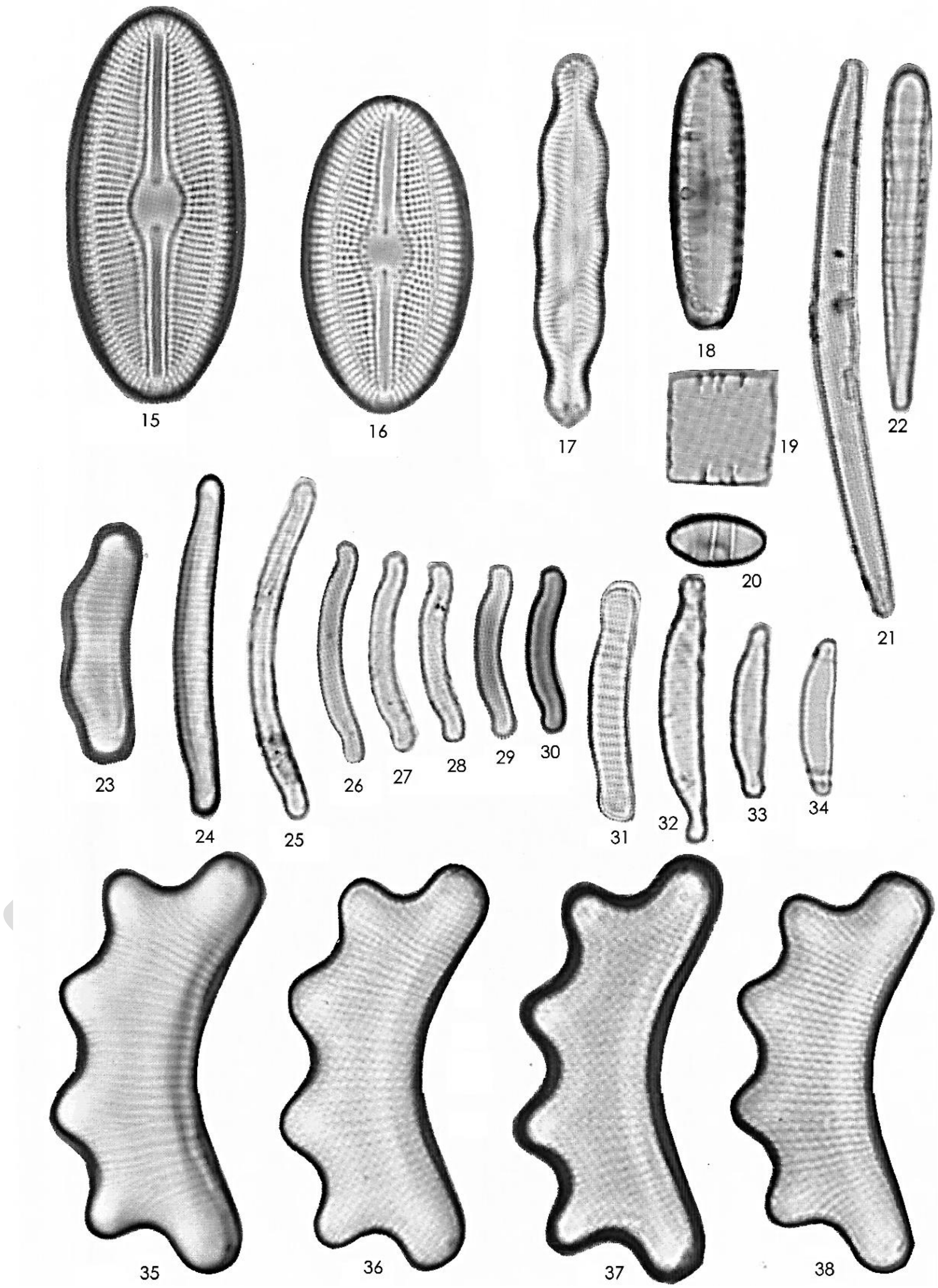


Plate I. Selected diatom taxa (1000x): **1.** *Diatoma ehrenbergii* Kützing, **2.** *Stauroneis anceps* Ehrenberg, **3.** *Frustulia crassinervia* (Bréb. ex W.Smith) Lange-Bertalot & Krammer, **4.** *Caloneis silicula* (Ehrenberg) Cleve, **5.** *Neidium longiceps* (W.Gregory) R. Ross, **6.** *Neidium bisulcatum* (Lagerstedt) Cleve, **7-8.** *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg, **9.** *Stauroneis gracilis* Ehrenberg, **10.** *Cymbopleura naviculiformis* (Auerswald ex Heiberg) Krammer, **11-12.** *Encyonema neogracile* Krammer, **13-14.** *Cymbopleura cuspidata* (Kützing) Krammer.



<u>**Plate II.** Selected diatom taxa (1000x):</u> **15-16.** *Diploneis krammeri* Lange-Bertalot & E. Reichardt, **17.** *Pinnularia grunowii* Krammer, **18.** *Pinnularia borealis* Ehrenberg, **19-20.** *Odontidium mesodon* (Kützing) Kützing, **21.** *Hannaea arcus* (Ehrenberg) R.M.Patrick in R.M.Patrick & C.W.Reimer, **22.** *Meridion circulare* (Greville) C.Agardh, **23.** *Eunotia bigibba* Kützing, **24.** *Eunotia valida* Hustedt, **25.** *Eunotia mucophila* Lange-Bertalot in Metzeltin & Lange-Bertalot, **26-30.** *Eunotia nymanniana* Grunow in Van Heurck, 31**.** *Eunotia arcus* Ehrenberg, **32.** *Eunotia boreoalpina* Lange-Bertalot & Nörpel-Schempp **33-34.** *Eunotia incisa* W.Smith ex W.Gregory, **35-38.** *Eunotia tetraodon* Ehrenberg.

H. Results of indicator values of diatoms

Indicator values were used (Van Dam et al., 1994) for the purpose of understanding the complex environmental conditions. Comparative overview of results of the indicator value of diatoms determined in freshwater oligotrophic habitats on Vranica Mountain is shown in table (Table XX) (Table 8).

Table 8. Comparative overview of results of indicator value of diatoms determined in freshwater oligotrophic habitats on Vranica Mountain [M-moisture aerophile, N-nitrogen uptake, P-pH requirements, O-oxygen requirements, S1-salinity, S2-Saprobity and T-trophic state]

Habitat types	M	N	P	O	S1	S2	T
H1	2.66	1.53	3.33	1.66	1.78	1.74	3.69
H2	2.58	1.49	3.63	1.57	1.88	1.82	3.95
H3	2.38	1.63	3.61	1.88	1.87	1.95	4.05
H4	2.42	1.42	3.45	1.77	1.79	1.80	4.29
H5	2.92	1.25	2.88	1.40	1.57	1.42	2.90
Min	2.38	1.25	2.88	1.40	1.57	1.42	2.90
Max	2.92	1.63	3.63	1.88	1.88	1.95	4.29
Average	2.59	1.46	3.38	1.66	1.78	1.75	3.78

A comparison between the spectrum of indicator values of studied freshwater oligotrophic habitat types on Vranica Mountain reveals important differences, such as:

1. Values for humidity ranged from 2.42 (H4) to 2.92 (H5). The obtained results indicate that habitats H2, H3 and H4 inhabit diatom species which have optimum of their development in the water and that partially tolerate short periods of drying. Habitats H1 and H5 inhabit real aquatic taxa, but these habitats also inhabit species that tolerate longer drying periods, i. e. they can live in aerophile conditions.
2. Values for nitrogen uptake ranged from 1.25 (H5) to 1.63 (H3). The obtained results indicate that habitats H2, H4 and H5 inhabit species that are tolerant to the presence of nitrogen, while habitats H1 and H3 inhabit species that require nitrogen.
3. Values for pH requirements ranged from 2.88 (H5) to 3.63 (H2). The obtained results indicate that habitat H5 (peatlands) is inhabited mostly by acidophilic species, while other habitats are inhabited mostly by neutrophilic species.
4. Values for oxygen requirements ranged from 1.4 (H5) to 1.88 (H3). Habitats with lower values are inhabited mostly by species which need more oxygen for their development, while habitats with higher values are inhabited mostly by species which need less oxygen for their development. In studied habitat types species, which need more oxygen for their development are abundant (75% to 100%).
5. Values for salinity ranged from 1.57 (H5) to 1.88 (H3). The obtained results indicate that freshwater oligotrophic habitat types are inhabited mostly by oligohalobous diatom taxa.
6. All studied habitats are inhabited diatom taxa which indicate oligosaprobous condition. Values for saprobity ranged from 1.42 (H5) to 1.95 (H3).
7. Values for trophic state ranged from 2.90 (H5) to 4.29 (H4). The obtained results indicate that the studied habitats are inhabited by diatom taxa which indicate oligo-mesotrophic or meso-eutrophic state. The lowest values for the trophic state are determined in mountain peatlands, while the highest values for the trophic state are determined in Prokoško Lake.

I. Differentiation of diatom assemblages in the studied habitat types

In order to differentiate the composition of diatom assemblages in relation to the gradient of the measured physical-chemical parameters, the method of PCoA analysis was used. Results of PCoA differentiation of the analysed diatom assemblages are shown in Figure 15.

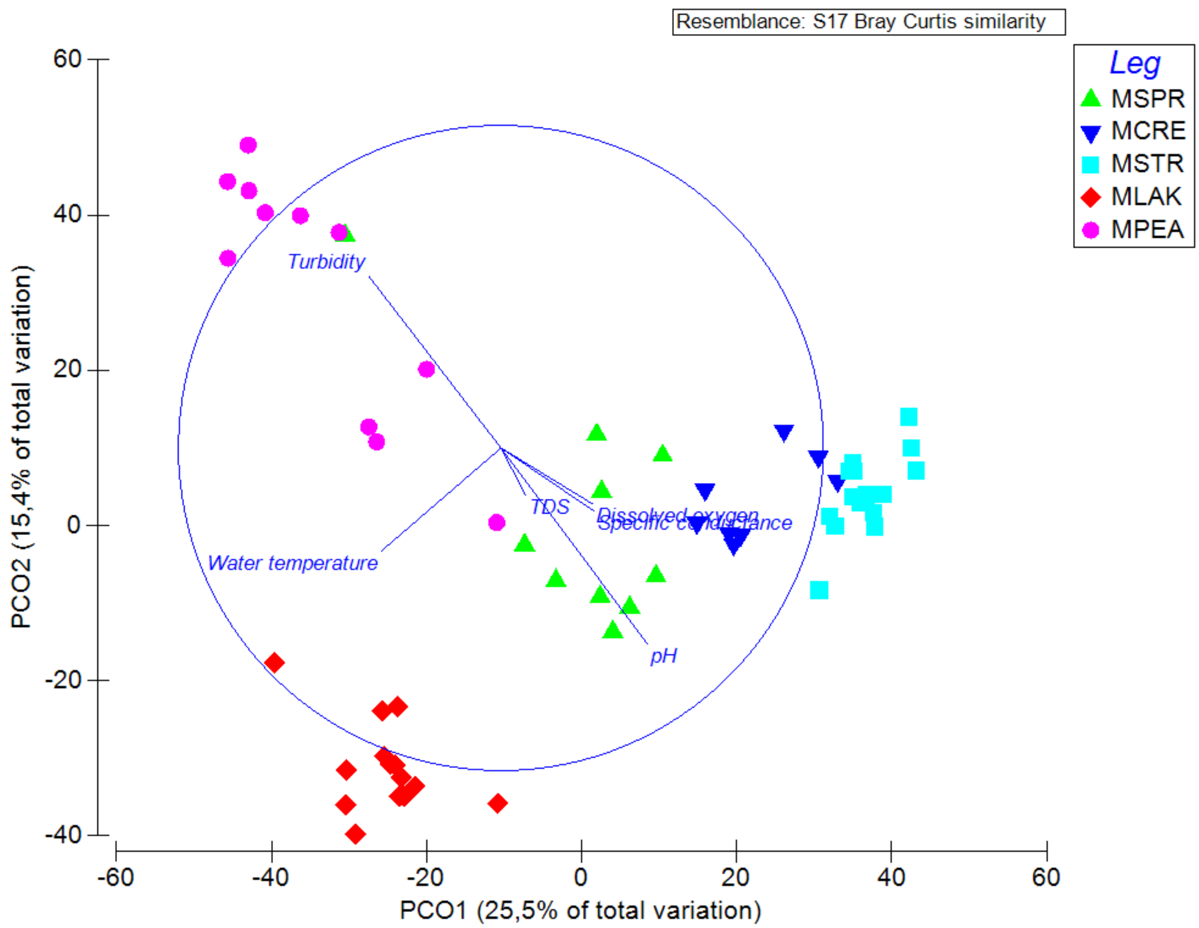


Figure 15. PCO differentiation of the analysed diatom assemblages based on Bray-Curtis similarity matrix with vector overlay showing measured physical-chemical parameters (Pearson's coefficient of correlation with PCoA axes)

The first PCoA axis explained 25.5% total variation, while the second PCoA axis explained 15.4% of the total variation. PCoA analysis clearly shows the differentiation of studied phytobenthos samples on three groups. The first and the largest group consists of samples collected from springs, creeks and streams, the second group consists of samples collected from the lake and the third group consists of samples collected from the peatlands. It is interesting to note that on the left side of the figure are samples collected from standing water, while on the left side of the figure are samples collected from running water. Within the peatlands, the maximum turbidity of water and the lowest pH value were measured. The highest temperature of the water was measured in Prokoško Lake, while the other physical and chemical parameters had significantly lower values. High values of dissolved oxygen and specific electric conductivity were measured in springs, creeks and streams.

REMARKS AND CONCLUSIONS

Based on the conducted study, following remarks and conclusions can be derived:

1. Research for this study was carried out in the wider area of Vranica Mountain. In all studied sites, the physical condition of habitat and physical and chemical parameters of water were measured. Also, algae of phytobenthos were sampled.
2. In addition to the basic physical and chemical parameters, water for selected heavy metals was taken and measured in laboratory condition. Basic physical and chemical parameters in studied sites ranged respectively, and correspond with literature data. The highest value for water temperature was measured in Prokoško Lake, and the lowest pH values were measured within mountain peatlands.
3. The highest values of metals were measured in mountain peatlands, while the lowest values were measured within mountain springs. In all studied freshwater oligotrophic habitat types the following metals were identified: potassium (K), Ca (Calcium), Mg (Magnesium), Cu (Copper) and Pb (Lead). From a total of 12 analysed metals, copper, nickel and lead had extremely low values, while cadmium was not detected in the studied habitat types.
4. Diatomaceous indices (IPS, SLA, TID and SID) have confirmed the good ecological state of mounting springs, creeks, streams and peatlands, while the moderate ecological state is confirmed for Prokoško Lake. In order to conclude about the real ecological state of freshwater oligotrophic habitat types on Vranica Mountain, the establishment of long-term monitoring is necessary. This kind of monitoring would be also good for the conservation of biological diversity within these target habitat types. The conducted study represents a good basis for future monitoring of ecological state, but also for monitoring specific ecological phenomena which are in direct connection with water ecosystems (e.g. eutrophication, acidification etc).
5. The highest number of taxa was identified within the class Bacillariophyceae, Chlorophyceae, Conjugatophyceae, while taxa of other classes occurred with a smaller numerical value. The largest number of taxa was found in Prokoško Lake (121 species or 27.10%), while the smallest number of taxa was recorded in the stream Jezernica (61 species or 13.62%). In all studied habitat types, algae from class Bacillariophyceae were the most dominant. The largest number of species was found in springs (82 species or 24.70%), while the smallest number of species was found in Prokoško Lake (52 species or 15.66%).
6. A total of 174 taxa belonging to 57 genera were determined in the material. Genera with the highest number of species were *Eunotia* (22), *Gomphonema* (14), *Pinnularia* (12), *Encyonema* (7), *Stauroneis* (6), *Cocconeis* (5), *Cymbopleura* (5), *Navicula* (5), *Neidium* (5) and *Nitzschia* (5). Altogether 169 diatom taxa mostly belonging to pennate diatoms were found and five centric diatoms. The most common centric diatoms were *Aulacoseira crenulata*, *Orthoseira roeseana*, *Aulacoseira alpigena*, *Ellerbeckia arenaria* and *Melosira varians*.
7. The largest number of rare and endangered diatom taxa was determined in mountain peatlands (44 taxa or 59,45%), while the smallest number was determined in mountain creeks (16 taxa) and mountain lake (16 taxa).
8. Ecological values of diatom taxa have confirmed the oligotrophic state of mountain spring, creeks, streams and peatlands, while the ecological state of Prokoško Lake has been changed. Unfortunately, due to entering of organic matter, but also because of the process of natural eutrophication there was overgrowing of coastal part of the lake with macrophytes vegetation. As a result of this overgrowing, total surface of Prokoško Lake is

decreasing. If this trend continues, the faith of Prokoško Lake is similar to neighbouring Suho Lake.

9. With PCoA analysis the most important variables for differentiation between studied freshwater habitats types are identified. Prokoško Lake is characterized by high water temperature, peatlands are characterized by the lowest pH and highest turbidity of water, while mountain springs, creeks and streams are characterized by the highest values of remaining parameters of water.
10. Based on the conducted research it can be concluded that the freshwater oligotrophic habitats on Vranica Mountain are in good ecological state and that they represent a "*hot spot of biodiversity*". We cannot forget that the studied freshwater habitats are under strong anthropogenic influences. From that point of view, it is important to notice that Prokoško Lake and mountain peatlands which cover only a small part of Vranica Mountain are the most fragile and the most sensitive habitat types in this area. It is very important to notice that other habitat types should not be excluded. Because of the very unique diversity in these habitat types, in the future, it is necessary to pay special attention, especially in regard to their restoration and enhancement of protection measures.
11. Through this study, an extensive work on inventorisation of cyanobacteria and algae was done. For the first time, we have identified and mapped freshwater oligotrophic habitat types on Vranica Mountain. These habitat types will be subject of future monitoring of ecological state, as well as their biodiversity. It is also important to highlight that the concentration of heavy metals is measured, as well as other different physical and chemical parameters. Using these measured parameters of water, basic variable for differentiation of freshwater oligotrophic habitat types were identified.
12. Future planned activities which are related directly to this project would be connected with the establishment of long-term monitoring of biodiversity within freshwater oligotrophic habitats on the wide area of Vranica Mountain. In order to conduct this monitoring, 20 representative sites have been selected. During the next period of time, abiotic and biotic parameters will be monitored during vegetation season at least once a month. In addition to cyanobacteria and algae as bioindicator organisms, diversity of aquatic macrophytes will be also taken into account. As a basis for this monitoring, EDGG methodology[1] of phytocoenological relevés will be taken into account as well. Current and future results will serve as a good basis for establishing a long-term monitoring of these very sensitive habitat types and for their better protection.
13. In cooperation with the competent institution in the future, it is necessary to work more on the protection of these specific habitat types, especially in the mountain area. This cooperation will be particularly relevant with the municipality of Fojnica, Federal Ministry of Environment and Tourism of the Federation of Bosnia and Herzegovina, as well as a number of interested non-governmental associations whose interests of research are the protection of nature and particular groups of organisms.
14. The obtained results of the research will provide a good basis for the preparation of future experts when it comes to these issues. Due to a large number of freshwater oligotrophic habitats in the area of Vranica Mountain and their good ecological state, and the extraordinary degree of biodiversity of cyanobacteria and algae, as well as other groups of organisms, it will be possible to write future high-quality diploma and master's thesis. The above-mentioned works and future planned projects will greatly improve the protection, but also the ecological state of these very unique habitat types.

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[1] Dengler, Jürgen, Steffen Boch, Goffredo Filibeck, Alessandro Chiarucci, Iwona Dembicz, Riccardo Guarino, B. Henneberg et al. "Assessing plant diversity and composition in grasslands across spatial scales: the standardized EDGG sampling methodology." *Bulletin of the Eurasian Dry Grassland Group* 32 (2016): 13-33.

REFERENCES

- Anderson M. J, Gorley R. N, Clarke K. R 2008: PERMANOVA + for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth. 214 p.
- Barudanović, S., Mašić, E., Macanović, A. (2017). Tresetišta na bosanskim planinama. Prirodno-matematički fakultet. Štamparija "Fojnica" d.d. Fojnica. pp 1-184.
- Basler, Đ., Benac, A. (1979). Praistorija jugoslavenskih zemalja: Neolitsko doba. Akademija Nauka i Umjetnosti Bosne i Hercegovine, Centar za Balkanološka Ispitivanja.
- Bey, M-Y., Ector, L. (2013a). Atlas des diatomées des course d'eau de la région Rhône-Alpes. Tome 1: Centrique: Monoraphidées. Avec la collaboration de Rémy Chavaux et Patrick Béranger. DREAL Rhône-Alpes. 181 p. Bey, M-Y., Ector, L. 2013b) Atlas des diatomées des course d'eau de la région Rhône-Alpes. Tome 2: Araphidées, Brachyraphidées. Avec la collaboration de Rémy Chavaux et Patrick Béranger. DREAL Rhône-Alpes. 330 p.
- Bey, M-Y., Ector, L. (2013c). Atlas des diatomées des course d'eau de la région Rhône-Alpes. Tome 3: Naviculacées, Naviculoidées (a). Avec la collaboration de Rémy Chavaux et Patrick Béranger. DREAL Rhône-Alpes. 530 p.
- Bey, M-Y., Ector, L. (2013d). Atlas des diatomées des course d'eau de la région Rhône-Alpes. Tome 4: Naviculacées, Naviculoidées (b). Avec la collaboration de Rémy Chavaux et Patrick Béranger. DREAL Rhône-Alpes. 750 p.
- Bey, M-Y., Ector, L. (2013e). Atlas des diatomées des course d'eau de la région Rhône-Alpes. Tome 5: Naviculacées, Cymbelloidées, Gomphonématoidées. Avec la collaboration de Rémy Chavaux et Patrick Béranger. DREAL Rhône-Alpes. 972 p.
- Bey, M-Y., Ector, L. (2013f). Atlas des diatomées des course d'eau de la région Rhône-Alpes. Tome 5: Bacillariacées, Rhopalodiacées, Surirellacées. Avec la collaboration de Rémy Chavaux et Patrick Béranger. DREAL Rhône-Alpes. 1182 p.
- Buczkó, K., & Wojtal, A. (2005). Moss inhabiting siliceous algae from Hungarian peat bogs. *Studia botanica hungarica*, *36*, 21-42.
- Butchard et al. 2010. Global biodiversity: indicators of recent declines. *Science*, 328: 1164-1168.
- Cantonati, M., & Spitale, D. (2009). The role of environmental variables in structuring epiphytic and epilithic diatom assemblages in springs and streams of the Dolomiti Bellunesi National Park (south-eastern Alps). *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, 174(2), 117-133.
- Cantonati, M., Corradini, G. I. O. R. G. I. A., Juttner, I. N. G. R. I. D., & Cox, E. J. (2001). Diatom assemblages in high mountain streams of the Alps and the Himalaya. *Nova Hedwigia Beiheft*, 123, 37-62.
- Cantonati, M., Kelly, G. M., Lange-Bertalot, H. (2017). Freshwater Benthic Diatoms of Central Europe: Over 800 Common Species Used in Ecological Assesment. English edition with updated taxonomy and added species. Koeltz Botanical Book. 942 p.
- Coste, M. (1982). Étude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Cemagref QE Lyon-AF Bassin Rhône Méditerranée Corse.
- Drešković, N., Mirić, R. (2017). Regionalna geografija Bosne i Hercegovine. Univerzitetu u Sarajevu, Prirodno-matematički fakultet. Štamparija "Fojnica" d.o.o. Fojnica. pp 1-436.
- Falasco, E., Bona, F. (2011). Diatom community biodiversity in an Alpine protected area: a study in the Maritime Alps Natural Park.
- Falasco, E., Ector, L., Ciaccio, E., Hoffmann, L., & Bona, F. (2012). Alpine freshwater ecosystems in a protected area: a source of diatom diversity. Hydrobiologia, 695(1), 233-251.
- Fránková, M., Bojková, J., Poulíčková, A., & Hájek, M. (2009). The structure and species richness of the diatom assemblages of the Western Carpathian spring fens along the gradient of mineral richness. *Fottea*, 9(2), 355-368.

- Guiry, M.D. & Guiry, G.M. 2019. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 09 April 2019.
- Hammer, Ø. 2017: PAST PAleontological STatistics Version 3.18. Reference manual. 259 p.
- Hofman, G., Lange-Bertalot, H., Werum, M. (2013). Diatomen im Süsswasser-Benthos von Mitteleuropa. Koeltz Scientific Book. 908 p.
- Hustedt, F. 1930: Bacillariophyta (Diatomae). In: Die Üsserwasser-flora Mitteleuropas, Heft 10. Jena. 466 p.
- Jahn, R. & Kusber, W.-H., 2019 (AlgaTerra Information System). http://www.algaterra.org/.
- John, D. M., Whitton, B. A., Brook, A. J. (2003). The freshwater Algae Flora of the British Isle. An Identification Guide to Freshwater and Terrestrial Algae. The Natural History Museum and the British Phycology Society, 2002, Cambridge. 714 p.
- John, J. (2015). A Beginner's guide to diatoms. Revised second edition. Koeltz Scientific Books. 174 p.
- Jüttner, I., Bennion, H., Carter, C., Cox, E.J., Ector, L., Flower, R., Jones, V., Kelly, M.G., Mann, D.G., Sayer, C., Turner, J.A., Williams, D.M., 2019 (Freshwater Diatoms Flora of Britain and Ireland).
- Kamberović, J., Kišić, A., Hafner, D., Plenković-Moraj, A. (2016). Comparative analysis of epilithic diatom assemblages of springs and streams in the Konjuh mountain (Bosnia and Herzegovina). *Works of the Faculty of Forestry, University of Sarajevo*, 2: 54-67.
- Kapetanović, T., & Hafner, D. (2007). Diatoms of wet habitats in the subalpine belt of Mt. Vranica (Bosnia and Herzegovina). In Proceedings of the 1st Central European Diatom Meeting, Berlin-Dahlem (pp. 73-78).
- Körner, C. H. (1995). Alpine plant diversity: a global survey and functional interpretations. In Arctic and alpine biodiversity: Patterns, causes and ecosystem consequences (pp. 45-62). Springer, Berlin, Heidelberg.
- Krammer, K. (1997a). Die cymbelloiden Diatomen. Eine Monographie der weltweit bekannten Arten. Teil 1. Allgemeines und Encyonema Part. Bibliotheca Diatomologica, 36. 382 p.
- Krammer, K. (1997b). Die cymbelloiden Diatomen. Eine Monographie der weltweit bekannten Arten. Teil 2. Encyonema part., Encyonopsis und Cymbellopsis. *Bibliotheca Diatomologica* 37. 469 p.
- Krammer, K. (2000). The genus Pinnularia. Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Edited by Horst Lange-Bertalot. Volume 1. A.R.G. Ganter Verlag K. G. 294 p.
- Krammer, K. (2001). Cymbella. Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Edited by Horst LangeBertalot. Volume 3. A.R.G. Ganter Verlag K. G. 584 p.
- Krammer, K. (2003). Cymbopleura, Delicata, Navicymbulla, Gomphocymbellopsis, Afrocymbella. Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Edited by Horst Lange-Bertalot. Volume 4. A.R.G. Ganter Verlag K. G. 530 p.
- Krammer, K., Lange-Bertalot, H. (1986). Süsswasserflora von Mitteleuropa. Bacillariophyceae, 1. Teil: Naviculaceae. VEB Gustav Ficher Verlag, Jena. 876 p.
- Krammer, K., Lange-Bertalot, H. (1988). Süsswasserflora von Mitteleuropa. Bacillariophyceae, 2. Teil: Bacillariophyceae, Epithemiaceae, Surirellaceae. VEB Gustav Ficher Verlag, Jena. 596 p.
- Krammer, K., Lange-Bertalot, H. (1991). Süsswasserflora von Mitteleuropa. Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. VEB Gustav Ficher Verlag, Jena. 576 p.
- Krammer, K., Lange-Bertalot, H. (1991). Süsswasserflora von Mitteleuropa. Bacillariophyceae, 4. Teil: Achnanthaceae, Kritische Erganzungen zu Navicula

(Lineolatae), und Gomphonema Gasamliteraturerzeichnis, Teil 1-4. VEB Gustav Ficher Verlag, Jena. 248 p.

- Lange-Bartalot, H. (2001). Navicula sensu stricto. 10 Genera Separated from Navicula sensu lato Frustulia. Diatoms of the European Inland Waters and Comparable Habitats. Edited by Horst LangeBertalot. Volume 4. A.R.G. Ganter Verlag K. G. 526 p.
- Lange-Bertalot, H. (1993). 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süsswasserflora von Mitteleuropa Vol. 2/1-4. *Bibliotheca Diatomologica*, 27. 455 p.
- Lange-Bertalot, H., Metzeltin, D. (1996). Oligotrophie-Indicatoren. 800 Taxa repräsentative fur drei diverse See-Typen. *Iconographia Diatomologica*, 2. 390 p.
- Lange-Bertalot, H., Steindorf, A. (1996). Rote liste der limnischen Kieselalgen (Bacillariophyceae) Deutschlands. *Schr. R.f.Vegetationskunde*, 28: 633-677.
- Lange-Bertalot, H., Steindorf, A. (1996). Rote liste der limnischen Kieselalgen (Bacillariophyceae) Deutschlands. Schr. R.f.Vegetationskunde, 28: 633-677.
- Lange-Bertalot, H., Wojtal, A. Z. (2014). Diversity in species complexes of Placoneis clementis (Grunow) Cox and Paraplaconeis placentula (Ehrenberg) Kulikovskiy, Lange-Bertalot & Metzeltin. *Nova Hedwigia*. Beiheft 143: 403-420.
- Lecointe, C., Coste, M., Prygiel, J. (1993). "Omnidia": sofware for taxonomy, calculation of diatom indices and inventories management. H. van Dam (ed.), Twelfth International Diatom Symposium. *Hydrobiologia*. 269/270: 509-513.
- Legendre, P., Legendre, L. (1998). Numerical Ecology. 2nd English edition. Elsevier, Amsterdam.
- Levkov, Z., Ector, L. (2010). A comparative study of Reimeria species (Bacillariophyceae). Nova Hedwigia, 90 (3/4): 469-489.
- Levkov, Z., Mihalić, K. C., Ector, L. (2010). A taxonomical study of Rhoicosphenia Grunow (Bacillariophyceae) with a key for identification of selected taxa. Fottea, 10 (2): 145-200.
- Levkov, Z., Williams, D. M. (2012). Checklist of diatoms (Bacillariophyta) from Lake Ohrid and Lake Prespa (Macedonia), and their watersheds. *Phytotaxa*, 45: 1-76.
- Mašić, E., Žero, S., Barudanović, S., Memić, M. (2018). Effect of heavy metals on phytobenthos assemblages in mine pit lakes of Bosnia and Herzegovina. Uticaj teških metala na sastav fitobentosa u kopovskim jezerima Bosne i Hercegovine. *Biologica Nyssana*, 9 (2), December 2018. pp 103-118.
- Pavlov, A., Jovanovska, E., Wetzel, C. E., Ector, L., Levkov, Z. (2016). Freshwater Mastogloia (Bacillariophyceae) taxa from Macedonia, with a description of the epizoic M. sterijovskii sp. nov. *Diatom Research*, 31 (2): 85-112.
- Pavlov, A., Levkov, Z. (2013. Diversity and distribution of taxa in the genus Eunotia Ehrenberg (Bacillariophyta) in Macedonia. *Phytotaxa*, 86 (1): 1-117.
- Pavlov, A., Levkov, Z., Williams, D. M., Edlund, M.B. (2013). Observations on Hippodonta (Bacillariophyceae) in selected ancient lakes. *Phytotaxa*, 90 (1): 1-53.
- Protić, DŽ. (1926). Hidrobiološke i plankton-studije na jezerima Bosne i Hercegovine. III dio - Glečerska jezera. *Glasnik Zemaljskog muzeja*. Knjiga 1. 47-78.
- Redžić, S. (2007). Syntaxonomic diversity as an indicator of ecological diversity - case study Vranica Mts in the Central Bosnia. *Biologia*, 62(2), 173-184.
- Reichardt, E. (1999). Zur Revision der Gattung Gomphonema. Die Arten um G. affine/G. insigne, G. angustatum/micropus, G. acuminatum sowie gomphonemoide Diatomeen aus dem Oberoligozän in Böhmen. *Iconographia Diatomologica*, 8: 1-203.
- Rott E. (ed.). (1999). Teil, 2: Trophieindikation Sowie Geochemische Präferenz; Taxonomische und Toxikologische Anmerkungen. Indikationslisten für Aufwuchsalgen in österreichischen Fliessgewässern, Wien.
- Rott E., Hofmann G., Pall K., Pfister P., Pipp E. (1997). Teil, 1: Saprobielle Indikation. Indikationslisten für Aufwuchsalgen in österreichischen Fliessgewässern, Wien.

- Schmidt, R., Kamenik, C., Lange-Bertalot, H., Klee, R. (2004). Fragilaria and Staurosira (Bacillariophyceae) from sediment surface of 40 lakes in the Austrian Alps in relation to environment variables, and their potential for paleoclimatology. *Journal of Limnology*, 63 (2): 171-189.
- Sládeček, V. (1986). Diatoms as indicators of organic pollution. *Acta hydrochimica et hydrobiologica*, 14(5), 555-566.
- Spahić, M. (2001). Prirodna jezera Bosne i Hercegovine: Limnološka monografija. Harfgraf, Tuzla. pp 1-170.
- Spaulding, S.A., Lubinski, D.J. and Potapova, M. 2019 (Diatoms of the United States). https://diatoms.org/.
- Van Dam, H., Mertens, A., Sinkeldam, J. (1994). A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Neth. J. Aquat. Ecol*. 28: 117-133.
- Witkowski, A., Lange-Bertalot, H., Kociolek J. P., Ruppel, M., Wawrzyniak-Wydrowska, B., Bak, M., Brezezinska, A. (2004). Four new species of *Nitzschia* sect. *Tryblionella* (*Bacillariophyceae*) resmbling *N. parvula*. *Phycologia*, 43 (5): 579-595.
- Wojtal, A. (2003). Diatoms of the genus *Gomphonema* Ehr. (*Bacillariophyceae*) from karstic stream in the Krakowsko-Czestochowska upland. *Acta Societatis Botanicorum poloniae*, 72 (3): 213-220.
- Wojtal, A. Z. (2009). The diatoms of Kobylanka Stream near Krakow (Wyzyna KrakowskoCzestochowska upland, S Poland). *Polish Botanical Journal*, 54 (2): 129-330.
- Wojtal, A. Z., Ector, L., Van de Vijver, B., Morales, E. A., Blanco, S., Piatek, J., Smieja, A. (2011). The *Achnanthidium minutissimum* complex (Bacillariophyceae) in southern Poland. Algological Studies, 136/137: 211-238.
- Wojtal, A. Z., Kwandras, J. (2006). Diatoms of the Wyzyna Krakowsko-Czestochowska upland (S Poland) - Coscinodiscophyceae (Thalassiosirophycideae). *Polish Botanica*. Journal, 51 (2): 177-207.