Belg. J. Bot. **141** (1) : 39-50 (2008)

DIATOM ASSEMBLAGES AND WATER QUALITY ASSESSMENT IN THE DUERO BASIN (NW SPAIN)

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Received 17 May 2007; accepted 9 October 2007.

Abstract. — In order to determine water quality status in the Duero basin (NW Spain), epilithic diatom samples were collected and analyzed in 137 stations in August 2004 following normalized standard protocols. The floristic particularities of the diatom assemblages in the basin are discussed. A total of 429 diatom taxa were identified in the basin and 90 taxa (21%) were new for the Iberian Peninsula. Especially noticeable was the presence of *Achnanthidium rivulare*, only known until now from North America. Ordination revealed the existence of five species assemblages related to the following environmental factors: 5-Day Biochemical Oxygen Demand (BOD_5), [PO_4^{3-}], and conductivity. Light and scanning electron microscopy micrographs are provided for some common, infrequent or exotic diatom species present in the Duero basin. Three diatom indices (Specific Pollution Index SPI, European Index CEC and Biological Diatom Index BDI) were applied. All of them correlated significantly with water physical and chemical variables, but SPI achieved the best correlations, and is therefore recommended as the reference diatom index for this Mediterranean basin.

Key words. — Bacillariophyta, bioindicators, diatom indices, Mediterranean Basin, Pollution Sensivity Index, Water Framework Directive.

INTRODUCTION

During the past three decades diatoms have been used worldwide to determine the ecological quality of freshwaters (see e.g., Whitton *et al.* 1991, Whitton & Rott 1996, Prygiel *et al.* 1999). Diatoms fulfill the requirements of good indicator organisms in aquatic ecosystems. They occur in a wide variety of environments and show a broad range of tolerance along several gradients of abiotic factors, while individual species have specific water chemistry requirements (Round 1991). Finally, sampling and processing are relatively simple and cost-effective.

Standardized phytobenthos-based monitoring networks are one of the targets of the Water Framework Directive (WFD, European Parliament & European Council 2000), a legislative set that aims at assessing and restoring the ecological status of inland water bodies throughout EU countries. This ecological status is defined as a deviation measurement between the characteristic structure of aquatic biota and the reference conditions of the same parameter. The reference conditions correspond to a water system with no or minor anthropogenic impacts (Ector & Rimet 2005).

The use of benthic diatoms for biomonitoring purposes usually implies the application of diatom indices. Until now, several methods have been proposed, mainly based on the ZELINKA & MARVAN (1961) formula, i.e., assigning a pair of individual values (pollution tolerance and stenoecy degree) to a certain set of common taxa. Among these, the Specific Pollution Index (SPI, CEMAGREF 1982), the Biological Diatom Index (BDI, AFNOR 2000) and the European Index (CEC, DESCY & COSTE 1991) have been routinely applied for European streams. As these indices are based on diatom collections from relatively homogeneous watercourses in Central and Western Europe, problems may arise when applied elsewhere. Adding to the presumable biogeographical constraints in the species dataset, the ecological particularities of the study area, such as those characteristic of the Mediterranean bioclimate, may introduce a source of bias in water quality estimation. Despite this, several authors have studied the applicability of diatom-based methods for biomonitoring Iberian rivers. GOMÀ *et al.* (2004, 2005), BLANCO *et al.* (2007) and OSCOZ *et al.* (2007) reported a good performance of the SPI index in both mountain and plateau Spanish rivers. However, it failed to diagnose the effects of oil-spillage pollution in a Mediterranean stream (DAMÁSIO *et al.* 2007). SABATER *et al*. (1987), LEIRA & SABATER (2005) and TORNÉS *et al*. (2007) stressed the relevancy of physiographical factors in the distribution of diatom assemblages in some Pyrennean and Mediterranean streams, adressing the need for a careful development of data sets in order to understand the processes that are structuring diatom assemblages in these ecosystems.

This paper reports the first extensive diatom survey carried out in the Duero basin (NW Spain). We analyze the applicability of three common diatom indices for the evaluation of water quality, thus providing a scientific basis for the implementation of the WFD in the Iberic-Macaronesian region.

STUDY AREA

The Duero basin (97 290 km^2) is the largest one in the Iberian Peninsula and covers part of Spain and Portugal. This study was performed in the Spanish area of the basin (78 952 km^2), located in the north-west of Spain (Fig. 1). The drainage basin, crossed transversally by the Duero River (895 km), covers an extensive plateau (~750 m a.s.l.) mostly formed by Tertiary and Quaternary (alluvial or colluvial) materials, divided in two sub-systems: a) a western plain, basically constituted by Paleozoic granites, and b) the central and eastern sedimentary catchment area, composed of calcareous and marly terrains, with several evaporitic inclusions. The surrounding mountains consist of heterogeneous siliceous materials, except for the limestones in the central part of the Cantabric Range. The largest part of this endorheic basin is located within the Mediterranean bioclimate, here

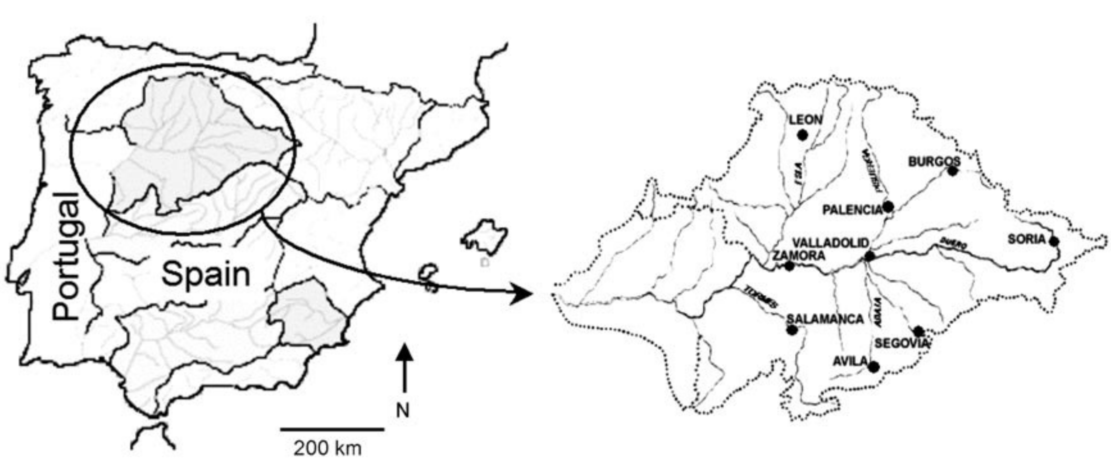

Fig. 1. Geographical location of the Duero basin in Spain.

characterized by a strong continentality (air temperature ranging between -25ºC and 45ºC). Mean annual precipitation is 620 mm, concentrated in autumn and winter, while the potential evapotranspiration is ~700 mm $year^{-1}$. A network of 80 large reservoirs regulates the flow in the main streams of the Duero basin. Main land uses are agriculture and stockbreeding, industrial activities being of less importance. Population density is one of the lowest in Europe (26 hab-km^{-2}); however, its aquatic ecosystems have suffered historically intense anthropogenic impacts. A more detailed description of the study area is available in GARCÍA & GONZÁLEZ (1986) and CEBALLOS *et al.* (2003).

MATERIAL AND METHODS

The survey was conducted in August 2004 in 137 sites along the rivers of the Spanish side of the Duero basin, randomly selected from the Water Quality Surveillance Network of the Duero Basin Water Agency (CHD). Water physical and chemical parameters were analyzed according to APHA (1995) and provided by the CHD automatic water analysis stations in each site. Diatom samples were taken and processed following Spanish standards (AENOR 2004, 2005). Epilithon was sampled on submerged stones in the flow, euphotic zone of each stream, using a toothbrush, and preserved in 4% v/v formaldehyde. Clean frustule suspensions were obtained by oxidizing organic matter with hot hydrogen peroxide 30% v/v. Carbonate inclusions were removed adding a few drops of hydrochloric acid. Permanent microscopic slides were mounted using a refractive resin (Naphrax®). At least 400 valves were identified and counted under 1000x light microscopy (Leica DM-RB). Taxonomy and nomenclature followed common reference works (KRAMMER & LANGE-BERTALOT 1986-1991, LANGE-BERTALOT 1995-2007, 2000-2002). Diatom indices SPI, BDI and CEC were calculated using OMNIDIA ver. 4.2 (LECOINTE *et al.* 1993). We selected these indices because of their widespread use in Spain, Portugal and other European countries. SPI takes into account all known taxa, and has been considered a reference index (DESCY & COSTE 1991). It is based on the ZELINKA & MARVAN (1961) method. BDI is based on 209 common taxa, and is the standard method in France (AFNOR 2000). The CEC index is based on a twofold quality grid, and considers only 208 taxa. The results of these indices are numeric values standardized from 1 to 20, representing the theoretical minimal and maximal water quality statuses, respectively. These values were classified in the quality categories established by the WFD: bad = [1-5[, poor = [5-9[, moderate = [9-13[, good = [13-17[, and high quality = [17-20].

The relationships between physical and chemical parameters, and diatom indices, were established by calculating Pearson's correlation coefficients. Data (except for pH) were previously log-transformed to fit normality. In order to identify the main diatom species assemblages according to their occurrence in the sampling stations, an ordination dendrogram was performed (Bray-Curtis similarity index, unweighted pair-grouping method, SNEATH & SOKAL 1973). Finally, the species data and the physical, chemical and physiographical parameters were related by means of a Canonical Correspondence Analysis (CCA) using CANOCO ver. 4.5 (TER BRAAK & ŠMILAUER 2002). Variables with inflation factors over 20 were excluded from the analysis. A forward selection method was used to identify the environmental variables that significantly explained variance in species data (Monte Carlo test, 199 unrestricted permutations). Statistical differences between the groups formed in the CCA plots were assessed by means of Analysis of Similarity (ANOSIM, CLARKE 1993). The last two analyses were performed upon a restricted list of the most frequent taxa (≥ 10 % in abundance in the whole dataset).

RESULTS

WATER PHYSICAL AND CHEMICAL PARAMETERS

Waters were circumneutral throughout the whole catchment area (Table 1), with a certain tendency towards alkalinity in the northern ranges. The highest conductivity values correspond to the chalky substrate rivers in the northeastern regions, not necessarily related to high pollutant concentrations. Oxygenation was generally close to saturation; the lowest values are due to wastewater or reservoir hypolimnetic water inflows. Parameters indicating the degree of eutrophication (nutrient concentrations, organic matter) were also highly variable. The most oligotrophic sites were located in high mountain watercourses, while inner, downstream stations were generally more polluted, becoming eutrophic or even hypereutrophic.

Table 1. Main physical, chemical and physiographical characteristics measured in the Duero basin during summer 2004 (means of the values measured on 8 July 2004 and 3 August 2004 in 137 sampling stations).

Variable and units	Mean	Variation range
Altitude (m a.s.l.)	840.3	334.0 - 1575.0
Water temperature (ºC)	17.7	9.6 - 26.3
pH	7.8	6.4 - 8.3
Conductivity (µS cm^{-1})	282.4	24.5 - 1140.0
Total suspended solids (mg L^{-1})	13.7	0.0 - 135.6
[O_2] (mg L^{-1})	7.8	2.2 - 12.2
[O_2] (% saturation)	80.7	24.0 - 130.4
BOD_5 (mg O_2 L^{-1})	0.9	0.0 - 8.1
COD (mg O_2 L^{-1})	3.2	0.0 - 11.4
[NH_4^+-N] (mg L^{-1})	0.5	0.0 - 28.3
[NO_2^--N] (mg L^{-1})	0.1	0.0 - 0.7
[NO_3^--N] (mg L^{-1})	4.4	0.0 - 27.5
[PO_4^{3-}-P] (mg L^{-1})	0.3	0.0 - 4.4
[Total P] (mg L^{-1})	0.1	0.0 - 0.5
[Cl^-] (mg L^{-1})	11.6	0.0 - 89.0

DIATOM ASSEMBLAGES

A total of 429 diatom taxa were identified, 184 of them reaching a relative abundance of over 1% in at least one inventory. Ninety taxa (21%) were new for the Iberian phycoflora (Appendix S1, Supplementary material; Fig. 2). The ordination dendrogram (Fig. 3) revealed the presence of five homogeneous species assemblages (Bray-Curtis similarity index > 0.01). Each of them was composed of taxa that share similar ecological characteristics according to the literature (DENYS 1991, HOFMANN 1994, VAN DAM *et al.* 1994). Group I consisted of *Fragilaria* species, intolerant to high eutrophication or low oxygenation levels. Group II comprised taxa indicating low trophic and oligosaprobic conditions such as *Achnanthidium pyrenaicum* or *Gomphonema rhombicum*. Two planktonic, alcaliphilous and eutrophilous species (*Aulacoseira granulata* and *Stephanodiscus neoastrea*) formed group III. Taxa related to meso- or eutrophic conditions were included in group IV. Finally, *Navicula recens* and *Rhoicosphenia abbreviata*, two somewhat rheophilous species inhabiting relatively high conductivity streams appeared in group V. Canonical correspondence analysis significantly discriminated the same groups (one-way ANOSIM on CCA coordinates, $P < 0.05$), and related them to environmental factors (Fig. 4). The first two axes accounted for 37.6% of the total variance explained in the species dataset. Monte Carlo test identified BOD_5, [PO_4^{3-}] and conductivity as the environmental variables ($P < 0.05$) to significantly explain this variance. The first axis very likely arranges the species along a gradient of rheophily, while the second axis clearly expresses a gradient of eutrophication. The distribution of the taxa assemblages was consistent with these explanations, since the species groups typical of polluted waters tended to appear in the positive end of the second axis and vice-versa, while limnophilous and planktonic taxa were represented on the right end of the first axis. Moreover, sampling stations show an analogous ordination in the CCA plot (Fig. 4 c), where altitude discriminates several significantly different groups of sampling points (one-way ANOSIM on CCA coordinates, $P < 0.01$). Hence, high-altitude, low-impacted stations are associated to taxa groups (Fig. 4 a) indicative of good biological water quality conditions, and vice-versa.

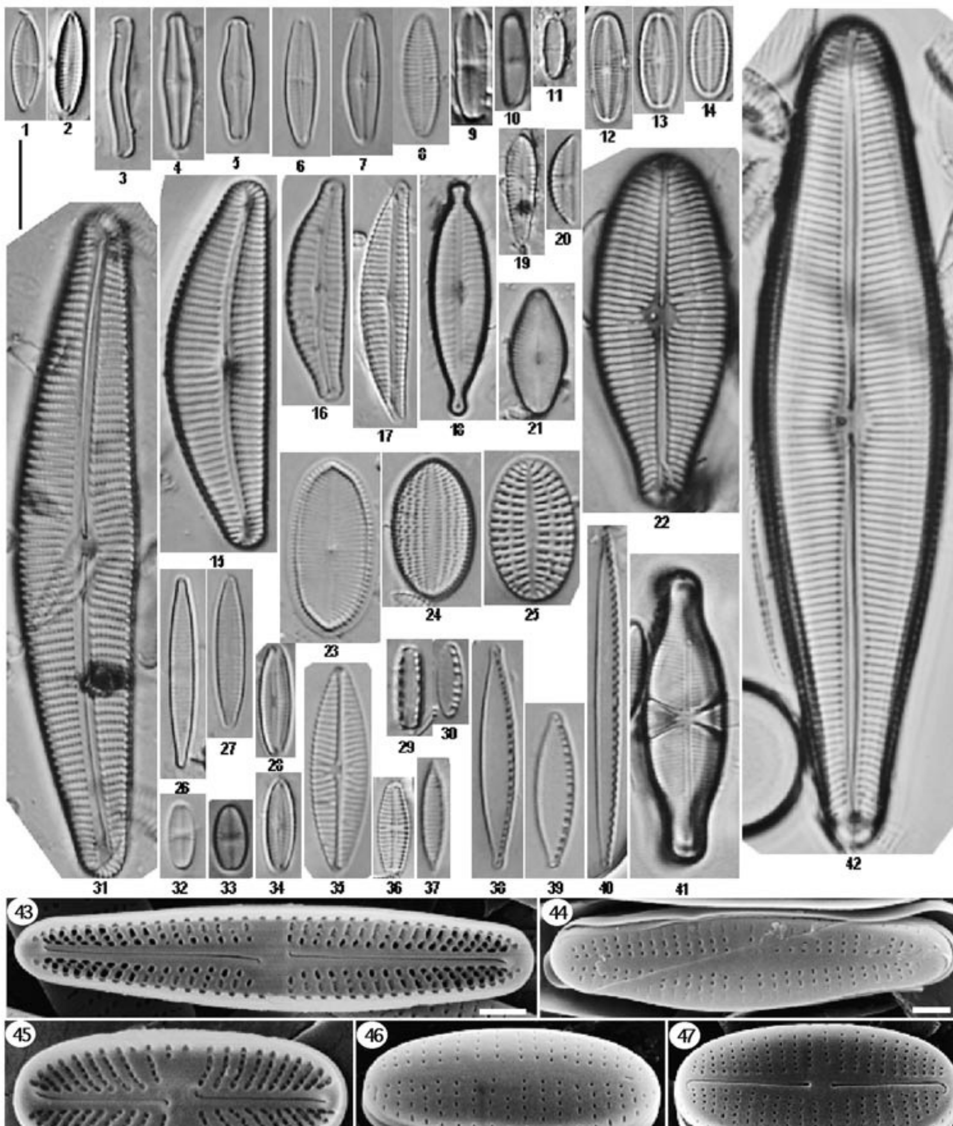

Fig. 2. Some characteristic diatom species in the Duero basin (LM: scale bar = 10 µm; SEM: scale bar = 1 µm). 1, 2: *Achnanthes subhudsonis*. 3-5, 43, 44: *Achnanthidium minutissimum*. 6-8: *A. pyrenaicum*. 9-11, 45, 46: *A. atomoides*. 12-14, 47-50: *A. rivulare*. 15: *Cymbella compacta.* 16: *C. excisiformis*. 17: *Encyonema perminutum*. 18: *Gomphonema lagenula*. 19: *Gomphosphenia lingulatiformis.* 20: *Amphora pediculus.* 21: *Diadesmis confervacea.* 22: *Gomphoneis eriensis* var. *variabilis*. 23, 24: *Cocconeis euglypta*. 25: *C. disculus.* 26, 27: *Fragilaria rumpens.* 28, 34: *Eolimna comperei*. 29, 30: *Nitzschia inconspicua.* 31: *Cymbella lange-bertalotii*. 32, 33: *Eolimna minima.* 35: *Navicula cryptotenella*. 36: *Hippodonta subrhombica*. 37: *Simonsenia delognei.* 38-39: *Nitzschia fonticola*. 40: *N. radicula.* 41: *Capartogramma crucicula.* 42: *Gomphoneis minuta.*

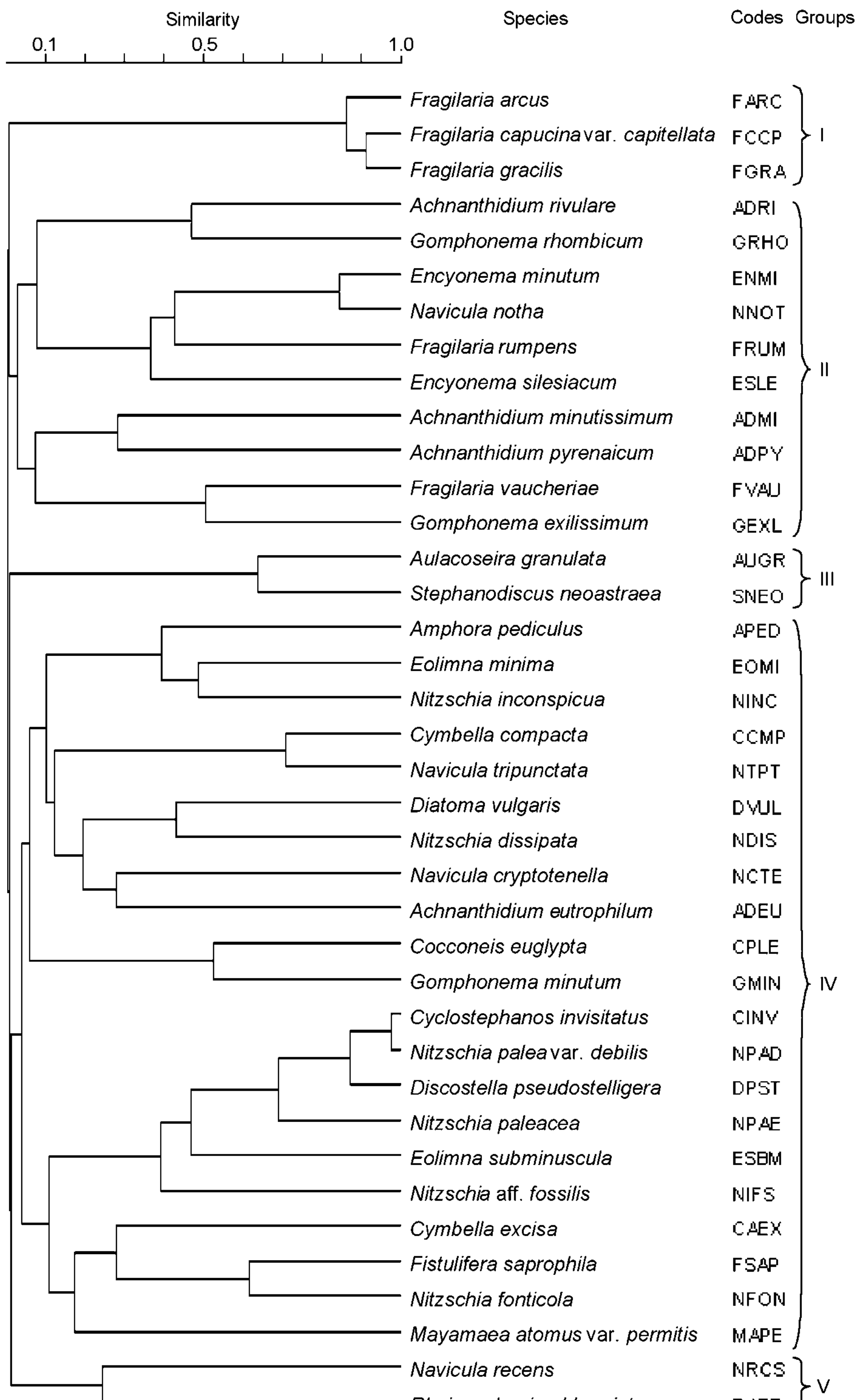

Fig. 3. Ordination dendrogram of the most frequent taxa and clusters formed at similarity > 0.01.

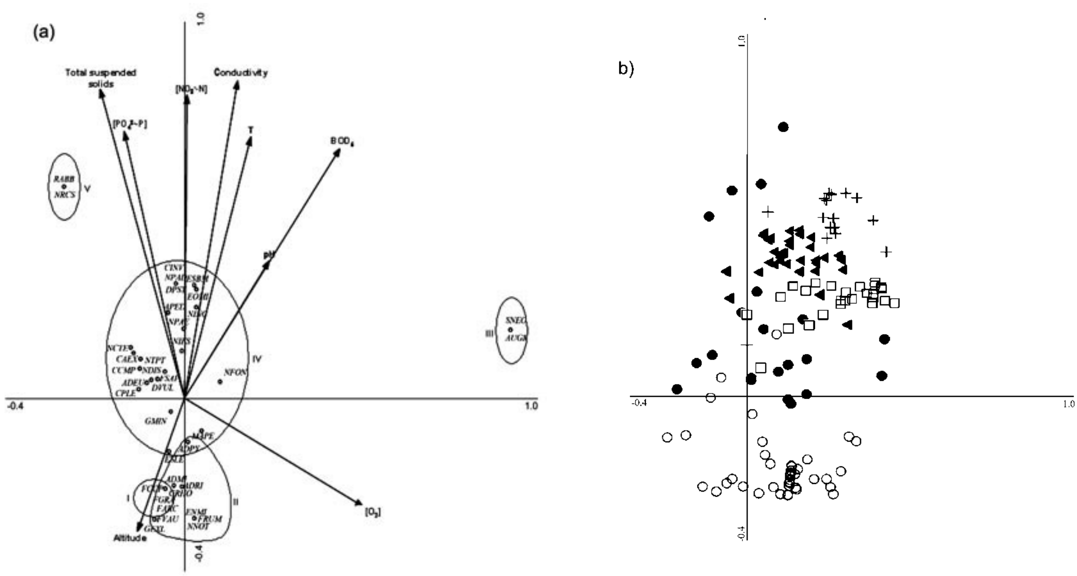

Fig. 4. Ordination plots of benthic diatom assemblages with respect to environmental variables based on Canonical Correspondence Analysis (CCA). Diatom taxa and environmental variables (a) and sampled sites (b) are displayed on CCA axes in separated plots. Sampling sites are classified according to their altitude; white circles: < 700 m; black circles: 700-800 m; squares: 800-900 m; triangles: 900-1000 m; crosses: > 1000 m a.s.l. Clusters formed in the ordination dendrogram (see Fig. 3) are also displayed (a). See taxa codes in Fig. 3.

Diatom indices and water quality

The results provided by the three diatom indices applied were rather different from each other, as seen in the distribution of the sampling stations in five quality classes (Fig. 5). BDI evaluated water quality differently from the two others, and tended to overemphasize the average quality classes. However, all of them correlated significantly with the physical and chemical parameters indicating water quality (Table 2). The most significant correlations were found using SPI. Following the results provided by this index, 63.5% of the studied stations reached the "good" or "high" water quality level, almost all of them located in the northern watercourses (Fig. 6). Only three stations (2.2%) had a "bad" water quality status: Alija de la Ribera (Bernesga River, downstream from León city, SPI = 4.0), Medina del Campo (Zapardiel River, downstream from Medina del Campo city, SPI = 2.4) and Villavieja de Yeltes (Yeltes River, SPI = 2.5). These heavily polluted waters were to the result of untreated urban (Alija de la Ribera), industrial (Medina del Campo) or farm (Villavieja de Yeltes) wastewater inflows. Epilithic diatom communities in these stations were characterized by their low diversity, with species typical from hypereutrophic and polysaprobic waters such as *Fistulifera saprophila*, *Nitzschia capitellata* or *N. palea*.

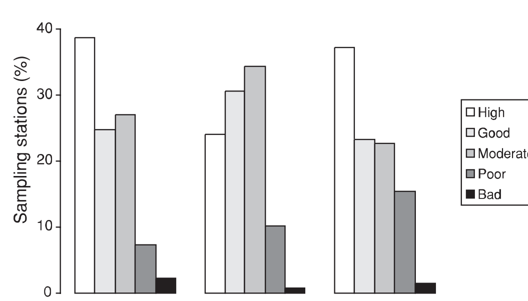

Fig. 5. Distribution of the Duero basin sampling stations among the five quality classes for the three diatom indices applied: SPI, BDI and CEC.

Table 2. Pearson's correlation coefficients (*r*) between diatom indices and physical and chemical variables (n = 137).

Variable	Diatom index		
	CEC	BDI	SPI
Conductivity	-0.31**	-0.37***	-0.42***
Total suspended solids	-0.24*	-0.34***	-0.33**
$[O_2]$ ($mg \cdot L^{-1}$)	0.46***	0.51***	0.52***
$[O_2]$ (% saturation)	0.31***	0.34***	0.39***
BOD_5	-0.54***	-0.58***	-0.57***
COD	-0.68***	-0.68***	-0.70***
$[NH_4^+$-N]	-0.49***	-0.38**	-0.53***
$[NO_2^-$-N]	-0.54***	-0.45***	-0.56***
$[NO_3^-$-N]	-0.34**	-0.56***	-0.49***
$[PO_4^{3-}$-P]	-0.69***	-0.48**	-0.73***
[Total P]	-0.72***	-0.62***	-0.64***
$[Cl^-]$	-0.54***	-0.69***	-0.72***

Note. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

DISCUSSION

From a floristic point of view, the present work reveals the presence of a high diatom taxa richness in the Duero basin. The considerable extension of the catchment area, together with the strong lithological, hydrodynamic and landscape heterogeneity in its watercourses contributed to this diversity. So far, the diatom flora of this basin has been poorly known and only a few and local studies have been published (see ABOAL *et al.* 2003 and references therein). This explains the relatively high number of new citations (21% of the taxa list, see Appendix S1, Supplementary material) for the Iberian algal flora (ALMEIDA *et al.* 1999, ALMEIDA & GIL 2001, ABOAL *et al.* 2003, NUNES *et al.* 2003, BLANCO *et al.* 2004, GOMÀ *et al.* 2004, 2005, LEIRA & SABATER 2005, TORNÉS *et al.* 2007). Among them, especially noticeable was the presence of *Achnanthidium rivulare*. To our knowledge, this is the second record of this species outside America, where it was first described (POTAPOVA & PONADER 2004). In 2004 it was found dominant in several rivers of the Massif Central in Limousin region, France (ECTOR *et al.* 2005). In the Duero basin it inhabits similar habitats (oligotrophic, softwater rivers), reaching subdominant levels in some places. This taxon is probably widely distributed in European streams, but it has been very likely confused until now with morphologically similar species, e.g., *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *A. biasolettianum* Grunow in Cleve & Grunow or *A. deflexum* (Reimer) Kingston (PONADER & POTAPOVA 2007). However, its autoecological spectrum together with its ultrastructural features (Fig. 2) lead to an unequivocal identification. We also noticed the presence of species such as *Capartogramma crucicula*, *Diadesmis confervacea*, *Encyonema triangulum*, *Gomphoneis eriensis* var. *variabilis*, *Gomphoneis minuta* or *Gomphonema lagenula*, considered as rare or exotic for European freshwaters (COSTE & ECTOR 2000), but actually not uncommon in Mediterranean watercourses.

According to the cluster and canonical correspondence analyses, the main factor controlling the distribution of the principal diatom species in the Duero basin was trophic level, which integrates several related parameters ($[PO_4^{3-}]$, $[NO_3^-]$, conductivity). This result allows the use of diatom communities to assess the chemical quality of the water, particularly as bioindicators of nutrient concentrations (e.g., LOWE & PAN 1996, STEVENSON & PAN 1999, ECTOR & RIMET 2005). Among the diatom indices applied, the SPI is the only one based on the autoecological parameters of all known taxa.

In addition, this index produced minimal residuals in the correlation analyses (Table 2). In contrast, CEC and, particularly, BDI, tended to under- or overestimate the quality values in some stations, owing both to the relative abundance of taxa not considered in the respective datasets (e.g., *Achnanthidium rivulare*, *Achnanthes subhudsonis*), and to the inaccurate autoecological parameters assigned. For instance, several taxa such as *Achnanthidium minutissimum* var. pl. or the group of *Encyonopsis microcephala* s.l. are pooled as ecological synonyms in the BDI method, though they have rather different environmental spectra. This behavior has also been observed in other Mediterranean basins (Gomà *et al.* 2004). Moreover, SPI has been used successfully in non-European watercourses (Gómez & Licursi 2001, De la Rey *et al.* 2004). Our results are consistent with the findings of Gomà *et al.* (2004, 2005) and Oscoz *et al.* (2007) who successfully used SPI for monitoring rivers in other Iberian basins.

The application of SPI to the Duero basin revealed the presence of up to 50 sampling stations (36.5%) with moderate, poor or bad water quality, therefore not reaching the WFD thresholds. Almost all of these sites are located in the basin's central plain (Fig. 7) and their water quality very likely reflects diffuse pollutant inflows, mainly of agricultural fertilizers. Environmental policies should priorize the water management in the three sites detected with critically low quality values (SPI < 5).

CONCLUSIONS

Diatom-based indices led to successful water quality assessment in the Duero basin, suggesting that such biological methods are valuable tools for the development of environmental surveillance networks in Mediterranean watercourses. The following conclusions can be drawn from our results: 1. Despite being an index developed from Central European diatom surveys, SPI seems to be robust enough against presumable differences in the ecological parameters of the species used. 2. These results lead us to recommend the SPI as the reference index for the Duero basin and the routine application of this index for the assessment of water quality in Iberian rivers until new local, national or European indices are available. Recent

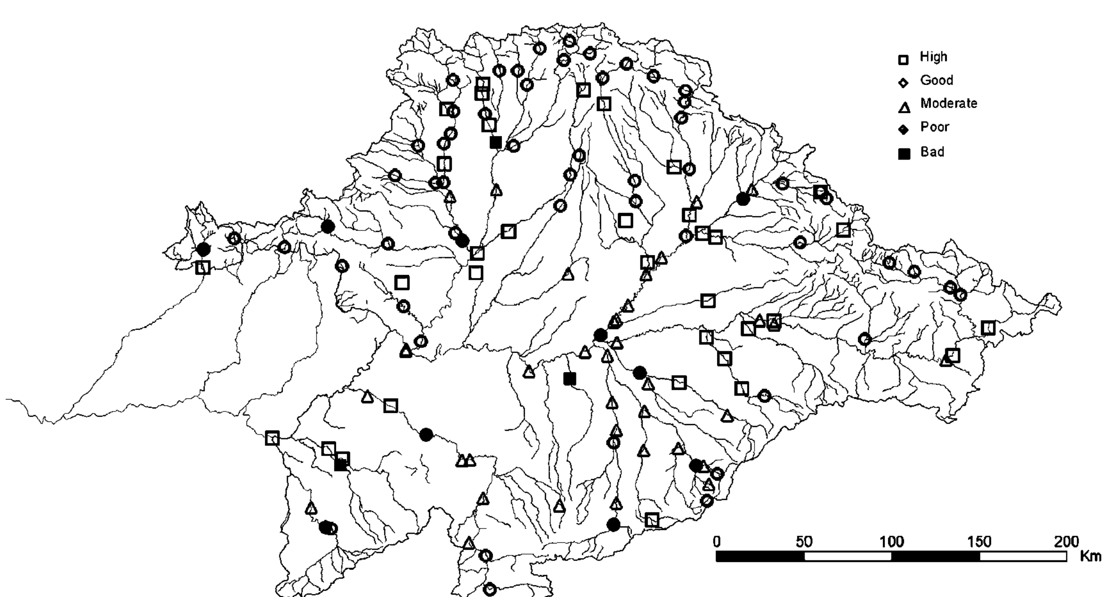

Fig. 6. Water quality in the sampling stations of the Spanish part of the Duero basin according to the results of SPI.

works in other Spanish basins (GOMÀ *et al.* 2004, 2005) came to similar conclusions. 3. Further taxonomic and ecological research is also needed in order to determine more accurate autoecological parameters within common diatom indices. 4. In any case, a more integrative approach, by means of the application of a wide range of different bioindicators, is needed to assess the 'ecological status' of this Mediterranean and other European basins, as required by the WFD.

ACKNOWLEDGEMENTS

This study was funded by the Duero Basin Water Agency (Confederación Hidrográfica del Duero, CHD). S. Blanco was supported by a grant of the Public Research Center - Gabriel Lippmann (Luxembourg). Authors are grateful to A. Oliveira for her correction of the English text, and P. Albaladejo and C. Bouillon for their technical and SEM assistance. Two anonymous reviewers are thanked for their valuable comments on the manuscript.

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Supplementary material

The Appendix S1 is available as supplementary material in the electronic version of this article, which is available at:
http://www.ingentaconnect.com/content/rbsb/bjb/2008/00000141/00000001/art00004

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SUPPLEMENTARY MATERIAL: APPENDIX S1

Checklist of diatom taxa found in the Duero Basin. Most frequent taxa (relative abundance ≥ 1% in at least one sampling station) are in bold. Asterisks mark new taxa for the Iberian Peninsula.

Achnanthes sp.
**Achnanthes exigua* var. *elliptica* Hustedt
***Achnanthes inflata* (Kützing) Grunow**
**Achnanthes obliqua* (Gregory) Hustedt
**Achnanthes sphacelata* J.R. Carter
***Achnanthes subhudsonis* Hustedt**
Achnanthidium affine (Grunow) Czarnecki
***Achnanthidium atomoides* Monnier, Lange-Bertalot et Ector**
Achnanthidium catenatum (Bílý et Marvan) Lange-Bertalot
****Achnanthidium eutrophilum* (Lange-Bertalot) Lange-Bertalot**
***Achnanthidium exiguum* (Grunow) Czarnecki**
***Achnanthidium exilis* (Kützing) Round et Bukhtiyarova**
**Achnanthidium gracillimum* (F. Meister) Czarnecki
Achnanthidium jackii Rabenhorst
***Achnanthidium macrocephalum* (Hustedt) Round et Bukhtiyarova**
***Achnanthidium minutissimum* (Kützing) Czarnecki**
Achnanthidium pusillum (Grunow) Czarnecki
***Achnanthidium pyrenaicum* (Hustedt) H. Kobayasi**
****Achnanthidium rivulare* Potapova et Ponader**
***Achnanthidium saprophilum* (H. Kobayasi et Mayama) Round et Bukhtiyarova**
Achnanthidium subatomus (Hustedt) Lange-Bertalot
***Adlafia minuscula* (Grunow) Lange-Bertalot**
***Adlafia minuscula* var. *muralis* (Grunow) Lange-Bertalot**
***Amphora copulata* (Kützing) Schoeman et Archibald**
***Amphora inariensis* Krammer**
Amphora montana Krasske
Amphora oligotraphenta Lange-Bertalot
***Amphora ovalis* (Kützing) Kützing**
***Amphora pediculus* (Kützing) Grunow ex A. Schmidt**
***Amphora veneta* Kützing**
Aneumastus stroesei (Østrup) D.G. Mann
***Asterionella formosa* Hassall**
Aulacoseira sp.
***Aulacoseira ambigua* (Grunow) Simonsen**
Aulacoseira distans (Ehrenberg) Simonsen
***Aulacoseira distans* var. *nivalis* (W. Smith) E.Y. Haworth**
***Aulacoseira granulata* (Ehrenberg) Simonsen**
***Aulacoseira granulata* var. *angustissima* (O. Müller) Simonsen**
Aulacoseira lirata (Ehrenberg) R. Ross
****Aulacoseira muzzanensis* (Meister) Krammer**
***Aulacoseira subarctica* (O. Müller) E.Y. Haworth**
Bacillaria paxillifer (O.F. Müller) Hendey
***Brachysira neoexilis* Lange-Bertalot**
Brachysira vitrea (Grunow) R. Ross
***Caloneis bacillum* (Grunow) Cleve**
Caloneis lepidula (Grunow) Cleve
***Caloneis silicula* (Ehrenberg) Cleve**
Capartogramma crucicula (Grunow et Cleve) R. Ross
Cavinula cocconeiformis (Gregory ex Greville) D.G. Mann et Stickle
***Cocconeis disculus* (Schumann) Cleve**
***Cocconeis euglypta* Ehrenberg**
***Cocconeis lineata* Ehrenberg**
Cocconeis neodiminuta Krammer
Cocconeis neothumensis Krammer
***Cocconeis pediculus* Ehrenberg**
***Cocconeis pseudolineata* (Geitler) Lange-Bertalot**
**Cocconeis pseudothumensis* E. Reichardt
Craticula accomoda (Hustedt) D.G. Mann

Craticula ambigua (Ehrenberg) D.G. Mann
Craticula cuspidata (Kützing) D.G. Mann
Craticula minusculoides (Hustedt) Lange-Bertalot
***Craticula molestiformis* (Hustedt) Lange-Bertalot**
Ctenophora pulchella (Ralfs ex Kützing) D.M. Williams et Round
***Cyclostephanos dubius* (Fricke) Round**
***Cyclostephanos invisitatus* (Hohn et Hellerman) Theriot, Stoermer et Håkansson**
* *Cyclostephanos tholiformis* Stoermer, Håkansson et Theriot
***Cyclotella atomus* Hustedt**
****Cyclotella atomus* var. *gracilis* Genkal et Kiss**
* *Cyclotella cyclopuncta* Håkansson et J.R. Carter
Cyclotella distinguenda Hustedt
* *Cyclotella distinguenda* var. *unipunctata* (Hustedt) Håkansson et J.R. Carter
* *Cyclotella krammeri* Håkansson
Cyclotella meduanae H. Germain
***Cyclotella meneghiniana* Kützing**
Cyclotella ocellata Pantocsek
***Cyclotella striata* (Kützing) Grunow**
***Cymatopleura elliptica* (Brébisson) W. Smith**
Cymatopleura solea (Brébisson) W. Smith
****Cymbella affiniformis* Krammer**
***Cymbella amphicephala* Nägeli**
Cymbella aspera (Ehrenberg) H. Peragallo
* *Cymbella cantonatii* Lange-Bertalot
****Cymbella compacta* Østrup**
Cymbella cymbiformis C. Agardh
Cymbella delicatula Kützing
***Cymbella excisa* Kützing**
* *Cymbella excisa* var. *procera* Krammer
****Cymbella excisiformis* Krammer**
Cymbella hustedtii Krasske
****Cymbella kolbei* Hustedt var. *angusta* Krammer**
Cymbella lanceolata (C. Agardh) C. Agardh
* *Cymbella lange-bertalotii* Krammer
* *Cymbella neocistula* Krammer
***Cymbella parva* (W. Smith) Wolle**
Cymbella subaequalis Grunow
****Cymbella subleptoceros* Krammer**
Cymbella tumida (Brébisson) Van Heurck
Cymbella turgidula Grunow
* *Cymbella vulgata* Krammer
Cymbopleura naviculiformis (Auerswald) Krammer et Lange-Bertalot
***Denticula tenuis* Kützing**
Diadesmis biceps (Grunow) Arnott ex Grunow
****Diadesmis confervacea* Kützing**
Diadesmis perpusilla (Grunow) D.G. Mann
***Diatoma ehrenbergii* Kützing**
Diatoma hyemalis (Roth) Heiberg
***Diatoma mesodon* (Ehrenberg) Kützing**
***Diatoma moniliformis* Kützing**
***Diatoma vulgaris* Bory**
***Diploneis elliptica* (Kützing) Cleve**
Diploneis marginestriata Hustedt
Diploneis oblongella (Nägeli) A. Cleve
***Discostella pseudostelligera* (Hustedt) Houk et Klee**
***Discostella stelligera* (Cleve et Grunow) Houk et Klee**
****Discostella woltereckii* (Hustedt) Houk et Klee**
***Ellerbeckia arenaria* (Moore) R.M. Crawford**
***Encyonema caespitosum* Kützing**
****Encyonema lange-bertalotii* Krammer**
***Encyonema minutum* (Hilse) D.G. Mann**
****Encyonema perminutum* Krammer**
***Encyonema prostratum* (Berkeley) Kützing**
***Encyonema reichardtii* (Krammer) D.G. Mann**
***Encyonema silesiacum* (Bleisch) D.G. Mann**
* *Encyonema triangulum* (Ehrenberg) Kützing
****Encyonema ventricosum* (C. Agardh) Grunow**
* *Encyonopsis krammeri* E. Reichardt
***Encyonopsis microcephala* (Grunow) Krammer**
****Encyonopsis microcephala* var. *robusta* (Hustedt) Krammer**
* *Encyonopsis minuta* Krammer et E. Reichardt
* *Encyonopsis subminuta* Krammer et E. Reichardt
Encyonopsis tavirana Krammer
****Eolimna comperei* Ector, M. Coste et Iserentant**
***Eolimna minima* (Grunow) Lange-Bertalot**
***Eolimna subminuscula* (Manguin) Moser, Lange-Bertalot et Metzeltin**
***Epithemia adnata* (Kützing) Brébisson**
***Epithemia sorex* Kützing**
***Epithemia turgida* (Ehrenberg) Kützing**
Eucocconeis flexella Hustedt
* *Eucocconeis ninckei* (Guermeur et Manguin) Lange-Bertalot
Eunotia bilunaris (Ehrenberg) Souza
Eunotia bilunaris var. *mucophila* Lange-Bertalot, Nörpel et Alles

Eunotia exigua (Brébisson ex Kützing) Rabenhorst
Eunotia formica Ehrenberg
***Eunotia implicata* Nörpel, Lange-Bertalot et Alles**
Eunotia incisa Gregory
***Eunotia minor* (Kützing) Grunow**
Eunotia paludosa Grunow
Eunotia pectinalis var. *undulata* (Ralfs) Rabenhorst
***Eunotia soleirolii* (Kützing) Rabenhorst**
**Eunotia thumii* Hustedt
***Fallacia lenzii* (Hustedt) D.G. Mann**
Fallacia monoculata (Hustedt) D.G. Mann
Fallacia pygmaea (Kützing) Stickle et D.G. Mann
***Fallacia subhamulata* (Grunow) D.G. Mann**
**Fallacia sublucidula* (Hustedt) D.G. Mann
Fistulifera pelliculosa (Brébisson ex Kützing) Lange-Bertalot
***Fistulifera saprophila* (Lange-Bertalot et Bonik) Lange-Bertalot**
Fragilaria sp.
***Fragilaria arcus* (Ehrenberg) Cleve**
***Fragilaria austriaca* (Grunow) Lange-Bertalot**
***Fragilaria bidens* Heiberg**
***Fragilaria capucina* Desmazières**
***Fragilaria capucina* var. *capitellata* (Grunow) Lange-Bertalot**
***Fragilaria capucina* var. *distans* (Grunow) Lange-Bertalot**
***Fragilaria crotonensis* Kitton**
***Fragilaria gracilis* Østrup**
Fragilaria mesolepta Rabenhorst
***Fragilaria montana* (Krasske) Lange-Bertalot**
***Fragilaria neoproducta* Lange-Bertalot**
***Fragilaria rumpens* (Kützing) Carlson**
Fragilaria tenera (W. Smith) Lange-Bertalot
***Fragilaria vaucheriae* (Kützing) J.B. Petersen**
Fragilariforma bicapitata (Mayer) D.M. Williams et Round
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer
Frustulia rhomboides (Ehrenberg) De Toni
***Frustulia vulgaris* (Thwaites) De Toni**
Geissleria sp.
**Geissleria acceptata* (Hustedt) Lange-Bertalot et Metzeltin
**Geissleria cummerowi* (Kalbe) Lange-Bertalot
Geissleria decussis (Hustedt) Lange-Bertalot et Metzelin
**Gomphoneis eriensis* var. *variabilis* Kociolek et Stoermer
***Gomphoneis minuta* (Stone) Kociolek et Stoermer**
Gomphonema sp.
***Gomphonema acidoclinatum* Lange-Bertalot et E. Reichardt**
Gomphonema acuminatum Ehrenberg
Gomphonema affine Kützing
Gomphonema angustatum (Kützing) Rabenhorst
Gomphonema angustum C. Agardh
Gomphonema augur Ehrenberg
**Gomphonema bavaricum* E. Reichardt et Lange-Bertalot
**Gomphonema bohemicum* (Reichelt et Fricke) A. Cleve
Gomphonema clavatum Ehrenberg
***Gomphonema exilissimum* (Grunow) Lange-Bertalot et E. Reichardt**
***Gomphonema gracile* Ehrenberg**
***Gomphonema italicum* Kützing**
***Gomphonema lagenula* Kützing**
**Gomphonema lateripunctatum* E. Reichardt et Lange-Bertalot
Gomphonema micropus Kützing
***Gomphonema minutum* (C. Agardh) C. Agardh**
***Gomphonema olivaceum* (Hornemann) Brébisson**
**Gomphonema olivaceum* var. *olivaceoides* (Hustedt) Lange-Bertalot
***Gomphonema parvulius* Lange-Bertalot et E. Reichardt**
***Gomphonema parvulum* (Kützing) Kützing**
***Gomphonema productum* (Grunow) Lange-Bertalot et E. Reichardt**
***Gomphonema pumilum* (Grunow) E. Reichardt et Lange-Bertalot**
***Gomphonema pumilum* var. *elegans* E. Reichardt et Lange-Bertalot**
***Gomphonema pumilum* var. *rigidum* E. Reichardt et Lange-Bertalot**
***Gomphonema rhombicum* M. Schmidt**
***Gomphonema subclavatum* Grunow**
***Gomphonema tergestinum* Fricke**
***Gomphonema truncatum* Ehrenberg**
***Gomphosphenia lingulatiformis* (Lange-Bertalot et E. Reichardt) Lange-Bertalot**

***Gyrosigma acuminatum* (Kützing) Rabenhorst**
***Gyrosigma attenuatum* (Kützing) Rabenhorst**
***Gyrosigma nodiferum* (Grunow) Reimer**
Hantzschia abundans Lange-Bertalot
***Hantzschia amphioxys* (Ehrenberg) Grunow**
***Hippodonta* sp.**
***Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski**
Hippodonta costulata (Grunow) Lange-Bertalot, Metzeltin et Witkowski
****Hippodonta lueneburgensis* (Grunow) Lange-Bertalot, Metzeltin et Witkowski**
***Karayevia clevei* (Grunow) Round et Bukhtiyarova**
**Karayevia laterostrata* (Hustedt) Round et Bukhtiyarova
Karayevia amoena (Hustedt) Bukhtiyarova
**Karayevia kolbei* (Hustedt) Bukhtiyarova
***Karayevia ploenensis* var. *gessneri* (Hustedt) Bukhtiyarova**
***Lemnicola hungarica* (Grunow) Round et Basson**
Luticola cohnii (Hilse) D.G. Mann
***Luticola goeppertiana* (Bleisch) D.G. Mann**
***Luticola mutica* (Kützing) D.G. Mann**
***Martyana martyi* (Héribaud) Round**
Mayamaea atomus (Kützing) Lange-Bertalot
**Mayamaea atomus* var. *alcimonica* (E. Reichardt) E. Reichardt
***Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot**
**Mayamaea lacunolaciniata* (Lange-Bertalot et Bonik) Lange-Bertalot
***Melosira varians* C. Agardh**
***Meridion circulare* (Greville) C. Agardh**
Meridion constrictum (Ralfs) Van Heurck
**Microcostatus kuelbsii* (Lange-Bertalot) Lange-Bertalot
Navicula sp.
***Navicula angusta* Grunow**
****Navicula antonii* Lange-Bertalot**
Navicula arvensis Hustedt
****Navicula associata* Lange-Bertalot**
***Navicula capitatoradiata* H. Germain**
Navicula catalanogermanica Lange-Bertalot et Hofmann
***Navicula caterva* Hohn et Hellerman**
***Navicula cryptocephala* Kützing**
***Navicula cryptotenella* Lange-Bertalot**
****Navicula cryptotenelloides* Lange-Bertalot**
**Navicula densilineolata* (Lange-Bertalot) Lange-Bertalot
Navicula exilis Kützing
****Navicula germainii* Wallace**
***Navicula gregaria* Donkin**
****Navicula heimansioides* Lange-Bertalot**
**Navicula hintzii* Lange-Bertalot
***Navicula lanceolata* (C. Agardh) Ehrenberg**
**Navicula leptostriata* Jørgensen
Navicula microcari Lange-Bertalot
Navicula modica Hustedt
***Navicula notha* Wallace**
***Navicula phyllepta* Kützing**
Navicula pseudoarvensis Hustedt
Navicula radiosa Kützing
***Navicula recens* (Lange-Bertalot) Lange-Bertalot**
***Navicula reichardtiana* Lange-Bertalot**
Navicula rhynchocephala Kützing
***Navicula rostellata* Kützing**
***Navicula subrotundata* Hustedt**
***Navicula symmetrica* R.M. Patrick**
***Navicula tripunctata* (O.F. Müller) Bory**
Navicula trivialis Lange-Bertalot
**Navicula vandamii* Schoeman et Archibald
***Navicula veneta* Kützing**
***Navicula viridula* (Kützing) Ehrenberg**
**Navicula viridulacalcis* Lange-Bertalot
Neidium dubium (Ehrenberg) Cleve
***Nitzschia abbreviata* Hustedt**
***Nitzschia acicularis* (Kützing) W. Smith**
***Nitzschia acidoclinata* Lange-Bertalot**
Nitzschia agnita Hustedt
***Nitzschia amphibia* Grunow**
Nitzschia angustatula Lange-Bertalot
***Nitzschia archibaldii* Lange-Bertalot**
**Nitzschia bacillum* Hustedt
***Nitzschia capitellata* Hustedt**
Nitzschia denticula Grunow
***Nitzschia dissipata* (Kützing) Grunow**
***Nitzschia dissipata* var. *media* (Hantzsch) Grunow**
Nitzschia draveillensis M. Coste et Ricard
***Nitzschia filiformis* (W. Smith) Van Heurck**
Nitzschia filiformis var. *conferta* (Richter) Lange-Bertalot

***Nitzschia fonticola* Grunow**
****Nitzschia* aff. *fossilis* Grunow**
***Nitzschia frustulum* (Kützing) Grunow**
***Nitzschia fruticosa* Hustedt**
***Nitzschia gessneri* Hustedt**
Nitzschia graciliformis Lange-Bertalot et Simonsen
***Nitzschia gracilis* Hantzsch**
***Nitzschia hantzschiana* Rabenhorst**
Nitzschia heufleriana Grunow
**Nitzschia incognita* Legler et Krasske
***Nitzschia intermedia* Hantzsch ex Cleve et Grunow**
Nitzschia lacuum Lange-Bertalot
Nitzschia linearis (C. Agardh) W. Smith
Nitzschia linearis var. *tenuis* (W. Smith) Grunow
Nitzschia microcephala Grunow
Nitzschia nana Grunow
***Nitzschia palea* (Kützing) W. Smith**
***Nitzschia palea* var. *debilis* (Kützing) Grunow**
***Nitzschia paleacea* (Grunow) Grunow**
***Nitzschia paleaeformis* Hustedt**
***Nitzschia perminuta* (Grunow) M. Peragallo**
***Nitzschia pura* Hustedt**
Nitzschia pusilla Grunow emend. Lange-Bertalot
Nitzschia radicula Hustedt
Nitzschia recta Hantzsch
Nitzschia scalpelliformis (Grunow) Grunow
Nitzschia sigmoidea (Nitzsch) W. Smith
Nitzschia sinuata (Thwaites) Grunow
Nitzschia sinuata var. *tabellaria* Grunow
***Nitzschia sociabilis* Hustedt**
***Nitzschia solgensis* A. Cleve**
Nitzschia solita Hustedt
***Nitzschia subacicularis* Hustedt**
Nitzschia subtilis Grunow
***Nitzschia suchlandtii* Hustedt**
***Nitzschia supralitorea* Lange-Bertalot**
***Nitzschia* aff. *tropica* Hustedt**
Nitzschia tubicola Grunow
Nitzschia umbonata (Ehrenberg) Lange-Bertalot
Nitzschia valdestriata Aleem et Hustedt
***Nitzschia vermicularis* (Kützing) Hantzsch**
****Opephora mutabilis* (Grunow) Sabbe et Vyverman**
Parlibellus protractus (Grunow) Witkowski, Lange-Bertalot et Metzeltin
Pinnularia sp.
**Pinnularia anglica* Krammer
Pinnularia acrosphaeria W. Smith
***Pinnularia divergentissima* (Grunow) Cleve**
**Pinnularia frequentis* Krammer
***Pinnularia gibba* Ehrenberg**
***Pinnularia microstauron* (Ehrenberg) Cleve**
Pinnularia obscura Krasske
**Pinnularia sinistra* Krammer
**Pinnularia transversa* (A. Schmidt) Mayer
****Pinnularia viridiformis* Krammer**
Placoneis elginensis (Gregory) E.J. Cox
***Placoneis pseudanglica* (Lange-Bertalot) E.J. Cox**
**Placoneis signata* (Hustedt) Mayama
**Planothidium biporomum* (Hohn et Hellerman) Lange-Bertalot
***Planothidium delicatulum* (Kützing) Round et Bukhtiyarova**
***Planothidium dubium* (Grunow) Round et Bukhtiyarova**
Planothidium ellipticum (Cleve) Round et Bukhtiyarova
***Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot**
Planothidium granum (Hohn et Hellerman) Lange-Bertalot
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot
***Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot**
Planothidium peragallii (Brun et Héribaud) Round et Bukhtiyarova
***Planothidium rostratum* (Østrup) Lange-Bertalot**
Platessa conspicua (Mayer) Lange-Bertalot
Platessa hustedtii (Krasske) Lange-Bertalot
Pleurosira laevis (Ehrenberg) Compère
**Psammodictyon constrictum* (Gregory) D.G. Mann
***Psammothidium bioretii* (H. Germain) Bukhtiyarova et Round**
***Psammothidium curtissimum* (J.R. Carter) Aboal**
***Psammothidium daonense* (Lange-Bertalot) Lange-Bertalot**
***Psammothidium helveticum* (Hustedt) Bukhtiyarova et Round**
****Psammothidium lauenburgianum* (Hustedt) Bukhtiyarova et Round**

Psammothidium oblongellum (Oestrup) Vijver
Psammothidium sacculum (J.R. Carter) Bukhtiyarova et Round
***Psammothidium subatomoides* (Hustedt) Buhktiyarova et Round**
***Pseudostaurosira binodis* (Ehrenberg) M.B. Edlund**
***Pseudostaurosira brevistriata* (Grunow) D.M. Williams et Round**
Pseudostaurosira parasitica (W. Smith) Morales
Pseudostaurosira pseudoconstruens (Marciniak) D.M. Williams et Round
***Puncticulata radiosa* (Lemmermann) Håkansson**
***Reimeria sinuata* (Gregory) Kociolek et Stoermer**
***Reimeria uniseriata* Sala, Guerrero et Ferrario**
***Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot**
***Rhopalodia gibba* var. *minuta* Krammer**
***Rossithidium petersenii* (Hustedt) Round et Bukhtiyarova**
Rossithidium pusillum (Grunow) Round et Bukhtiyarova
Sellaphora bacillum (Ehrenberg) D.G. Mann
***Sellaphora mutata* (Krasske) Lange-Bertalot**
***Sellaphora parapupula* Lange-Bertalot**
***Sellaphora pupula* (Kützing) Mereschkowsky**
***Sellaphora seminulum* (Grunow) D.G. Mann**
****Simonsenia delognei* (Grunow) Lange-Bertalot**
Stauroneis anceps Ehrenberg
Stauroneis phoenicenteron (Nitzsch) Ehrenberg
***Staurosira construens* Ehrenberg**
***Staurosira elliptica* (Schumann) D.M. Williams et Round**
***Staurosira subsalina* (Hustedt) Lange-Bertalot**
***Staurosira venter* (Ehrenberg) Cleve et Möller**
**Staurosirella lapponica* (Grunow) D.M. Williams et Round
***Staurosirella leptostauron* (Ehrenberg) D.M. Williams et Round**
***Staurosirella pinnata* (Ehrenberg) D.M. Williams et Round**
***Stephanodiscus hantzschii* Grunow**
***Stephanodiscus hantzschii* f. *tenuis* (Hustedt) Håkansson et Stoermer**
***Stephanodiscus minutulus* (Kützing) Cleve et Möller**
***Stephanodiscus neoastraea* Håkansson et Hickel**
Stephanodiscus parvus Stoermer et Håkansson
**Stephanodiscus vestibulis* Håkansson, Theriot et Stoermer
Surirella angusta Kützing
Surirella birostrata Hustedt
Surirella brebissonii Krammer et Lange-Bertalot
Surirella brebissonii var. *kuetzingii* Krammer et Lange-Bertalot
Surirella linearis W. Smith
Surirella ovalis Brébisson
***Surirella roba* L. Leclercq**
Surirella subsalsa W. Smith
* *Surirella suecica* Grunow
Surirella tenera Gregory
Synedrella subconstricta (Grunow) Round et Maidana
Tabellaria fenestrata (Lyngbye) Kützing
***Tabellaria flocculosa* (Roth) Kützing**
**Tabellaria ventricosa* Kützing
***Tabularia fasciculata* (C. Agardh) D.M. Williams et Round**
***Thalassiosira lacustris* (Grunow) Hasle**
Thalassiosira pseudonana Hasle et Heimdal
Thalassiosira weissflogii (Grunow) Fryxell et Hasle
Tryblionella apiculata Gregory
Tryblionella hungarica (Grunow) D.G. Mann
Tryblionella levidensis W. Smith
***Ulnaria acus* (Kützing) Aboal**
***Ulnaria delicatissima* var. *angustissima* (Grunow) Aboal**
***Ulnaria ulna* (Nitzsch) Compère**