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

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Background. The ecological state of surface waters is typically assessed by a multiaspect 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 (EIV), it is possible to create diatom indexes 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. 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, water 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 changed, ecological indicator values, according to Van Dam et al., of species of diatoms characteristic of three post-mining aquatic ecosystems **Methods.** In total, 36 species were identified that were characteristic of three water ecosystems: a salt water complex, 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 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.

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- Critical multi-stranded approach to determining the
- 2 tolerance and ecological values of diatoms in unique
- 3 aquatic ecosystems of anthropogenic origin

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Abstract

16

- 17 **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
- 20 values (EIV), it is possible to create diatom indexes which serve as the basic tool in assessing the
- 21 ecological status of surface waters. These ecological indicator values are set according to
- 22 classification systems, such as the Van Dam et al. system, which classifies species of diatoms
- 23 according to seven different ecological factors. However, recent studies on the autecology of
- 24 diatoms have shown the need to verify and establish new ecological indicator values. To this
- 25 end, water ecosystems are good environments to observe the range of tolerance of benthic
- 26 diatoms to environmental conditions due to their unique physical and chemical parameters. The
- 27 aim of the present study was to propose the establishment of new, or changed, ecological
- 28 indicator values, according to Van Dam et al., of species of diatoms characteristic of three post-
- 29 mining aquatic ecosystems
- 30 **Methods.** In total, 36 species were identified that were characteristic of three water ecosystems:
- 31 a salt water complex, a mined iron ore reservoir and a mined lignite reservoir. Their ecological
- 32 indicator values were calculated using OMNIDIA software, and the environmental conditions
- prevailing in the studied ecosystems were determined. Of the 36 characteristic species, 16
- 34 lacking at least one assigned ecological indicator value were analysed further. The analysis
- 35 identified three groups of selected characteristic species which showed a correlation, or lack of
- such, to the tested physical and chemical parameters.
- 37 **Results.** Based on this multistage study of the autecology of characteristic diatoms, comprising
- an analysis of environmental conditions, literature analysis and reference indicator values of
- 39 other species, it is proposed that 32 ecological indicator values be established or adjusted for 16
- 40 species, and that *Planothidium frequentissimum* be excluded from water quality assessments.

41 Introduction

- 42 Diatoms (Bacillariophyta) are one of the main biotic elements used in the biological assessment
- 43 of the ecological state of surface waters (Water Framework Directive 2000). Due to the fact that
- 44 many countries are obliged to continually engage in biomonitoring, there is a clear need to
- 45 develop flawlessly functioning methods based on the standardised use of diatoms as
- 46 bioindicators (Kahlert et al. 2016; Poikane, Kelly & Cantonati 2016; Szczepocka & Żelazna-
- 47 Wieczorek 2018). Diatom indexes and ecological systems based on the bioindication values of
- 48 particular diatom species, derived from various environmental parameters, constitute a
- 49 fundamental tool in the biological assessment of environments. Diatom indexes have been
- 50 commonly used to assess flowing and standing water for over 20 years (*Kelly et al. 2008*;
- 51 Harding & Taylor, 2014; Szczepocka et al. 2014; Hutorowicz & Pasztaleniec 2014; Holms &
- 52 Taylor 2015; Żelazna-Wieczorek & Nowicka-Krawczyk, 2015; Kolada et al. 2016).
- 53 Currently, many countries use the OMNIDIA program (Lecointe et al. 1993) as a biological
- assessment tool. Its latest version (version 6.0.6) allows the calculation of 18 diatom indexes, and
- 55 the determination of seven environmental parameters for eight ecological systems. However, the



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

Materials & Methods

88 Study area

86

- 89 The study was performed on three hydrological objects created through exploration for mineral
- 90 deposits due or were formed by the closure of mines. All three are located in the Łódzkie and
- 91 Wielkopolskie voivodeships, central Poland. The first object, Pełczyska (PE), is situated in the
- 92 village of Pełczyska, between Łódź and Łęczyca (Łódzkie voivodeship) (Fig. 1). As the local
- area is characterized by the presence of salt deposits, numerous wells were sunk in the eighteenth
- 94 century to obtain brine. Currently, salt water flows out of one of them. This area has been studied



- 95 by biologists and hydrobiologists since the 1960s (Olaczek 1963; Pliński 1966; Pliński 1969;
- 96 Pliński 1971a; Pliński 1971b; Pliński 1971c; Pliński 1973; Żelazna-Wieczorek 1996; Żelazna-
- 97 Wieczorek 2002; Żelazna-Wieczorek, Olszyński & Nowicka-Krawczyk 2015; Żelazna-Wieczorek
- 98 & Olszyński 2016). The objects chosen for our research form the PE hydrological complex
- 99 located in the vicinity of farmland; it comprises the salt water outflow, a drainage ditch and a
- 100 pond, which acts as the receiver of the water.
- 101 The second object is the Łęczyca (LE) urban reservoir located within the city of Łęczyca
- 102 (Łódzkie voivodeship) (Fig. 1). The reservoirs were created following the flooding the open-cast
- iron ore mine in the 1990s. This area is rich in syderite deposits, which are accompanied by other
- minerals. The complex consists of three connected reservoirs: two are directly connected to each
- other (LEP1 and LEP2), and the third (LEP3) is connected to LEP2 via a water drainage ditch
- 106 (Olszyński & Żelazna-Wieczorek 2018). All three are located in an area with houses, garden plots
- and partly-wooded areas.
- 108 The third hydrological object is the Bogdałów reservoir (BO), created by the flooding of an
- opencast brown coal mine. It is located in the village of Bogdałów (Wielkopolskie voivodeship)
- in an area rich in lignite deposits (Fig. 1). Lignite from quaternary deposits was exploited from
- 111 1977 to a depth of 50 meters. Due to the specific construction of the open-pit area, being
- 112 characterised by the thickest layer of poorly permeable boulder clay in the region, it was
- transformed into a storage site for quarried rocks in Koźmin. Finally, in 1993/1994, the drainage
- and runoff of surface waters was blocked to form a water reservoir with a depth of about 12
- meters surrounded by forest (Gabryś-Godlewska et al. 2004; Gadomska et al. 2007; Orlikowski
- 116 & Szwed 2009).
- 117 *Samples*
- 118 Samples of benthic diatoms and water samples were collected from each hydrological facility for
- the analysis of physical and chemical parameters. The water temperature, pH and electrolytic
- 120 conductivity were measured *in situ* (Elmetron CP-401 and CC-401 devices). The following
- 121 sampling points were established:
- 122 Pełczyska (51°58'34.47"N, 19°14'21.11"E) outflow (PESB.), ditch (D.PEDB.) and pond
- 123 (D.PEPB.); samples were collected quarterly in the period July 2013 to March 2014;
- 124 Leczyca (52°3'5.30"N; 19°11'50.24"E) reservoir 1 (D.LEP1.), reservoir 2 (D.LEP2.) and
- reservoir 3 (D.LEP3.), samples were collected quarterly in the period March 2014 to December
- 126 2015;
- 127 Bogdałów (52°2'51.29"N; 18°35'51.49"E) reservoir (D.BOZB.), samples were collected
- 128 quarterly in the period March 2015 to December 2016.
- 129 The water samples were subjected to chemical and physical analysis at the Laboratory of
- 130 Geology at the Faculty of Geographical Sciences, University of Lodz.
- 131 In total, 44 benthic samples were collected. The permanent slides were created according to
- 132 Żelazna-Wieczorek (2011).



- 133 Qualitative and quantitative analysis of diatoms was performed using a Nikon Eclipse 50i light
- microscope (LM) under $1000 \times \text{magnification}$ (plan oil-immersion objective $100 \times / 1.25$): the
- diatoms were identified and counted for up to 500 valves in each preparation. Light
- photomicrographs were taken with an OPTA-TECH digital camera.
- 137 In the case of diatoms that were difficult to be identified using LM were subjected to scanning
- electron microscope (SEM) analysis utilize a Phenom Pro X (gold layer of 8 and 20 nm, at 10
- 139 kV) SEM at the Laboratory of Microscopy Imaging and Specialist Biological Techniques,
- 140 Faculty of Biology and Environmental Protection, University of Lodz.
- 141 Mathematical analysis
- 142 The average percentage (AP) for a given species was determined based on the percentage
- 143 contribution of the species in the samples tested for a given hydrological object (Żelazna-
- 144 Wieczorek 2011). Species whose AP was greater than or equal to 5% for each hydrological
- object were identified as dominant.
- 146 The incidence was determined according to the Tümpling & Friedrich (1999) coefficient
- according to the range values: 100%–75% euconstant taxa (EC), 75%–50% constant taxa (CN),
- 148 50%–25% accessory taxa (AC) and 25%–1% accidental taxa (AD) (Tümpling & Friedrich
- 149 *1999*).
- 150 Multidimensional scaling analysis (MDS) based on Bray-Curtis similarity coefficients was used
- to identify natural groupings of samples. The results are given as a 3D diagram in which the
- degree of similarity is represented as the distances between particular points (samples), with
- 153 greater distances indicating a lower degree of similarity. The reliability of the ordering of the
- assemblage is represented by the stress value, which reflects how well the ordination summarizes
- the observed distances among the samples. The best MDS fits are those with stress values near
- 156 zero (*Clarke & Gorlev 2015*).
- 157 The Shade Plot analysis, based on the Bray-Curtis similarity coefficient, was used to identify the
- diatom species that have the strongest influence on the similarities between the samples
- demonstrated in the MDS analysis. Shade Plot analysis compares two data matrices with each
- other and then groups them on two levels, according to the similarity of the samples and the
- 161 factors affecting their similarity, i.e. diatom species. The results are represented graphically by
- shading individual cells: the intensity of the shading indicates the degree of the influence of a
- 163 given factor (species) on the position of its sample within a given similarity cluster. The range of
- 164 the shading was determined on the basis of $\log (x+1)$.
- 165 The SIMPER analysis was used to determine the characteristic species distinguishing the studied
- ecosystems. This method examines the participation of each variable in the overall similarity
- between groups of samples, thus indicating the species with the greatest influence on the degree
- of similarity, or dissimilarity, between particular samples and hydrological objects. This analysis
- is also based on the Bray-Curtis similarity coefficient; however, unlike the MDS method, in
- which one trial is compared to all the other samples, the SIMPER analysis compares a single
- sample to each subsequent sample (*Żelazna-Wieczorek 2011*). The results indicate the species



- which most strongly differentiated a sampled site from the others, and to what extent. A species
- was regarded as being characteristic of the studied ecosystem if it was characterized by a mean
- dissimilarity greater than or equal to 2 according to the SIMPER analysis, and an mean
- abundance greater in one ecosystem than the other.
- 176 In total, 19 physical and chemical parameters of water were measured in the studied ecosystems.
- 177 The results of the correlation analysis found 15 physical and chemical parameters to be
- indicative of environmental conditions. The selected parameters were subjected to principal
- 179 component analysis (PCA) to determine which had the strongest effect on the studied diatom
- 180 habitats.
- 181 Using the information from the OMNIDIA database, the environmental conditions for each
- sampling point were determined according to Van Dam et al. (1994). Following this, the
- percentage share of diatom species included in each ecological value class was indicated. For
- species found to be characteristic of the studied ecosystems, classes of ecological values were
- assembled. Taxa which had at least one value of 0 (unknown) were selected for further analysis.
- The selected ecological values according to Van Dam et al. (1994) were verified, or new ones
- 187 established, for the species found to be characteristic of the studied ecosystems according to
- three premises: previous literature reports about ecological indicator values of those species,
- 189 chemical and physical conditions analysis, and the classification of the environmental conditions
- 190 according to Van Dam et al. (1994).
- 191 The analyses were performed using PRIMER 7.0.13, OMNIDIA 6.0.6 and STATISTICA 13
- 192 software.

193 Results & Discussion

- 194 *Chemical analysis of water samples*
- 195 The mean values and range of all tested parameters are given in Table 1.
- 196 The PE hydrological complex was characterized by elevated values of electrolytic conductivity,
- 197 reaching as high as 9230 µS L⁻¹. The pH changed with the direction of water outflow: a slightly
- acidic reaction was observed in the outflow and an alkaline one in the pond. Due to the
- 199 geological profile of the region, the water flowing out of the well contained a high concentration
- of chloride ions, whose gradient decreased with the flow of water through the ditch to the pond.
- 201 In addition, higher concentrations of the cations Mg²⁺, Ca²⁺, Na⁺ and K⁺ were observed
- 202 compared to other ecosystems, as well as the anions HCO³⁻, PO₄³⁻ and SO₄²⁻.
- 203 Each of the sampling points in the PE complex was characterized by different chemical
- 204 parameters, resulting in differences between the habitats. The highest electrolytic conductivity
- was noted in the outflow, which was mainly influenced by the concentrations of Cl-, Na⁺ and
- 206 HCO³⁻ ions. The maximum concentration of HCO³⁻ ions was recorded in D.PESB.250314; in the
- 207 other locations, it did not exceed 410 mg L⁻¹.
- 208 Low concentrations of K⁺ ions were observed throughout the entire studied PE complex;
- 209 however, maximum values were recorded in the locations characterised by the highest HCO³- ion
- 210 content. The highest concentration of Ca⁺ ions of all ecosystems was recorded in the outflow.



- 211 The ditch represented an intermediate section between the PE sampling points. However, as it is
- 212 susceptible to periodic drying, limited chemical data was collected from this habitat and hence it
- 213 was not possible to assess its chemical and physical nature.
- 214 The lowest electrolytic conductivity was found in the pond, which displayed lower
- 215 concentrations of Cl⁻, Na⁺ and, to a lesser degree, HCO³⁻. The pH of the water never dropped
- below 8, except in one case in March 2014. In the pond, the concentration of K⁺ remained
- 217 relatively unchanged, which could be related to the fact that the reservoir was also a receiver of
- 218 waters flowing from the surrounding arable fields. The pond was also characterized by the
- lowest concentration of Ca^{2+} and Mg^{2+} . In the summer periods, a significant reduction in the
- 220 water table level and occasional drying of the reservoir were noted.
- 221 The urban reservoirs in Łęczyca were characterized by a slightly alkaline water reaction, which
- was similar in all reservoirs during the course of the study. No elevated concentrations of Fe^{2+}
- 223 $^{3+}$ and Mn³⁺ ions were observed. The content of SO_4^{2-} anions was not higher than in other
- ecosystems studied. The concentration of HCO³⁻ ions was lower than that observed in BO and
- 225 PE. No significant differences in chemical and physical parameters were observed between the
- 226 individual sampling points constituting LE
- 227 The Bogdałów reservoir was characterized by an alkaline reaction. It K⁺, Cl⁻ and NH⁴⁺ ion
- 228 content was the lowest of the studied ecosystems.
- 229 Diatom samples
- 230 A total of 381 diatom taxa were identified in 44 benthic samples: 139 in PE, 192 in LE and 188
- 231 in BO. The dominant species in PE were *Navicula veneta*, and *Nitzschia frustulum*, in LE
- 232 Cyclostephanos dubius and Stephanodiscus hantzschii, in BO Achnanthidium minutissimum,
- 233 Pantocsekiella ocellata and Mastogloia smithii. In the examined ecosystems, the most
- commonly identified classes were accidental (PE-84; LE-111; BO-86), accessory (PE-25; LE-
- 235 35) and euconstant taxa (BO-39) (Fig. 2).
- 236 MDS analysis
- 237 MDS analysis identified the variation between samples for each studied hydrological object (Fig.
- 238 3). The samples taken from BO constitute a separate cloud, with the samples demonstrating high
- 239 similarity with each other, whereas the samples of D.LEP1, D.LEP2 and D.LEP3 constitute a
- 240 distinct group, with no clear differentiation into individual reservoirs. In the case of PE, the pond
- 241 group (D.PEPB) was found to be clearly distinct from the others.
- 242 Shade Plot
- 243 Shade Plot analysis identified 50 species which had the strongest influence on the degree of
- similarity, or non-similarity, between the samples in the studied ecosystems. Of these taxa, the
- three that most strongly influenced the similarity between the samples in at least two ecosystems
- 246 were Navicula veneta, N. cincta, N. gregaria, Nitzschia frustulum, N. inconspicua, N. palea



- 247 Planothidium frequentissimum, Amphora pediculus, Cyclotella meneghiniana, Fragilaria
- 248 radians and Achnanthidium minutissimum (Fig. 4).
- 249 SIMPER analysis
- 250 SIMPER analysis allowed 36 species characteristic of the tested hydrological objects to be
- 251 distinguished. These included Cocconeis placentula, Craticula buderi, Fragilaria sopotensis,
- 252 Gomphonema parvulum, Hippodonta hungarica, Navicula cincta, Nitzschia palea and
- 253 Planothidium frequentissimum for D.PEPB; Chamaepinnularia krookiformis, C. plinskii,
- 254 Craticula halophila, Navicula veneta, Nitzschia frustulum, N. inconspicua, N. liebethruthii, N.
- 255 perminuta and N. tubicola for D.PEDB and Fragilaria famelica, Navicula veneta, Nitzschia
- 256 frustulum, N. inconspicua, Planothidium delicatulum, and Tabularia fasciculata for D.PESB
- 257 Characteristic species for LA are: Achnanthidium minutissimum, Amphora pediculus,
- 258 Cyclostephanos dubius, C. invisitatus, Cyclotella meneghiniana, Navicula gregaria, N. moskalii,
- 259 Nitzschia palea, Stephanodiscus binatus, S. hantzschii and S. parvus. For BO: : Achnanthidium
- 260 minutissimum, Diatoma moniliformis, Encyonopsis subminuta, Mastogloia smithii, Nitzschia
- 261 dissipata var. media, Pantocsekiella ocellata and P. pseudocomensis.
- 262 .In addition, two species were found to be as characteristic of two different ecosystems:
- 263 Achnanthidium minutissimum for LE and BO, and Nitzschia palea for PE and LE.
- 264 Ecological analysis based on Omnidia software
- 265 The ecological analysis of diatom assemblages based on data obtained from the OMNIDIA
- 266 program database, indicated the following:
- pH requirements: while alkaliphilic species predominate in PE (63%), a large percentage in
- 268 D.PEPB are unknown (24%) or neutrophilic species (23%). The LE reservoirs were dominated
- by alkalibiontic (45%) and alkaliphilic (24%) organisms. In D.LEP1, 25% of species were
- 270 unknown. BO was dominated by alkaliphilic (39%) and neutrophilic (29%) species, and 26 % of
- 271 species were unknown (Fig 5a):
- salinity: the PE complex was characterized by the occurrence of halophilic (43%).
- oligohalobous (30%) and mesohalobous species (16%); the greatest proportion of the
- 274 mesohalobous species were found in D.PESB (28%). The LE reservoirs were dominated by
- oligohalobous (44%) and halophilic species (42%). BO was dominated by oligohalobous (43%),
- 276 halophobe (24%) and unknown species (23%) (Fig 5b).
- nitrogen uptake: the most common species in the PE complex N-autotrophic tolerant (39%)
- 278 followed by unknown (25%). The largest percentage of unknown species (28%) was recorded in
- 279 D.PEPB and D.PEDB. In the LE reservoirs, the most common groups of species were N-
- autotrophic (57%) and unknown (25%). In BO, 51% species were unknown, 24% were N-
- autotrophic tolerant and 22% N-autotrophic sensitive (Fig 5c);
- oxygen requirements: in PE, the largest groups of species were low oxygen (30%), unknown
- 283 (27%) and moderate oxygen (24%). In LE, oxybiontic species were most common (43%)



- followed by unknown (25%). In BO, unknown (46%) and polyoxybiontic species (42%)
- 285 predominated (Fig 5d);
- sensitivity to saprobity: in PE, the largest group of taxon were α -meso: polysabrobe (28%) and
- unknown (23%). In D.PEPB, the most abundant was α -meso-polysabrobe (34%) followed by β -
- 288 mesosaprobe (31%) and unknown (27%). LE primarily included taxa from the α -mesosaprobe
- 289 group (47%) and unknown (23%). In BO, unknown (34%), β-mesosaprobe (31%) and
- 290 oligosaprobe taxa (28%) predominated (Fig 5e);
- trophic status: in PE, the largest group of diatoms were eutrophic (50%) and unknown taxa
- 292 (25%), LE had the highest percentage (61%) of eutrophic species but also unknown (15%) and
- 293 hypetrophic (13%) were present. In BO, the most abundant species were unknown (42%),
- 294 indifferent (19%) and meso-eutrophic (16%) (Fig 5f);
- moisture aerophily: in PE, the largest group was aquatic to aerophilic (56%), representing 66%
- of species in D.PESB, 61% in D.PEDB, and 42% in D.PEPB. The second largest group was
- 297 unknown (23%), constituting 32% of taxa in D.PEPB. In LE, 37% of the species were aquatic
- 298 (24% of taxa in D.LEP2), 54% were occasionally aerophilic and 22% were unknown. In BO, the
- predominant groups of species were unknown (44%) and aquatic to aerophilic (33%) (Fig 5g).
- 300 Characteristic species: OMNIDIA and PCA analysis
- 301 The analysis of species characteristic of the tested ecosystems, determined according to Van
- Dam et al. (1994), identified 16 taxa classified as 0 in at least one category (Table 2). The next
- 303 step determined the percentage contribution of each of these species classified as class 0 for the
- ecological parameters defined by Van Dam et al. (1994) at each sample point (Table 3).
- Based on the PCA analysis of the 16 characteristic taxa and selected physical and chemical
- 306 parameters, the following relationships were demonstrated:
- 307 Group A: Chamaepinnularia krookiformis, C. plinskii, Nitzschia liebethruthii and Planothidium
- 308 delicatulum demonstrate a negative correlation with pH and a positive correlation with a
- decrease in the concentrations of HCO³⁻ $Ca^{2+} Fe^{2+/3+}$ (Fig. 6).
- 310 Group B: Craticula buderi, Planothidium frequentissimum and Navicula cincta did not
- demonstrate any relationship with any water parameters (Fig. 6).
- 312 Group C: Navicula moskalii, Cyclostephanos invisitatus, Stephanodiscus parvus, S. binatus,
- 313 Diatoma moniliformis, Nitzschia dissipata var. media, Mastogloia smithii, Pantocsekiella
- 314 pseudocomensis and Encyonopsis subminuta demonstrated a negative correlation with a decrease
- in electrolyte conductivity, as well as with the concentrations of K⁺, Mg²⁺, Na⁺, SO₄²⁻, Cl⁻, PO₄³⁻
- and Mn³⁺ and water pigments (Fig. 6).
- 317 *Ecological values of characteristic species*
- 318 Characteristic species for PE
- 319 Planothidium delicatulum (PTDE) (Figs 7. A1-A6)



- 320 Planothidium delicatulum is a euconstant taxon for PE and an accidental taxon for LE. Its mean
- percentage share in PE was 2%, and constituted 5% in D.PESB.
- 322 Planothidium delicatulum does not currently have six ecological values according to Van Dam et
- 323 al. (1994).
- 324 This species was more abundant in environments such as D.PESB, which was also characterized
- by the highest concentration of Cl⁻ (up to 2976 mg L⁻¹), elevated electrolytic conductivity, and
- 326 decreased K⁺ concentration. The pH of the water in which this species was observed did not
- 327 exceed 7.
- 328 Planothidium delicatulum was mainly recorded in salty and brackish environments with neutral
- 329 or slightly alkaline conditions (Campeau, Pienitz & Héquette 1999; Gell et al. 2005; Caballero
- 330 et al. 2013; Yamamoto, Chiba & Tuji 2017; Van de Vijver et al. 2018).
- Based on our findings, we suggest changing the following ecological values in the Van Dam et
- al. (1994) classification for *Planothidium delicatulum*:
- pH requirements: 3 (neutrophilic) (changing from 5 to 3);
- salinity: 5 (brackish-marine) (changing from 4 to 5)
- 335 Chamaepinnularia krookiformis (CHKF) (Figs 7. B1-B5) and Chamaepinnularia plinskii
- 336 (CHPL) (Figs 7. C1-C5)
- 337 In 2016 Chamaepinnularia krookiformis was divided into two separate taxa: C. krookiformis and
- 338 C. plinskii (Żelazna-Wieczorek & Olszyński 2016). Both species were very often recorded
- 339 together in the same ecosystem. However, the publications which identified *C. krookiformis*
- often do not provide appropriate photographic documentation or photos of individual specimens
- 341 (Witkowski 1994, Bak et al. 2006; Wojtal 2008; Peszek et al. 2015). Currently available
- 342 documentation is insufficient to determine whether C. krookiformis and C. plinskii are both
- 343 present simultaneously in a given environment or whether just one of these species exists.
- Chamaepinnularia krookiformis is an accessory taxon for PE (a constant taxon for D.PEDB), C.
- 345 plinskii is a constant taxon for PE (a euconstant taxon for D.PEDB). The mean share of C.
- 346 krookiformis was 1.6% in all PE samples, 4% in D.PEDB; for C. plinskii, this amounted to 2.7%
- 347 in PE, 6% in D.PEDB.
- 348 Currently, C. krookiformis lacks three assigned ecological values. For PE, it constitutes 6% of
- 349 the *unknown* group in nitrogen *uptake*, 6% in oxygen requirements and 6% in trophic state
- 350 (respectively for D.PEDB: 16%, 16% and 15%). *C. plinskii* has no assigned ecological values
- and represents 26% of the *unknown* group for pH requirements, 19% for salinity, 12% for
- 352 nitrogen uptake, 10% for oxygen requirements, 14% for saprobity, 11% for trophic state and
- 353 10% for moisture (respectively for D.PEDB: 60%, 25%, 24%, 23%, 32%, 23% and 28%).
- 354 The conditions of the environments in which both species have been recorded indicate that they
- are class 3 with regard to pH range (neutrophilic). Both species were the most abundant in
- 356 locations subjected to periodic drying and characterised by high concentrations of chloride ions
- 357 (up to 1006 mg L⁻¹) indicating a brackish environment (*Żelazna-Wieczorek, Olszyński &*
- 358 Nowicka-Krawczyk 2015)



- On the basis of our findings and those of previous studies (*Krammer & Lange–Bertalot 1986*;
- 360 Krammer 1992; Witkowski 1994; Bak, Witkowski & Lange-Bertalot 2006; Wojtal 2009; Peszek
- 361 et al. 2015; Żelazna-Wieczorek & Olszyński 2016), we propose the following changes to the
- ecological values according to Van Dam et al. (1994) for Chamaepinnularia krookiformis and C.
- 363 plinskii:
- 364 pH requirements: 3 (neutrophilic) for both species;
- salinity: 4 (mesohalobous) established for C. plinskii, and changing from 3 to 4 for C.
- 366 krookiformis;
- trophic state: 5 (eutrophic) for both species;
- moisture aerophily: 4 (aerophilic) established for *C. plinskii* and changing from 3 to 4 for *C.*
- 369 krookiformis.
- saprobity: from 2 to 4 (β-mesosaprobe to α -meso-polysabrobe) for both species. Due to the
- 371 specific conditions and locations of the studied objects, they were exposed to large fluctuations
- in the inflow of organic matter, mainly from runoff from arable fields and pollution caused by
- animal grazing. These impurities were manifested as elevated concentrations of K⁺ ions.
- 374 Therefore, we believe that classifying *C. krookiformis* as an oligosaprobe is inappropriate.
- 375 Further tests are needed to determine the optimum occurrence of these species in areas subjected
- 376 to organic matter loads.

377 Nitzschia liebethruthii (NLBT) (Figs 7. D1-D4)

- 378 Nitzschia liebethruthii is a euconstant taxon for PE. Its mean percentage share was 4% in the PE
- samples, and 10% in the D.PEDB samples. It was most numerous in the sample D.PEDB.301113
- 380 (19%). This species has two specific ecological values. The ecological value analysis for PE
- 381 found N. liebethruthii to represent 17% of the unknown group in nitrogen uptake, 16% in oxygen
- requirements, 18% in saprobity, 17% in trophic state and 21% in moisture (respectively for
- 383 D.PEDB: 37%, 37%, 43%, 36% and 49%).
- 384 Nitzschia liebethruthii occurred in environments subjected to periodic drying with a pH close to
- 385 7 and high concentration of chloride ions.
- 386 This species was noted in environments with increased salinity, electrolytic conductivity and
- 387 high pH value (Rumrich, Lange-Bertalot & Rumrich 2000; Witkowski, Lange-Bertalot &
- 388 *Metzeltin 2000; Lange-Bertalot et al. 2017; Földi et al. 2018*).
- We propose the following changes to the ecological values according to Van Dam et al. (1994)
- 390 assigned to *Nitzschia liebethruthii*:
- pH requirements: 3 (neutrophilic) (changing from 5 to 3);
- saprobity: from 2 to 4 (β-mesosaprobe to α -meso-polysabrobe) as in the case of *C. krookiformis*
- 393 and C. plinskii;
- 394 trophic state: 5 (eutrophic);
- 395 moisture aerophily: 4 (aerophilic).
- 396 Craticula buderi (CRBU) (Figs 7. E1-E5)



- 397 Craticula buderi is a euconstant taxon for PE and an accidental taxon for LE. Its mean
- 398 percentage share was 4.3% in all samples for PE, and 12% for D.PEPB. This species has no
- 399 recorded ecological values. The ecological value analysis for the PE found C. buderi to
- 400 constitute 19% of the *unknown* group in pH requirements, 28% in salinity, 16% in nitrogen
- 401 uptake, 16% in oxygen requirements, 16% in saprobity, 19% in trophic state and 15% in
- 402 moisture (respectively for D.PEPB: 53%, 73%, 46%, 46%, 46%, 54% and 41%).
- 403 Although Craticula buderi was classified into group B, it was found to be most abundant in
- 404 environments with an elevated concentration of Cl⁻ ions, ranging from 685 to 1090 mg L⁻¹, (all
- samples from D.PEPB and one sample from D.PEDB in which the concentration of chloride
- 406 ions was 1006 mg L⁻¹). However, its numbers were decreased in the D.PEPB sample, which was
- 407 characterised by a chloride ion content of over 1500 mg L⁻¹. Interestingly, the concentration of
- 408 K⁺ ions exceeded 100 mg L⁻¹ at Cl⁻ concentrations below 1500 mg L⁻¹; therefore, it is possible
- 409 that the decline of this species could be related to the concentration of K⁺ ions alone. Our
- observations indicate that the population of C. buderi from D.PEPB favours a concentration of
- 411 chloride ions from 500 to 1006 mg L⁻¹ which coincides with a K⁺ ions concentration from 50 to
- 412 70 mg L⁻¹.
- 413 *Craticula buderi* is widespread throughout the world and recognized as cosmopolitan (*Rumrich*,
- 414 Lange-Bertalot & Rumrich 2000; Lange-Bertalot 2001; Bahls 2009; Soltanpour-Gargari,
- 415 Lodenius & Hinz 2011, Żelazna-Wieczorek 2011; Cichoń 2016). This species was found to be
- 416 dominant in environments characterised by increased electrolytic conductivity and an alkaline
- 417 water reaction (*Holmes & Taylor 2015*). Holmes and Taylor (2015) place C. buderi in the Bad in
- 418 Water Quality class. Their recorded values of diatom indices indicate that the environment was
- 419 eutrophic.
- 420 We therefore propose the following classes of ecological values according to Van Dam et al.
- 421 (1994) for Craticula buderi:
- 422 pH requirements: 4 (alkaliphilic);
- 423 sabrobity: from 2 to 4 (β-mesosaprobe to α-meso-polysabrobe) as in the case of C. krookiformis
- 424 and *C. plinskii*;
- 425 trophic state: 5 (eutrophic):
- 426 salinity: 4 (mesohalobous)
- moisture aerophily: 3 (aquatic to aerophilic).
- 428 Navicula cincta (NCCA) (Figs 7. F1-F5)
- 429 Navicula cincta is a euconstant taxon for PE, a constant taxon for LE and an accessory taxon for
- BO. The mean percentage share of this species for PE is 3%, of which 7% was found in D.PEPB
- 431 samples.
- 432 Currently this species has been assigned two ecological values. The ecological values analysis
- 433 for the PE found *N. cincta* to constitute 24% of the *unknown* group in pH requirements, 13% in
- and 12% in oxygen requirements, 13% in saprobity, and 12% in moisture
- 435 (respectively for D.PEPB: 28%, 25%, 25%, 26% and 23%).



- 436 An analysis of the physical and chemical data and the variability of occurrence did not show any
- clear relationships between environmental parameters and the percentage share of *Navicula*
- 438 *cincta* in the tested samples. This lack of dependence is also confirmed by the PCA analysis.
- 439 Navicula cincta has been recorded in various types of ecosystems, although mainly in eutrophic
- ones with high conductivity. It also tolerates elevated levels of organic matter. This species was
- also observed in habitats subjected to periodic drying (Lange-Bertalot & Genkal 1999; Rumrich,
- 442 Lange-Bertalot & Rumrich 2000; Witkowski, Lange-Bertalot & Metzeltin 2000; Lange-Bertalot
- 443 2001; Żelazna-Wieczorek 2011; Wojtal 2013; Lange-Bertalot, Hofmann & Werum 2017).
- However, several new species from the group *N. cincta* s.l. have been described, and it can be
- assumed that each of these individual species in this group may be associated with narrower
- 446 optimal ecological conditions (Cantonati et al. 2016).
- Based on our present findings, and those of previous studies, we believe that it is not appropriate
- 448 to classify *Navicula cincta* as an oligohalobous species with regard to *salinity*: it has been
- recorded in fresh (Żelazna-Wieczorek 2011; Wojtal 2013), brackish (Żelazna-Wieczorek,
- 450 Olszyński & Nowicka-Krawczyk 2015; Żurek et al. 2018) and salt waters (Witkowski, Lange-
- 451 Bertalot & Metzeltin 2000). We propose the following ecological value according to Van Dam et
- 452 al. (1994) for Navicula cincta s.l.:
- 453 moisture aerophily: 3 (aquatic to aerophilic).
- 454 Shade Plot analysis found that the presence of *N. cincta* s.l. can falsely indicate high similarity
- between samples from different environments, thus distorting the results of any environmental
- 456 analysis. Therefore, with regard to the unclear taxonomic status of *Navicula cincta* s.l. and the
- 457 current lack of knowledge regarding its activities, we recommend this taxon be excluded from
- 458 the biological assessment of surface water quality.

459 Planothidium frequentissimum (PLFQ) (Figs 7. G1-G5)

- 460 Planothidium frequentissimum is a euconstant taxon for PE and LE. The mean percentage of this
- species for PE is 2.7%, of which 5% was found in D.PEPB samples. It was most numerous in the
- D.PEDB.301113 sample (19%). The species has currently six established ecological values. The
- ecological values analysis for PE found *P. frequentissimum* to constitute 12% of the *unknown*
- group in moisture aerophily (for D.PEPB 14% and D.PESB: 20%).
- No relationship was observed between percentage share of *Planothidium frequentissimum* and
- the changes in chemical and physical parameters in the tested samples. This lack of relationship
- was confirmed by PCA analysis.
- 468 Planothidium frequentissimum is an eurytopic species that occurs globally in a variety of habitat
- 469 types, from natural springs to rivers in urban areas with high levels of pollution. Its value as an
- 470 indicator is low, as confirmed by the Shade Plot analysis, which found it to significantly affect
- 471 the degree of similarity observed between samples from different environments. We therefore
- 472 recommend that *P. frequentissimum* be excluded from the biological assessment of surface water
- 473 quality (Siver, et al. 2005; Levkov et al. 2007; Żelazna-Wieczorek 2011; Kulikovskiy, Lange-
- 474 Bertalot & Kuznestova 2015; Szczepocka, Nowicka-Krawczyk & Kruk 2018).



- 475 Characteristic species for LE
- 476 Cyclostephanos invisitatus (CINV) (Figs 8. A1-A6)
- 477 *Cyclostephanos invisitatus* is a euconstant taxon for LE. Its percentage share for LE was 4.8%.
- 478 *C. invisitatus* currently has two ecological values assigned. The ecological values analysis for LE
- found it to constitute 24% of the *unknown* group in pH requirements, 17% in nitrogen uptake,
- 480 18% in oxygen requirements, 19% in saprobity, and 20% in moisture.
- 481 Cyclostephanos invisitatus occurs in diverse environments, however, it is most frequently
- 482 reported in aquatic ecosystems subjected to high human impact characterised by an alkaline
- 483 reaction and increased conductivity (Reavie & Smol 1998; Yang et al. 2005; Wojtal &
- 484 Kwandrans, 2006; Kiss et al. 2012; Houk, Klee & Tanaka 2014; Reavie & Kireta 2015;
- 485 Olszyński & Żelazna-Wieczorek 2018).
- We therefore propose that the following classes of ecological values according to Van Dam et al.
- 487 (1994) be established for *Cyclostephanos invisitatus*:
- 488 pH requirements: 4 (alkaliphilic);
- 489 moisture aerophily: 1 (aquatic);
- 490 Navicula moskalii (NMOK) (Figs 8. B1-B6)
- 491 Navicula moskalii is an accessory taxon for LE. Its mean percentage share for LE was 1.5%. Its
- 492 incidence was greatest in sample D.LEP1.250315 (26%).
- 493 Navicula moskalii has no assigned ecological values. The ecological value analysis for LE found
- 494 it to constitute 5% of the *unknown* group in pH requirements, 7% in salinity, 5% in nitrogen
- 495 uptake, 5% in oxygen requirements, 5% in saprobity, 6% in trophic state and 5% in moisture.
- The greatest occurrence of *N. moskalii* was observed in samples with the highest concentrations
- 497 of Ca^{2+} (143.6 mg L^{-1}), HCO^{3-} (338.6 mg L^{-1}), SO_4^{2-} (146.9 mg L^{-1}) and with high Mg^{2+} content.
- 498 Navicula moskalii was observed in a number of ecosystems (Metzeltin & Witkowski 1996:
- 499 Lange-Bertalot 2001; Żelazna-Wieczorek 2011; Noga et al. 2016; Lange-Bertalot, Hofmann &
- Werum 2017), particularly in eutrophic waters with an elevated level of Ca²⁺ and HCO³⁻ ions.
- 501 Żelazna-Wieczorek (2011) report a significant number of *N. moskalii* in springs with high levels
- of eutrophication, however with Ca²⁺, SO₄²⁻, HCO³⁻ and Mg²⁺ concentrations lower than those in
- the LE samples.
- We therefore propose that the following classes of ecological values according to Van Dam et al.
- 505 (1994) be established for Navicula moskalii:
- 506 pH requirements: 4 (alkaliphilic);
- 507 salinity: 2 (oligohalobous);
- 508 trophic state: 7 (indifferent)
- 509 Stephanodiscus binatus (SBNT) (Figs 8. C1-C5)



- 510 Stephanodiscus binatus is a euconstant taxon for LE. Its percentage share for LE was 4.3%. S.
- binatus has no recorded ecological values. The ecological value analysis for LE found it to
- 512 constitute 25% of the *unknown* group in pH requirements, 47% in salinity, 18% in nitrogen
- 513 uptake, 18% in oxygen requirements, 20% in saprobity, 29% in trophic state and 21% in
- 514 moisture.
- The largest percentage share of *S. binatus* was recorded in the spring months and the lowest in
- autumn. Its abundance was found to be elevated in December 2014 and 2015; the same samples
- demonstrated the highest concentrations of Ca²⁺, Mg²⁺ and the highest pH (above 8).
- 518 Stephanodiscus binatus has been recorded in various water ecosystems ranging from
- oligotrophic to eutrophic; however, all are characterised by elevated pH value (Stoermer &
- 520 Håkansson 1984; Håkansson & Kling 1990; Houk, Klee & Tanaka 2014; Olszyński & Żelazna-
- 521 *Wieczorek 2018*).
- We therefore propose that the following classes of ecological values according to Van Dam et al.
- 523 (1994) be established for Stephanodiscus binatus:
- 524 pH requirements: 4 (alkaliphilic);
- 525 salinity: 2 (oligohalobous);

526 Stephanodiscus parvus (SPAV) (Figs 8. D1-D5)

- 527 Stephanodiscus parvus is a euconstant taxon for LE and an accidental taxon for PE. Its
- percentage share for LE was 2.4%. It was most abundant in the D.LEP3.260714 sample (22%).
- 529 This species has three assigned ecological values. The ecological values analysis for LE found
- 530 the taxon to constitute 9% of the *unknown* group in nitrogen uptake, 9% in oxygen requirements,
- 531 10% in saprobity, and 10% in moisture.
- 532 S. parvus is noted mainly in eutrophic hypertrophic ecosystems with elevated electrolytic
- 533 conductivity. It is also a good indicator of waters with a strong anthropogenic impact (Reavie &
- 534 Smol 1998; Reavie & Kireta, 2015; Olszyński & Żelazna-Wieczorek 2018; Reavie & Cai 2019).
- Based on our findings and literature data, we propose the following changes in ecological values
- according to Van Dam et al. (1994) for Stephanodiscus parvus:
- 537 pH requirements: from 5 to 4 (alkaliphilic);
- 538 Characteristic species for BO

539 Diatoma moniliformis (DMOF) (Figs 9. A1-A6)

- 540 Diatoma moniliformis is a euconstant taxon for BO. Its mean percentage share for BO was 3.9%.
- 541 It currently has no assigned ecological values. According to the ecological values analysis for
- BO, this taxon constituted 10% of the *unknown* group in pH requirements, 11% in salinity, 8% in
- 543 nitrogen uptake, 8% in oxygen requirements, 9% in saprobity, 9% in trophic state and 8% in
- 544 moisture.
- 545 D. moniliformis was found in 87.5 % of samples from BO. Interestingly, it constituted 28% of
- 546 the share in one sample from December 2016 (D.BOZB.091216); however, its share was below



- 547 2% in the previous season, and was not higher than 1-2% in the other samples from December
- 548 2016. The chemical and physical characteristics of D.BOZB.091216 did not differ significantly
- 549 from those of the other samples.
- This species is also found in fresh and salt water, as well as the Baltic and arctic areas with high
- 551 conductivity (Potapova & Snoeijs 1997; Rumrich, Lange-Bertalot & Rumrich 2000; Levkov et
- 552 al. 2007; Pniewski & Sylwestrzak 2018).
- One of the factors that influences the abundance of *D. moniliformis* is the water temperature.
- 554 Studies indicate that temperatures above 10-15°C (*Potapova & Snoeijs 1997; Pniewski &*
- 555 Sylwestrzak 2018) are associated with population growth. However, populations have been
- observed in freshwater streams and lakes in arctic areas, in which the temperature of the water is
- below 10°C (Antoniades Douglas & Smol 2005). Population growth was also observed at 4.7°C
- in sample D.BOZB.091216; therefore, low temperature may have an influence on the abundance
- of this species.

560 Encyonopsis subminuta (ESUM) (Figs 9. B1-B6)

- 561 Encyonopsis subminuta is a euconstant taxon in BO, where its mean percentage share was 4.1%.
- Presently, E. subminuta has been assigned five ecological values. Ecological values analysis for
- BO found it to constitute 7% of the *unknown* group in *nitrogen uptake* and 8% in *moisture*.
- *Encyonopsis subminuta* was found to be most abundant in sample D.BOZB.041115. The sample
- was also characterised by an elevated concentration of $Fe^{2+/3+}$ ions and the lowest pH value. In
- subsequent samples, when the concentration of Fe ions dropped, the abundance of E. subminuta
- 567 also decreased.
- 568 E. subminuta is regarded as a cosmopolitan taxon, occurring in the temperate and boreal zone. It
- is most abundant in oligo- to mesotrophic waters with electrolytic conductivity between 190-250
- 570 μS L⁻¹ (*Krammer 1997; Noga et al. 2014; Novais et al. 2014; Feret, Bouchez & Rimet 2017*).
- 571 E. subminuta may be sensitive to the concentration of Fe ions; however, the increase of these
- 572 ions is associated with a drop in pH. Our research confirms that the optimal pH for population
- 573 size is close to 7.

574 Mastogloia smithii (MSMI) (Figs 9. C1-C7)

- 575 Mastogloia smithii is a euconstant taxon for BO. Its mean percentage share for BO was 6.3%. it
- 576 was found in greatest numbers in D.BOZB.300615 (22%) and D.BOZB.261016 (15%). M.
- 577 *smithii* has been assigned four ecological values. The ecological values analysis for the BO
- found this species to constitute 13% of the *unknown* group in nitrogen uptake, 14% in oxygen
- 579 requirements and 15% in trophic state.
- The environment in BO regarding salinity was classified according to Vam Dam et al. (1994) as
- oligohalobus (43% species); however, 7% of the mesohalobous species were represented by one
- 582 species: Mastogloia smithii.
- This species is recorded in fresh, brackish and salt water (Witkowski, Lange-Bertalot & Metzeltin
- 584 2000; Busse & Snoeijs 2003; Weckström & Juggins 2005; Martinzes-Goss & Evangelista 2011;



- 585 Lange-Bertalot, Hofmann & Werum 2017). Its presence in environments with varying degrees of
- salinity may suggest that this does not have an significant influence on population size.
- 587 Based on our present findings and literature data, we propose the following changes in the
- ecological values according to Van Dam et al. (1994) for Mastogloia smithii:
- salinity: from 4 to 3 (halophilic).

590 Nitzschia dissipata var. media (NDME) (Figs 9. D1-D5)

- 591 Nitzschia dissipata var. media is a euconstant taxon for BO. Its mean percentage share for BO
- was 3.8%, and the highest proportion (20%) was found in D.BOZB.041115. *N. dissipata* var.
- 593 *media* has been assigned two ecological values. Ecological values analysis for BO found it to
- constitute 9% of the *unknown* group in nitrogen uptake, 10% in oxygen requirements, 11% in
- saprobity, 11% in trophic state and 9% in moisture.
- The growth in occurrence of *Nitzschia dissipata* var. *media* is associated with an increase in the
- level of $Fe^{2+/3+}$ ions, similar to *Encyonopsis subminuta*. In addition, it was found in the
- ecosystem, i.e. BO, with the lowest concentrations of ions indicative of the presence of organic
- 599 pollutants in the environment, such as K⁺ and NH⁴⁺.
- Although Nitzschia dissipata var. media is found sporadically, it is commonly found in oligo- to
- mesotrophic waters with a pH between 7 and 8 (Van der Vijver, Frenot & Beyens 2002;
- 602 Antoniades, Douglas & Smol 2005; Żelazna-Wieczorek 2011; Lange-Bertalot, Hofmann &
- 603 Werum 2017).
- Based on our findings and literature data, we propose the following ecological values according
- 605 to Van Dam et al. (1994) for Nitzschia dissipata var. media:
- 606 saprobity: 2 (β-mesosaprobe)

607 Pantocsekiella pseudocomensis (PPCS) Figs (9. E1-E7)

- 608 Pantocsekiella pseudocomensis is a euconstant taxon for BO. Its mean percentage share for BO
- 609 was 4.4%. It was most abundant in D.BOZB.250315 (9%) and in D.BOZB.220616 (10%). P.
- 610 pseudocomensis has not been assigned any ecological values according to Van Dam et al. (1994).
- The ecological values analysis for BO found it to represent 22% of the *unknown* group in pH
- requirements, 25% in salinity, 10% in nitrogen uptake, 11% in oxygen requirements, 18% in
- 613 saprobity, 12% in trophic state and 13% in moisture.
- The greatest amount of *P. pseudocomensis* was found in samples characterised by the highest
- 615 levels of ammonium ions. Its percentage share was lowest in samples with the lowest water
- 616 temperature, apart from D.BOZB.250315.
- 617 Currently, Pantocsekiella pseudocomensis is assigned to the P. comensis complex, with P.
- 618 comensis and P. costei. We believe that assigning ecological values for particular species of the
- 619 P. comensis complex is unjustified at the current state of knowledge, and that all species within
- 620 the complex should be assigned the same provisional ecological values until their individual
- properties are better understood (Houk, Klee & Tanaka 2010; Kistenich et al. 2014; Duleba et al.
- 622 *2015*).



Conclusions

623

- The water ecosystems created in the post-mining areas create a complex of conditions that are
- not found in other natural ecosystems, and the benthic diatom species present in such
- environments are very often present in higher numbers than in other habitats. The specific hydro-
- 627 geological conditions prevailing in the post-production reservoirs provide a unique opportunity
- 628 to observe interspecies differences and intra-species variability, allowing for the verification or
- 629 isolation of new taxa and a greater insight into their autecology (Żelazna-Wieczorek & Olszyński
- 630 2016; Olszyński & Żelazna-Wieczorek 2018).
- The identification of species characteristic of the studied ecosystems may foster further growth
- of ecological research and increase the reliability of surface water quality assessment, as such
- knowledge is needed to verify their ecological indicator values, and hence calculate
- 634 diatomaceous indexes with greater accuracy.
- Ecological values as set out by Van Dam et al. (1994) are utilized in many ecological works
- describing the ecological conditions of the studied ecosystems. These ecological values form the
- basis for calculating diatomaceous indexes describing the ecological state of surface waters. It is
- 638 therefore necessary to constantly update and establish new ecological indicator values for
- 639 particular diatom species.
- Many authors who describe new species, or encounter existing species in new ecosystems,
- regularly propose updates for individual ecological indicator values. However, these findings,
- 642 may not be introduced and updated in the OMNIDIA program for a number of years. The
- 643 OMNIDIA system is used by state institutions in many countries around the world to assess
- 644 surface water quality, (Campeau, Pienitz & Héquette 1999; Rumrich, Lange-Bertalot & Rumrich
- 645 2000; Witkowski, Lange-Bertalot & Metzeltin 2000; Gell et al. 2005; Potapova & Ponader
- 646 2008; Caballero et al. 2013; Żelazna-Wieczorek & Olszyński 2015; Yamamoto, Chiba & Tuji
- 647 2017; Lange-Bertalot, Hofmann & Werum 2017; Földi et al. 2018; Van de Vijver, Wetzel &
- 648 Ector 2018). Clearly, if these assessments are based on incomplete or outdated data, assessments
- of aquatic environments may be fraught with error.
- 650 The present study used three principles to identify proposed changes in the classification of
- ecological indicator values for characteristic species according to the Van Dam et al. (1994)
- 652 system, or to establish new values which were previously absent: the analysis of environmental
- 653 conditions prevailing in the studied ecosystems, the analysis of relevant literature data, and
- references to the ecological indicator values of other species. This mode of research 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



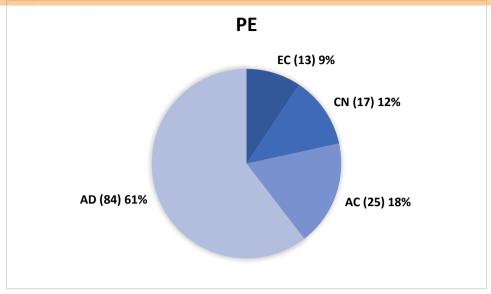


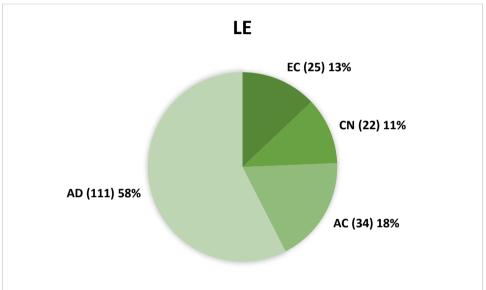
Figure 2(on next page)

Percentage share of diatoms in individual classes of prevalence according to the Tümpling & Friedrich factor (1999)

PE – Pełczyska complex, LE – Łęczyca reservoirs, BO – Bogdałów reservoir. EC – euconstant taxa, CN – constant taxa, AC – accessory taxa AD – accidental taxa. The number of species is shown in brackets







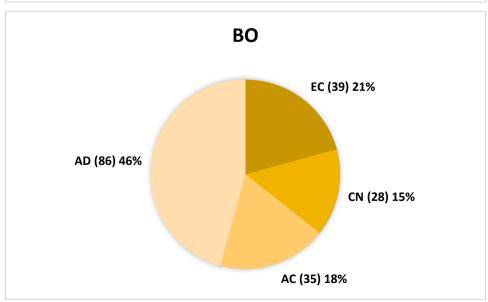




Figure 3

MDS 3D analysis. The diagram shows three distinct clouds of samples which are grouping coincides with three hydrological objects

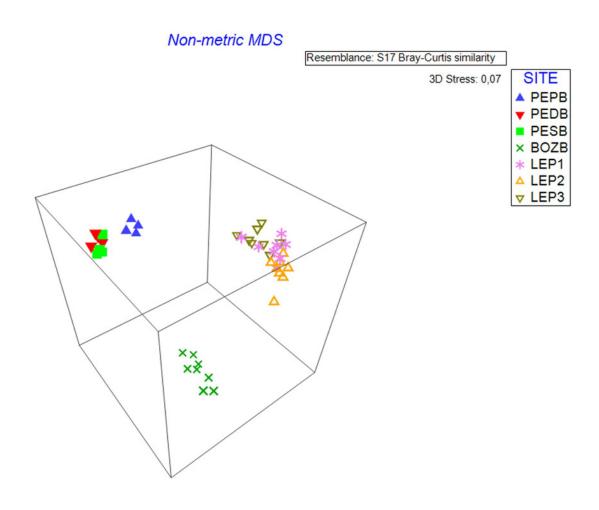




Figure 4

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



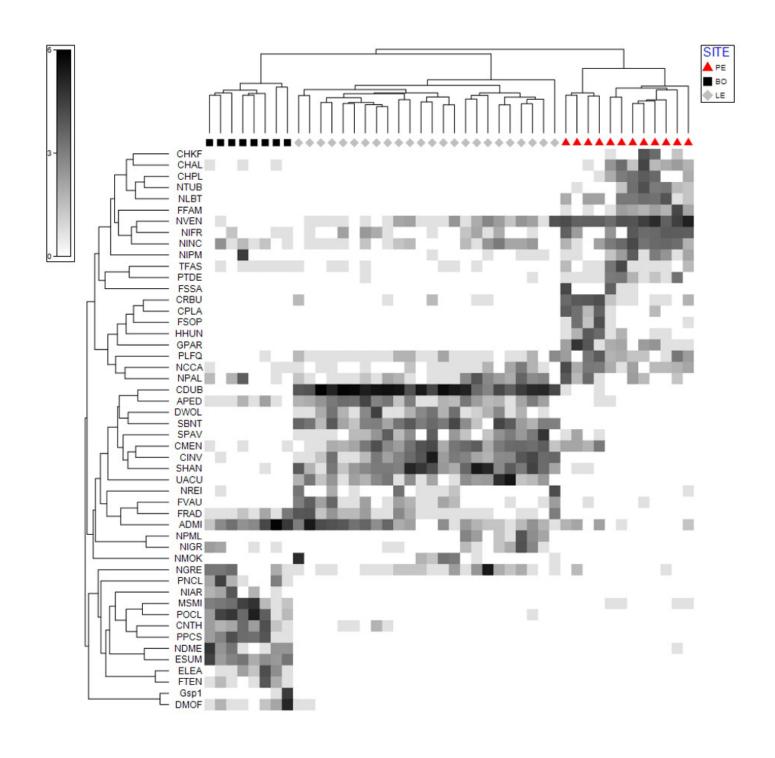
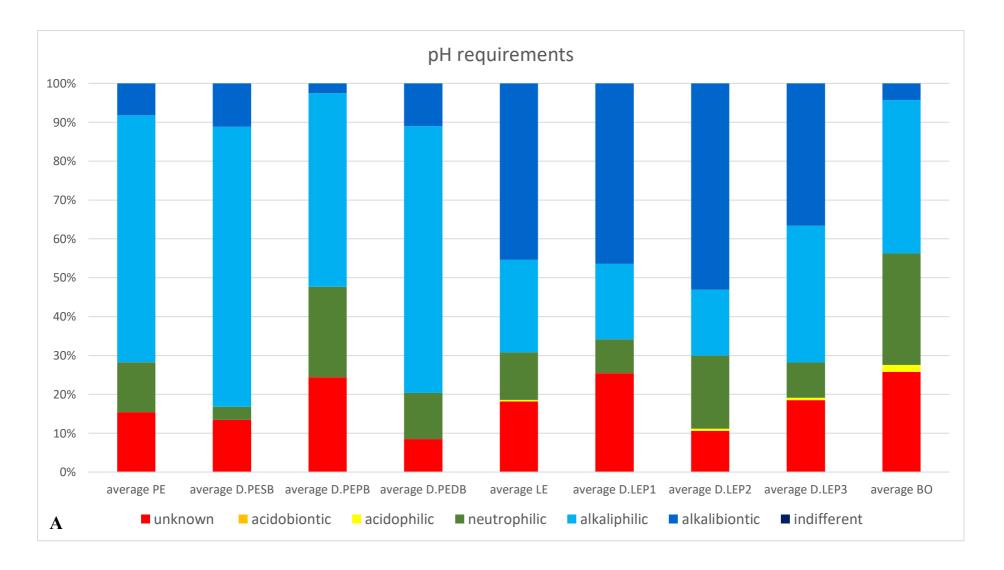


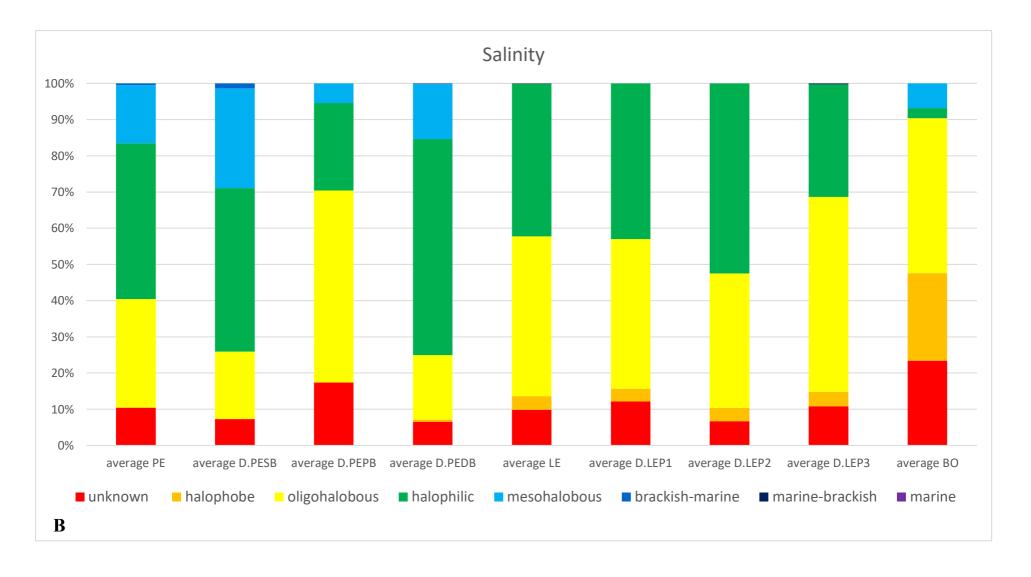


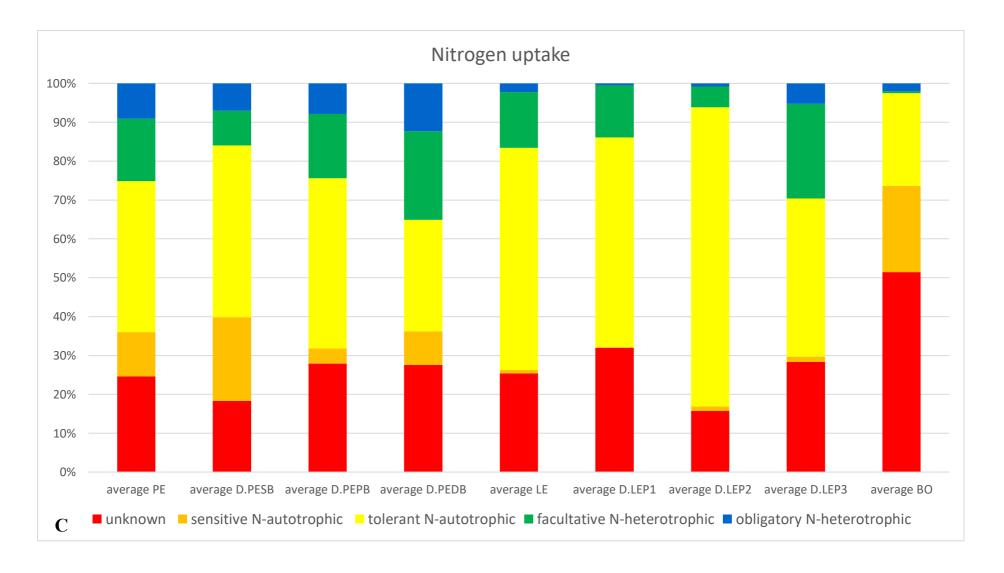
Figure 5(on next page)

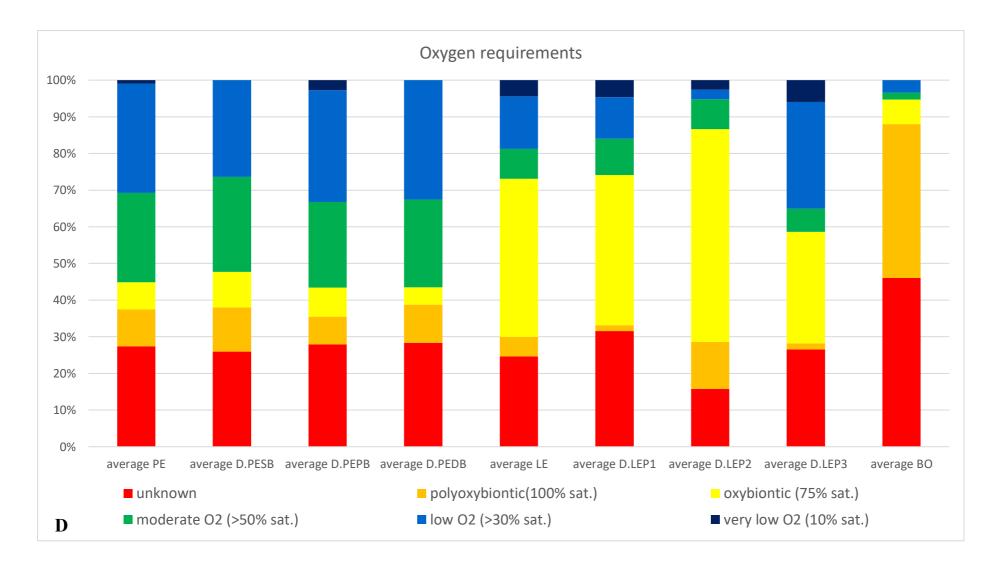
Average percentages of diatom species in individual classes of ecological values according to Van Dam et al. 1994 based on OMNIDIA 6.0.6 software.

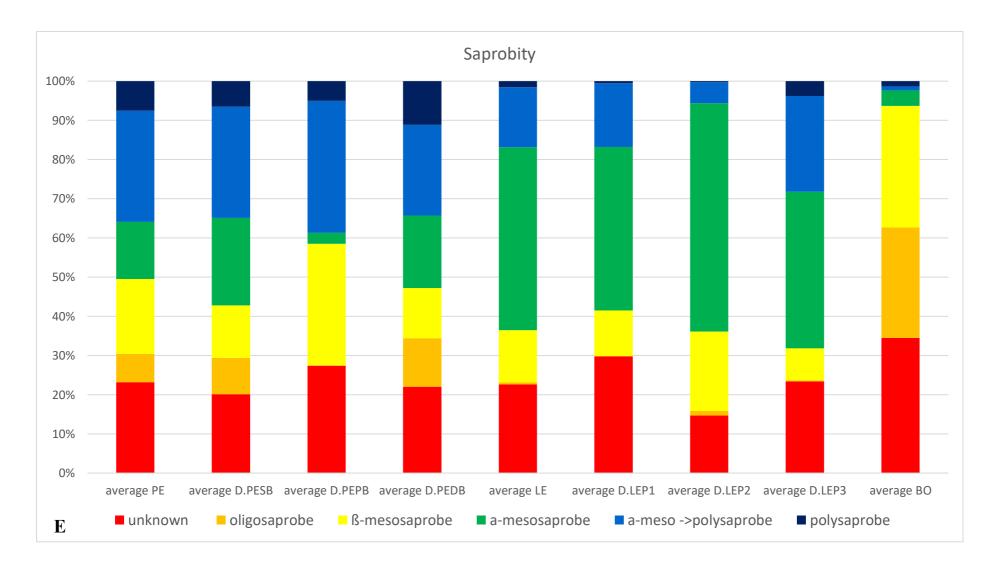
A. pH requirements. B. Salinity. C. Nitrogen uptake. D. Oxygen requirements. E. Saprobity. F. Trophic state. G. Moisture aerophily

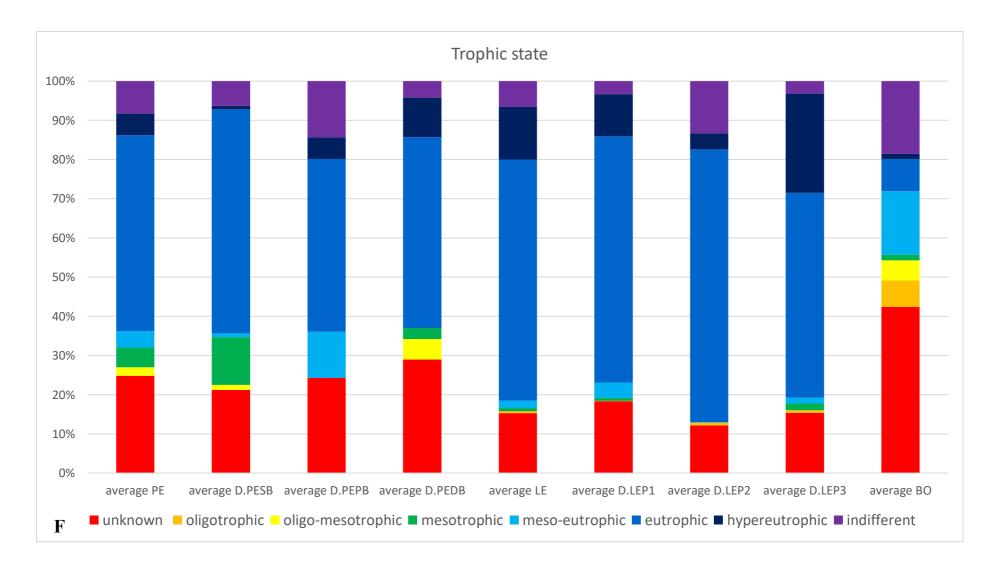


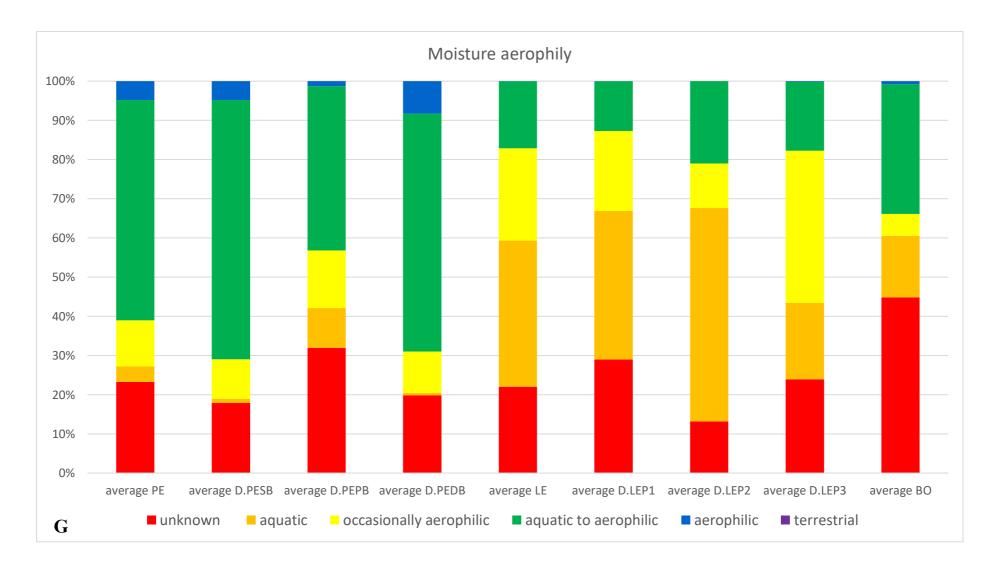






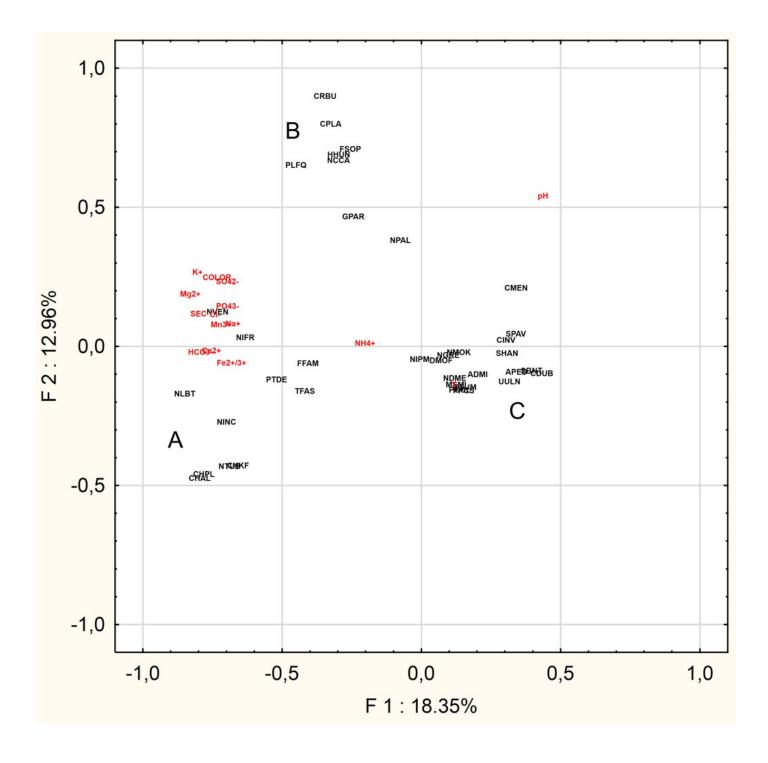








PCA analysis. The diagram presents three groups of species A, B and C, whose occurrence can be correlated with selected physical and chemical factors

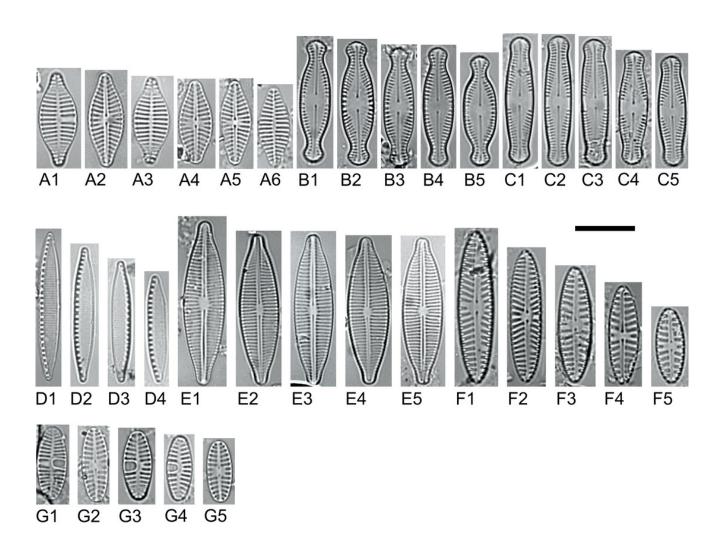




LM microphotographs of characteristic species for Pełczyska

A1-A6. Planothidium delicatulum. B1-B5. Chamaepinnularia krookiformis. C1-C5. Chamaepinnularia plinskii. D1-D4. Nitzschia liebethruthii. E1-E5. Craticula buderi. F1-F5. Navicula cincta. G1-G5. Planothidium frequentissimum. Scale bar = 10 µm





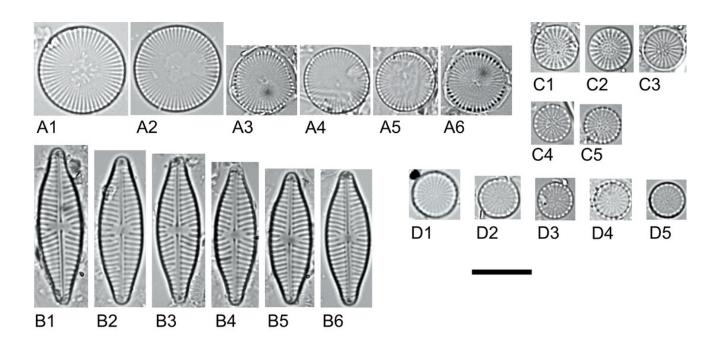


LM microphotographs of characteristic species for Łęczyca

A1-A6. Cyclostephanos invisitatus. B1-B6. Navicula moskalii. C1-C5. Stephanodiscus binatus.

D1-D5. Stephanodiscus parvus. Scale bar = $10 \mu m$







LM microphotographs of characteristic species for Bogdałów

A1-A6. Diatoma moniliformis. B1-B6. Encyonopsis subminuta. C1-C7. Mastogloia smithii (C5-C7. Same specimen, different focal plane). D1-D5. Nitzschia dissipata var. media. E1-E7. Pantocsekiella pseudocomensis



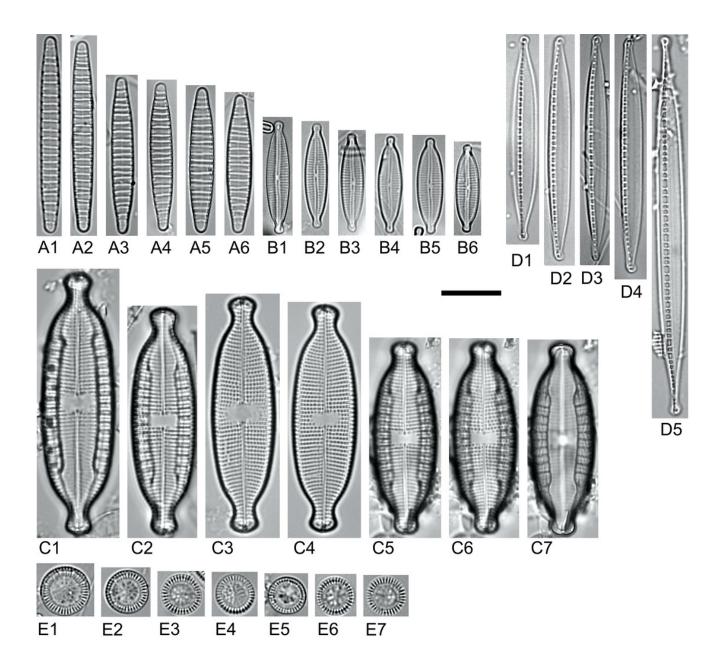




Table 1(on next page)

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

Table 1. Physical and chemical parameters in the examined habitats. The minimum, maximum and mean values.

	Pelczyska (PE)								Łeczyca (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.
pН	6,6	7,7	7,0	6,4	6,4	6,4	7,8	9,6	8,7	7,9	8,6	8,1	7,5	8,6	8,1	7,5	8,8	8,3	7,9	8,3	8,1
SEC [µs]	4450	9230	6699	5170	5170	5170	2645	5150	3646	657	865	758	558	836	719	472	778	659	505	734	623
T [°c]	6,8	13,9	9,1	7,8	7,8	7,8	1,1	17,3	7,1	1,6	20,6	10,3	0,6	21,1	10,3	2,2	15,2	7,8	4,7	22,7	11,4
НСО3-	345	744	475	610	610	610	284	451	352	211	339	260	168	275	210	183	290	241	174	369	270
$\mathrm{CO}_2^{[\mathrm{HCO3-}]}$	124	268	171	220	220	220	102	163	126	76	122	94	61	99	75	66	105	87	63	133	96
Cl-	1585	2976	2426	1006	1006	1006	685	1524	1053	57	93	78	60	93	76	67	106	78	41	52	45
N_{NH4}	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⁺	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-	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}	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 -	176	198	188	165	165	165	151	197	176	71	147	107	67	122	101	63	91	75	110	147	123
S_{SO4}	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	25	160	81	140	140	140	50	120	78	10	60	27	9	60	27	12	60	31	4	10	6
Mn ³⁺	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
$Fe^{2+/3+}$	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+}	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⁺	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 ⁺	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 ⁺	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



Table 2(on next page)

Species of diatoms in the studied hydrological objects and index values assigned to them according to Van Dam et al. 1994, on the basis of OMNIDIA 6.0.6 data



Table 2. Species of diatoms in the studied hydrological objects and index values assigned to them according to Van Dam et al. 1994, on the basis of OMNIDIA 6.0.6 data.

Species	Code	Moisture aerophity	Nitrrogen 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

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Table 3(on next page)

Percentage of characteristic species in the unknown (total) class according to Van Dam et al. (1994) at each sample point

Percentage of individual characteristic species constituting at least 10% of the 'unknown' class at each sampling point

Table 3. Percentage of characteristic species in the unknown (total) class according to Van Dam et al. (1994) at each sample point. Percentage of individual characteristic species constituting at least 10% of the 'unknown' class at each sampling point.

	рН	Salinity	Nitrogen uptake	Oxygen requirements	Saprobity	Trophic state	Moisture aerophily
	total 33%	total 44%	total 34%	total 48%	total 30%	total 22%	total 43%
D.PESB.	CHPL (19%) NCCA (11%)	CHPL (33%) CHKF (10%)	CHPL (11%) NCCA (10%)	PTDE (25%)	CHPL (11%)	CHPL (10%)	PLFQ (20%)
	total 92%	total 25%	total 82%	total 82%	total 80%	total 74%	total 85%
D.PEDB.	CHPL (60%) NCCA (32%)	CHPL (25%)	NLBT (37%) CHPL (25%) CHKF (16%)	NLBT (37%) CHPL (16%) CHKF (16%)	NLBT (43%) CHPL (32%)	NLBT (365) CHPL (23%) CHKF (15%)	NLBT (49%) CHPL (285)
	total 81%	total 73%	total 78%	total 78%	total 79%	total 60%	total 84%
D.PEPB.	CRBU (53%) NCCA (27%)	CRBU (73%)	CRBU (46%) NCCA (25%)	CRBU (46%) NCCA (25%)	CRBU (46%) NCCA (26%)	CRBU (54%)	CRBU (41%) NCCA (23%) PLFQ (14%)
D.LEP1.	total 76%	total 79%	total 67%	total 68%	total 71%	total 54%	total 78%
	CINV (36%) SBNT (23%) NMOK (16%)	SBNT (56%) NMOK (21%)	CINV (29%) SBNT (19%) NMOK (14%)	CINV (29%) SBNT (19%) NMOK (14%)	CINV (31%) SBNT (20%) NMOK (14%)	SBNT (36%) NMOK (17%)	CINV (32%) SBNT (22%) NMOK (14%)
D.LEP2.	total 39%	total 44%	total 35%	total 35%	total 28%	total 23%	total 44%
	SBNT (27%) CINV (11%)	SBNT (44%)	SBNT (18%)	SBNT (18%)	SBNT (20%)	SBNT (23%)	SBNT (22%) SPAV (11%) CINV (11%)
D.LEP3.	total 57%	total 41%	total 50%	total 53%	total 60%	total 29%	total 57%
	SBNT (25%) CINV (24%)			SBNT (17%) CINV (16%) SPAV (14%)	SBNT (20%) CINV (18%) SPAV (15%)	SBNT (29%)	SBNT (20%) CINV (17%) SPAV (15%)
	total 33%	total 36%	total 47%	total 44%	total 38%	total 47%	total 38%
D.BOZB.	PPCS (22%) DMOF (10%)	PPCS (25%) DMOF (11%)	MSMI (13%) PPCS (10%)	MSMI (14%) PPCS (11%) NDME (10%)	PPCS (18%) NDME (11%)	MSMI (15%) PPCS (12%) NDME (11%)	PPCS (13%)