Critical multi-stranded approach for determining the ecological values of diatoms in unique aquatic ecosystems of anthropogenic origin

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Abstract

Background. The ecological state of surface waters is typically assessed by a multi-aspect approach based on a determination of its chemical and physical parameters, by hydromorphology and the use of indicator organisms such as benthic diatoms. By assigning ecological indicator values, it is possible to create diatom indices which serve as the basic tool in assessing the ecological status of surface waters. These ecological indicator values are set according to classification systems, such as the Van Dam et al. 1994 system, which classifies species of diatoms according to seven different ecological factors. However, recent studies on the autecology of diatoms have shown the need to verify and establish new ecological indicator values. To this end, aquatic ecosystems are good environments to observe the range of tolerance of benthic diatoms to environmental conditions due to their unique physical and chemical parameters. The aim of the present study was to propose the establishment of new, or altered, ecological indicator values, according to Van Dam et al. classification, of species of diatoms characteristic of three post-mining aquatic ecosystems.

Methods. In total, 36 species were identified that were characteristic of three waterbodies: a salt aquatic complex (water outflow, a drainage ditch and a pond), a mined iron ore reservoir and a mined lignite reservoir. Their ecological indicator values were calculated using OMNIDIA software, and the environmental conditions prevailing in the studied ecosystems were determined. Of the 36 characteristic species, 16 lacking at least one assigned ecological indicator value were analysed further. The analysis identified three groups of selected characteristic species which showed a correlation, or lack of such, to the tested physical and chemical parameters.

Results. Based on this multistage study of the autecology of characteristic diatoms, comprising an analysis of environmental conditions, literature analysis and reference ecological indicator values of other species, it is proposed that 32 ecological indicator values be established or adjusted for 16 species, and that *Planothidium frequentissimum* be excluded from water quality assessments.

Introduction

Diatoms (Bacillariophyta) are one of the main biotic elements used in the biological assessment of the ecological state of surface waters (*Water Framework Directive, European Union 2000*). Due to the fact that many countries are obliged to continually engage in biomonitoring, there is a clear need to develop flawlessly functioning methods based on the standardised use of diatoms as bioindicators (*Kahlert et al. 2016; Poikane, Kelly & Cantonati 2016; Szczepocka & Żelazna-Wieczorek 2018*). Diatom indices and ecological systems based on the bioindication values of particular diatom species, derived from various environmental parameters, constitute a fundamental tool in the biological assessment of environments. Diatom indices have been commonly used to assess flowing and standing water for over 20 years (*Kelly et al. 2008; Harding & Taylor 2014; Szczepocka et al. 2014; Hutorowicz & Pasztaleniec 2014; Holms & Taylor 2015; Żelazna-Wieczorek & Nowicka-Krawczyk 2015; Kolada et al. 2016*).

59 Currently, many countries use the OMNIDIA program (*Lecointe et al. 1993*) as a biological
60 assessment tool. Its latest version (version 6.0.6) allows the calculation of 18 diatom indices, and
61 the determination of seven environmental parameters for eight ecological systems. However, the
62 specific ecological indicator values of many of the species given in the OMNIDIA database are
63 absent or have not been updated in response to recent research. To complete these missing
64 values, and to verify existing ones, further studies are needed of the ecological optima and
65 tolerance of diatom species in different types of aquatic ecosystems.
66 Due to their specific environmental conditions, post-mining reservoirs represent an extremely
67 valuable source of information for the study of ecological diatom tolerance ranges. Some studies
68 of these environments have been performed, but these have addressed diatom paleoecology and
69 their role as indicators of past climatic or environmental change (*de Haan et al. 1993; Rakowska*
70 *1996; Thomas & John 2006; Sienkiewicz & Gąsiorowski 2016*). Until now, the autecology of
71 diatoms in post-mining reservoirs has rarely been studied (*Van Landingham 1968; de Haan et al.*
72 *1993; Rakowska 1996; Ferreira da Silva et al. 2009; Luís et al. 2009; Luís et al. 2016;*
73 *Sienkiewicz & Gąsiorowski 2016*).
74 The present study examines the diatom assemblages present in three post-mining reservoirs of
75 various geological origins. Due to variations in their environmental parameters, these bodies of
76 water serve as specific and unique habitats for the development of these algae. The diatom
77 assemblages quickly adapt to the currently prevailing conditions, which is manifested in the
78 presence of taxa characteristic of these specific parameters. Considering their large share of the
79 assemblage, the index values of the assemblages constitute the most important component in the
80 calculation of diatom indices. These species are therefore of the greatest importance for surface
81 water biomonitoring.
82 The aim of the present study was to identify the species of diatoms characteristic of the three
83 studied types of post-mine reservoirs. Following this, taxa that did not have at least one
84 ecological indicator value specified in the OMNIDIA database, according to the environmental
85 parameters given by Van Dam et al. (*1994*), were identified. New ecological indicator values
86 were proposed based on the relationship between the occurrence of the individual species and
87 certain selected physical and chemical parameters, or existing ones were verified.
88 The Van Dam et al. (*1994*) ecological system is one of the main systems on which the
89 OMNIDIA programme is based. It describes the ecological indicator values of diatoms according
90 to pH, salinity, nitrogen uptake metabolism, oxygen requirement, saprobity, trophic state and
91 moisture aerophily. These values play a key role in calculating diatom indices, and hence need to
92 be kept up to date to enable accurate routine biomonitoring.

93 **Materials & Methods**

94 *Study area*

95 The study was performed on three waterbodies created through exploration for mineral deposits
96 or were formed by the closure of mines. All three are located in the Łódzkie and Wielkopolskie
97 voivodeships, Central Poland.

98   The first complex of waterbodies - Pełczyska (PE), is situated in the village of Pełczyska,
99   between Łódź and Łęczyca (Łódzkie voivodeship) (Fig. 1). As the local area is characterized by
100   the presence of salt deposits, numerous wells were sunk in the eighteenth century to obtain brine.
101   Currently, salt water flows out of one of them. This area has been studied by biologists and
102   hydrobiologists since the 1960s (*Olaczek 1963; Pliński 1966; Pliński 1969; Pliński 1971a;*
103   *Pliński 1971b; Pliński 1971c; Pliński 1973; Żelazna-Wieczorek 1996; Żelazna-Wieczorek 2002;*
104   *Żelazna-Wieczorek, Olszyński & Nowicka-Krawczyk 2015; Żelazna-Wieczorek & Olszyński*
105   *2016*). The waterbodies chosen for our research form the PE hydrological complex located in the
106   vicinity of farmland; it comprises the salt water outflow, a drainage ditch and a pond, which acts
107   as the receiver of the water.
108   The second complex of waterbodies - Łęczyca (LE), urban reservoirs located within the city of
109   Łęczyca (Łódzkie voivodeship) (Fig. 1). The reservoirs were created following the flooding the
110   open-cast iron ore mine in the 1990s. This area is rich in syderite deposits, which are
111   accompanied by other minerals. The complex consists of three connected reservoirs: two are
112   directly connected to each other (LEP1 and LEP2), and the third (LEP3) is connected to LEP2
113   via a water drainage ditch (*Olszyński & Żelazna-Wieczorek 2018*). All three are located in an
114   area with houses, garden plots and partly-wooded areas.
115   The third waterbody - Bogdałów reservoir (BO), created by the flooding of an opencast brown
116   coal mine. It is located in the village of Bogdałów (Wielkopolskie voivodeship) in an area rich in
117   lignite deposits (Fig. 1). Lignite from quaternary deposits was exploited since 1977 until 1991 to
118   a depth of 50 meters. Due to the specific construction of the open-pit area, being characterised by
119   the thickest layer of poorly permeable boulder clay in the region. This pit was later transformed
120   into a storage site for quarried rocks in Koźmin. Finally, in 1993/1994, the drainage and runoff
121   of surface waters were blocked to form a reservoir with a depth of about 12 meters surrounded
122   by forest (*Gabryś-Godlewska et al. 2004; Gadomska et al. 2007; Orlikowski & Szwed 2009;*
123   *Kasztelewicz 2011*).

124   *Samples*

125   Samples of benthic diatoms from sediments and water samples were collected quarterly (once in
126   any season) from each hydrological waterbodies. Analysis of all water samples (Ca^{2+}, Mg^{2+}, Na^+,
127   K^+, $Fe^{2+/3+}$, Mn^{3+}) involved flame absorption spectrophotometry SpectrAA 300 (Varian, Palo
128   Alto, CA, USA) (detection limit is 0.05 mg/L) and UV-vis spectrophotometry S.330 (Marcel,
129   Poland) in the case of NH_4^+ (d.l. is 0.001 mg/L) and PO_4^{3-} (d.l. is 0.01 mg/L). SO_4^{2-} was
130   determined by the gravimetric method (PN-C-04566-09), Cl^- by Mohr's method (PN-ISO 9297).
131   The chemical analyses were performed in the Laboratory of the Department of Geology at the
132   Faculty of Geographical Sciences, University of Łódź and the Laboratory of Computer and
133   Analytical Techniques at the Faculty of Biology and Environmental Protection, University of
134   Łódź. The water temperature, pH and electric conductivity were measured *in situ* (Elmetron CP-
135   401 and CC-401 devices). The following sampling points were established:

136   Pełczyska (51°58'34.47"N; 19°14'21.11"E) – outflow (P.ESB.) (4 samples, both water and
137   benthic), ditch (D.PEDB.) (1 water and 4 benthic samples) and pond (D.PEPB.) (4 samples, both
138   water and benthic); samples were collected quarterly from July 2013 to March 2014;
139   Łęczyca (52°3'5.30"N; 19°11'50.24"E) – reservoir 1 (D.LEP1.), reservoir 2 (D.LEP2.) and
140   reservoir 3 (D.LEP3.), samples were collected quarterly from March 2014 to December 2015 (6
141   water and 8 benthic samples from each reservoirs);
142   Bogdałów (52°2'51.29"N; 18°35'51.49"E) – reservoir (D.BOZB.), samples were collected
143   quarterly from March 2015 to December 2016 (8 samples, both water and benthic).
144   In total, 44 benthic samples were collected. The permanent slides were prepared according to
145   Żelazna-Wieczorek (*2011*). To obtain pure diatom frustule the material was chemically treated
146   using a H_2SO_4 and $H_2Cr_2O_7$. The cleaned diatom precipitate was mounted on permanent slides
147   using Naphrax® synthetic resin.
148   Qualitative and quantitative analysis of diatoms was performed using a Nikon Eclipse 50i light
149   microscope (LM) under 1000× magnification (plan oil-immersion objective 100×/1.25): the
150   diatoms were identified and counted for up to 500 valves in each permanent slide. Light
151   photomicrographs were taken with an OPTA-TECH digital camera.
152   When diatoms were difficult to identify using LM they were subjected to scanning electron
153   microscope (SEM) analysis using a Phenom Pro X (gold layer of 8 and 20 nm, at 10 kV, low
154   vacuum mode) at the Laboratory of Microscopy Imaging and Specialist Biological Techniques,
155   Faculty of Biology and Environmental Protection, University of Lodz.

156   *Data processing & statistical analysis*

157   The average percentage (AP) for a given species was determined based on the percentage
158   contribution (%) of the species in the samples tested for a given hydrological object (*Żelazna-*
159   *Wieczorek 2011*). Species whit AP greater than or equal to 5% for each hydrological object were
160   identified as dominant.
161   The incidence was determined according to the Tümpling & Friedrich (*1999*) coefficient
162   according to the range values: 100%–75% euconstant taxa (EC), 75%–50% constant taxa (CN),
163   50%–25% accessory taxa (AC) and 25%–1% accidental taxa (AD) (*Tümpling & Friedrich*
164   *1999*).
165   Multidimensional scaling analysis (nMDS) based on Bray-Curtis similarity coefficients was used
166   to identify natural groupings of samples. The results are given as a 3D diagram in which the
167   degree of similarity is represented as the distances between particular points (samples), with
168   greater distances indicating a lower degree of similarity. The reliability of the ordering of the
169   assemblage is represented by the stress value, which reflects how well the ordination summarizes
170   the observed distances among the samples. A 3-dimensional presentation, whose stress value is
171   lower, is likely to be more satisfactory than a 2-dimensional one (*Clarke & Gorley 2015*).
172   The Shade Plot analysis, based on the Bray-Curtis similarity coefficient, was used to identify the
173   diatom species that have the strongest influence on the similarities between the samples
174   demonstrated in the nMDS analysis. Shade Plot analysis compares two data matrices with each

175 other and then groups them on two levels, according to the similarity of the samples and the
176 factors affecting their similarity, i.e. diatom species. The results are represented graphically by
177 shading individual cells: the intensity of the shading indicates the degree of the influence of a
178 given factor (species) on the position of its sample within a given similarity cluster. The range of
179 the shading was determined on the basis of log(x+1) (x–number of valves).
180 The SIMPER analysis was used to determine the characteristic species distinguishing the studied
181 ecosystems. This method examines the participation of each variable in the overall similarity
182 between groups of samples, thus indicating the species with the greatest influence on the degree
183 of similarity, or dissimilarity, between particular samples and hydrological objects. This analysis
184 is also based on the Bray-Curtis similarity coefficient; however, unlike the nMDS method, in
185 which one trial is compared to all the other samples, the SIMPER analysis compares a single
186 sample to each subsequent sample (*Żelazna-Wieczorek 2011*). The results indicate the species
187 which most strongly differentiated a sampled site from the others, and to what extent. A species
188 was regarded as being characteristic of the studied ecosystem if it was characterized by a mean
189 dissimilarity greater than or equal to 2 according to the SIMPER analysis, and a higher mean
190 abundance greater in one ecosystem than the other.
191 In total, 19 physical and chemical parameters of water were measured in the studied ecosystems.
192 The results of the correlation analysis found 15 physical and chemical parameters indicating an
193 environmental conditions. The parameters were subjected to principal component analysis (PCA)
194 to determine which had the strongest effect on the selected species.
195 Using the information from the OMNIDIA database, the environmental conditions for each
196 sampling point were determined according to Van Dam et al. (*1994*) (Table S1). Following this,
197 the percentage share of diatom species included in each ecological indicator value class was
198 indicated. For species found to be characteristic of the studied ecosystems, classes of ecological
199 indicator values were assembled. Taxa which had at least one value of 0 (unknown) were
200 selected for further analysis.
201 The selected ecological indicator values according to Van Dam et al. (*1994*) were verified, or
202 new ones established, for the species found to be characteristic of the studied ecosystems
203 according to three premises: previous literature reports about ecological indicator values of those
204 species, chemical and physical conditions analysis, and the classification of the environmental
205 conditions according to Van Dam et al. (*1994*).
206 The analyses were performed using PRIMER 7.0.13 (nMDS, Shade Plot, SIMPER), OMNIDIA
207 6.0.6 and STATISTICA 13 (PCA), software.

208 **Results**

209 *Chemical analysis of water samples*

210 The mean values and range of all tested parameters are given in Table 1.
211 The PE hydrological complex was characterized by elevated values of electric conductivity,
212 reaching as high as 9230 μS cm^{-1}. The pH changed with the direction of water outflow: a slightly
213 acidic reaction was observed in the outflow and an alkaline one in the pond. Due to the

214   geological profile of the region, the water flowing out of the well contained a high concentration
215   of chloride ions, whose gradient decreased with the flow of water through the ditch to the pond.
216   In addition, higher concentrations of the cations Mg^{2+}, Ca^{2+}, Na^+ and K^+ were observed
217   compared to other ecosystems, as well as the anions HCO_3^-, PO_4^{3-} and SO_4^{2-}.
218   The K^+ concentration is acknowledged parameter coming from agricultural activity, in particular
219   animal husbandry, or municipal wastes (*Macioszczyk & Dobrzyński 2002*).
220   Each of the sampling points in the PE complex was characterized by different chemical
221   parameters, resulting in differences between the habitats. The highest electrolytic conductivity
222   was noted in the outflow, which was mainly influenced by the concentrations of Cl^-, Na^+ and
223   HCO_3^- ions. The maximum concentration of HCO_3^- ions was recorded in Pełczyska ouflow in
224   March 2014 (D.PESB.250314); in the other locations, it did not exceed 410 mg L^{-1}.
225   Low concentrations of K^+ ions were observed throughout the entire studied PE complex;
226   however, maximum values were recorded in the locations characterised by the highest HCO_3^- ion
227   content. The highest concentration of Ca^+ ions of all ecosystems was recorded in the outflow.
228   The ditch represented an intermediate section between the PE sampling points. However, as it is
229   susceptible to periodic drying, limited chemical data was collected from this habitat and hence it
230   was not possible to assess its chemical and physical nature.
231   The lowest electrolytic conductivity was found in the pond, which displayed lower
232   concentrations of Cl^-, Na^+ and, to a lesser degree, HCO_3^-. The pH of the water never dropped
233   below 8, except in one case in March 2014. In the pond, the concentration of K^+ remained
234   relatively unchanged, which could be related to the fact that the reservoir was also a receiver of
235   waters flowing from the surrounding arable fields. The pond was also characterized by the
236   lowest concentration of Ca^{2+} and Mg^{2+}. In the summer periods, a significant reduction in the
237   water table level and occasional drying of the reservoir were noted.
238   The urban reservoirs in Łęczyca (LE) were characterized by a slightly alkaline water reaction,
239   which was similar in all reservoirs during the course of the study. No elevated concentrations of
240    $Fe^{2+/3+}$ and Mn^{3+} ions were observed. The content of SO_4^{2-} anions was not higher than in other
241   waterbodies studied. The concentration of HCO_3^- ions was lower than that observed in BO and
242   PE. No significant differences in chemical and physical parameters were observed between the
243   individual sampling points constituting LE.
244   The Bogdałów (BO) reservoir was characterized by an alkaline reaction. It's K^+, Cl^- and NH^{4+}
245   ion content was the lowest of the studied ecosystems.

246   *Diatom samples*

247   A total of 381 diatom taxa were identified in 44 benthic samples: 139 in PE, 192 in LE and 188
248   in BO. The dominant species in PE were *Navicula veneta*, and *Nitzschia frustulum*, in LE
249   *Cyclostephanos dubius* and *Stephanodiscus hantzschii*, in BO *Achnanthidium minutissimum*,
250   *Pantocsekiella ocellata* and *Mastogloia smithii*. In the examined ecosystems, the most
251   commonly identified classes were accidental (PE-84; LE-111; BO-86), accessory (PE-25; LE-
252   35) and euconstant taxa (BO-39) (Fig. S1).

253   *nMDS analysis*

254   nMDS analysis (stress level = 0.07) identified the variation between samples for each studied
255   hydrological object (Fig. 2). The samples taken from BO constitute a separate cloud, with the
256   samples demonstrating high similarity with each other, whereas the samples of D.LEP1, D.LEP2
257   and D.LEP3 constitute a distinct group, with no clear differentiation into individual reservoirs. In
258   the case of PE, the pond group (D.PEPB) was found to be clearly distinct from the others.

259   *Shade Plot*

260   Shade Plot analysis identified 50 species which had the strongest influence on the degree of
261   similarity, or non-similarity, between the samples in the studied ecosystems. Of these taxa, the
262   11 that most strongly influenced the similarity between the samples in at least two ecosystems
263   were *Navicula veneta* (NVEN)*, N. cincta* (NCCA)*, N. gregaria* (NGRE)*, Nitzschia frustulum*
264   (NIFR)*, N. inconspicua* (NINC)*, N. palea* (NPAL)*, Planothidium frequentissimum* (PLFQ)*,
265   *Amphora pediculus* (APED)*, Cyclotella meneghiniana* (CMEN)*, Fragilaria radians* (FRAD) and
266   *Achnanthidium minutissimum* (ADMI) (Fig. 3).

267   *SIMPER analysis*

268   SIMPER analysis allowed 36 species characteristic of the tested hydrological objects to be
269   distinguished (Table 2). In addition, two species were found to be characteristic of two different
270   ecosystems: *Achnanthidium minutissimum* for LE and BO and *Nitzschia palea* for PE and LE.

271   *Ecological analysis based on Omnidia software*

272   The ecological analysis of diatom assemblages based on data obtained from the OMNIDIA
273   program database, indicated the following:
274   - pH requirements: while alkaliphilic species predominate in PE (63%), a large percentage in
275   D.PEPB are unknown (24%) or neutrophilic species (23%). The LE reservoirs were dominated
276   by alkalibiontic (45%) and alkaliphilic (24%) organisms. In D.LEP1, 25% of species were
277   unknown. BO was dominated by alkaliphilic (39%) and neutrophilic (29%) species, and 26 % of
278   species were unknown (Fig. S2);
279   - salinity: the PE complex was characterized by the occurrence of halophilic (43%),
280   oligohalobous (30%) and mesohalobous species (16%); the greatest proportion of the
281   mesohalobous species were found in D.PESB (28%). The LE reservoirs were dominated by
282   oligohalobous (44%) and halophilic species (42%). BO was dominated by oligohalobous (43%),
283   halophobe (24%) and unknown species (23%) (Fig. S2);
284   - nitrogen uptake: the most common species in the PE complex N-autotrophic tolerant (39%)
285   followed by unknown (25%). The largest percentage of unknown species (28%) was recorded in
286   D.PEPB and D.PEDB. In the LE reservoirs, the most common groups of species were N-
287   autotrophic (57%) and unknown (25%). In BO, 51% species were unknown, 24% were N-
288   autotrophic tolerant and 22% N-autotrophic sensitive (Fig. S2);

289   - oxygen requirements: in PE, the largest groups of species were low oxygen (30%), unknown
290   (27%) and moderate oxygen (24%). In LE, oxybiontic species were most common (43%)
291   followed by unknown (25%). In BO, unknown (46%) and polyoxybiontic species (42%)
292   predominated (Fig. S2);
293   - sensitivity to saprobity: in PE, the largest group of taxa were α-meso: polysabrobe (28%) and
294   unknown (23%). In D.PEPB, the most abundant was α-meso-polysabrobe (34%) followed by β-
295   mesosaprobe (31%) and unknown (27%). LE primarily included taxa from the α-mesosaprobe
296   group (47%) and unknown (23%). In BO, unknown (34%), β-mesosaprobe (31%) and
297   oligosaprobe taxa (28%) predominated (Fig. S2);
298   - trophic status: in PE, the largest group of diatoms were eutrophic (50%) and unknown taxa
299   (25%), LE had the highest percentage (61%) of eutrophic species but also unknown (15%) and
300   hypereutrophic (13%) were present. In BO, the most abundant species were unknown (42%),
301   indifferent (19%) and meso-eutrophic (16%) (Fig. S2);
302   - moisture aerophily: in PE, the largest group was aquatic to aerophilic (56%), representing 66%
303   of species in D.PESB, 61% in D.PEDB, and 42% in D.PEPB. The second largest group was
304   unknown (23%), constituting 32% of taxa in D.PEPB. In LE, 37% of the species were aquatic
305   (24% of taxa in D.LEP2), 54% were occasionally aerophilic and 22% were unknown. In BO, the
306   predominant groups of species were unknown (44%) and aquatic to aerophilic (33%) (Fig. S2).

307   *Characteristic species: OMNIDIA and PCA analysis*

308   The analysis of species characteristic of the tested ecosystems, determined according to Van
309   Dam et al. (*1994*), identified 16 taxa classified as 0 in at least one category (Table 3). The next
310   step determined the percentage contribution of each of these species classified as class 0 for the
311   ecological parameters defined by Van Dam et al. (*1994*) at each sampling point (Table S2).
312   The principal components analysis (PCA) was performed to find the relationships between the
313   abiotic parameters and the characteristic species (n =36) (Fig. 4). The Eigenvalues Plot method
314   given eigenvalues above 1%, showed that 12 factors account for 83,2% of the total variance. The
315   first two factors account for 31,3% of the total variance. Based on the PCA analysis for of the 16
316   characteristic taxa mentioned above and physical and chemical parameters, the following
317   relationships were demonstrated:
318   - Group A: *Chamaepinnularia krookiformis*, *C. plinskii*, *Nitzschia liebethruthii* and *Planothidium*
319   *delicatulum* demonstrate a negative correlation with pH and a positive correlation with a
320   decrease in the concentrations of HCO^{3-}, Ca^{2+} and $Fe^{2+/3+}$ (Fig. 4).
321   - Group B: *Craticula buderi*, *Planothidium frequentissimum* and *Navicula cincta* did not
322   demonstrate any relationship with any water parameters (Fig. 4).
323   - Group C: *Navicula moskalii*, *Cyclostephanos invisitatus*, *Stephanodiscus parvus*, *S. binatus*,
324   *Diatoma moniliformis*, *Nitzschia dissipata* var. *media*, *Mastogloia smithii*, *Pantocsekiella*
325   *pseudocomensis* and *Encyonopsis subminuta* demonstrated a negative correlation with a decrease
326   in electrolyte conductivity, as well as with the concentrations of K^+, Mg^{2+}, Na^+, SO_4^{2-}, Cl^-, PO_4^{3-}
327   and Mn^{3+} and water pigments (Fig. 4).

Discussion

329 Verification and establishing new ecological indicator values is a key step in standard
330 biomonitoring procedure (*Szczepocka & Żelazna-Wieczorek 2018*). To specify ecological
331 indicator values or establish new ones we have determined characteristic species. For these
332 species we performed analysis of environmental condition of the ecosystem where they were
333 noted, previous published data and co-occurring species.

Planothidium delicatulum (PTDE) (Figs 5. A-E)

335 *Planothidium delicatulum* is a euconstant taxon for PE and an accidental taxon for LE. Its mean
336 percentage share in PE was 2%, and constituted 5% in D.PESB.
337 *Planothidium delicatulum* does not currently have one ecological indicator value (oxygen
338 requirements) according to Van Dam et al. (*1994*).
339 This species was more abundant in environments such as D.PESB, which was also characterized
340 by the highest concentration of Cl^- (up to 2976 mg L^{-1}), elevated electrolytic conductivity, and
341 decreased K^+ concentration. The pH of the water in which this species was observed did not
342 exceed 7.
343 *Planothidium delicatulum* was mainly recorded in salty and brackish environments with neutral
344 or slightly alkaline conditions (*Campeau, Pienitz & Héquette 1999; Gell et al. 2005; Caballero*
345 *et al. 2013; Yamamoto, Chiba & Tuji 2017; Van de Vijver, Wetzel & Ector 2018*).
346 Based on our findings, we suggest changing the following ecological indicator values in the Van
347 Dam et al. (*1994*) classification for *P. delicatulum*:
348 - pH requirements: 3 (neutrophilic) (changing from 5 to 3);
349 - salinity: 5 (brackish-marine) (changing from 4 to 5).

Chamaepinnularia krookiformis (CHKF) (Figs 5. F-I) and *Chamaepinnularia plinskii*
(CHPL) (Figs 5. J-M)

352 In 2016 *Chamaepinnularia krookiformis* was divided into two separate taxa: *C. krookiformis* and
353 *C. plinskii* (*Żelazna-Wieczorek & Olszyński 2016*). Both species were very often recorded
354 together in the same ecosystem. However, the publications which identified *C. krookiformis*
355 often do not provide appropriate photographic documentation or photos of individual specimens
356 (*Witkowski 1994; Bąk, Witkowski, Lange-Bertalot 2006; Wojtal 2008; Peszek et al. 2015*).
357 Currently available documentation is insufficient to determine whether *C. krookiformis* and *C.*
358 *plinskii* are both present simultaneously in a given environment or whether just one of these
359 species exists.
360 *Chamaepinnularia krookiformis* is an accessory taxon for PE (a constant taxon for D.PEDB), *C.*
361 *plinskii* is a constant taxon for PE (a euconstant taxon for D.PEDB). The mean share of *C.*
362 *krookiformis* was 1.6% in all PE samples, 4% in D.PEDB; for *C. plinskii,* this amounted to 2.7%
363 in PE, 6% in D.PEDB.

364   Currently, *C. krookiformis* lacks three assigned ecological indicator values. For PE, it constitutes
365   6% of the unknown group in nitrogen *uptake*, 6% in oxygen requirements and 6% in trophic state
366   (respectively for D.PEDB: 16%, 16% and 15%). *C. plinskii* has no assigned ecological indicator
367   values and represents 26% of the unknown group for pH requirements, 19% for salinity, 12% for
368   nitrogen uptake, 10% for *oxygen requirements*, 14% for saprobity, 11% for trophic state and
369   10% for moisture (respectively for D.PEDB: 60%, 25%, 24%, 23%, 32%, 23% and 28%).
370   The conditions of the environments in which both species have been recorded indicate that they
371   are class 3 with regard to pH range (neutrophilic). Both species were the most abundant in
372   locations subjected to periodic drying and characterised by high concentrations of chloride ions
373   (up to 1006 mg L^{-1}) indicating a brackish environment (*Żelazna-Wieczorek, Olszyński &*
374   *Nowicka-Krawczyk 2015*).
375   On the basis of our findings and those of previous studies (*Krammer & Lange–Bertalot 1986;*
376   *Krammer 1992; Witkowski 1994; Bąk, Witkowski & Lange-Bertalot 2006; Wojtal 2009; Peszek*
377   *et al. 2015; Żelazna-Wieczorek & Olszyński 2016*), according to Van Dam et al (*1994*) ecological
378   indicator system we propose:
379   established ecological indicator values for *Chamaepinnularia plinskii*
380   - pH requirements: 3 (neutrophilic);
381   - salinity: 4 (mesohalobous);
382   - trophic state: 5 (eutrophic);
383   - moisture aerophily: 4 (aerophilic);
384   - saprobity: 4 (α-meso-polysabrobe);
385   for *C. krookiformis*
386   - trophic state: 5 (eutrophic);
387   and the following changes for *Chamaepinnularia krookiformis*
388   - salinity: from 3 to 4 (mesohalobous);
389   - moisture aerophily: from 3 to 4 (aerophilic);
390   - saprobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe). Due to the specific conditions
391   and locations of the studied objects, they were exposed to large fluctuations in the inflow of
392   organic matter, mainly from runoff from arable fields and pollution caused by animal grazing.
393   These impurities were manifested as elevated concentrations of K^{+} ions. Therefore, our results
394   suggest that classifying *C. krookiformis* as an oligosaprobe is inappropriate. Further tests are
395   needed to determine the optimum occurrence of these species in areas subjected to organic
396   matter loads.

397   ***Nitzschia liebethruthii* (NLBT) (Figs 5. N-Q)**

398   *Nitzschia liebethruthii* is a euconstant taxon for PE. Its means percentage share was 4% in the PE
399   samples, and 10% in the D.PEDB samples. It was most numerous in the sample from November
400   2013 (19%). This species has two specific ecological indicator values. The ecological indicator
401   value analysis for PE found *N. liebethruthii* to represent 17% of the unknown group in nitrogen

402 uptake, 16% in oxygen requirements, 18% in saprobity, 17% in trophic state and 21% in
403 moisture (respectively for D.PEDB: 37%, 37%, 43%, 36% and 49%).
404 *Nitzschia liebethruthii* occurred in environments subjected to periodic drying with a pH close to
405 7 and high concentration of chloride ions.
406 This species was noted in environments with increased salinity, electrolytic conductivity and
407 high pH value (*Rumrich, Lange-Bertalot & Rumrich 2000; Witkowski, Lange-Bertalot &*
408 *Metzeltin 2000; Lange-Bertalot et al. 2017; Földi et al. 2018*).
409 We propose established new ecological indicator values according to Van Dam et al. (*1994*)
410 assigned to *N.liebethruthii*:
411 - trophic state: 5 (eutrophic);
412 - moisture aerophily: 4 (aerophilic);
413 and following changes:
414 - pH requirements: from 5 to 3 (alkalibiontic to neutrophilic);
415 - saprobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe).

416 ***Craticula buderi* (CRBU) (Figs 5. R-U)**

417 *Craticula buderi* is a euconstant taxon for PE and an accidental taxon for LE. Its mean
418 percentage share was 4% in all samples for PE, and 12% for D.PEPB. This species has no
419 recorded ecological indicator values. The ecological indicator value analysis for the PE found *C.*
420 *buderi* to constitute 19% of the unknown group in pH requirements, 28% in salinity, 16% in
421 nitrogen uptake, 16% in oxygen requirements, 16% in saprobity, 19% in trophic state and 15% in
422 moisture (respectively for D.PEPB: 53%, 73%, 46%, 46%, 46%, 54% and 41%).
423 Although *Craticula buderi* was classified into group B (PCA), it was found to be most abundant
424 in environments with an elevated concentration of Cl^- ions, ranging from 685 to 1090 mg L^{-1}, (all
425 samples from D.PEPB and one sample from D .PEDB in which the concentration of chloride
426 ions was 1006 mg L^{-1}). However, relative abundance was lower in the D.PEPB sample, which
427 was characterised by a chloride ion content of over 1500 mg L^{-1}. Interestingly. the concentration
428 of K^+ ions exceeded 100 mg L^{-1} at Cl^- concentrations below 1500 mg L^{-1}; therefore, it is possible
429 that the decline of this species could be related to the concentration of K^+ ions alone. Our
430 observations indicate that the population of *C. buderi* from D.PEPB favours a concentration of
431 chloride ions from 500 to 1006 mg L^{-1} which coincides with a K^+ ions concentration from 50 to
432 70 mg L^{-1}.
433 *Craticula buderi* is widespread throughout the world and recognized as cosmopolitan (*Rumrich,*
434 *Lange-Bertalot & Rumrich 2000; Lange-Bertalot 2001; Bahls 2009; Soltanpour-Gargari,*
435 *Lodenius & Hinz 2011; Żelazna-Wieczorek 2011; Cichoń 2016*). This species was found to be
436 dominant in environments characterised by increased electrolytic conductivity and alkaline water
437 (*Holmes & Taylor 2015*). Holmes and Taylor (*2015*) place *C. buderi* in the Bad water quality
438 class. Their recorded values of diatom indices indicate that the environment was eutrophic.
439 We therefore propose the following classes of ecological indicator values according to Van Dam
440 et al. (*1994*) for *Craticula buderi*:

441   - pH requirements: 4 (alkaliphilic);
442   - trophic state: 5 (eutrophic);
443   - salinity: 4 (mesohalobous);
444   - moisture aerophily: 3 (aquatic to aerophilic);
445   and following change:
446   - sabrobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe).

447   ***Navicula cincta* (NCCA) (Figs 5. V-Z)**

448   *Navicula cincta* is a euconstant taxon for PE, a constant taxon for LE and an accessory taxon for
449   BO. The mean percentage share of this species for PE is 3%, of which 7% was found in D.PEPB
450   samples.
451   Currently this species has been assigned two ecological indicator values. The ecological
452   indicator values analysis for the PE found *N. cincta* to constitute 24% of the unknown group in
453   pH requirements, 13% in nitrogen uptake, 12% in oxygen requirements, 13% in saprobity, and
454   12% in moisture (respectively for D.PEPB: 28%, 25%, 25%, 26% and 23%).
455   An analysis of the physical and chemical data and the variability of occurrence did not show any
456   clear relationships between environmental parameters and the percentage share of *Navicula*
457   *cincta* in the tested samples. This lack of dependence is also confirmed by the PCA analysis.
458   *Navicula cincta* has been recorded in various types of ecosystems, although mainly in eutrophic
459   ones with high conductivity. It also tolerates elevated levels of organic matter. This species was
460   also observed in habitats subjected to periodic drying (*Lange-Bertalot & Genkal 1999; Rumrich,*
461   *Lange-Bertalot & Rumrich 2000; Witkowski, Lange-Bertalot & Metzeltin 2000; Lange-Bertalot*
462   *2001; Żelazna-Wieczorek 2011; Wojtal 2013; Lange-Bertalot, Hofmann & Werum 2017*).
463   However, several new species from the group *N. cincta* s.l. have been described, and it can be
464   assumed that each of these individual species in this group may be associated with narrower
465   optimal ecological conditions (*Cantonati et al. 2016*).
466   Based on our present findings, and those of previous studies, in our opinion that it is not
467   appropriate to classify *Navicula cincta* as an oligohalobous species with regard to *salinity*: it has
468   been recorded in fresh (*Żelazna-Wieczorek 2011; Wojtal 2013*), brackish (*Żelazna-Wieczorek,*
469   *Olszyński & Nowicka-Krawczyk 2015; Żurek et al. 2018*) and salt waters (*Witkowski, Lange-*
470   *Bertalot & Metzeltin 2000*). We propose the following ecological indicator value according to
471   Van Dam et al. (*1994*) for *Navicula cincta* s.l.:
472   - moisture aerophily: 3 (aquatic to aerophilic).
473   Shade Plot analysis found that the presence of *N. cincta* s.l. can falsely indicate high similarity
474   between samples from different environments, thus distorting the results of any environmental
475   analysis. Therefore, with regard to the unclear taxonomic status of *Navicula cincta* s.l. and the
476   current lack of knowledge regarding its activities, we recommend this taxon be excluded from
477   the biological assessment of surface water quality.

478   ***Planothidium frequentissimum* (PLFQ) (Figs 5. AA-FF)**

479   *Planothidium frequentissimum* is a euconstant taxon for PE and LE. The mean percentage of this
480   species for PE is 2.7%, of which 5% was found in D.PEPB samples. It was most numerous in the
481   D.PEDB.301113 sample (19%). The species has currently six established ecological indicator
482   values. The ecological indicator values analysis for PE found *P. frequentissimum* to constitute
483   12% of the unknown group in moisture aerophily (for D.PEPB 14% and D.PESB: 20%).
484   No relationship was observed between percentage share of *Planothidium frequentissimum* and
485   the changes in chemical and physical parameters in the tested samples. This lack of relationship
486   was confirmed by PCA analysis.
487   *Planothidium frequentissimum* is an eurytopic species that occurs globally in a variety of habitat
488   types, from natural springs to rivers in urban areas with high levels of pollution. Its value as an
489   indicator is low, as confirmed by the Shade Plot analysis, which found it to significantly affect
490   the degree of similarity observed between samples from different environments (*Siver, et al.*
491   *2005; Levkov et al. 2007; Żelazna-Wieczorek 2011; Kulikovskiy, Lange-Bertalot & Kuznestova*
492   *2015; Szczepocka, Nowicka-Krawczyk & Kruk 2018*).
493   Recently *P. frequentissimum* was divided into several different species. In studied samples we
494   observe several species which belong to *P. frequentissimum* s. l. (Fig. 5. AA-FF) (*Wetzel et al.*
495   *2019*). A light microscope (LM) is still used to identify species in ecological research and
496   biological assessment of aquatic ecosystems. Due to the likeness of the basic morphological
497   features of the newly described species observed in LM, especially in the case of *P.*
498   *frequentissimum* s.s. and *P. straubianum*, distinguishing them will be difficult or limited, which
499   may lead to errors in the assessment. We therefore recommend that *P. frequentissimum* s. l. be
500   excluded from the biological assessment of surface water quality.

501   **Cyclostephanos invisitatus (CINV) (Figs 5. GG-JJ)**

502   *Cyclostephanos invisitatus* is a euconstant taxon for LE. Its percentage share for LE was 4.8%.
503   *Cyclostephanos invisitatus* currently has two ecological indicator values assigned. The ecological
504   indicator values analysis for LE found it to constitute 24% of the unknown group in pH
505   requirements, 17% in nitrogen uptake, 18% in oxygen requirements, 19% in saprobity, and 20%
506   in moisture.
507   *Cyclostephanos invisitatus* occurs in diverse environments, however, it is most frequently
508   reported in aquatic ecosystems subjected to high human impact, alkaline and high conductivity
509   (*Reavie & Smol 1998; Yang et al. 2005; Wojtal & Kwandrans, 2006; Kiss et al. 2012; Houk,*
510   *Klee & Tanaka 2014; Reavie & Kireta 2015; Olszyński & Żelazna-Wieczorek 2018*).
511   We therefore propose that the following classes of ecological indicator values according to Van
512   Dam et al. (*1994*) be established for *Cyclostephanos invisitatus*:
513   - pH requirements: 4 (alkaliphilic);
514   - moisture aerophily: 1 (aquatic).

515   **Navicula moskalii (NMOK) (Figs 5. KK-NN)**

516 *Navicula moskalii* is an accessory taxon for LE. Its mean percentage share for LE was 1.5%. Its
517 incidence was greatest in sample D.LEP1.250315 (26%).
518 *Navicula moskalii* has no assigned ecological indicator values. The ecological indicator value
519 analysis for LE found it to constitute 5% of the unknown group in pH requirements, 7% in
520 salinity, 5% in nitrogen uptake, 5% in oxygen requirements, 5% in saprobity, 6% in trophic state
521 and 5% in moisture. The greatest occurrence of *N. moskalii* was observed in samples with the
522 highest concentrations of Ca^{2+} (143.6 mg L^{-1}), HCO^{3-} (338.6 mg L^{-1}), SO_4^{2-} (146.9 mg L^{-1}) and
523 with high Mg^{2+} content.
524 *Navicula moskalii* was observed in a number of ecosystems (*Metzeltin & Witkowski 1996;*
525 *Lange-Bertalot 2001; Żelazna-Wieczorek 2011; Noga et al. 2016; Lange-Bertalot et al. 2017*),
526 particularly in eutrophic waters with an elevated level of Ca^{2+} and HCO^{3-} ions. Żelazna-
527 Wieczorek (*2011*) report a significant number of *N. moskalii* in springs with high levels of
528 eutrophication, however with Ca^{2+}, SO_4^{2-}, HCO^{3-} and Mg^{2+} concentrations lower than those in
529 the LE samples.
530 We therefore propose that the following classes of ecological indicator values according to Van
531 Dam et al. (*1994*) be established for *Navicula moskalii*:
532 - pH requirements: 4 (alkaliphilic);
533 - salinity: 2 (oligohalobous);
534 - trophic state: 7 (indifferent).

535 **Stephanodiscus binatus (SBNT) (Figs 5. OO-RR)**

536 *Stephanodiscus binatus* is a euconstant taxon for LE. Its percentage share for LE was 4.3%.
537 *Stephanodiscus binatus* has no recorded ecological indicator values. The ecological indicator
538 value analysis for LE found it to constitute 25% of the unknown group in pH requirements, 47%
539 in salinity, 18% in nitrogen uptake, 18% in oxygen requirements, 20% in saprobity, 29% in
540 trophic state and 21% in moisture.
541 The largest percentage share of *Stephanodiscus binatus* was recorded in the spring months and
542 the lowest in autumn. Its abundance was found to be elevated in December 2014 and 2015; the
543 same samples demonstrated the highest concentrations of Ca^{2+}, Mg^{2+} and the highest pH (above
544 8).
545 *Stephanodiscus binatus* has been recorded in various water ecosystems ranging from
546 oligotrophic to eutrophic; however, all are characterised by elevated pH value (*Stoermer &*
547 *Håkansson 1984; Håkansson & Kling 1990; Houk, Klee & Tanaka 2014; Olszyński & Żelazna-*
548 *Wieczorek 2018*).
549 We therefore propose that the following classes of ecological indicator values according to Van
550 Dam et al. (*1994*) be established for *Stephanodiscus binatus*:
551 - pH requirements: 4 (alkaliphilic);
552 - salinity: 2 (oligohalobous);

553 **Stephanodiscus parvus (SPAV) (Figs 5. SS-VV)**

554   *Stephanodiscus parvus* is a euconstant taxon for LE and an accidental taxon for PE. Its
555   percentage share for LE was 2.4%. It was most abundant in the D.LEP3.260714 sample (22%).
556   This species has three assigned ecological indicator values. The ecological indicator values
557   analysis for LE found the taxon to constitute 9% of the unknown group in nitrogen uptake, 9% in
558   oxygen requirements, 10% in saprobity, and 10% in moisture.
559   *Stephanodiscus parvus* is noted mainly in eutrophic hypereutrophic ecosystems with elevated
560   electrolytic conductivity. It is also a good indicator of waters with a strong anthropogenic impact
561   (*Reavie & Smol 1998; Reavie & Kireta, 2015; Olszyński & Żelazna-Wieczorek 2018; Reavie &*
562   *Cai 2019*).
563   Based on our findings and literature data, we propose the following change in ecological
564   indicator values according to Van Dam et al. (*1994*) for *Stephanodiscus parvus*:
565   - pH requirements: from 5 to 4 (alkalibiontic to alkaliphilic).

566   **_Diatoma moniliformis_ (DMOF) (Figs 5. WW-AAA)**

567   *Diatoma moniliformis* is a euconstant taxon for BO. Its mean percentage share for BO was 3.9%.
568   It currently has no assigned ecological indicator values. According to the ecological indicator
569   values analysis for BO, this taxon constituted 10% of the unknown group in pH requirements,
570   11% in salinity, 8% in nitrogen uptake, 8% in oxygen requirements, 9% in saprobity, 9% in
571   trophic state and 8% in moisture.
572   *Diatoma moniliformis* was found in 87.5 % of samples from BO. Interestingly, it constituted
573   28% of the share in one sample from December 2016 (D.BOZB.091216); however, its share was
574   below 2% in the previous season, and was not higher than 1-2% in the other samples from
575   December 2016. The chemical and physical characteristics of D.BOZB.091216 did not differ
576   significantly from those of the other samples.
577   This species is also found in fresh and salt water, as well as the Baltic and arctic areas with high
578   conductivity (*Potapova & Snoeijs 1997; Rumrich, Lange-Bertalot & Rumrich 2000; Levkov et*
579   *al. 2007; Pniewski & Sylwestrzak 2018*).
580   One of the factors that influences the abundance of *D. moniliformis* is the water temperature.
581   Studies indicate that temperatures above 10-15°C (*Potapova & Snoeijs 1997; Pniewski &*
582   *Sylwestrzak 2018*) are associated with population growth. However, populations have been
583   observed in freshwater streams and lakes in arctic areas, in which the temperature of the water is
584   below 10°C (*Antoniades Douglas & Smol 2005*). Population growth was also observed at 4.7°C
585   in sample D.BOZB.091216; therefore, low temperature may have an influence on the abundance
586   of this species.

587   **_Encyonopsis subminuta_ (ESUM) (Figs 5. BBB-GGG)**

588   *Encyonopsis subminuta* is a euconstant taxon in BO, where its mean percentage share was 4.1%.
589   Presently, *E. subminuta* has been assigned five ecological indicator values. Ecological indicator
590   values analysis for BO found it to constitute 7% of the unknown group in nitrogen uptake and
591   8% in moisture.

592 *Encyonopsis subminuta* was found to be most abundant in sample D.BOZB.041115. The sample
593 was also characterised by an elevated concentration of $Fe^{2+/3+}$ ions and the lowest pH value. In
594 subsequent samples, when the concentration of Fe ions dropped, the abundance of *E. subminuta*
595 also decreased.
596 *Encyonopsis subminuta* is regarded as a cosmopolitan taxon, occurring in the temperate and
597 boreal zone. It is most abundant in oligo- to mesotrophic waters with electrolytic conductivity
598 between 190-250 μS cm^{-1} (*Krammer 1997; Noga et al. 2014; Novais et al. 2014; Feret, Bouchez*
599 *& Rimet 2017*).
600 *Encyonopsis subminuta* may be sensitive to the concentration of Fe ions; however, the increase
601 of these ions is associated with a drop in pH. Our research confirms that the optimal pH for
602 population size is close to 7.

603 **_Mastogloia smithii_ (MSMI) (Figs 5. HHH-MMM)**

604 *Mastogloia smithii* is a euconstant taxon for BO. Its mean percentage share for BO was 6.3%. it
605 was found in greatest numbers in D.BOZB.300615 (22%) and D.BOZB.261016 (15%).
606 *Mastogloia smithii* has been assigned four ecological indicator values. The ecological indicator
607 values analysis for the BO found this species to constitute 13% of the unknown group in nitrogen
608 uptake, 14% in oxygen requirements and 15% in trophic state.
609 The environment in BO regarding salinity was classified according to Vam Dam et al. (*1994*) as
610 oligohalobus (43% species); however, 7% of the mesohalobous species were represented by one
611 species: *Mastogloia smithii*.
612 This species is recorded in fresh, brackish and salt water (*Witkowski, Lange-Bertalot & Metzeltin*
613 *2000; Busse & Snoeijs 2003; Weckström & Juggins 2005; Martinzes-Goss & Evangelista 2011;*
614 *Lange-Bertalot et al. 2017*). Its presence in environments with varying degrees of salinity may
615 suggest that this does not have an significant influence on population size.
616 Based on our present findings and literature data, we propose the following change in the
617 ecological indicator values according to Van Dam et al. (*1994*) for *Mastogloia smithii*:
618 - salinity: from 4 to 3 (mesohalobous to halophilic).

619 **_Nitzschia dissipata_ var. _media_ (NDME) (Figs 5. NNN-QQQ)**

620 *Nitzschia dissipata* var. *media* is a euconstant taxon for BO. Its mean percentage share for BO
621 was 3.8%, and the highest proportion (20%) was found in D.BOZB.041115. *N. dissipata* var.
622 *media* has been assigned two ecological indicator values. Ecological indicator values analysis for
623 BO found it to constitute 9% of the unknown group in nitrogen uptake, 10% in oxygen
624 requirements, 11% in saprobity, 11% in trophic state and 9% in moisture.
625 The increase in occurrence of *N. dissipata* var. *media* is associated with an increase in the level
626 of $Fe^{2+/3+}$, similar to *Encyonopsis subminuta*. In addition, it was found in the ecosystem, i.e. BO,
627 with the lowest concentrations of ions indicative of the presence of organic pollutants in the
628 environment, such as K^+ and NH_4^+.

629 Although *Nitzschia dissipata* var. *media* is found sporadically, it is commonly found in oligo- to
630 mesotrophic waters with a pH between 7 and 8 (*Van der Vijver, Frenot & Beyens 2002;*
631 *Antoniades, Douglas & Smol 2005; Żelazna-Wieczorek 2011; Lange-Bertalot, Hofmann &*
632 *Werum 2017*).
633 Based on our findings and literature data, we propose the following ecological indicator values
634 according to Van Dam et al. (*1994*) for *Nitzschia dissipata* var. *media*:
635 - saprobity: 2 (β-mesosaprobe)

636 ***Pantocsekiella pseudocomensis* (PPCS) (Figs 5. RRR-XXX)**

637 *Pantocsekiella pseudocomensis* is a euconstant taxon for BO. Its mean percentage share for BO
638 was 4.4%. It was most abundant in D.BOZB.250315 (9%) and in D.BOZB.220616 (10%). *P.*
639 *pseudocomensis* has not been assigned any ecological indicator values according to Van Dam et
640 al. (*1994*). The ecological indicator values analysis for BO found it to represent 22% of the
641 unknown group in pH requirements, 25% in salinity, 10% in nitrogen uptake, 11% in oxygen
642 requirements, 18% in saprobity, 12% in trophic state and 13% in moisture.
643 The greatest amount of *P. pseudocomensis* was found in samples characterised by the highest
644 levels of ammonium ions. Its percentage share was lowest in samples with the lowest water
645 temperature, apart from D.BOZB.250315.
646 Currently, *Pantocsekiella pseudocomensis* is assigned to the *P. comensis* complex, with *P.*
647 *comensis* and *P. costei*. In our opinion that assigning ecological indicator values for particular
648 species of the *P. comensis* complex is unjustified at the current state of knowledge, and that all
649 species within the complex should be assigned the same provisional ecological indicator values
650 until their individual properties are better understood (*Houk, Klee & Tanaka 2010; Kistenich et*
651 *al. 2014; Duleba et al. 2015*).

Conclusions

653 The water ecosystems created in the post-mining areas create a complex of conditions that are
654 not found in other natural ecosystems, and the benthic diatom species present in such
655 environments are very often present in higher numbers than in other habitats. The specific hydro-
656 geological conditions prevailing in the post-production reservoirs provide a unique opportunity
657 to observe interspecies differences and intra-species variability, allowing for the verification or
658 isolation of new taxa and a greater insight into their autecology (*Żelazna-Wieczorek & Olszyński*
659 *2016; Olszyński & Żelazna-Wieczorek 2018*).
660 The identification of species characteristic of the studied ecosystems may foster further growth
661 of ecological research and increase the reliability of surface water quality assessment, as such
662 knowledge is needed to verify their ecological indicator values, and hence calculate diatoms
663 indices with greater accuracy.
664 Ecological indicator values as set out by Van Dam et al. (*1994*) are utilized in many ecological
665 works describing the ecological conditions of the studied ecosystems. These ecological indicator
666 values form the basis for calculating diatoms indices describing the ecological state of surface

667 waters. It is therefore necessary to constantly update and establish new ecological indicator
668 values for particular diatom species.
669 Many authors who describe new species, or encounter existing species in new ecosystems,
670 regularly propose updates for individual ecological indicator values. However, these findings,
671 may not be introduced and updated in the OMNIDIA program for a number of years. The
672 OMNIDIA system is used by state institutions in many countries around the world to assess
673 surface water quality (*Campeau, Pienitz & Héquette 1999; Rumrich, Lange-Bertalot & Rumrich*
674 *2000; Witkowski, Lange-Bertalot & Metzeltin 2000; Gell et al. 2005; Potapova & Ponader*
675 *2008; Wojtal & Sobczyk 2012; Caballero et al. 2013; Żelazna-Wieczorek & Olszyński 2015;*
676 *Yamamoto, Chiba & Tuji 2017; Lange-Bertalot, Hofmann & Werum 2017; Földi et al. 2018;*
677 *Van de Vijver, Wetzel & Ector 2018*). Clearly, if these assessments are based on incomplete or
678 outdated data, assessments of aquatic environments may be fraught with error.
679 The present study used three principles to identify proposed changes in the classification of
680 ecological indicator values for characteristic species according to the Van Dam et al. (*1994*)
681 system, or to establish new values which were previously absent: the analysis of environmental
682 conditions prevailing in the studied ecosystems, the analysis of relevant literature data, and
683 references to the ecological indicator values of other species (Table 4). This mode of research
684 can serve as a model for updating databases used to assess surface water quality.

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Figure 1

Location of sampling points in the Łódzkie and Wielkopolskie voivodships. Poland.

A. Pełczyska (PE). B. Łęczyca (LE). C. Bogdałów (BO).

Figure 2

nMDS 3D analysis. The diagram shows three distinct clouds of samples which coincide with the three hydrological objects.

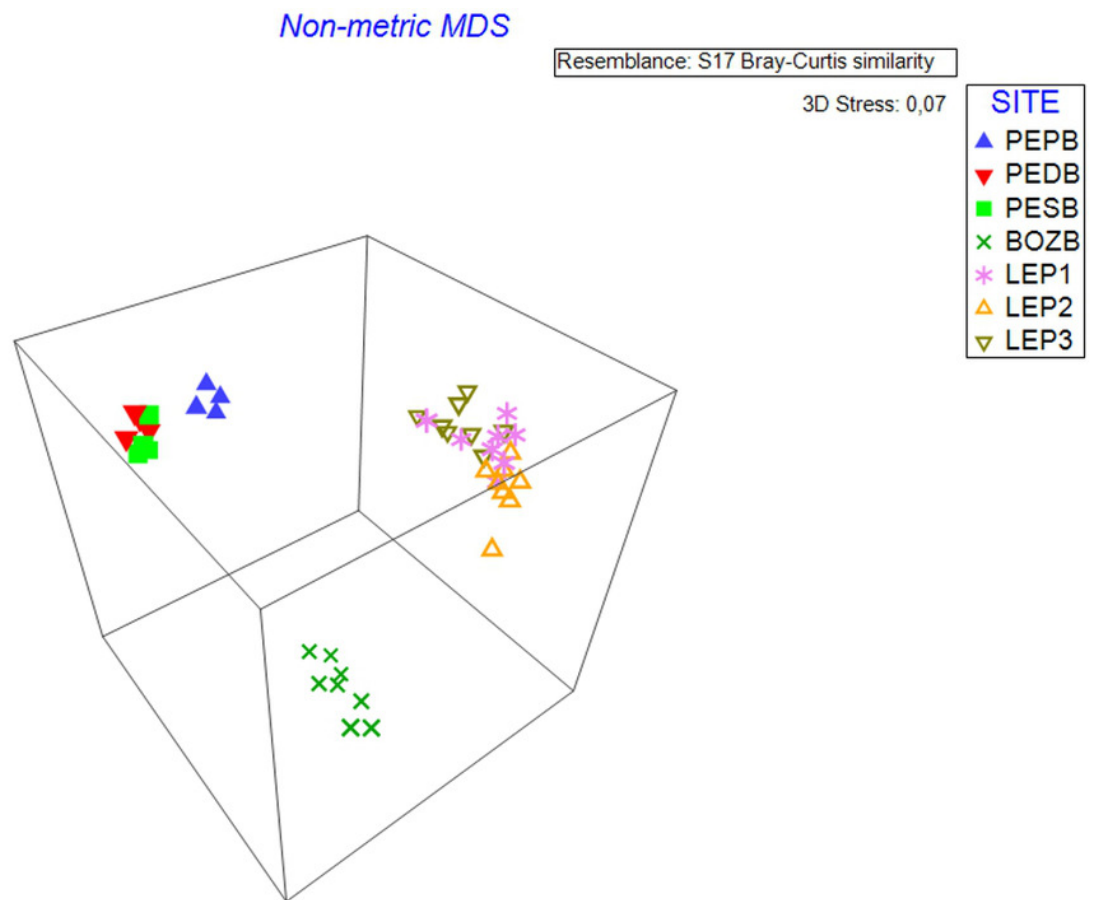



Figure 3

Shade Plot analysis. The diagram shows the strength of the factor (taxon) affecting the similarity between the samples.

Upper dendrogram - samples divided according to hydrological object. Left dendrogram - 50 taxa of diatoms which have the strongest influence on the similarity between the samples.

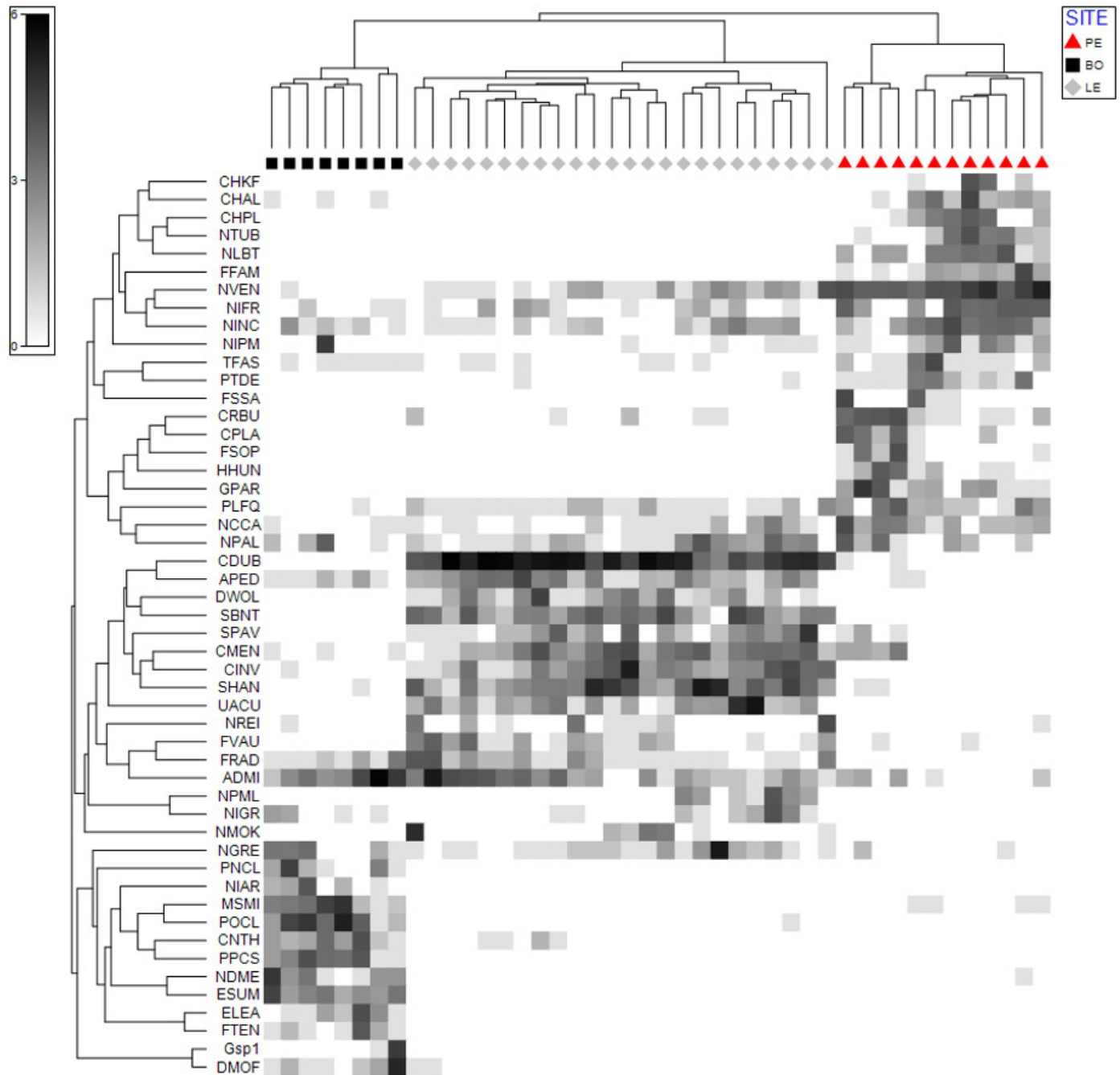



Figure 4

Principal Component Analysis.

The diagram presents three groups of species A, B and C, whose occurrence can be correlated with selected physical and chemical factors.

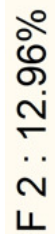
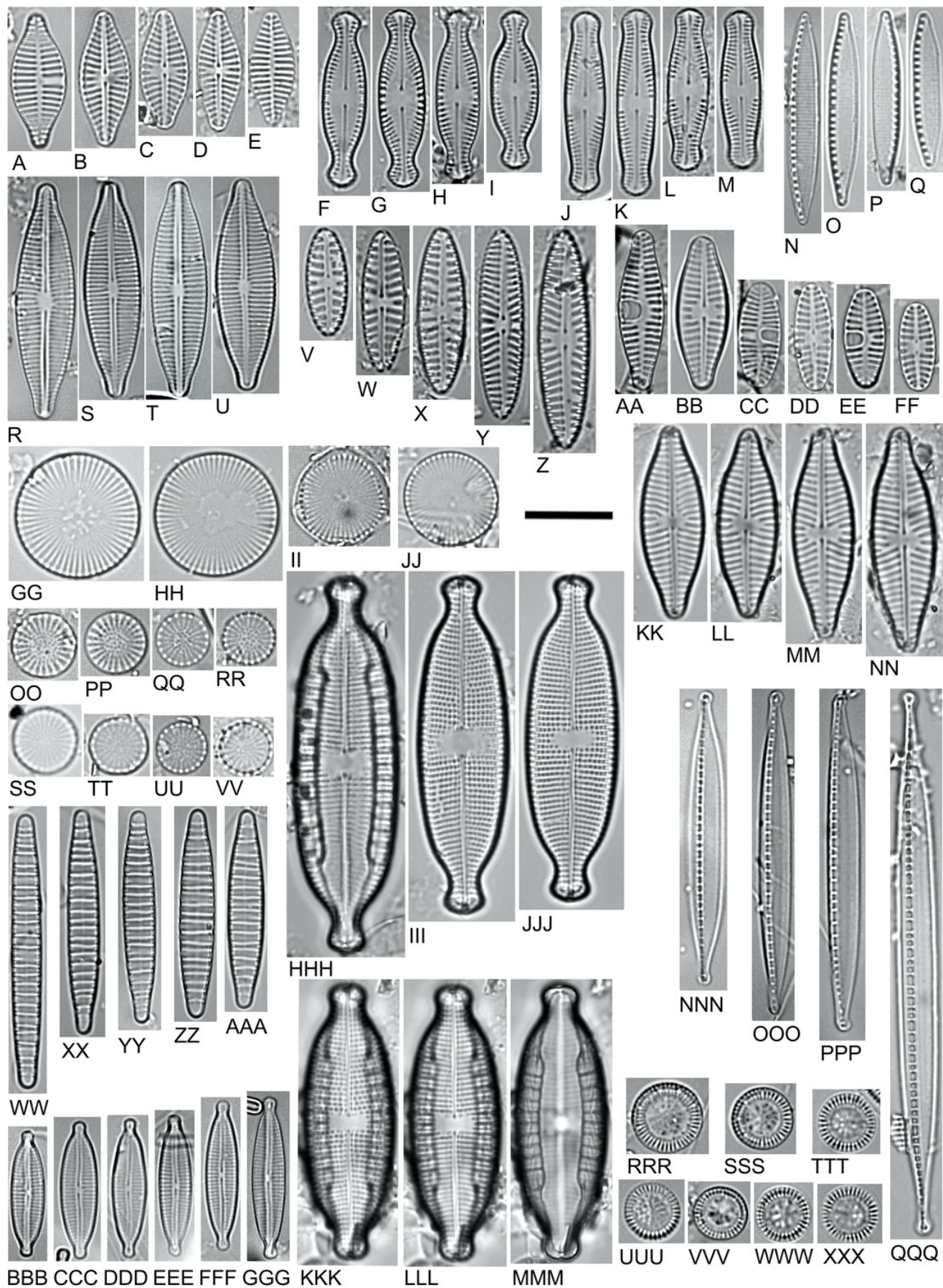



Figure 5

LM microphotographs of characteristic diatom species.

A-E. *Planothidium delicatulum*. F-I. *Chamaepinnularia krookiformis*. J-M. *Chamaepinnularia plinskii*. N-Q. *Nitzschia liebethruthii.* R-U. *Craticula buderi*. V-Z. *Navicula cincta*. AA-FF. *Planothidium frequentissimum*. GG-JJ. *Cyclostephanos invisitatus*. KK-NN. *Navicula moskalii*. OO-RR. *Stephanodiscus binatus*. SS-VV. *S. parvus*. WW-AAA. *Diatoma moniliformis*. BBB-GGG. *Encyonopsis subminuta*. HHH-MMM. *Mastogloia smithii* (KKK-MMM. Same specimen, different focal plane). NNN-QQQ. *Nitzschia dissipata* var. *media*. RRR-XXX. *Pantocsekiella pseudocomensis.* Scale bar = 10μm.

Physical and chemical parameters in the examined sampling sites. The minimum, maximum and mean values.

| | Pełczyska (PE) | | | | | | | | | Łęczyca (LE) | | | | | | | | | Bogdałów (BO) | | |
| | outflow (D.PESB) | | | ditch (D.PEDB) | | | pond (D.PEPB) | | | L1 (D.LEP1) | | | L2 (D.LEP2) | | | L3 (D.LEP3) | | | reservoir (D.BOZB) | | |
	min	max	avar.	min	max	avar.	min	max	avar.	min	max	avar.	min	max	avar.	min	max	avar.	min	max	avar.
pH	6.6	7.7	7.0	6.4	6.4	6.4	7.8	9.6	8.7	7.9	8.6	8.2	7.5	8.6	8.2	7.5	8.8	8.3	7.9	8.3	8.1
Conductivity [μS cm^{-1}]	4450	9230	6699	5170	5170	5170	2645	5150	3646	657	865	743	558	836	728	472	778	680	505	734	623
T [ºc]	6.8	13.9	9.1	7.8	7.8	7.8	1.1	17.3	7.1	1.6	20.6	11.1	0.6	21.1	11.1	2.2	15.2	9.2	4.7	22.7	11.4
HCO^{3-} [mg L^{-1}]	345	744	475	610	610	610	284	451	352	211	339	260	168	275	210	183	290	241	174	369	270
CO$_2$[HCO3-] [mg L^{-1}]	124	268	171	220	220	220	102	163	126	76	122	94	61	99	75	66	105	87	63	133	96
Cl$^-$ [mg L^{-1}]	1585	2976	2426	1006	1006	1006	685	1524	1053	57	93	78	60	93	76	67	106	78	41	52	45
N$_{NH4}$ [mg L^{-1}]	0.02	1.63	0.45	0.15	0.15	0.15	0.00	0.36	0.11	0.02	0.71	0.22	0.03	0.53	0.26	0.00	1.16	0.32	0.00	0.07	0.02
NH$_4^+$ [mg L^{-1}]	0.03	2.09	0.71	0.19	0.19	0.19	0.00	0.46	0.14	0.03	0.55	0.22	0.03	0.54	0.28	0.00	0.90	0.31	0.00	0.09	0.03
PO$_4^{3-}$ [mg L^{-1}]	0.60	12.46	3.73	8.57	8.57	8.57	0.85	9.10	3.99	0.22	0.53	0.36	0.05	0.57	0.41	0.09	0.57	0.34	0.27	0.45	0.34
P$_{PO4}$ [mg L^{-1}]	0.20	4.11	1.23	2.83	2.83	2.83	0.28	3.00	1.32	0.07	0.17	0.12	0.02	0.19	0.13	0.03	0.19	0.11	0.09	0.15	0.12
SO$_4^{2-}$ [mg L^{-1}]	176	198	188	165	165	165	151	197	176	71	147	107	67	122	101	63	91	75	110	147	123
S$_{SO4}$ [mg L^{-1}]	58.8	66.2	62.9	55.1	55.1	55.1	50.3	65.7	58.7	23.5	49.0	35.8	22.3	40.8	33.8	21.0	30.3	25.1	37.9	49.2	41.5
COLOR [mgPt dm^{-3}]	25	160	81	140	140	140	50	120	78	10	60	27	9	60	27	12	60	31	4	10	6
Mn^{3+} [mg L^{-1}]	0.17	0.51	0.36	0.14	0.14	0.14	0.03	0.25	0.13	0.00	0.05	0.02	0.01	0.03	0.02	0.01	0.07	0.03	0.00	0.01	0.00
Fe$^{2+/3+}$ [mg L^{-1}]	0.25	0.41	0.32	0.25	0.25	0.25	0.05	0.24	0.11	0.03	0.27	0.09	0.00	0.13	0.04	0.01	0.07	0.03	0.01	0.09	0.02
Mg^{2+} [mg L^{-1}]	39.5	48.7	45.0	35.9	35.9	35.9	25.6	37.0	32.1	11.6	19.1	15.2	10.3	18.3	15.1	9.5	15.3	12.5	9.6	15.7	11.7
Ca^{2+} [mg L^{-1}]	171.7	216.2	195.2	165.0	165.0	165.0	75.8	139.8	121.1	58.0	143.6	89.0	59.5	117.6	79.1	52.6	86.4	65.5	77.7	117.6	86.3
Na$^+$ [mg L^{-1}]	500.7	1537.4	1227.1	453.3	453.3	453.3	277.3	681.8	455.9	15.6	40.4	30.9	20.3	42.4	28.3	22.0	42.4	33.0	24.0	43.1	32.6
K$^+$ [mg L^{-1}]	8.5	124.8	42.4	109.8	109.8	109.8	58.6	68.8	63.5	4.5	9.5	7.2	5.1	9.3	7.3	5.6	10.7	8.2	0.1	3.6	1.5

Characteristic species according to SIMPER analysis for studied waterbodies.

EC – euconstant taxa, CN – constant taxa, CN – accessory taxa (Tümpling & Friedrich 1999).

Pełczyska dich D.PEDB	Łęczyca reservoirs LE	Bogdałów reservoir BO
Chamaepinnularia krookiformis (AC)	*Achnanthidium minutissimum* (EC)	*Achnanthidium minutissimum* (EC)
Chamaepinnularia plinskii (CN)	*Amphora pediculus* (EC)	*Diatoma moniliformis* (EC)
Cocconeis placentula (CN)	*Cyclostephanos dubius* (EC)	*Encyonopsis subminuta* (EC)
Craticula buderi (EC)	*Cyclostephanos invisitatus* (EC)	*Mastogloia smithii* (EC)
Craticula halophila (EC)	*Cyclotella meneghiniana* (EC)	*Nitzschia dissipata* var. *media* (EC)
Fragilaria famelica (EC)	*Navicula gregaria* (EC)	*Pantocsekiella ocellata* (EC)
Fragilaria sopotensis (CN)	*Navicula moskalii* (AC)	*Pantocsekiella pseudocomensis* (EC)
Gomphonema parvulum (EC)	*Nitzschia palea* (EC)	
Hippodonta hungarica (CN)	*Stephanodiscus binatus* (EC)	
Navicula cincta (EC)	*Stephanodiscus hantzschii* (EC)	
Navicula veneta (EC)	*Stephanodiscus parvus* (EC)	
Nitzschia frustulum (EC)		
Nitzschia inconspicua (EC)		
Nitzschia liebethruthii (EC)		
Nitzschia palea (CN)		
Nitzschia perminuta (EC)		
Nitzschia tubicola (CN)		
Planothidium delicatulum (EC)		
Planothidium frequentissimum (EC)		
Tabularia fasciculata (EC)		

Characteristics species with classification of ecological indicators values by Van Dam et al. (*1994*).

Species	Code	Moisture aerophity	Nitrogen uptake	pH requirements	Oxygen requirements	Salinity	Saprobity	Trophic state
Achnanthidium minutissimum	ADMI	3	2	3	1	2	2	7
Amphora pediculus	APED	3	2	4	2	2	2	5
Chamaepinnularia krookiformis	CHKF	3	**0**	3	**0**	3	1	**0**
Chamaepinnularia plinskii	CHPL	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Cocconeis placentula	CPLA	2	2	4	3	2	2	5
Craticula buderi	CRBU	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Craticula halophila	CHAL	2	2	4	2	4	3	5
Cyclostephanos dubius	CDUB	1	2	5	2	3	3	5
Cyclostephanos invisitatus	CINV	**0**	**0**	**0**	**0**	2	**0**	5
Cyclotella meneghiniana	CMEN	2	3	4	5	3	4	5
Diatoma moniliformis	DMOF	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Encyonopsis subminuta	ESUM	**0**	**0**	3	1	1	1	1
Fragilaria famelica	FFAM	3	1	4	1	2	1	3
Fragilaria sopotensis	FSOP	1	2	4	1	2	2	4
Gomphonema parvulum	GPAR	3	3	3	4	2	4	5
Hippodonta hungarica	HHUN	3	2	4	3	2	2	4
Mastogloia smithii	MSMI	3	**0**	4	**0**	4	2	**0**
Navicula cincta	NCCA	**0**	**0**	**0**	**0**	2	**0**	7
Navicula gregaria	NGRE	3	2	4	4	3	3	5
Navicula moskalii	NMOK	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Navicula veneta	NVEN	3	2	4	4	3	4	5
Nitzschia dissipata var. *media*	NDME	**0**	**0**	4	**0**	2	**0**	**0**
Nitzschia frustulum	NIFR	3	4	4	3	3	2	5
Nitzschia inconspicua	NINC	3	3	4	3	3	3	5
Nitzschia liebethruthii	NLBT	**0**	**0**	5	**0**	4	**0**	**0**
Nitzschia palea	NPAL	3	4	3	4	2	5	6
Nitzschia perminuta	NIPM	3	1	4	1	2	1	2
Nitzschia tubicola	NTUB	2	3	4	4	3	5	6
Pantocsekiella ocellata	POCL	1	1	4	1	1	1	4
Pantocsekiella pseudocomensis	PPCS	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Planothidium delicatulum	PTDE	3	1	5	**0**	4	5	3
Planothidium frequentissimum	PLFQ	**0**	2	4	3	2	4	7
Stephanodiscus binatus	SBNT	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Stephanodiscus hantzschii	SHAN	2	3	5	4	2	4	6
Stephanodiscus parvus	SPAV	**0**	**0**	5	**0**	2	**0**	6
Tabularia fasciculata	TFAS	3	2	4	3	4	3	5

Selected 16 characteristic species with the new or altered (bold) ecological indicator values according to Van Dam et al. (*1994*).

Species	Code	Moisture aerophity	Nitrogen uptake	pH requirements	Oxygen requirements	Salinity	Saprobity	Trophic state
Chamaepinnularia krookiformis	CHKF	~~3~~ **4**	0	3	0	~~3~~ **4**	~~1~~ **4**	~~0~~ **5**
Chamaepinnularia plinskii	CHPL	~~0~~ **4**	0	~~0~~ **3**	0	~~0~~ **4**	~~0~~ **4**	~~0~~ **5**
Craticula buderi	CRBU	~~0~~ **3**	0	~~0~~ **4**	0	~~0~~ **4**	~~0~~ **4**	~~0~~ **5**
Cyclostephanos invisitatus	CINV	~~0~~ **1**	0	~~0~~ **4**	0	2	0	5
Diatoma moniliformis	DMOF	0	0	0	0	0	0	0
Encyonopsis subminuta	ESUM	0	0	3	1	1	1	1
Mastogloia smithii	MSMI	3	0	4	0	~~4~~ **3**	2	0
Navicula cincta	NCCA	~~0~~ **3**	0	0	0	2	0	7
Navicula moskalii	NMOK	0	0	~~0~~ **4**	0	~~0~~ **2**	0	~~0~~ **7**
Nitzschia dissipata var. *media*	NDME	0	0	4	0	2	~~0~~ **2**	0
Nitzschia liebethruthii	NLBT	~~0~~ **4**	0	~~5~~ **3**	0	4	~~0~~ **4**	~~0~~ **5**
Pantocsekiella pseudocomensis	PPCS	0	0	0	0	0	0	0
Planothidium delicatulum	PTDE	3	1	~~5~~ **3**	0	~~4~~ **5**	5	3
Planothidium frequentissimum	PLFQ	0	2	4	3	2	4	7
Stephanodiscus binatus	SBNT	0	0	~~0~~ **4**	0	~~0~~ **2**	0	0
Stephanodiscus parvus	SPAV	0	0	~~5~~ **4**	0	2	0	6