

## Diatom communities of the Kisielina River: Assessment of water quality based on diatom indices

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### ABSTRACT

The aim of the study was to investigate diatom communities in the Kisielina River and assess water quality based on selected diatom indices: SPI, GDI, TDI, and %PT. The Kisielina River is a right-site tributary of the Vistula River, with a length of 42.34 km. During two-season studies conducted in 2023, the waters of Kisielina were characterized by neutral or slightly acidic pH and low to medium values of conductivity. A total of 600 diatom taxa were identified at 7 sites along the river's course, 44 of which were classified as dominant species. At most sites, the water was characterized by moderate ecological status (Class III) based on the SPI and GDI indices. In September, the index values slightly deteriorated (poor ecological status, Class IV), especially in the upper and near-mouth sections. The trophic index at most sites indicated eutrophic conditions. In Kisielina, alkaliphilous and pH-neutral diatoms dominated, as well as  $\beta$ - and  $\alpha$ -mesosaprobious species (Class II and III) and eutrophic or wide-spectrum trophic species (oligo- to eutraphentic).

**Keywords:** generic diatom index, ecological status, trophic index, flowing waters, diatom indices.

### INTRODUCTION

The water resources have always been regarded as essential for human life, as well as for industrial and economic purposes. The increase in population and the increasingly rapid pace of civilization development have led to the degradation of many river ecosystems. Various forms of pollution continue to enter rivers, either directly or indirectly, and the waters have become recipients of wastewater, both treated and untreated, including from households.

The ongoing population explosion increasingly burdens the environment, with aquatic ecosystems being particularly vulnerable to pollution in this regard. In response, the European Union has taken concrete steps to improve the state of waters and limit further degradation. As a result, the Water Framework Directive (WFD, 2000/60/EC) was introduced in 2000, changing

the approach to water protection and management. Surface waters began to be regarded in environmental terms, recognizing that they serve as habitats for various plant and animal species, including many endangered and ecologically valuable taxa, as well as key species. According to the Water Framework Directive, phytobenthos is one of the elements that must be obligatory considered when classifying the ecological status of surface water bodies. Among algae, diatoms are the most preferred group, dominating phytobenthos in terms of species number (Kawecka, Eloranta, 1994, Rakowska, 2001, Ruszczyńska, Picińska-Fałtynowicz, 2013). In assessing water quality, it is important to differentiate between species that are sensitive, tolerant, and resistant to pollution, along with their quantitative contribution (Rakowska, 2001, Bąk et al., 2012). Currently, monitoring studies using diatoms are being conducted on a large scale in many countries. Special

computer programs (e.g., OMNIDIA) are being developed, containing ecological and taxonomic databases based on indicator values and the sensitivity levels of individual taxa to pollutants (Lecointe et al., 1993). Such monitoring studies are being conducted in many countries, both in Europe and worldwide (e.g., CEMAGREF 1982, Coste, Ayphassorho, 1991, Prygiel, Coste, 1993, Kelly, Whitton, 1995, Kelly et al., 1995, 2008, 2018, Eloranta, 1999, Prygiel, 2002, Blanco et al., 2007, 2008, Stenger-Kovács et al., 2007, Álvarez-Blanco et al., 2013, Poikane et al., 2016, Karaouzas et al., 2019, Solak et al., 2020, Dalu et al., 2020, Masouras et al., 2021, Mbaio et al., 2022, Blanco, 2024). In Poland as well, benthic diatoms have been used for many years in water quality assessment (e.g., Kwandrans et al., 1998, Kawecka, Kwandrans, 2000, Bogaczewicz-Adamczak et al., 2001, Rakowska, 2001, Bogaczewicz-Adamczak, Dziengo, 2003, Żelazowski et al., 2004, Dumnicka et al., 2006, Rakowska, Szczepocka, 2011, Noga et al., 2013a,b,c, 2015, 2016, Szczepocka et al., 2019).

In 2003, during studies on the ichthyofauna composition and the ecological status of the Vistula River and its tributaries, ichthyological studies were conducted at three sites in the Kisielina River, and diatom analyses were performed at only one site, located near the lower section of river (Dumnicka et al., 2006).

The aim of this study was to examine the diatom communities occur in the Kisielina River (at seven sites designated from the source to the lower section) and to assess the water quality based on the identified taxa and their assigned indicative values (diatom indices SPI, GDI, TDI, %PT).

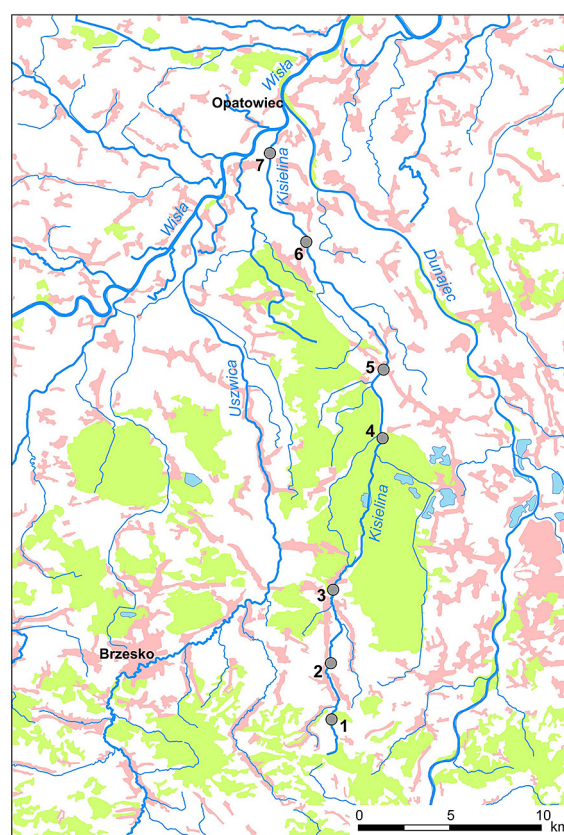
## STUDY AREA

The Kisielina River flows in northeastern Małopolska and is a right-bank tributary of the Vistula River, with a length of 42.34 km and a catchment area of 166.2 km<sup>2</sup>. Its source is located on the northern slopes of Wilkówka Hill, at an elevation of 340.7 m above sea level, near the villages of Łysa Góra and Grabno. It flows into the Vistula at the 160th kilometer of the river's course, in the village of Nowopole, at an elevation of 170.6 m a.s.l. It belongs to the category of small lowland streams of the 17th abiotic type.

Until the beginning of the 20th century, the Kisielina River was not a direct tributary of the

Vistula River but flowed into the Dunajec River, which is a tributary of the Vistula. During regulatory works at the start of the 20th century, the course of the river was altered, redirecting its mouth to the Vistula River. As a result, the river's channel was significantly shortened and regulated. In the stretch of the river near the villages of Brzeźnica and Wola Radłowska, wooden piles can still be found in the riverbed, remnants of these regulatory works. Until 1997, 50% of the river in the Radłów municipality was embanked. A severe flood in 1997, which caused widespread flooding in the Kisielina River valley, led to the decision to further regulate the river and construct new embankments. Between 2002 and 2005, the river underwent partial channel regulation along the Sufczyn–Biadolinie Szlacheckie section. Currently, the river is also embanked in the villages of Wola Radłowska, Wał Ruda, Zabawa, and Przybysławice (Przybysz-Lawnicka et al., 2008, 2010).

The river flows mainly through rural areas, including built-up zones built-up zones, croplands, and meadows. Its sources are located in a small forested area, while in its middle course, it



**Figure 1.** The distribution of study sites with designation of land management in the Kisielina River basin; (green colour – forest areas, pink colour – built-up areas, grey point with number – study site)

passes through a large forest complex known as the Puszcza Radłowska (Fig. 1). In recent years, increased beaver (*Castos fiber*) activity has been observed in forested and wooded areas along the river valley (in the village of Brzeźnica, beaver dams have been recorded on the river) (Przybysz-Ławnicka et al., 2008, 2010).

## METHODS

Samples for the study were collected in April and September 2023 at seven research sites (Fig. 1) along the entire length of the river (from source to mouth), from all available habitat types (stone, silt, aquatic plants). Additionally, water samples for chemical analyses were taken at each site and analyzed at the Department of Soil Science, Environmental Chemistry, and Hydrology at the University of Rzeszów, using a Thermo Scientific DIONEX ICS–5000+DC ion chromatograph. In the field, water temperature, pH, and electrical conductivity were measured directly.

The laboratory processing of diatoms was carried out using methods employed by, among others, Kawecka (2012) and Noga et al. (2015). To obtain clean diatom frustules and digest organic matter, samples were macerated in chromic acid (a mixture of sulfuric acid and potassium dichromate in a 3:1 ratio), followed by washing and centrifugation using a laboratory centrifuge.

Permanent microscopic preparations were sealed in synthetic resin Pleurax. Diatom identification was performed at a magnification of 1000× using a Carl Zeiss Axio Imager A2 light microscope, employing differential interference contrast (DIC) and a planapochromatic objective with a numerical aperture of 1.4. Diatoms were identified using specialized literature: Reichardt (1999), Lange-Bertalot (2001), Krammer (2000), Lange-Bertalot et al. (2011), Houk et al. (2010, 2014, 2017), and Levkov et al. (2013, 2016), Lange-Bertalot et al., (2017).

Diatom taxa abundance was obtained by counting all frustules fields of view under the microscope until a total of approximately 300 was obtained. Species with a percentage share of 5% or more were considered dominant.

Based on the list by Van Dam and co-authors (1994), the ecological preferences of the identified diatom species were determined based on indicators: pH, trophic status, and saprobity. The compiled lists of taxa were used to calculate diatom

indices: specific pollution sensitivity index (SPI), calculated based on species, generic diatom index (GDI), calculated based on genera, trophic diatom index (TDI), and percentage pollution tolerant valves (%PT), using the specialized software OMNIDIA, version 4.2 (Lecointe et al., 1993), which also contains ecological and taxonomic databases (Prygiel, Coste, 1993). The SPI and GDI indices range from 1 to 20 (the higher the index value, the better the water quality), while the TDI index ranges from 1 to 100 (the higher the index value, the worse the water quality). The PT index ranges from 1 to 100%. Values of the index > 20% indicate the possibility of slight organic pollution in the water, while values exceeding 60% indicate strong organic pollution and eutrophication (CEMAGREF, 1982, Coste, Ayphassorho, 1991, Kelly, Whitton, 1995, Kelly et al., 2001). The range of ecological water quality classes and their corresponding ecological and trophic status was adopted from Żelazowski et al. (2004) and Dumnicka et al. (2006), and was also applied in the work by Noga et al. (2016). Statistical calculations with graphical interpretation were performed in Canoco program (version 5.03) based on dominant taxa.

To examine the differences between sites and habitats in two research seasons, a DCA (Detrended Correspondence Analysis) was performed. This analysis was chosen due to the gradient length (3.7 SD). Subsequently, an RDA (Redundancy Analysis) was conducted to analyze the influence of physicochemical parameters on the diversity and development of diatom communities in the Kisielina River at the various sites (with a gradient length of 1.6 SD). The statistical significance of the canonical axes was determined using the Monte Carlo test (Ter Braak, Šmilauer, 2012). Data were considered statistically significant if the p-value was below 0.05.

## RESULTS

The waters of the Kisielina River were characterized by neutral or slightly acidic reaction (pH 6.2–7.2) and low to moderate values of electrolyte content (115–320 µS/cm). Most measured chemical parameters indicated very good chemical status of the waters (I quality class), except for NO<sup>3-</sup>, which corresponded to good chemical status (most often in April 2023) or below good chemical status (mainly in September 2023). The



highest  $\text{NO}_3^-$ -values (7.37–8.09 mg/l) were consistently found at site 3.

In the upper section of the Kisielina River (sites 1–3), the waters exhibited the highest values for most of the studied parameters, with sites 2 and 3 showing the greatest divergences from the standards corresponding to class I water quality, especially in September 2023 (Table 1).

During the studies conducted in the Kisielina River at seven sites in 2023, a total of 600 diatom taxa were identified. Each site had between 115 and 274 taxa. A total of 44 diatom taxa were classified as dominant ( $> 5\%$ ), with 23 identified in April and 34 in September. The species that dominated in both research seasons were: *Achnanthes minutissimum*, *Amphora pediculus*, *Cyclotella choctawhatcheeana*, *Mayamaea perminis*, *Meridion circulare*, *Navicula gregaria*, *N. lanceolata*, *Nitzschia inconspicua*, *N. linearis*, *Planorbulina mediterranensis*, *Rhoicosphenia abbreviata*, and *Surirella lacrimula*. The most numerous populations in the assemblage included *Cocconeis euglypta* (87.7% in September at site 6), *Achnanthes minutissimum* ( $> 60\%$  in April at site 7), and *Navicula lanceolata* ( $> 50\%$  in April at site 3). The diatoms identified at each site, along with their abundance values, were entered into the OMNIDIA software to calculate the values of the SPI, GDI, TDI, and %PT indices, which were used to assess the ecological and trophic status of the waters in the Kisielina River (Table 2). At most sites, the water was

characterized by a moderate ecological status (Class III) based on the SPI and GDI indices. In September, the index values slightly decreased, indicating more frequently a poor ecological status (Class IV), especially in the upper section (sites 1–4) and the mouth (site 7). The highest index values, indicating a good ecological status (Class II), were recorded in April at site 7 (based on the SPI and GDI indices) and at site 1 (based on the GDI index) – Table 2.

At most of the study sites, based on the TDI index, a trophic status corresponding to eutrophication was observed. Only the upstream section (site 1) and the downstream section (site 7) exhibited low TDI index values ( $< 40$ ) in April 2023, indicating an oligotrophic/mesotrophic status. The highest TDI index values were recorded in September at site 2 and in April at site 3 ( $> 75$ ), indicating a hypereutrophic trophic status (Table 2). The PT index, which determines the percentage of taxa tolerant to organic pollution, was below 20% at most sites. Values exceeding 20%, indicating slight organic pollution, were noted in September at sites 1 and 2 (22% and 28%, respectively), as well as in April at site 4 (25.5%) (Table 2). Based on the analyses of diatom communities, the ecological preferences regarding three parameters: reaction, trophic status, and saprobity were assessed according to the classification by Van Dam et al. (1994). At most of the studied sites, alkaliphilic taxa predominated, occurring most abundantly ( $> 70\%$ ) at sites 2 and 3 in both

**Table 1.** Physico-chemical parameters on studied sites (1–7) in the Kisielina River (April and September 2023); T – temperature, C – conductivity.

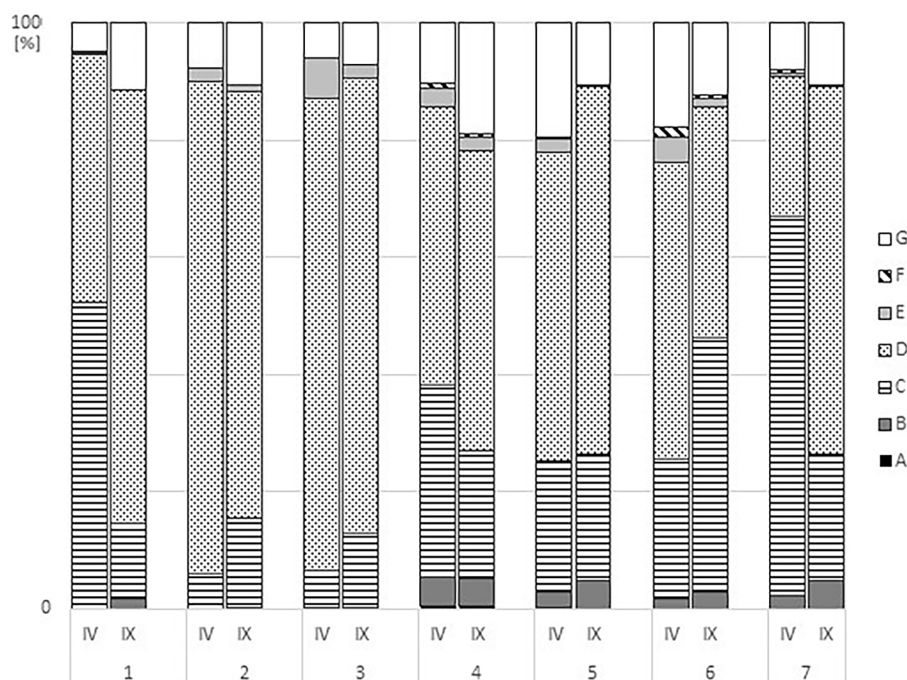
| Site | Date  | T [°C] | pH  | C [ $\mu\text{S}/\text{cm}$ ] | Cl <sup>-</sup> [mg/l] | $\text{SO}_4^{2-}$ [mg/l] | $\text{NO}_3^-$ [mg/l] | $\text{NH}_4^+$ [mg/l] | $\text{Mg}^{2+}$ [mg/l] | $\text{Ca}^{2+}$ [mg/l] |
|------|-------|--------|-----|-------------------------------|------------------------|---------------------------|------------------------|------------------------|-------------------------|-------------------------|
| 1    | 04.23 | 2.6    | 6.2 | 245                           | 13.50                  | 35.47                     | 4.32                   | 0.04                   | 6.08                    | 38.84                   |
|      | 09.23 | 15.0   | 7.2 | 232                           | 18.17                  | 37.74                     | 2.31                   | 0.07                   | 7.24                    | 47.30                   |
| 2    | 04.23 | 1.5    | 6.6 | 273                           | 20.25                  | 37.66                     | 5.69                   | 0.19                   | 8.68                    | 37.92                   |
|      | 09.23 | 15.2   | 7.2 | 320                           | 21.80                  | 38.22                     | 4.52                   | 0.10                   | 10.74                   | 35.88                   |
| 3    | 04.23 | 2.5    | 6.7 | 249                           | 17.91                  | 36.05                     | 8.09                   | 0.20                   | 7.96                    | 32.10                   |
|      | 09.23 | 14.1   | 7.1 | 295                           | 28.94                  | 45.63                     | 7.37                   | 0.15                   | 11.86                   | 34.44                   |
| 4    | 04.23 | 4.5    | 6.7 | 115                           | 13.27                  | 40.32                     | 3.61                   | 0.08                   | 4.46                    | 21.07                   |
|      | 09.23 | 14.7   | 7.2 | 163                           | 18.52                  | 25.61                     | 3.30                   | 0.09                   | 6.60                    | 25.24                   |
| 5    | 04.23 | 5.0    | 6.6 | 153                           | 14.57                  | 31.53                     | 4.25                   | 0.05                   | 5.05                    | 24.99                   |
|      | 09.23 | 15.1   | 6.9 | 170                           | 11.56                  | 22.47                     | 2.69                   | 0.11                   | 3.59                    | 16.36                   |
| 6    | 04.23 | 4.6    | 6.6 | 160                           | 11.79                  | 22.43                     | 3.80                   | 0.02                   | 3.42                    | 16.70                   |
|      | 09.23 | 15.4   | 6.8 | 165                           | 14.44                  | 28.58                     | 2.83                   | 0.14                   | 5.03                    | 25.88                   |
| 7    | 04.23 | 6.5    | 6.8 | 140                           | 10.55                  | 20.33                     | 2.95                   | 0.04                   | 3.22                    | 17.01                   |
|      | 09.23 | 16.0   | 7.0 | 180                           | 16.35                  | 32.09                     | 2.75                   | 0.16                   | 5.43                    | 26.51                   |

**Table 2.** The values of diatomaceous indices IPS, GDI, TDI and %PT calculated for individual sites in the Kisielina River (1–7) in two studied seasons 2023 (the index values were assumed according to Dumnicka et al., 2006)

| Site  | Date         | IPS               | GDI         | TDI       | %PT          |
|---|--------------|-------------------|-------------|-----------|--------------|
| 1   | 04.23        | 13.8              | 14.2        | 37.3      | 7.3          |
|   | 09.23        | 10.3              | 10.9        | 71.0      | 22.0         |
| 2   | 04.23        | 13.7              | 10.2        | 67.5      | 6.7          |
|   | 09.23        | 9.0               | 10.0        | 78.2      | 28.0         |
| 3   | 04.23        | 13.6              | 12.3        | 75.9      | 16.0         |
|   | 09.23        | 10.2              | 12.3        | 69.6      | 14.7         |
| 4   | 04.23        | 12.3              | 12.8        | 65.5      | 25.5         |
|   | 09.23        | 11.6              | 11.2        | 62.3      | 15.7         |
| 5   | 04.23        | 12.0              | 11.6        | 65.1      | 18.4         |
|   | 09.23        | 12.1              | 12.0        | 70.0      | 16.5         |
| 6   | 04.23        | 12.9              | 12.3        | 63.2      | 11.7         |
|   | 09.23        | 13.8              | 13.8        | 49.0      | 13.0         |
| 7   | 04.23        | 15.1              | 14.9        | 38.1      | 13.2         |
|   | 09.23        | 10.2              | 12.3        | 69.6      | 14.7         |
| Legend  |              |                   |             |           |              |
| Ecological and trophic status and water quality classes | High         | Good              | Moderate    | Poor      | Bad          |
|   | Oligotrophic | Oligo/mesotrophic | Mesotrophic | Eutrophic | Hypertrophic |
|   | I            | II                | III         | IV        | V            |

research seasons, as well as at site 1 in September. The second most numerous group consisted of pH-neutral diatoms, which developed most abundantly (>50%) at sites 1 and 7 in April (Fig. 2).

Most of the studied sites were characterized by a large development of eutrophic diatoms, which often dominated the community (e.g., at site 3 in September, they constituted as much as

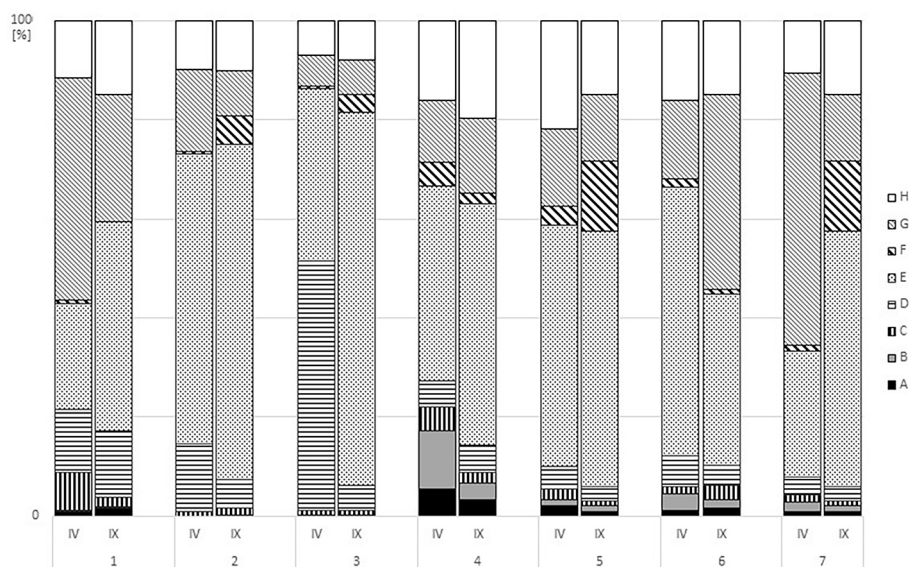
**Figure 2.** Classification of ecological indicator values (according to Van Dam et al., 1994) on studied sites (1–7), in two studied seasons (IV – April, IX – September) in 2023; pH range: A – acidobiontic, B – acidophilous, C – neutral, D – alkaliphilous, E – alkalibiontic, F – indifferent, no apparent optimum, G – unknown

75%). In the lower section of the Kisielina River (sites 6 and 7) and in the headwaters (site 1), there were numerous taxa ranging from oligo- to eutraphentic, indicating a wide trophic spectrum. In April, the dominant group at site 3 consisted of meso-eutraphentic taxa (Fig. 3).

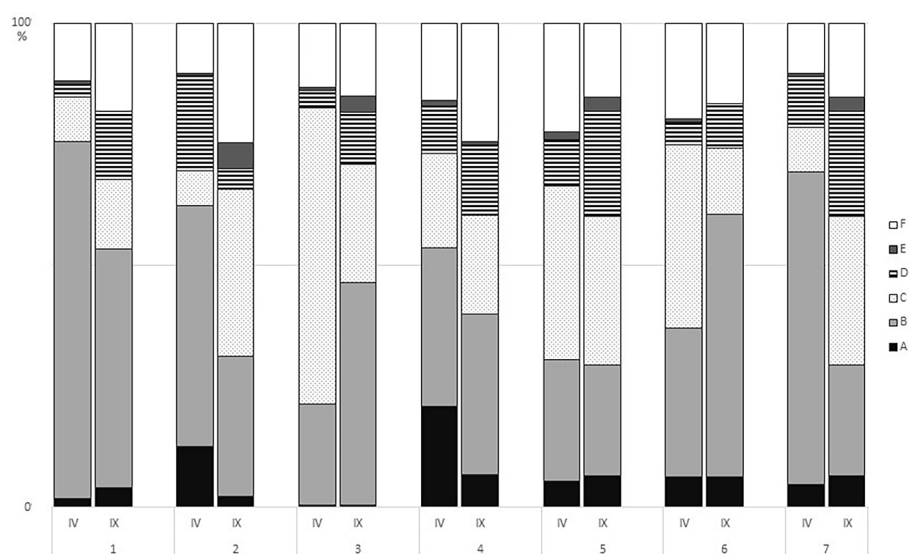
The saprobic parameter indicated that the most prevalent diatoms in the waters of the Kisielina were  $\beta$ -mesosaprobic taxa, which were most numerous (> 50%) in the upper and lower

sections of the river (sites 1, 2, 6, and 7). A significant group also consisted of  $\alpha$ -mesosaprobic diatoms, which were most abundant (> 35%) in April at sites 3, 5, and 6. In April, oligosaprobic diatoms developed significantly (> 20%) at site 4, making them the second most numerous group in the assemblage (Fig. 4).

The statistical analysis conducted using Canoco software was based on two methods: DCA and RDA. The DCA analysis allowed for the



**Figure 3.** Classification of ecological indicator values (according to Van Dam et al., 1994), on studied sites (1–7), in two studied seasons (IV – April, IX – September) in 2023; Trophic state range: A – oligotraphentic, B – oligo-mesotraphentic, C – mesotraphentic, D – meso-eutraphentic, E – eutraphentic, F – hypereutraphentic, G – oligo- to eutraphentic, H – unknown



**Figure 4.** Classification of ecological indicator values (according to Van Dam et al. 1994), on studied sites (1–7), in two studied seasons (IV – April, IX – September) in 2023; Saprobity range: A – oligosaprobous, B –  $\beta$ -mesosaprobous, C –  $\alpha$ -mesosaprobous, D –  $\alpha$ -meso-polysaprobous, E – polysaprobous, F – unknown.

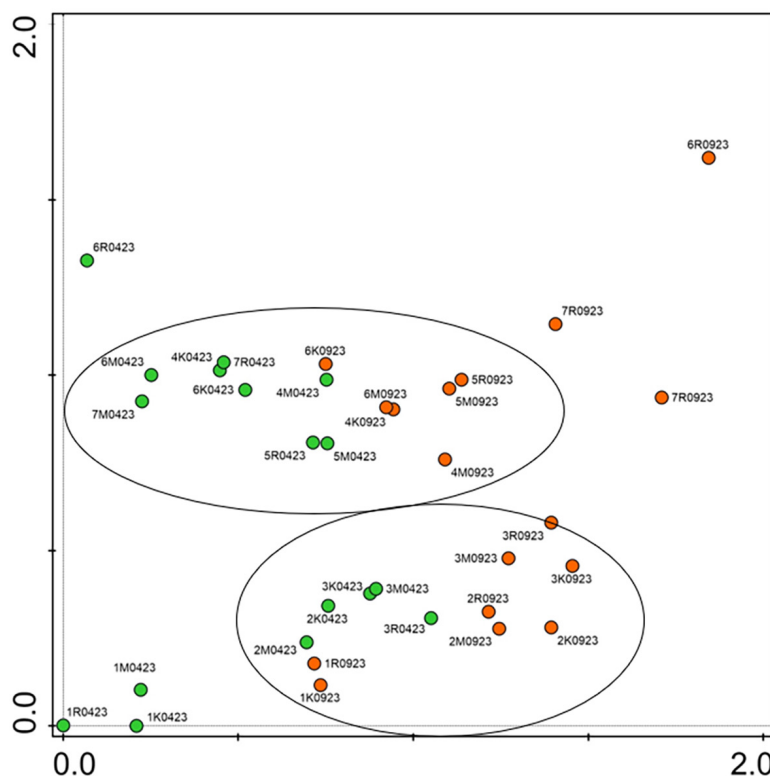
identification of two groups of sites based on the dominance structure in different habitat types (stones, silt, plants). The first group (located in the middle part of the graph) included assemblages from all habitats collected from sites 4 and 5 in both research seasons, as well as additional samples from sites 6 and 7 (excluding most assemblages developing on plants). Sites 4 and 5 consistently exhibited the highest species richness of diatoms. The second group comprised communities from all habitat types developing at sites 2 and 3, along with additional samples from site 1 during the autumn season (Fig. 5).

Statistical analyses conducted using the RDA method revealed statistically significant correlations between environmental factors and the structure of diatom assemblages ( $F = 1.3$ ,  $p = 0.016$ ). The RDA analysis showed that the first axis explained 29.2% of the fitted variation, two axes explained 55.8%, three axes accounted for 72.2%, and four axes explained 84.5%. The most statistically significant influence on the differentiation of diatom assemblages was attributed to  $\text{Cl}^-$  ions ( $p = 0.002$ ), explaining 16.5% of the variability, followed by conductivity ( $p = 0.002$ ; 15.6%) and  $\text{Ca}^{2+}$  ( $p = 0.002$ ; 15.5%). The influence of reaction,

temperature, and sulfate ions on the differentiation of diatom assemblages was smaller ( $p = 0.016$ – $0.05$ ; 11.2–12.4%), but these factors were also statistically significant. Temperature showed a very strong correlation with pH ( $r = 0.82$ ) – this parameter correlated with the occurrence of, among others: *Navicula erifuga* (NAER), *N. cryptotenella* (NACR), *N. tripunctata* (NATR), and to a lesser extent *Rhoicosphenia abbreviata* (RABB), *Sellaphora atomoides* (SATO), and *Navicula gregaria* (NAGR). Conductivity also demonstrated a strong correlation with  $\text{Cl}^-$  ions ( $r = 0.77$ ) and  $\text{SO}_4^{2-}$  ( $r = 0.63$ ), which in turn correlated with the occurrence of *Amphora pediculus* (AMPE) and, to a lesser extent, *Navicula trivialis* (NTRI) – Figure 6.

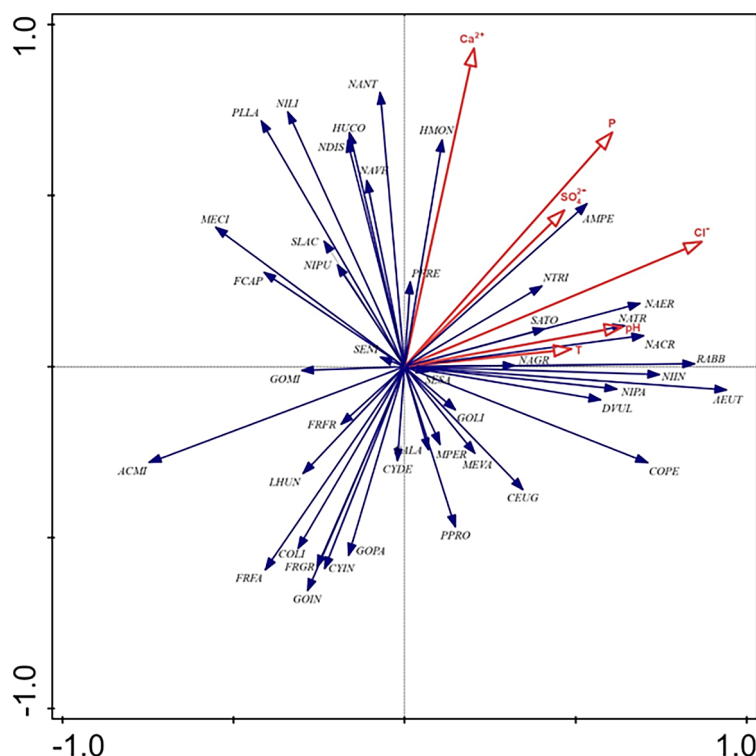
## DISCUSSION

Due to the continuous decline in the quality of surface waters in Europe, the European Parliament established the Water Framework Directive (WFD) (2000/60/EC). EU Member States (MS) were obligated to achieve “good ecological status” and “good chemical status” for surface



**Figure 5.** DCA analysis diagram of the arrangement of sites based on species similarity of dominant diatom assemblages; green – samples collected in spring (04.23), orange – samples collected in autumn (09.23), K – stone, R – plant, M – mud





**Figure 6.** RDA diagram showing the influence of physicochemical parameters on the occurrence of dominant diatom assemblages; (abbreviation designations – ACMI: *Achnanthes minutissimum*, AEUT: *A. eutrophilum*, AMPE: *Amphora pediculus*, CEUG: *Cocconeis euglypta*, COLI: *C. lineata*, COPE: *C. pediculus*, CYIN: *Cyclotella invisitata*, CYDE: *C. delicatus*, DVUL: *Diatoma vulgare*, FCAP: *Fragilaria capucina*, FRFA: *F. famelica*, FRFR: *F. fragilarioides*, FRGR: *F. gracilis*, GOLI: *Gomphonella olivacea*, GOIN: *Gomphonema innocens*, GOMI: *G. micropus*, GOPA: *G. parvulum*, HALA: *Halophora montana*, HUCO: *Humidophila contenta*, LHUN: *Lemnicola hungarica*, MPER: *Mayamaea peris*, MEVA: *Melosira varians*, MECI: *Meridion circulare*, NAGR: *Navicula gregaria*, NALA: *N. lanceolata*, NANT: *N. antonii*, NACR: *N. cryptotenella*, NAER: *N. erifuga*, NATR: *N. tripunctata*, NTRI: *N. trivialis*, NVEN: *N. veneta*, NIIN: *Nitzschia inconspicua*, NILI: *N. linearis*, NDIS: *N. dissipata*, NPAL: *N. palea*, NIPU: *N. pusilla*, PFRE: *Planorhynchus frequentissimus*, PLLA: *P. lanceolatus*, PPRO: *Prestauroneis protractoides*, RABB: *Rhoicosphenia abbreviata*, SATO: *Sellaphora atomoides*, SENA: *S. nigrii*, SESA: *S. saugeresii*, SLAC: *Surirella lacrimula*)

waters by 2015. However, it turned out that this goal was difficult to achieve for many water bodies, and as a result, the European Commission extended the deadline to 2027 or even beyond (European Commission, 2000, 2012). The assessment of ecological status aims at an integrated evaluation of waters, based on biological, physicochemical, and hydromorphological quality. Benthic diatoms (phytobenthos) are classified, in addition to phytoplankton, macrophytes, benthic invertebrates and fish, as major biological elements (BQE). They are an excellent tool for use in water quality in rivers, including, among others, for the application of eutrophication, organic and acidification (Masouras et al., 2021, Blanco, 2024). Diatoms quickly on the effects that have been applied are triggered by development cycles in detail to macroinvertebrates and fish. Thanks

to this possibility, they can be detected by warnings, triggering, among others, the detection of residue growth (Mbao et al., 2022). Poland has implemented methods for assessing the quality of rivers based on diatom phytobenthos and they are used in the monitoring of surface waters within the framework of the State Environmental Monitoring. The current method for classifying the ecological status of flowing waters is the Multimetric Diatom Index for Rivers (IO). It is used to classify both natural streams and dammed reservoirs of all abiotic types. Biological methods for assessing the ecological status of waters have been used since 2007, and the first results obtained showed that in 2007–2009 only 19.1% of rivers met the most important requirements of the WFD (very good and good ecological status). The majority of the studied river sections in Poland



were classified at that time as moderate ecological status (Błachuta, Picińska-Fałtynowicz, 2010, Picińska-Fałtynowicz, Błachuta, 2010, Ciecierska, Dynowska, 2013, Zgrundo et al., 2020).

Currently, the most well-known tool (based on diatoms) used for water quality assessment and biomonitoring is the OMNIDIA software. This program contains information on the tolerance of individual diatom taxa to environmental parameters and is constantly updated and expanded with new data on diatoms (Lecointe et al. 1993, Szczepocka, Żelazna-Wieczorek, 2018, Masouras et al., 2021).

The assessment of the ecological status of flowing waters in Europe is often carried out based on diatom indices such as SPI, GDI and TDI. These indices are well suited for water assessment, especially the SPI index, which responds best to water quality fluctuations. It is one of the most commonly used diatom-based indices, both in European countries and on other continents. Although SPI was originally prepared based on diatom materials collected from rivers in Central Europe, it is successfully used all over the world. SPI provides a realistic assessment of water quality, taking into account organic pollutants, eutrophication and salinity (CEMAGREF 1982, Prygiel, Coste, 1993, Triest et al., 2012, Szczepocka, Żelazna-Wieczorek, 2018, Blanco, 2024). SPI is also best suited for assessing the quality of surface waters in Poland (Żelazowski et al., 2004, Noga et al., 2013a,b,c, 2014, 2015, Szczepocka et al., 2014, Szczepocka, Żelazna-Wieczorek, 2018).

The Kisielina River is characterized by a large diversity of habitats, because on a section of over 40 km it flows through urbanized areas, meadows, farmlands, a large forest complex, the so-called Radłowska Forest, and also near a large-scale gravel mine. In addition, the marshes and water bodies occurring in the forest areas are conducive to the settlement of beavers. All this together means that the river also contains species of standing waters, especially the numerously identified centric diatoms. The Kisielina is distinguished by its enormous species richness of diatoms (600 taxa). Studies conducted in the last 15 years, including on the Biała Tarnowska (Noga et al., 2015) and Wisłoka (Noga et al., 2016) rivers, have shown that the aforementioned rivers are characterized by a much lower species richness of diatoms (205–238 taxa). Diatom communities at the sites chosen for research in the Kisielina River

didn't differ significantly in the research seasons (i.e. spring and autumn). This was also confirmed by the DCA statistical analysis, which distinguished two groups of diatoms. The first group included communities from sites 4 and 5 from all habitats and seasons and some from sites 6 and 7 (mainly from stones and mud). These were mostly sites characterized by the highest species richness of diatoms. Diatom communities from sites 2 and 3 formed the second group and were the most similar to each other. The highest values of chemical parameters were also recorded at these sites, mainly nitrates, sulphates and chlorides.

Among the dominant diatoms in the waters of the Kisielina River species of the genus *Navicula* were frequently observed, including: *N. antonii*, *N. cryptotenella*, *N. erifuga*, *N. gregaria*, *N. lanceolata*, *N. tripunctata*, *N. trivialis* and *N. veneta*. All of them belong to cosmopolitan species in Europe and very often develop in various types of standing and flowing waters. Apart from *N. cryptotenella*, all of them also prefer waters with increased trophicity and are highly tolerant to organic pollutants. *Navicula cryptotenella* develops primarily in alkaline rivers, in conditions of low trophicity and with high electrolyte content. Some of them, including *N. gregaria* and *N. erifuga*, also develop in brackish waters, or even have their optimum occurrence there, e.g. *N. erifuga* (Lange-Bertalot, 2001, Hofmann et al., 2011, Bąk et al., 2012, Lange-Bertalot et al., 2017). An often dominant in both seasons, apart from species of the genus *Navicula*, was *Achnanthes minutissimum* (especially in material collected from plants). This species very often occurs in all types of waters in Central Europe, including Poland. It's characterized by a very wide ecological amplitude, tolerates a wide range of trophic conditions (from oligo- to eutrophic) and is classified as a pioneer species (Hofmann et al., 2011, Bąk et al., 2012, Kawecka, 2012, Lange-Bertalot et al., 2017). The largest percentage share (87.7% in autumn at site 6) in the diatom community was achieved by *Cocconeis euglypta*. Less numerous, but still dominant, were *C. lineata* and *C. pediculus*. All the species mentioned occur commonly and in large numbers throughout Central Europe. Both *Cocconeis euglypta* and *C. lineata* still have poorly understood ecological requirements, due to their previous inclusion in one *C. placentula* complex. Both species develop on various substrates, i.e. stones, among mosses and vascular plants (Lange-Bertalot et al., 2017). In the Kisielina River,

they dominated in autumn, only in the material collected from plants. In autumn, the following species appeared among the dominants at the first site: *Halamphora montana*, *Humidophila contenta*, *Navicula veneta* and *Nitzschia pusilla*. They belong to aerophytic species, often eutrophic with a high electrolyte content and often developing on soils, in conditions of variable humidity (Levkov, 2009, Bąk et al., 2012, Lange-Bertalot et al., 2017). The numerous development of these species was probably caused by the almost complete drying of the river in the source section in September 2023. *Humidophila contenta*, *Halamphora montana* and *Nitzschia pusilla* were also identified in materials collected from mosses growing on the bark of tree (Rybak et al., 2018), while *H. montana* and *N. pusilla* were abundant in arable soils in southeastern Poland (Stanek-Tarkowska, Noga, 2012). In the Kisielina River, centric diatoms also dominated, including *Cyclotella meniscus* and *C. delicatus*. They occur in slowly flowing rivers (including the Danube and the Rhine), as well as in lakes, in conditions from meso- to eutrophic (Houk et al., 2014).

Diatomological studies in the Kisielina River have been conducted so far only at one site in the village of Jadowniki Mokre, in 2006 (Dumnicka et al., 2006). The results of the SPI, GDI and TDI indices obtained at that time indicated a moderate ecological status, and the PT index value was 17.1%. The waters of the Kisielina River were classified as eutrophic, with low oxygen content, belonging to the  $\beta$ - $\alpha$ -mesosaprobic zone. Among the dominant diatoms identified at that time were: *Cocconeis placentula* var. *euglypta* (currently *Cocconeis euglypta*), *Achnanthes minutissimum* and *A. rostrata* (currently *Planorbulina rostratoholarctica*).

Analyses carried out in 2023 using the OMNIDIA computer program (Lecointe et al., 1993) showed that the waters of the Kisielina River (in most of the studied sites) were characterized by moderate or poor ecological status (based on the SPI and GDI indices) and a trophic status corresponding to eutrophy (based on the TDI index). Only in spring at site 7 the values of the studied indicators (SPI, GDI) indicated good ecological status (water quality class II) and oligo-mesotrophic conditions based on the TDI trophic index (CEMAGREF, 1982, Coste, Ayphassorho, 1991, Kelly, Whitton, 1995). In autumn, in the upper and lower reaches of the river, there was a significant deterioration in the ecological status of the waters

of the Kisielina River, compared to the conditions prevailing in spring. The lowest values of SPI and GDI (poor ecological status) and the highest value of TDI (hypertrophy) were noted in autumn at site 2. This site also contained the largest number of taxa resistant to organic pollution (%PT = 28), which indicated deteriorating trophic conditions (Kelly, Whitton, 1995, Kelly et al., 2001).

During the research conducted in 2023, the nearest to the site in Jadowniki Mokre studied in 2006 by Dumnicka et al. (2006) was site 6 in the Wietrzychowice Village. The water at this site in both seasons was characterized by a moderate ecological state based on the values of the SPI and GDI indices. Since similar results were obtained in the assessment of the water quality of the lower section of the Kisielina River over nearly 20 years, it can be concluded that the quality of the waters of the studied river in the down section doesn't change. The differences are visible primarily in trophy (especially in the upper and lower reaches of the river), which clearly increases in autumn, after a period of low water levels often caused by long-term drought in the summer months. On the other hand, no large share of taxa tolerant to organic pollution was found in the studied sites (PT: 6.7–28%), and the obtained values rarely exceeded 20% (only in April at site 4 and in September at sites 1 and 2), indicating minor organic pollution (Kelly, Whitton, 1995). Much higher values of the PT index (> 50%) were noted in the spring of 2010 in the lower course of the Biała Tarnowska (Noga et al., 2015) and in the winter and spring of 2008 in the lower course of the Wisłok River (Noga et al., 2013a).

The rivers (Biała Tarnowska and Wisłoka) flowing in the vicinity of the area through which the Kisielina flows were characterized by a similar ecological state, and according to the SPI Index even poor, in some locations, compared to the Kisielina River. In turn, the trophic state was usually poor and corresponded to hypertrophy (Noga et al., 2015, 2016). Similarly, the waters in the middle and lower sections of the Wisłok River corresponded to a moderate or poor ecological state, while the trophic state always indicated hypertrophy. Only the upper course of the Wisłok was characterized by better water quality (Noga et al., 2013a).

Ecological analyses carried out based on the classification system of Van Dam et al. (1994) showed that alkaliphilic and neutral diatoms in terms of water pH predominate in the Kisielina River. This is also confirmed by the values of the

tested chemical parameters. The saprobic index, which provides information about water quality, showed a large share of  $\beta$ -mesosaprobic diatoms (quality class II) and  $\alpha$ -mesosaprobic diatoms (quality class III). In terms of trophic status, eutrophic species, characteristic of fertile waters, dominated in many locations (similar results were obtained based on the TDI index). In the upper and lower reaches of the Kisielina, oligo-eutrophic species, i.e. with a wide trophic spectrum, were the most numerous (Van Dam et al., 1994). A large share of alkaliphilic and/or neutral diatoms and a clear dominance of eutrophic taxa were also found in the Biała Tarnowska (Noga et al., 2015), Wisłoka (Noga et al., 2016) and Wisłok (Noga et al., 2013a) rivers.

Statistical analysis using the RDA method showed statistically significant correlations between environmental factors and the structure of diatom assemblages. The greatest statistically significant effect on the differentiation of diatom communities ( $p = 0.002$ ) had  $\text{Cl}^-$ , conductivity and  $\text{Ca}^{2+}$  ions (explaining 16.5–15.5% of the variability). Conductivity showed a strong correlation with  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  ions, which in turn were correlated with the occurrence of *Amphora pediculus* and, to a lesser extent, *Navicula trivialis*. Both species formed the most numerous populations at 2nd and 3rd site, where the highest values of chloride and sulphate ions were also measured (especially in the autumn season at site three). Both *Amphora pediculus* and *Navicula trivialis* are widespread in Central Europe in various types of waters and can develop in a wide range of trophic states (Lange-Bertalot, 2001, Bąk et al., 2012, Lange-Bertalot et al., 2017). *Amphora pediculus* is a common and abundant species in southeastern Poland, it was the most abundant dominant in the Wisłoka (Noga et al., 2016), and it was also a frequent and abundant dominant in the Wisłok (Noga, 2012, Noga et al., 2013a), Biała Tarnowska (Noga et al., 2015) and other smaller watercourses (Pajaczek et al., 2012).

Studies conducted along the course of the Kisielina River have proven that this stream is subject to significant anthropogenic pressure along its entire length, both directly, mainly through regulating measures, straightening the course of the river, lining the bottom with concrete slabs and building embankments, and also indirectly, through specific use of the catchment areas. Along the course of the river, in the immediate vicinity, mainly agricultural activities are carried out in the

stream valley. Residential buildings are also often located very close to the river. A significant part of the development of the Kisielina river valley still consists of non-sewered areas (Przybysz-Ławnicka et al., 2008, 2010).

Ecological analysis of diatom communities, also supported by statistical analysis, showed that in the Kisielina River taxa with a wide range of tolerance were found, also resistant to high trophic state, strongly correlated with increased chloride ions and higher salinity of water, indicating reduced water quality, classifying it into quality class II and III. It can therefore be stated that despite all the previously mentioned negative impacts, self-purification processes probably operate quite efficiently in the river, which is also evidenced by the high species diversity of diatoms.

The huge species richness identified during the research, including numerous endangered species and probably new species for Poland, results from the great diversity of habitats in the river valley. The ecological and statistical analyses of diatom communities, confirms the significant anthropogenic pressure affecting the entire course of the river, but at the same time they also confirm the efficiency of the self-purification processes taking place in it.

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