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Diatom biomonitoring – scientific foundations, commonly discussed issues and frequently made errors

by

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Abstract

Contemporary assessment of the ecological status of aquatic ecosystems is based on various groups of organisms, including diatoms. Biological assessment, implemented by the Water Framework Directive, has been applied worldwide for more than 15 years. Currently, the most basic "tool" used in biomonitoring are diatom indices, which are routinely applied in Europe and other countries around the world. In Poland, the diatom indices have been used for over 5 years, which is a period of time allowing to summarize and evaluate the effectiveness of these methods in the assessment of aquatic ecosystems. The purpose of this work was to analyse the use of diatom indices in the biomonitoring of surface water by collecting data on the possibilities and limitations of using diatom indices for the objective assessment of water quality. Attention was paid to mistakes made in the course of biological assessment performed with the use of diatom phytobenthos, which have a significant impact on the obtained results. The paper also contains suggestions for introducing several important changes in biological monitoring, which will improve its quality and efficiency in assessing the ecological status of various aquatic ecosystems.

Key words: diatoms, diatom indices, indicator values, Water Framework Directive

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Introduction

In Europe, routine monitoring of aquatic ecosystems was initiated following the implementation of the Water Framework Directive, adopted by the European Parliament and the Council of the European Union in 2000 and related to ecology and water conservation. Since then, European countries have been gradually incorporating this method of water quality assessment into their legal systems. From the very beginning, diatoms have been considered a priority group, because they are perceived as extremely efficient indicator organisms (Round et al. 1990; Kelly et al. 2008; 2012). Some countries have developed their own diatom indices, adjusted to local hydrobiological and hydrogeomorphological conditions, while others have modified the existing indices used in other regions of the world (Gomez, Licursi 2001; Fore, Grafe 2002; Torrsi et al. 2010; Holms, Taylor 2015).

The standardisation of diatom-based methods of biological assessment is a crucial stage in the development of efficiently and faultlessly functioning principles in various countries. Attempts have been made across Europe to systematise and regulate the methods used to assess the ecological status of aquatic ecosystems in order to guarantee the high quality of their application (Kahlert et al. 2016). In Poland, diatoms have been used in the routine biomonitoring of flowing-water and standing-water ecosystems, which constitutes a foundation for scientific discussion upon its correct and accurate course. There are panels of experts who supervise the procedures of sampling and assay of diatom phytobenthos. Due to the vast diversity of aquatic ecosystems in Poland – from those typical for mountain regions to lowlands with various geological beds, we have had the opportunity to test and verify the applicability and suitability of miscellaneous diatom indices for different types of waters, which involves extensive experience in the application of biological methods.

The application of biological methods to assess water quality using the indicator values of diatoms has been a long-standing procedure. Since the 1970s, the bioindicative role of diatoms has been presented in the works of numerous renown researchers such as Kadłubowska (1964), Schoemann (1976), Coste (1976), Descy (1979), Round (1981), Sládeček (1986), Lange-Bertalot (1979), Hofmann (1994), and Van Dam et al. (1994).

The history of diatom indices began with saprobic indicators, the most recognized of which are two indices by Pantle & Buck (1955) and Zelinka & Marvan (1961). The latter has become the main equation for contemporary diatom indices. The very first diatom index based on the Zelinka-Marvan equation was applied in Belgium to assess the Sambre and Meuse Rivers (Descy 1979; Prygiel et al. 1999). This solution was called the Specific Pollution Sensitive Index (IPS). Soon afterwards, a new index came into use in Belgium, namely the Generic Diatom Index (GDI) (Coste, Ayphassorho 1991), which – following an update implemented in 1995 by Leclercq – included the bioindicator values of 403 diatom species.

In the late 20th century, new indices were introduced in Europe based on the autecology of diatoms. In France, the following indices were developed: the Artois-Picaedie Diatom Index (IDAP) (Prygiel et al. 1996) and the Biological Diatom Index (IBD) (Lenoir, Coste 1996), where the latter included as many as 1028 species of diatoms. In 1995, Kelly & Whitton offered the Trophic Diatom Index (TDI) (Kelly, Whitton 1995) to assess the trophic state of waters in England and Scotland. A year later, Dell'Uomo developed the Eutrophication/Pollution Index (EPI) in Italy (Dell'Uomo 1996), while in other European countries the biological assessment was also performed using such indices as the Leclercq and Maquet Index (LMI) (Leclerc, Maquet 1987) and the Sládeček Index (SLA) Sládeček 1986).

The European diatom indices were incorporated into the OMNIDIA software (Lecointe et al. 1993), which uses the indicative properties of diatoms. The software has been continuously upgraded and extended with new diatom-related data. The latest version, 6.0, contains a taxonomical and ecological database that includes 720 genera and 21 000 species of diatoms, and can calculate 18 diatom indices and 33 ecological statistics (www.omnidia.fr). The software also offers information on the environmental preferences of individual diatom taxa.

Biological assessment and biomonitoring performed with the use of diatom indices and the OMNIDIA software were conducted on a large scale, mainly in France (Prygiel 2002), Great Britain (Kelly et al. 1995; 2008; Kelly 2003) and Finland (Eloranta, Soininen 2002).

Nevertheless, diatom indices have also been applied outside Europe, after the introduction of new indices that allowed for environmental conditions typical for specific regions, which can be illustrated with the example of such indices as the River Diatom Index (RDI) prepared for the USA (Fore, Grafe 2002), the Eastern Canadian Diatom Index (IDEC) in Canada (Lavoie et al. 2006; 2014), the Pampean Diatom Index (IDP) in Argentina (Gomez, Licursi 2001) and the South African Diatom Index (SADI) for South Africa (Harding, Taylor 2011). In addition to the aforementioned new

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indices, various countries have also decided to apply European indices such as IPS, GDI and IBD to assess the ecological status of their aquatic ecosystems, e.g. in Africa (Rey et al. 2004; Harding et al. 2005; Taylor et al. 2007; Harding, Taylor 2014; Bere et al. 2014).

In Poland, scientific research in hydrobiology, ecology, algology and diatomology, which are the basis for monitoring of surface water quality, has a long tradition and history. A pioneering approach to diatoms as a "tool" to assess the water quality was applied by Kadłubowska in 1964. In her work entitled "Okrzemki rzeki Pilicy i ich znaczenie w ocenie jakości wody" ("Diatoms of the Pilica River and their importance in water quality assessment"), the author demonstrated the indicator value of diatoms resulting from their omnipresence in miscellaneous types of ecosystems and their varied species composition, which depends on the physiochemical parameters of water.

Decades of diatomaceous research on various ecosystems of surface water bodies in Poland (e.g. Rakowska 2001; Kawecka, Galas 2003; Wojtal, Sobczyk 2006; Kwandrans 2007; Żelazna-Wieczorek 2011; Kawecka 2012; Szczepocka et al. 2014; Żelazna-Wieczorek et al. 2015) and in other countries (e.g. Hoffman 1994; Van Dam et al. 1994; Jüttner et al. 1996; Lange-Bertalot, Metzeltin 1996; Cantonati 1998; Cantonati et al. 2001; 2006; Potapova, Charles 2007; Blanco et al. 2012; Venkatachalapathy et al. 2013; Holmes, Taylor 2015; Juggins et al. 2016) have advanced the knowledge of diatom autecology, which is the basis for biological assessment of water quality in Poland.

At present, surface water biomonitoring in Poland is chiefly based on diatom phytobenthos. Pursuant to the Regulation of the Minister of the Environment of 21 July 2016 (item 1187) on the classification of the ecological status, ecological potential and chemical status of surface water bodies, phytobenthos has been stipulated as one of the most fundamental criteria of biological assessment.

In Poland, "Polish" indices have been developed for the purpose of biological assessment based on diatom phytobenthos, i.e. the IO Index for rivers and the IOJ Index for lakes (Picińska-Fałtynowicz et al. 2006). The multimetric diatom index (IO) is a compilation of two indices: the trophic index TI (Rott et al. 1999) and the saprobic index SI (Rott et al. 1997). For the full assessment of the ecological status, this index also includes the species composition of diatom communities, the so-called abundance of reference species.

The European indices such as IPS, GDI, TDI, IBD, and EPI-D have also been used in Poland and their applicability in surface water quality assessment has been tested e.g. by Kwandrans et al. (1999), Bogaczewicz-Adamczak & Koźlarska (1999), Bogaczewicz-Adamczak et al. (2001), Bogaczewicz-Adamczak & Dziengo (2003), Rakowska (2001), Zgrundo & Bogaczewicz-Adamczak (2004), Żelazowski et al. (2004), Dumnicka et al. (2006), Szczepocka & Szulc (2009), Żelazna-Wieczorek & Ziułkiewicz (2009), Rakowska & Szczepocka (2011), and Żelazna-Wieczorek & Nowicka-Krawczyk (2015).

The purpose of this paper is to present and summarise the information on diatom biomonitoring in Poland and to draw the reader's attention to particular issues of its application, which still need to be defined more precisely. Not only is the implementation of several significant changes in the process of biomonitoring certain to result in its improvement, but also in the elimination of errors that impair the correct assessment of the ecological status of aquatic ecosystems. At the same time, however, suggestions regarding certain general issues such as field work, selection of appropriate sites for diatom sampling, reliable identification of species, etc. could become common practice in the course of any biological assessment conducted in other countries and regions of the world.

Methodology and identification

Fieldwork methodology and processing of diatom material – new recommendations for diatom research

The algal material used for the purpose of biological assessment is diatom phytobenthos, which can be found in various types of rivers. In Poland, river types have been defined and listed in Dziennik Ustaw (Journal of Laws) 2011, No. 257, item 1545 and in the Practical Guide published by Picińska-Fałtynowicz & Błachuta (2010). At present, there are 26 types of surface waters in Poland, divided into three categories depending on the landscape, i.e. mountain, upland and lowland. They have been categorised in regard to their hydrological parameters (so-called hydrological regime) such as water flow capacity and dynamics, connection with groundwater, and retention time. The second criterion that determines the types of rivers are morphological conditions, namely changes in the depth, width and shape of the river channel, the structure and shape of the bed, as well as the conditions and structure of the riverbank.

Depending on the type of riverbed, diatom phytobenthos can be divided into epipsammon

– diatom assemblages in sand, epipelon – diatom assemblages in mud, epilithon – diatoms attached to natural rocks, stones or man-made surfaces such as concrete, brick, etc., or epiphyton – diatom assemblages on aquatic vascular plants and macroscopic algae. The type of bed is of crucial importance as it determines the structure of species within diatom assemblages (Kawecka, Eloranta 1994; Blanco et al. 2004; Wojtal, Sobczyk 2006; Wojtal 2013). Therefore, the phytobenthos must only be collected from sediments of riverbeds, which are permanently submersed and dominant in a given type of river, i.e. the bed of mountain streams is mainly composed of stones, and therefore epilithon should be collected, while epipsammon should be collected in waterbodies with sandy beds. The following diatom species are usually found on the sandy bed: *Cocconeis* sp., *Planothidium* sp., *Psammothidium* sp, *Gomphonema* sp., *Karayevia* sp., in mud and silt: *Navicula* sp., *Pinnularia* sp., *Gyrosigma* sp., *Craticula* sp., *Cymatopleura* sp., on rocks: *Achnanthidium* sp., *Psammothidium* sp., *Diatoma* sp., *Navicula* sp., *Nitzschia* sp., while *Cocconeis* sp., *Cymbella* sp., *Encyonema* sp., *Cymbopleura* sp., and *Gomphonema* sp. are the most common epiphyton species (Fig. 1). Collection of diatom samples from an inadequate type of substrate, which is not representative of a given type of river, will result in an incorrect assessment of the ecological status, erroneous values of diatom indices, etc.

In Poland, diatom sampling and preparation of samples have been described in "Practical Guide: Sampling, Preparation and Processing of Diatom Phytobenthos Occurring in Rivers and Lakes" ("Przewodnik Metodyczny: Zasady poboru i opracowanie prób fitobentosu okrzemkowego z rzek i jezior" Picińska-Fałtynowicz, Błachuta 2010; Picińska-Fałtynowicz et al. 2006). This guide, however, needs to be supplemented with several crucial issues related to e.g. selection of sampling sites that must be representative of a given type of aquatic ecosystem, the timing and frequency of sampling procedures. The comments to the Guide have been published on the website of the Chief Inspectorate for Environmental Protection (www.gios.gov.pl) under the tab "Modification of phytobenthic methodology 2012". Nevertheless, this paper presents new recommendations for expanding, completing and improving certain crucial aspects related to the biological assessment, which can be applied in biomonitoring all over the world. As stipulated in the publication, diatom sampling sites should be located on a stretch of a riverbank between 50 and 100 m long and typical for a given type of river. The location must be characterized by a moderate water flow (locations with swift currents and still waterbodies should be avoided) and the greatest possible exposure to sunlight.

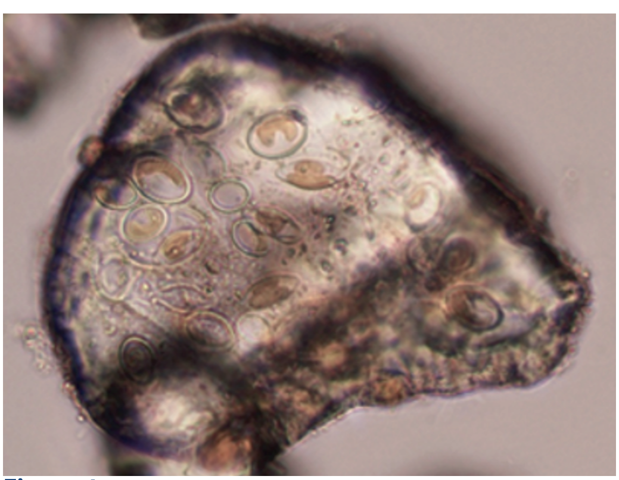

Figure 1

Diatom species of *Cocconeis* sp. and *Planothidium* sp. on a sand grain

What the Guide also fails to mention is the information about the selection of sampling sites. To assess the ecological status of any given aquatic ecosystem, it is crucial to select a representative location, i.e. a site that is not affected by any disturbances and anomalies. Therefore, the selected site should be situated far away from any infrastructure (buildings, bridges, motorways, roads, etc.) and outside the immediate vicinity of any ploughlands, where intense surface runoff may be anticipated (Fig. 2). All such factors interfere with the normal functioning of the river ecosystem, hence the assessment of its ecological status will always be distorted in such locations, returning results with errors. A place which is not directly exposed to such factors allows for the unobstructed development of a diatom assemblage most representative of a given type of river, including natural processes related to its self-purification capacity. The assessment based on such an assemblage is certain to return reliable and correct results.

Apart from the appropriate selection of sampling sites, it is also vital to opt for a correct sampling timing, which – according to the Guide – should be the winter season for mountain and foothill streams, while October and November for highland and lowland rivers. Additionally, the summer season is also recommended for the purpose of assessing any anthropogenic impact. These recommendations are burdened with errors, which prevent the appropriate assessment of the ecological condition of aquatic ecosystems, especially in the case of lowland and upland waters. Winter sampling, however, is not

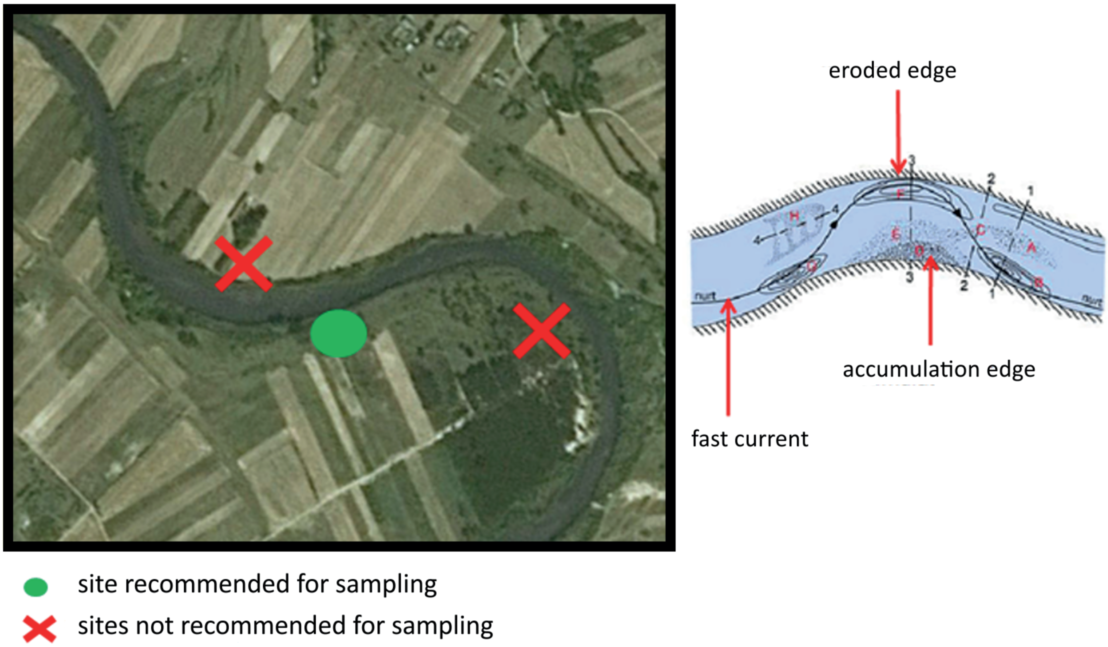

Figure 2

Example of selecting suitable and unsuitable sites for sampling of diatom phytobenthos in a lowland river

Map source: Google

representative due to low temperatures, excessive oxygen saturation of water (in the absence of ice cover), and the lack of vegetation, which translates into a low level of species diversity within diatom assemblages. On the other hand, summer sampling is affected by extensive surface runoff from agricultural areas, which frequently results in a short-term elevation of nutrient concentration in the water and a decrease in the concentration of oxygen, which – in turn – causes a rise in the trophic and saprobic state of water. When compared to the analyses performed in other seasons, the summer assessment of the ecological status of surface water indicates substantially lower water quality levels. In order to avoid errors, we suggest that sampling should be carried out at least twice a year, preferably during the two peaks of the diatom development cycle, i.e. in spring (April/May) and autumn (September/October). Sampling should be carried out on an annual basis over similar periods (+/– 2 weeks). Furthermore, meteorological and hydrological conditions must be taken into consideration, i.e. samples should be collected no sooner than 3 days after torrential rainfall, and never during periods of high or low water. The former increases the risk of erosion within the river channel. The latter dilutes suspended material and results in the accumulation of contaminants in ecosystems (Allan 1998). Such extreme conditions can disturb the structure of diatom assemblages, and thus falsify the assessment of the ecological status of any given ecosystem. Any correctly collected samples should contain diatom assemblages that reflect the long-term water quality typical of a given ecosystem (sampling performed outside the periods of extreme water levels). Mountain streams are an exception, for which winter is the best sampling time, as they are stable then in terms of water flow.

The collected phytobenthos is then chemically treated by means of appropriate lab technologies and the obtained diatom sediment is embedded in the Naphrax® resin. Such solid samples constitute the basis for qualitative and quantitative analyses. We wish to emphasize the importance of a preliminary viewing of live samples – an issue which is often ignored in the procedures of quantitative analysis. Duplicate samples should be collected from the same site and one of them should be kept fresh and unfixed until inspected in the laboratory after being stored in an appropriate temperature of 4 to 6°C. Evaluation of the vitality of a diatom sample is crucial, since an accurate assessment of the current state of the aquatic ecosystem can only be performed on the basis of live diatoms. Under optimal conditions, the ratio of live to dead cells should be 90 to 10%, respectively. Living diatom cells have chloroplasts that are golden in colour, whereas dead cells are green or without chloroplasts. If the majority of diatom cells are dead in a sample (in the form of so-called "empty frustules"), in all likelihood the sample was collected from deeper layers of sediment where only diatom frustules from previous seasons or – in the case of standing water – at least several-year-old frustules are present. The death of diatoms is primarily related to environmental changes that are detrimental to any given species. Any environmental change may be lethal for the populations of sensitive species.

Taxonomic identification of diatoms

Taxonomic identification of any given diatom sample is one of the most crucial stages in the biological assessment. A reliable taxonomic analysis is the most troublesome step due to the high diversity of diatoms and the growing interest in research into diatom taxonomy, which results in new species being described or new sub-species distinguished among the already described groups within the so-called species complexes (Rimet, Bouchez 2012). Diatom identification is mainly based on the analysis of morphological features of diatom cell walls that are characteristic for each taxon. Each cell wall is differentiated on the basis of features that can be easily determined, e.g. cell length and width, the number of striae within 10 μm, or traits that are more complex and include multiple constituents within the cell wall structure (so-called ornamentation), including raphe (raphe system), striae, ribs, crest puncta, areolae, spine and others (Pliński, Witkowski 2009). Some of these features are only visible through a scanning electron microscope (SEM). All constituents of the ornamentation form microstructures, unique to each species, on the basis of which the taxonomic identification is performed.

The minimum level of diatom identification necessary to study the ecological status of water requires the identification of their species. If diatoms are to be used for water quality assessment, it is of crucial importance to ensure that the identification is performed correctly. For the identification of uncertain taxa/species, a scanning electron microscope (SEM) is recommended. All necessary equipment should be available whenever verification involves specimens that are extremely difficult to identify. It should be possible to take large-scale, high-quality microscopic images and, in the case of difficulties or uncertainty regarding the identification of diatoms, it is highly advisable to contact experts in the field, with particular reference to professionals working for research centres specialising in this group of organisms.

Appropriate selection of literature is a key factor and the basis for the identification of diatoms. Updated iconographic identification keys should be used and the latest results of taxonomic studies as well as research in the autecology of diatoms should be followed. An important source is the series entitled "Diatoms of Europe" (Krammer 2000; 2002; 2003; Lange-Bertalot 2001; Levkov 2009; Lange-Bertalot et al. 2011; Levkov et al. 2013; Levkov et al. 2016), each volume of which is devoted to individual genera of diatoms, and the diatom identification key for benthos species occurring in fresh waters of Central Europe (Hofmann et al. 2011; 2013; Lange-Bertalot et al. 2017). Nowadays, such a key is also available in Polish and is used by Provincial Inspectorates for Environmental Protection in Poland to perform routine monitoring (Bąk et al. 2012). Other important publications indispensable to perform the successful identification of diatoms are Krammer & Lange-Bertalot 1991a,b, 1997a,b, Lange-Bertalot & Metzeltin 1996, Lange-Bertalot & Genkal 1999 and Żelazna-Wieczorek 2011. Another important aspect that we would like to refer to is the application of the current and updated diatom nomenclature, since – in the case of numerous already known species – new types have been distinguished and described as characterized by different habitat requirements, e.g. *Gomphonema truncatum* Ehrenberg has recently been divided into *G. truncatum* Ehrenberg, *G. italicum* Kützing, and *G. pala* Reichardt.

The basic measurement unit used to determine the quantitative ratio of individual taxa in a given sample is the number of cell valves. For example, in the case of the *Amphora* and *Halamphora* diatoms, two valves are often counted as one, which is a mistake. The evaluation of ratios of individual taxa in the sample should be based on the count of 300 to 500 units (valves). The tried and tested method, which is the most commonly used, is a sample size of 400 valves. We would like to emphasize that in order to eliminate the risk of calculating separated parts of broken valves or frustules, it is recommended to define a consistent course of action prior to commencing the research. The following options are available: (a) to include a damaged specimen in the total count if approximately three-quarters of its valve still exist, (b) to include a damaged specimen in the total count if at least one pole and the central area are preserved.

Lists of diatom indicator values as the basis of diatom indices

Diatom indices are an important tool for assessing the ecological status of aquatic ecosystems. Their mathematical formulas are based on, among others, indicator values of diatoms in relation to various environmental parameters. Due to the large-scale research on the taxonomy and autecology of diatoms, information on particular species is currently being verified. Species with different habitat requirements have been identified as new taxa in relation to those previously described, and in some areas new taxa have been identified for the first time, which resulted in complementing the information on their ecological preferences with respect to environmental conditions. Autecological studies are an important source of data on new diatom species and changes in indicator values of already existing taxa (e.g. Żelazna-Wieczorek et al. 2010; Rakowska, Szczepocka 2015; Żelazna-Wieczorek et al. 2015; Żelazna-Wieczorek, Olszyński 2016; Juggins et al. 2016). Lists of diatom species with indicator values used in biomonitoring should be updated periodically and in accordance with the latest research. Unfortunately, it is most common for indices to be based on preliminary lists, which were entered into spreadsheets during the process of developing a given index. This generates errors in the assessment of the ecological status of aquatic ecosystems, as illustrated by the IO index used in Poland, the initial list of which does not take into account changes in the diatom taxonomy introduced in recent years. In consequence, the IO index is not very reliable and, as evidenced by our research, inaccurately assesses the water quality, returning the same results for aquatic ecosystems that actually differ in water quality when measured by other diatom indices. A perfect illustration of the issue is the data obtained during the water quality analysis for the Czarna Staszowska River. Even though samples were collected in five different locations with different water quality, the assessment performed by the IO indicated class III water quality (moderate status) for the whole river (Szczepocka, Rakowska 2015).

At the same time, research has been conducted in Poland to adapt certain diatom indices that are applied as standard solutions by other European countries. The research consists mainly in the verification of the list of indicator diatom taxa regarding the hydrological and physicochemical conditions of the Polish surface waterbodies, i.e. rivers and lakes. The studies prove that the following indices are effective tools when assessing Polish rivers: IPS, GDI, TDI, IBD, EPI-D (Bogaczewicz-Adamczak et al. 2001; Bogaczewicz-Adamczak, Dziengo 2003; Zgrundo, Bogaczewicz-Adamczak 2004; Żelazowski et al. 2004; Dumnicka et al. 2006; Rakowska, Szczepocka 2011; Szulc, Szulc 2013; Szczepocka, Rakowska 2015; Żelazna-Wieczorek, Nowicka-Krawczyk 2015). The majority of the aforementioned indices differentiates the quality of the analysed rivers, taking into account changes in water quality, inflows of contaminants, surface runoffs from agricultural areas, etc.

Discussion

Monitoring conducted by means of diatom indices is an appropriate approach in the assessment of the ecological status of surface waters. Results obtained through the application of biological methods give a holistic view of the impact that physicochemical and hydrological factors have on the assemblages of organisms occurring in aquatic ecosystems. The previously applied methods of water quality assessment were restricted only to physicochemical parameters, and thus their results reflected the quality of water only at the time of sampling. Chemical parameters, on the other hand, may vary during the day, and thus the same water sample could be assigned to different quality classes. Biological methods are based on aquatic organisms that live in the aquatic ecosystem and are constantly affected by evolving environmental parameters. This allows for the development of assemblages typical for a given type of waterbody. The assessment based on indicator values of species contributing to those assemblages allows for a reliable assessment of the ecological status of waters. Physicochemical analyses provide the background for biological methods and are an integral part of the overall assessment. Therefore, a given ecosystem may not be recognized as representing excellent or good ecological status if the values of chemical parameters exceed the standard values assigned to a specific status (Picińska-Fałtynowicz, Błachuta 2010; OJ, item 1482 of 2014).

In Poland, diatom indices have been applied to assess hydromorphologically diverse aquatic ecosystems for over 15 years. The analysis of results obtained in our research and in the studies conducted by other Polish scientists reveals a number of positive aspects of biological methods. At the same time, however, we draw attention to the rules that must be strictly observed to avoid mistakes that lead to incorrect assessments of the ecological status of waters.

Diatom indices are used for the purpose of assessing the quality of lotic and lentic waters. Excellent effects are achieved when several indices are applied simultaneously to assess any given aquatic ecosystem (Harding, Taylor 2014; Vilmi et al. 2015), since such an assessment is comprehensive and demonstrates how individual indices react to changes in environmental conditions. As evidenced by the research conducted in Poland, indices offer different sensitivity to environmental parameters (Szczepocka et al. 2014; Szczepocka, Rakowska 2015). Commonly applied in surface water monitoring in Poland, the IO frequently fails to differentiate between various levels

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of water quality in samples collected at different study sites, which indicates its low sensitivity to changes caused by the inflow of contaminants (field research on the Linda River and the Czarna Staszowska River). In the case of this index, inaccuracy in the assessment of the ecological status of waters results from the lack of updates on the list of diatom indicator values and from the excessive number of widespread eurytopic taxa on which the index is based. Another issue related to the Polish IO index, which most likely results from errors on the lists, is the overestimation of the value of this index in the case of contaminated rivers (Rakowska, Szczepocka 2011; Szczepocka et al. 2016). Taxonomic and ecological studies of diatoms provide new information that should be applied at least once every two years to update checklists on which diatom indices are based. In recent years, numerous studies have been conducted in Poland regarding new diatom species in the Polish flora, the verification of indicator values, and recommendations for ecosystem assessment. However, this information has not been applied in the biomonitoring of surface waters (Żelazna-Wieczorek et al. 2010; Rakowska, Szczepocka 2015; Żelazna-Wieczorek et al. 2015; Żelazna-Wieczorek, Olszyński 2016; Rakowska et al. 2017).

As evidenced by numerous studies, the European IPS index is most effective for surface waters in Poland (Szczepocka, Szulc 2009; Noga et al. 2013; Szczepocka et al. 2014). The IPS index shows the best response to fluctuations in water quality and produces varied results depending on the location of sampling sites in rivers (Żelazna-Wieczorek, Nowicka-Krawczyk 2015; Szczepocka et al. 2016). It may, however, slightly underrate water quality in the case of non-contaminated waterbodies (Szczepocka et al. 2014). The TDI, customarily used for the assessment of the trophic state, usually proves very accurate when determining the trophic state of Polish river ecosystems (Szczepocka et al. 2016). The analysis of the IPS and TDI indices and their application in surface water biomonitoring worldwide shows that these two indices are the most accurate in the assessment of the ecological status of waters. Researches confirm their greater applicability and quite often greater accuracy in the biological assessment compared to indices developed for individual countries (Blanco et al. 2008).

European diatom indices such as IPS, GDI, EPI-D and TDI prove effective in the assessment of the ecological status of lotic waters. In Poland, attempts have been made to apply these indices in the assessment of other types of waters, e.g. springs, but the returned results were burdened with numerous errors. Spring ecosystems are characterized by very specific environmental conditions that favour the development of specific diatom assemblages. Compared to river ecosystems, there are relatively few studies that relate to correlations between diatom indices and chemical parameters of spring ecosystems. They include for example: Kwadrans et al. (1998), Torrisi & Dell'Uomo (2006), Żelazna-Wieczorek & Ziułkiewicz (2009). The assessment of the ecological status of water in spring ecosystems should be based on the autecology of selected diatom species in relation to hydrogeological parameters and the types of microhabitats (Cantonati et al. 2007; Żelazna-Wieczorek 2011; Żelazna-Wieczorek, Knysak 2017).

Another important issue is the type of substrate from which diatoms are sampled. Generally speaking, the principles stipulated in the chapter "Fieldwork methodology and processing of diatom material – new recommendations for diatom research" work well in the case of rivers with a low or medium level of degradation. Our research in highly degraded rivers, exposed to long-term anthropogenic pressure, shows that benthic samples composed of accumulated sediment are the most representative for biomonitoring, as they well reflect the entirety of environmental factors that shape diatom assemblages (Rakowska, Szczepocka 2011; Szczepocka et al. 2016). Even if a different type of substrate dominates in such waters, e.g. macrophytes, diatom assemblages that developed there still remain beyond the influence of many factors that are crucial for the process of assessing the ecological status of such an ecosystem.

The last issue we would like to emphasize is the application of diatom indices in the assessment of the ecological status of lotic waters with a disrupted model of the river continuum. Our research conducted in rivers with artificial reservoirs shows inconsistency in the assessment of the ecological status, due to the fact that a given river is periodically inhabited by diatom species typical of lentic waters (Żelazna-Wieczorek, Nowicka-Krawczyk 2015). Numerous species of centric diatoms have been found among the dominants. Disturbances in the species composition of river-specific diatom assemblages translate into errors in the assessment of their ecological status.

General recommendations

Since the biological assessment has been performed in Poland for several years now, it is possible to compile a summary and to emphasize certain important aspects that must be taken into consideration in order to obtain reliable and consistent results. As mentioned above, the following aspects are of major importance:

- Selection of an appropriate site for the purpose of diatom sampling. With the objective to perform the most reliable assessment of a given aquatic ecosystem, one must select a location outside the immediate vicinity of any facilities or beyond the range of factors that could disturb the natural functioning of the ecosystem, e.g. cultivated fields, bridges, road infrastructure, buildings, etc. Preservation of an appropriate distance enables the development of diatom assemblages that are representative for a given type of waterbody.

- Sampling should take place during the two peaks of the diatom development cycle, i.e. in spring (April/May) and autumn (September/October) and should be performed at least twice a year. The more often we collect the samples, however, the more accurate the assessment is bound to be, as it will then incorporate the natural developmental dynamics of the ecosystem as well as any regular or transient anthropogenic impacts.

- Diatom phytobenthos should be collected from a bed which is permanently immersed in water and predominant in a given type of river. Live samples should be viewed, as only live diatoms can serve the purpose of further analysis aimed at assessing the ecological status of a given aquatic ecosystem.

- Appropriate selection of literature is a key factor and the basis for identification of diatoms. Updated iconographic identification keys should be used and the latest research on the autecology of diatoms should be followed, which increases the knowledge about this group of organisms.

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