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Application of diatom biotic indices in the Guadalquivir River Basin, a Mediterranean basin. Which one is the most appropriated?

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Abstract The diatom community was studied in 110 sites within the Guadalquivir River catchment area, South Spain, in order to test the applicability of diatom biotic indices developed in other European regions to this site and to provide a useful tool for monitoring water quality in the river basin. We identified 399 taxa and calculated five diatomic indices (Specific Polluosensitivity Index (IPS), Biological Diatom Index, Trophic Diatom Index, Index of the European Economic Community, and Diatom-based Eutrophication Pollution Index (EPI-D)). Since the indices analyzed were highly correlated, their results could be compared. The indices that gave the best results were the EPI-D followed by the IPS, the latter being the most widely used index in Iberian catchments. Nevertheless, the EPI-D presented certain advantages: (1) this index correlated the best with the water chemistry in the catchment area; (2) EPI-D is not sensitive to the presence of taxa belonging to the *Achnanthidium minutissimum* complex frequently present in the Guadalquivir basin. Nevertheless, EPI-D retains its effectiveness and thus constitutes an easier index for application from a taxonomical standpoint. We estimated the general water quality of the entire basin on the basis of EPI-D. According to these results, 55% of the sites had either high or good water quality. The species that better characterized each water quality category in the study area were: *A. minutissimum* (high and good), *Amphora pediculus* (moderate), *Nitzschia frustulum* (poor), and *Nitzschia capitellata* (bad).

Keywords Biotic indices · Diatoms · Water quality · Rivers · Spain

Introduction

The implementation of the European Union’s Water Framework Directive (WFD, No. 60/2000; European Union 2000) requires the utilization of key biotic and abiotic elements that allow the assessment of the ecological status of different water bodies. The term *ecological status* has been defined as *the expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters* (European Union 2000). Particularly in rivers, this definition involves the conservation of the geomorphology of the river

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channel, the development of the vegetation (riparian and aquatic), and the water quality. A valid assessment of the ecological status of an ecosystem must necessarily entail an analysis of the relationships among different biological elements.

According to the WFD, the elements that should be evaluated for a consideration of biological quality in rivers are the composition and abundance of the aquatic flora, the benthic invertebrates, and the fish. Among the algal groups that have been used as indicators, the diatoms are the most suitable for the elaboration of quality indices because of their wide diversity, the ubiquitousness of the various species, the sensitivity to contamination of many of the taxa, and the ease of their transport and storage of the specimens. Several diatom indices for water quality assessment in rivers have been mainly used in Europe (cf. Montesanto et al. 1999; Sabater 2000; Prygiel 2002; Prygiel et al. 2002; Eloranta and Soininen 2002; Ács et al. 2005; Cappelletti et al. 2005; Torrisi and Dell'Uomo 2006) but also in other parts of the world (Wu 1999; Gómez 1999; Gómez and Licursi 2001; Wu and Kow 2002; Jüttner et al. 2003; Duong et al. 2006; Atazadeh et al. 2007). Most of these indices are based on relative abundance combined with the degree of sensitivity (or tolerance) shown by a group of selected taxa (generally at the species level). Some of these indices are: the Specific Polluosensitivity Index (IPS; CEMAGREF 1982), the Index of the European Economic Community (CEE; Descy and Coste 1991), the Trophic Diatom Index (TDI; Kelly 1998); the Diatom-Based Eutrophication Pollution Index (EPI-D; DellUomo et al. 1999); and the Biological Diatom Index (IBD; Prygiel and Coste 2000). Prygiel et al. (1999) described and analyzed some of these indices along with their application and stated that, despite their usefulness, none of them can be applied everywhere without some adaptation or modification. That limitation is why some countries even within Europe have either developed or are at present developing indices adapted to their particular geographical areas (Taylor et al. 2007; Kupe et al. 2007). In the Iberian Peninsula, no standard diatomic index has as yet been developed. This situation and the necessity of fulfilling the Directive's requirements compel investigators to apply indices generated in other European countries (Almeida 2001; Gomà et al. 2004, 2005; Oscoz et al. 2007; Penalta-Rodríguez and López-Rodríguez 2007; Camargo and Jiménez 2007; Blanco et al. 2008). Different Spanish river basins are being monitored through the use of European diatom indices (Agencia Catalana de l'Aigua 2003; Confederación Hidrográfica del Ebro 2003; Gobierno Vasco 2005; Confederación Hidrográfica del Duero 2007).

The present work is part of a project created in order to evaluate the ecological status of different sections of rivers within the Guadalquivir watershed, where microalgae and macroalgae have been studied together with the pertinent environmental information. The objective of this investigation was to evaluate whether or not diatomic indices developed in other European regions can be applied to this specific catchment area, so as to provide a practicable tool for the monitoring of water quality in this region up until such a time as a better one is generated.

Study area

The Guadalquivir River catchment area is located in the south of the Iberian Peninsula and extends over an area of 57,527 km^2. The entire basin is under a Mediterranean climate, receiving some oceanic influence in the lowest part. This climate is characterized by dry and hot summers with relatively mild temperatures in winter (annual average 16.8°C) and irregular and scant precipitations in wintertime or spring (annual average 630 mm). The high temperatures combined with the lack of precipitation in summer result in a marked water deficit during that season. This situation led to the necessity to regulate most rivers within the basin, with the result that some 26% of the total volume of water within the entire basin is presently stored in reservoirs.

The catchment area comprises three geological units: the mountains of Sierra Morena to the North, the Betic mountain chain to the South, and the Guadalquivir valley in between (Fig. 1). The watershed is delimited by chains of mountains with altitudes that range from 1,000 m to more than 3,000 m above sea level (asl). In contrast,

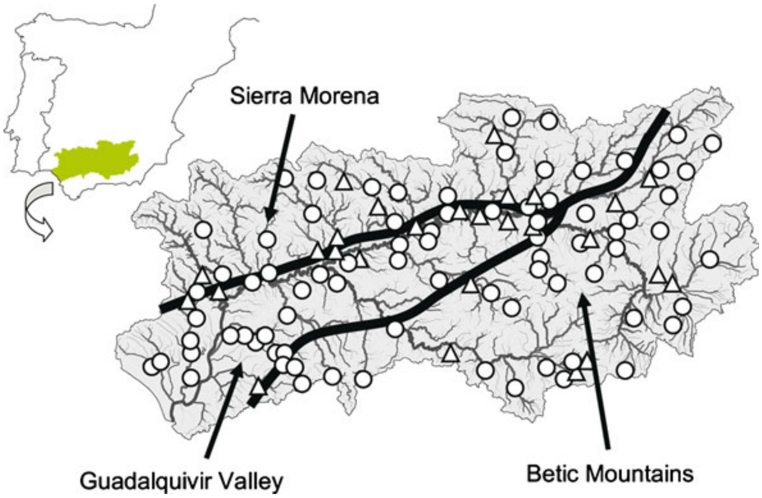

Fig. 1 Location of Guadalquivir River in the Iberian Peninsula and sampling stations; lithological zones are also shown. Sites situated downstream a reservoir (*triangles*)

the valley along which the Guadalquivir River runs is located at a lower altitude (average circa 150 m asl). This valley widens as it approaches the Atlantic Ocean.

The three geographical units have different lithological constitutions and tectonic structures, which characteristics influence the general relief of the landscape—principally, the resulting design of the drainage system, the precipitation pattern and water salinity, and the exposure to erosion. The principal human activities also differ in each of these units: the Sierra Morena is a wooded area dominated by extensive parkland (*dehesa*—i.e., wooded meadow) where livestock breeding is the principal activity. The rivers there have a low salinity (<250 mg/l of dissolved salts). The Betic mountain chain is less wooded than the Sierra Morena with a greater agricultural area, where olive monoculture predominates in most sectors, followed by irrigated crops and orchards in the mountainous parts of the land. The rivers along with their sources in this geographical unit are characterized by a variable range of dissolved salt content (250 mg/l–2 g/l). The Guadalquivir valley is an agricultural area where different irrigated crops are raised: olive (38%) semi-intensively, along with cotton and beetroot (16%) in combination with extensive summer crops such as corn and sunflower (up to 14%) plus extensive winter harvests including wheat and barley (up to 8%), vegetables, rice, and citrus fruits. The dissolved salt content of the river is intermediate owing to the admixture of the tributary waters flowing into it from both of its banks.

Agriculture demands 85% of the water resources within the catchment area which amount far exceeds the urban and industrial depletion. The main food and agricultural industries are furthermore seasonal and have a dispersed location, whereas other industries are grouped into sizeable complexes and function all year round. Among the food and agricultural industries, olive products and their derivatives are the most prominent, followed by sugar refineries, breweries, slaughterhouses, and alcoholic-beverage-producing enterprises. The textile industry and paper mills are also significant.

The majority of the human population (60%) is concentrated in a reduced number of cities, each with more than 20,000 inhabitants. Of the total volume of urban and industrial sewage, principally originated in small municipalities and industries that produce organic wastes, 30% is dumped into the Guadalquivir River without any attempt at purification.

Materials and methods

Selection of sampling points and sampling methodologies

During the spring months (March through June) of 2004 and 2005, we sampled 110 sites distributed in rivers and streams along the Guadalquivir basin (Fig. 1). In those sites situated near a reservoir, samples were taken downstream from the dam.

At each site, a segment of the river not heavily shadowed by riparian vegetation was chosen. The sampled segment—of 10-m length or more—was decided upon according to the observed habitat heterogeneity and substrate availability. Whenever possible, each sector was sampled within zones containing rapids, since stretches of river with very slow current (<20 cm/s) allow the buildup of loosely attached diatoms, silt, and other debris. Samples were collected at a distance from the riverbanks to avoid the pools and stagnant waters that would not reflect the characteristics of the site along with any substrata that had recently remained out of the water and could thus contain aerophilous diatoms.

At all the sites, the water conductivity and the pH were measured in situ through the use of the HANNA HI 9033 and HANNA HI 9025 probes, respectively, while a combined probe YSI 550[a] was employed to measure the dissolved oxygen and the temperature of the water. The current velocity was estimated by measuring the time that a small floating object needed to traverse a certain distance of at least 10 m.

The chemical analyses of the water samples were performed by the Confederación Hidrográfica del Guadalquivir in 50 set locations as part of the routine monitoring that the institution carries out in the basin. These samples were removed monthly in the majority of the sites, though only every 2 months in the rest. Nutrient data involving nitrogen compounds (nitrate, nitrite, and ammonia) were obtained at 71 sampling stations, and at 37 of these same sites, the levels of soluble reactive phosphorus (SRP) were also measured. By contrast, data on the levels of the herbicide atrazine and the total pesticides along with the concentrations of heavy metals and metalloids (Zn, Cd, Cu, Fe, Mn, Hg, and As) were acquired from 34 sites, though not necessarily included among the previous 37. The chemical analyses were performed according to the APHA (1998) guidelines.

The diatom sampling was done at all sites as indicated by the European Standard EN 13946 (2003) and Kelly et al. (1998). At each station, depending upon the availability, a single type of substrate was chosen according to the following order of preference: natural rocks, artificial surfaces (such as bridge pillars), artificial substrates, and plants. The artificial substrates had been placed approximately 2 months before the sampling, since the norm indicates a minimum of 1 month of prior immersion. Epiphyton samples were collected on the surface of the stems and leaves of helophytes. The sampling was carried out on natural rocks, artificial hard substrata, and plants in 75%, 18%, and 7% of the sites, respectively.

For the sampling of natural rocks, five to ten rocks were randomly collected and their top surface scraped off and removed with a toothbrush or first scraped and then sucked up with different elements depending upon whether or not the stones could be removed from the water. A scraper with a fitted collecting net of Nytal (pores of diameter 10 μm) was used in sites where there were vertical hard artificial surfaces, and the scraping was done at a depth of 30 cm in order to avoid the effects of water-level variations that might otherwise result in the collection of aerophilous species. Tiles and bricks were employed as artificial substrates because their rough surfaces favor epilithic diatom establishment. The artificial substrates were treated in the same way as the natural rocks. Finally, epiphyton was collected by gathering leaves and stem sections, discarding either those parts that had been recently out of the water or those that were near the bottom covered by sediment. Four or five macrophyte cuttings of 5-cm length were placed together in a bottle filled with tap water. At the laboratory, the stems and leaves were scraped gently with a coverslip.

A single sample from the most suitable substrate was taken at each site and the collected material homogenized and fixed with 4% (*v*/*v*) formaldehyde in all instances.

At all sites, we made an evaluation of the structural features of the riverbed and of the riparian vegetation and noted the human activities and their impacts in the ecosystem. Our objective in this evaluation was to investigate whether any relationship could be found between the human activities observed, either at the sampling sites or upstream from them, and the presence of specific toxicants in the water.

Treatment and analysis of diatom samples

The hydrogen peroxide treatment and preparation of permanent slides mounted in Naphrax™ were done according to the European Standard EN 13946 (2003). For scanning electron microscopy (SEM), part of the material was also mounted on glass stubs and then coated with gold palladium.

The identification and counting of taxa were carried out under a Nikon E-200 light microscope at a × 1,000 magnification. In accordance with the recommendations of Lobo et al. (1990) and Morales et al. (2001), SEM observations were made in samples where the most abundant species could be easily confused with others having different ecological requirements. The SEM was performed with a Jeol J.S.M. 6360 LV microscope at the Servicio de Microscopía Electrónica of Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata (La Plata, Argentina).

For the calculation of relative frequencies, according to the Confederación Hidrográfica del Ebro (2005), a minimum of 400 valves per sample were counted.

Species identifications were carried out following Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Germain (1981), Prygiel and Coste (2000), and other specific references on European diatoms.

Data analysis

The average value of each chemical parameter analyzed by the Confederación Hidrográfica del Guadalquivir between March and June was calculated for each site. Three principal component analyses (PCA) were performed on these data as well as the physical data obtained along with the diatom samples. A first, PCA was performed with the physical and chemical variables of the 71 stations (current velocity; water temperature; conductivity; pH; dissolved oxygen; and nitrate, nitrite, and ammonia concentrations). A second PCA was performed with the same variables plus the SRP concentration, but only in the 37 sites where the SRP was measured. The consistency within the groupings found in these analyses was tested through the Student *t* test with the sampling site coordinates on the first and second axes of the PCA. A third PCA was performed with the data for other chemicals that deteriorate water quality, such as atrazine, pesticides, metals, and metalloids. A Student *t* test was also applied to find out if a significant difference with respect to any of these parameters was found at sites under farming pressure relative to sites lacking such influences.

The diatom indices IBD, IPS, CEE, EPI-D, and TDI were calculated by means of the OMNIDIA 4.2 software. In order to facilitate comparisons among the results, the program automatically transformed them into a scale from 0 to 20, independent of the scale in which they had been expressed. The values for the indices were assigned to five categories of water quality symbolized by the colors specified by the WFD: high (blue), good (green), moderate (yellow), poor (orange), and bad (red). The numerical limit between two consecutive categories varied slightly among different indices.

The chemical and biological data were contrasted in sites where both types of analysis had been done.

In order to determine which index better reflected the presence of organic pollution and/or the trophic level in the basin, the physical and chemical data (ammonium, nitrate, nitrite, SRP, dissolved oxygen, water conductivity, and current velocity) as well as the coordinates of the sites on the first axis of the second PCA analysis were contrasted with the diatom indices by means of an *r*-Pearson correlation analysis. The coordinates of the first axis of the second PCA were considered as another chemical variable to test if the values of the indices were affected by a single variable or by a group of variables. In order to evaluate if the diatom indices were also influenced by other aspects of water quality, the same procedure was followed, but considering the toxicants used to perform the third PCA and the coordinates of sites present in it. The correlations between different indices were calculated to assess their comparability. Next, the *r*-Pearson correlations between the chemicals used in the third PCA and the percentage of valves of the pioneer genus *Achnanthidium* were calculated. All correlations, the PCA analyses, and the Student *t* tests were carried out by means of a Statistica 7 software package.

Once the relationships among the flora, indices, and water chemistry were evaluated and the effectiveness of some of the indices tested, the biological data from the sites without chemical data were used to infer certain conclusions about the biological quality at those locations.

Finally, to determine which species characterize each category of water quality, a Similarity Percentage analysis (SIMPER) was performed by means of the PRIMER 5 software package. This analysis also supplied information about the particular species that are responsible for the floristic similarity between sampling points bearing the same degree of water quality and those species that produce the separation of sites according to different categories of water quality.

Results

A chemical characterization of the entire Guadalquivir River basin with respect to water conductivity, dissolved oxygen levels, nutrients, and the presence of diverse toxicants, such as metals and pesticides, is summarized in Table 1.

The two PCA analyses performed with respect to nutrients grouped the study sites in relation to pH, salt concentration, dissolved oxygen, and nutrient content. The first PCA, which analysis comprised the majority of the sites but did not include the SRP, indicated that the dissolved oxygen levels, nitrite concentrations, and conductivity were the variables that principally contributed to the variance on the first axis, whereas the nitrate and ammonia concentrations were the significant parameters on the second axis (Table 2 and Fig. 2). The sites located on tributaries on the right and on the left bank of the river were segregated along the first axis, thus reflecting a gradient from poorly (right bank) to more richly mineralized (left bank) waters, respectively (Pearson $r = 0.67$; $p < 0.05$). This segregation along the first axis was also closely related to the nitrite concentrations (Pearson $r = 0.69$; $p < 0.05$) and dissolved oxygen levels (Pearson $r = -0.73$; $p < 0.05$), thus suggesting a generally higher trophic level, with more nitrite and less dissolved oxygen in the left riverbank. The sampling points situated along the Guadalquivir River itself have an intermediate position between these groups since the main river course collects water from tributaries on both sides. With regard to the second axis, the sampling sites on the tributaries of the river's left bank were widely distributed along the axis, since some of these locations are situated

Table 1 Average, standard deviation, X/SD, and maximum measured values of each of the considered water chemical parameters

	Average value (X)	Standard deviation (SD)	SD/X (%)	Maximum value
Conductivity (μS/cm)	1,017	1,136	112	6,870
Ammonium (mg N/l)	1.61	5.25	326	29.42
Nitrate (mg N/l)	1.85	2.25	122	11.46
Nitrite (mg N/l)	0.15	0.28	186	1.81
Dissolved oxygen (mg/l)	8.76	2.22	25	11.91
SRP (mg P/l)	0.27	0.30	111	1.21
As (μg/l)	2.00	4.13	206	15.35
Cd (μg/l)	0.31	1.20	387	5.18
Cu (μg/l)	3.92	9.19	234	40.00
Fe (μg/l)	349.93	601.51	171	3,165.50
Mn (μg/l)	161.96	265.64	164	1,079.00
Hg (μg/l)	0.03	0.13	433	0.71
Pb (μg/l)	6.32	37.41	591	221.33
Zn (μg/l)	96.04	337.02	351	1,642.75
Pesticides (μg/l)	0.12	0.42	350	1.98
Atrazine (μg/l)	0.00	0.01		0.07

Table 2 First, second, and third principal component analyses results

N	First PCA		Second PCA		Third PCA	
	71		37		34	
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
% explained variance	39.5	20.6	39.0	19.3	38.5	17.2
Ammonium	0.56	0.59	0.37	0.58		
Conductivity	0.67	−0.33	0.24	−0.44		
Nitrate	0.48	−0.72	0.31	−0.39		
Nitrite	0.69	−0.25	0.49	0.03		
Dissolved oxygen	−0.73	−0.39	−0.36	0.19		
pH	−0.61	−0.22	−0.26	0.41		
Soluble reactive phosphorus			0.51	0.34		
As					−0.001	−0.81
Cd					−0.90	0.26
Cu					−0.91	−0.07
Fe					−0.26	−0.59
Mn					−0.88	−0.27
Hg					0.12	−0.42
Zn					−0.93	0.19
Total pesticides					−0.17	−0.31
Atrazine					0.17	−0.29

within contaminated areas having significant industrial or agricultural activity, while other sites are located in mountainous zones far less anthropically impacted. The sampling stations on the tributaries of the river's right bank appeared less separated from each other in relation to the second axis because of their similar salinity and perturbation characteristics.

In the second PCA analysis performed considering only those sites where the SRP concentration had been registered, the parameters contributing to the most marked variance on the first axis were the SRP and nitrite concentrations and on the second axis the ammonia levels and the conductivity (Table 2 and Fig. 3). Likewise, we observed the same patterns mentioned above with respect to the first PCA: the sites situated on the tributaries of the right bank were more closely associated with less disturbed conditions, whereas the sampling areas located on the rivers flowing into the left bank appeared more scattered along the nutrient gradient determined by the axes and

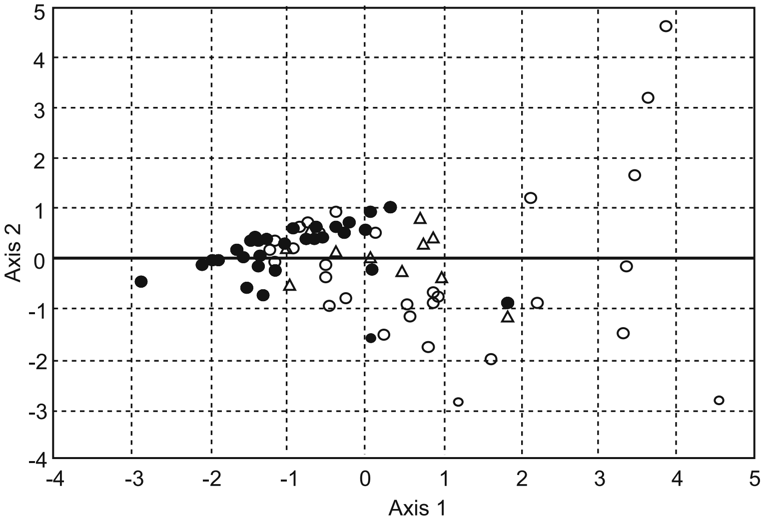

Fig. 2 First principal components analysis carried out using physical and chemical variables excepting phosphate concentration (71 sites). Stations on tributaries flowing into Guadalquivir River on the left bank (*empty circles*) and on the right bank (*filled circles*), as well as those on the principal channel (*triangles*), are differentiated

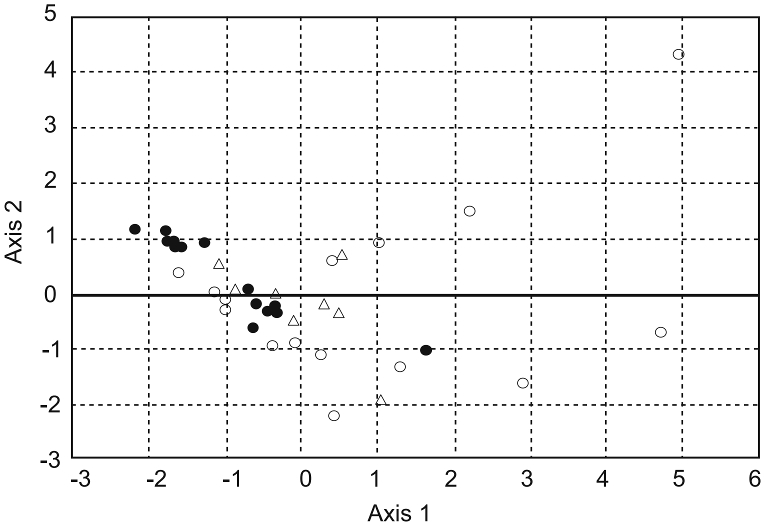

Fig. 3 Second principal components analysis including phosphate concentrations (37 sites). Stations on tributaries flowing into Guadalquivir River on the left bank (*empty circles*) and on the right bank (*filled circles*), as well as those on the principal channel (*triangles*), are differentiated

were thus situated in a more widely disturbed range.

The third PCA analysis (Table 2 and Fig. 4) showed that the sites affected by mining activities were clearly differentiated from the rest of the sampling stations because of their higher metal content in the water. We observed, however, no segregation between sites affected by pesticides related to farming activities and those free from agricultural influences. Moreover, the Student *t* test showed no significant difference ($p < 0.05$) between these two groups of sites for any of the chemical parameters examined.

We identified 399 diatom species plus varieties on the basis of the light microscopy and SEM analyses. Many of these taxa (33%) were not frequent, having been found at only one site. The most widespread species, present in more than 70% of the sites, were *Gomphonema parvulum* Kützing, *Achnanthidium minutissimum* (Kützing) Czarneki, and *Amphora pediculus* (Kützing) Grunow.

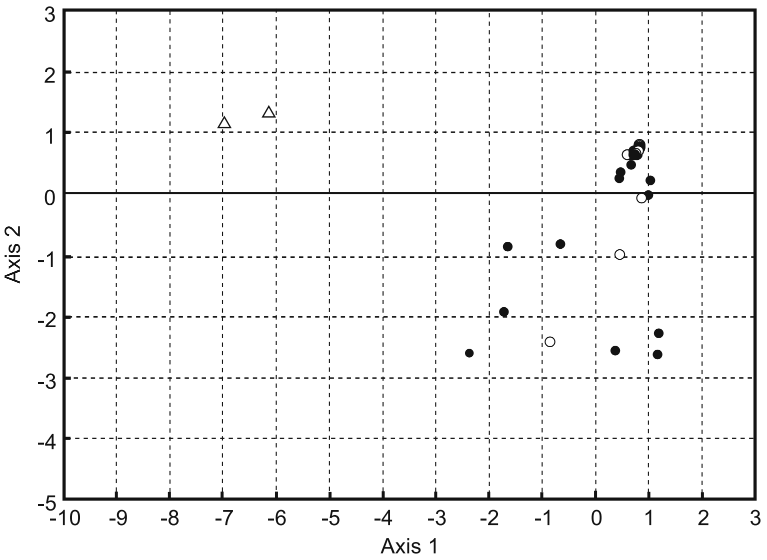

Fig. 4 Principal components analysis performed using metals, metalloids, and pesticides from 34 sites. Stations influenced by mining activities (*triangles*) and agriculture (*filled circles*) are differentiated from those free of them and located in wooded areas (*empty circles*)

Table 3 Pearson correlation coefficients between the different calculated diatom biotic indices

	TDI	CEE	IPS	IBD
CEE	−0.718			
IPS	−0.724	0.948		
IBD	−0.778	0.855	0.883	
EPI-D	−0.781	0.928	0.938	0.859

All correlations were significant ($p < 0.001$)
IPS Specific Polluosensitivity Index (CEMAGREF 1982), *IBD* Biological Diatom Index (Prygiel and Coste 2000), *CEE* Index of European Economic Community (Descy and Coste 1991), *TDI* Trophic Diatom Index (Kelly 1998), *EPI-D* Diatom-Based Eutrophication/Pollution Index (Dell'Uomo et al. 1999)

From the diatom indices that we analyzed, the best correlated—i.e., the ones with the highest *r*-Pearson correlation coefficients—were CEE, IPS, and EPI-D (Table 3). The *r*-Pearson correlation coefficients between the diatom indices and the different abiotic water quality indicators (conductivity, current velocity, ammonium, nitrate, nitrite, dissolved oxygen, SRP, some heavy metals plus metalloids, and pesticides) and the first-axis coordinates from the second and third PCAs (Table 4) suggest that the EPI-D index reflects a little more accurately the environmental conditions with respect to nutrient levels and salt contents. None of the tested indices showed good correlations with the current velocity.

On the basis of these results with the diatom indices, the water quality throughout the entire basin was estimated by means of the EPI-D (Fig. 5) according to a hierarchy consisting of five categories: bad [1–6), poor [6–9), moderate [9–12), good [12–15), and high [15–20]. This specific classification scale had been indicated in Torrisi and Dell'Uomo (2006). These authors also considered transition categories, comprising results falling within ±0.5 of the threshold values; but in this work, these transition categories have been ignored for the sake of simplicity. According to these five categories, 55% of the sites that we examined had either high or good water quality (Fig. 5).

We found no significant correlations between the levels of either atrazine or total pesticides and the percentages of *Achnanthidium* valves. On

Table 4 Pearson correlation coefficients between the calculated diatom biotic indices, different physical and chemical variables, and sampling station coordinates on the first axis of the second and third PCA

	TDI	CEE	IPS	IBD	EPI-D
1. Conductivity	0.445****	−0.550****	−0.575****	−0.534****	−0.582****
2. Current velocity	0.144	−0.025	−0.124	−0.039	−0.167
3. Ammonium	0.540****	−0.649****	−0.660****	−0.556***	−0.703****
4. Nitrate	0.436****	−0.364***	−0.394***	−0.361***	−0.386***
5. Nitrite	0.563****	−0.604****	−0.656****	−0.580****	−0.673****
6. Dissolved oxygen	−0.262**	0.313***	0.398***	0.366***	0.368***
7. SRP	0.422****	−0.659****	−0.680****	−0.367**	−0.703****
8. First axis 2nd PCA coordinates	−0.630****	0.639****	0.725****	0.545****	0.740****
9. As	0.438**	−0.560****	−0.554****	−0.416**	−0.498***
10. Cd	−0.219	0.227	0.228	0.404*	0.209
11. Cu	0.177	−0.280	−0.272	−0.119	−0.262
12. Fe	0.335*	−0.591****	−0.530***	−0.403*	−0.526***
13. Mn	0.341*	−0.462***	−0.410**	−0.303	−0.486***
14. Hg	0.089	−0.161	−0.176	−0.178	−0.086
15. Pb	0.272	−0.263	−0.289	−0.289	−0.289
16. Zn	−0.084	0.067	0.100	0.276	0.096
17. Pesticides	0.305	−0.400*	−0.468***	−0.330	−0.446**
18. Atrazine	−0.018	0.028	0.065	−0.020	−0.005
19. First axis 3rd PCA coordinates	−0.240	0.357*	0.315	0.155	0.355*

Values with no asterisks indicate nonsignificant correlation ($n = 71$ for variables 1 to 6; $n = 37$ for 7 to 8 and $n = 34$ for 9 to 19)
*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.001$, correlations significant at these levels

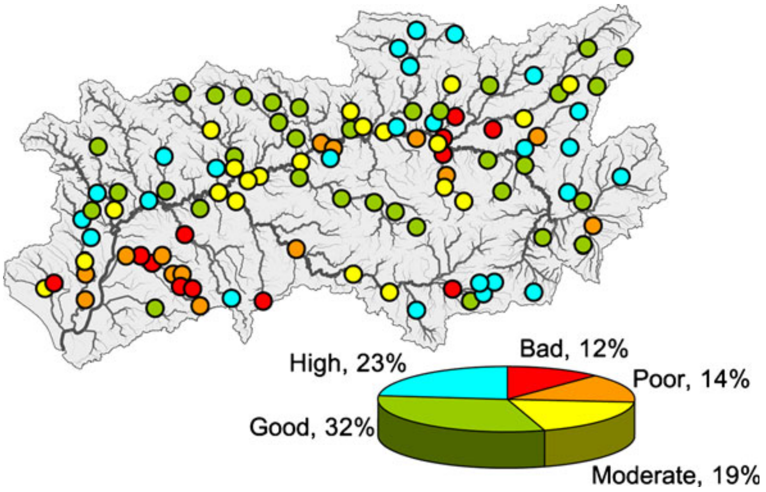

Fig. 5 Water quality of sampling sites determined by the EPI-D index. The *pie chart* indicates percentage of sites included in each water quality

the contrary, the presence of *Achnanthidium* was correlated with certain metals. Significant positive correlations occurred between Cd and both *A. biasolettianum* and the genus *Achnanthidium* as well as between Zn and *A. biasolettianum*; while *A. minutissimum* showed a negative correlation with Mn, Cu, As, Fe, Cd, and Zn (Table 5).

SIMPER analysis revealed the particular species that better characterized each of the water quality categories as calculated by the EPI-D index (Table 6). In the study area, the most characteristic species at sites of high water quality was *A. minutissimum*, although this species, together with *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow and *A. pediculus* (Kützing) Grunow, also indicated sites with simply good water quality.

A. pediculus and *Nitzschia inconspicua* Grunow were the best representatives of the moderate-water-quality category, although the former was also observed in samples having better water quality and the latter in those with worse. The species that most contributed to the similarity between the stations of poor water quality were *Nitzschia frustulum* (Kützing) Grunow and *N. inconspicua*. Despite their both being characteristic species present in these waters, *N. frustulum* showed a higher tendency for waters either more eutrophic or polluted than did *N. inconspicua*.

Bad water quality was fundamentally characterized by the presence of *Nitzschia capitellata* Hustedt, which accounted for more than 40% of the similarity between the sampling stations bearing this particular water quality. *Navicula veneta* Kützing and *N. frustulum* were important as well. These three species were furthermore registered under poor-water-quality conditions.

Discussion

Biotic indices have been developed particularly with an aim at the evaluation of water quality; and since a close correlation has accordingly been found between them and the abiotic indicators of water chemical composition, they can be regarded as useful tools for assessing the ecological status of water in a given area of interest. Although these indices have been designed to evaluate water quality with respect to trophic levels and/or the presence of organic pollution, they can also reflect other influences, such as heavy metals or acidity (Sabater 2000). In our study, Pearson's correlation analysis performed in relation to biotic indices and water physical and chemical variables enabled us to determine which of the former were more closely correlated with water chemistry and thus more adequate for application in the Guadalquivir basin. The high correlation between the diatom indices and the first axis coordinates of the second PCA suggested that these indices were more greatly influenced by a combination of the parameters affecting trophic levels than by a single one. Due to the existence of mining activities in the vicinity of some of the sampling sites and

Table 5 Pearson correlation coefficients between different species of the genus *Achnanthidium* and metals and pesticides

	As	Cd	Cu	Fe	Mn	Hg	Pb	Zn	Pesticides	Atrazine	3rd PCA coordinates
Achnanthidium affine	−0.005	−0.110	−0.193	−0.169	−0.091	0.257	−0.061	−0.128	−0.162	−0.110	0.139
Achnanthidium biasolettianum	−0.472*	0.479*	−0.004	−0.355*	−0.224	−0.215	−0.192	0.341*	−0.192	−0.124	0.027
Achnanthidium eutrophilum	0.100	−0.165	−0.290	0.039	−0.099	0.038	−0.092	−0.193	−0.243	0.315	0.232
Achnanthidium exiguum	−0.153	−0.075	−0.132	−0.192	−0.254	−0.075	−0.042	−0.088	−0.111	−0.075	0.139
Achnanthidium exilis	−0.106	−0.052	−0.092	−0.280	−0.177	−0.052	−0.029	−0.061	−0.078	−0.052	0.195
Achnanthidium jackii	0.076	−0.094	0.067	0.103	0.258	−0.094	−0.052	−0.110	0.187	0.291	−0.042
Achnanthidium minutissimum	−0.518*	−0.411*	−0.556*	−0.479*	−0.690*	−0.231	−0.231	−0.404*	−0.209	0.160	0.558
Achnanthidium saprophilum	−0.072	−0.165	0.169	0.201	0.111	−0.165	−0.092	−0.006	0.314	−0.165	−0.200
Genus *Achnanthidium*	−0.535*	0.352*	0.008	−0.305	−0.247	−0.309	−0.280	0.294	−0.115	0.049	0.102

$n = 35$ in all cases

*$p < 0.05$, correlations significant at this level

the role of intensive agriculture as a fundamental activity throughout the entire basin, some of the indices analyzed indicated significant correlations with the presence of heavy metals (mainly As and Mn), and the CEE, IPS, and EPI-D showed a significant negative correlation with pesticides. Although current velocity may exert a great influence on the species that become established in a given habitat (Ghosh and Gaur 1998; Navarro et al. 2000; Martín et al. 2004; Soininen 2005), within this study area, the index values were not at all influenced by this variable.

The EPI-D is an index that has given extremely good results in Italian Mediterranean rivers (Dell'Uomo et al. 1999; Torrisi and Dell'Uomo 2006) and as expected proved to be a good water quality indicator in the Guadalquivir basin, probably as a result of the climatic similarities between the two geographical areas. In contrast, the IPS offered the advantage of being extensively used in Iberian basins and thus enabled a comparison between the different catchment areas of this present region (Almeida 2001; Agencia Catalana de l'Aigua 2003; Confederación Hidrográfica del Ebro 2003; Gomà et al. 2004, 2005; Penalta-Rodríguez and López-Rodríguez 2007; Blanco et al. 2008). Nevertheless, the high correlation found between the EPI-D and IPS indices (0.938) suggest that the utilization of the EPI-D in this basin would not pose any limitation in making comparisons with the IPS values used in other catchment areas.

A particular complication in the calculation of indices has arisen concerning the identification of taxa related to *A. minutissimum* that are widely distributed throughout the whole basin. Potapova and Hamilton (2007) reported the difficulties in establishing taxonomical and ecological differences among *A. minutissimum* morphotypes, even when abundant information was available. *A. minutissimum* represents a complex of taxa that could have different ecological requirements and thus still need careful analysis. The differentiation of *A. minutissimum* from *A. biasolettianum*, *A. saprophilum*, *A. eutrophilum*, and other similar or transitional forms (many of which have been traditionally included in *Achnanthes minutissima* sensu lato) turns out to be extremely difficult without the aid of scanning

Table 6 SIMPER analysis results: group of species responsible for the 75% or more of the similarity among sampling stations having the same water quality level

	High	Good	Moderate	Poor	Bad
Achnanthidium biasolettianum	8.56				
Achnanthidium minutissimum	*68.62*	*25.95*	7.57	2.27	
Cocconeis placentula var. *euglypta*		*18.90*	6.25		
Amphora pediculus		*13.14*	*17.03*	2.00	
Nitzschia fonticola		5.30			
Cocconeis pediculus		3.05			
Nitzschia inconspicua		3.61	*12.98*	*14.09*	
Rhoicosphenia abbreviata		3.58	4.61		
Planothidium frequentissimum		2.06	3.07		
Navicula cryptotenella			3.62		
Navicula lanceolata			5.24		
Navicula gregaria			3.94	2.01	
Eolimna subminuscula			2.98	2.03	
Gomphonema parvulum			2.84	5.02	
Nitzschia frustulum			6.46	*19.70*	*11.67*
Navicula recens				3.89	
Nitzschia palea				3.40	
Cyclotella meneghiniana				3.70	
Nitzschia sigma				2.69	
Tryblionella hungarica				2.01	
Navicula veneta				6.00	*12.17*
Fistulifera saprophila				6.45	9.28
Nitzschia capitellata				2.21	*43.16*

Italicized results correspond to percentages higher than 10%

electron microscopy, a technique hardly applicable when performing routine screening work. Nonetheless, EPI-D and IPS have differing requirements with regard to the discrimination among these *taxa*. The estimation of the latter index requires the differentiation between *A. minutissimum* plus *A. biasolettianum*—considered indicators of good water quality—and *A. eutrophilum* plus *A. saprophilum*—reflecting bad water quality (Kobayasi and Mayama 1982). In contrast, such discrimination is not necessary when calculating EPI-D since *A. eutrophilum* and *A. saprophilum* become grouped under the collective umbrella of the category *A. minutissima* sensu lato. This independence of the results from an identification of these taxa gives the EPI-D index a clear advantage over the IPS.

At times, in sites that present a high percentage of the *Achnanthidium* genus, the IPS and EPI-D indices can give quite different results. This circumstance could arise in two main situations—when the substrata have been recently colonized or when toxicants are present:

(a) In the former situation (e.g., after a recent flood), the dominance of *A. minutissimum* can be more closely related to the age of the biofilm than to the quality of the water (Ács et al. 2004) since this species is an early colonizer (Sabater 2000). Casco and Toja (2003) found that *A. minutissimum* was dominant in the littoral zone of a reservoir within the Guadalquivir basin that suffered periodic desiccation and was recolonized each time by benthic algae. The authors suggested that the status of *A. minutissimum* as a pedunculate species could be one of the reasons for its ability as a colonizer. The facile breakage of the peduncle facilitates its movement towards other areas, while its rapid growth rate ensures a successful settlement. For this reason, it is necessary to let the biofilm settle and stabilize after a flood for as long as the sampling procedures recommend. This consideration is relevant to Guadalquivir River basin because of its Mediterranean climate and the great irregularity of the precipitations there. Considering that rainfall occurs mainly from the fall to the spring, the optimal time frame for diatom sampling would extend from late spring to early summer, before the small temporary streams become dry, as happens quite frequently in this basin. Nevertheless, since rivers there are highly regulated through human intervention, the artificial floods coming from the reservoirs in such instances should also be taken into account.

(b) *A. minutissimum* has been identified in the literature as being both pesticide and metal tolerant (Sabater 2000; Seguin et al. 2001). The presence of toxicants such as pesticides or heavy metals that would indirectly promote the dominance of the *Achnanthidium* genus can be masked since, on the basis of the above considerations, the indices can give spurious values that correspond to waters of either high or good quality. Our data do not allow the establishment of a relationship between the species within this genus and the presence of pesticides but do, however, indicate one between the members of *Achnanthidium* and contamination with metals. The finding of significant positive correlations between the abundance of *Achnanthidium* and the presence of certain metals (e.g., Cd and Zn) suggests that the dominance of members of this genus is positively influenced indirectly by these toxicants—not from being so much favored by the contaminants as less disfavored by them than other diatoms—so as to dominate under this type of pollution. In addition, since the negative correlation between *A. minutissimum* and the majority of the metals analyzed here is not consistent with the results of other authors, an elucidation of the source of this discrepancy will require further analysis.

Despite the drawbacks resulting from the dominance of *Achnanthidium* at certain sites, the use of an index that involves less taxonomic effort is more efficient from a pragmatic point of view. This consideration is especially relevant in water-quality-monitoring programs where a great bulk of samples has to be processed by a single analyst, or only few, within a short period of time. Such a circumstance constitutes a good reason to choose an index, such as the EPI-D, that avoids the identification of this conflictive genus. Nevertheless, further ecological and morphologic aspects pertaining to the *A. minutissimum* complex are presently being studied in this basin in order to calibrate the indices more completely and adapt them to the Spanish basins, as Poulickova et al. (2004) have already suggested.

With respect to taxa and degrees of water quality, few species were indicative of high or bad water quality as being clearly associated with one of those two categories. Because of their ubiquitousness and cosmopolitanism, the taxa living in intermediate ranges of water quality overlapped when they were considered as possible indicators of a particular type of water quality. Thus, *A. minutissimum*, which figured in the characterization of the high, good, moderate, and even poor-water-quality categories, is in fact adapted to live under a wide range of environmental conditions, although it does prefer the sites with good water quality. This preference could have its root in the organism's small size, which characteristic implies a high surface-to-volume ratio so as to provide a closer contact with the environment or in its characteristic growth on a peduncular stalk as opposed to being held fast to any substratum (Casco and Toja 2003). These features would favor a survival and proliferation in waters poorer in nutrients—i.e., with good trophic levels and minimal organic pollution.

Moderate and poor quality could not be characterized by any particular species but instead by a group of them, as taxa with different optimal growth rates live within this range of conditions. These species might nevertheless be assigned to a category in which a given taxon was more representative.

Conclusions

Although many of the species present in the Guadalquivir River basin are ubiquitous and cosmopolitan, there are certain taxa that better characterized each water quality level as defined by the diatom biotic index EPI-D, especially in the instance of the extreme categories (either very contaminated or very clean water), namely: *A. minutissimum* (high and good quality), *A. pediculus* (moderate quality), *N. frustulum* (poor quality), and *N. capitellata* (bad quality). These five categories of quality should be interpreted in terms of organic pollution and the trophic level. Other forms of pollution, such as pesticides and heavy metals, can sometimes be underestimated by these indices though still being important for river health.

According to our results in the Guadalquivir River basin, the application of the EPI-D diatom index is recommended because of its inherent advantages. First, this index achieved a good correlation with the general water chemistry of the basin. Second, EPI-D was highly correlated with the other indices—and especially with the IPS, which index has been extensively used in other Iberian basins. Finally, what appeared to be the most advantageous characteristic of the EPI-D index was that its calculation does not require the discrimination between certain problematical *Achnanthidium* species that are frequently encountered in this basin and are very similar to each other morphologically. In this way, the application of EPI-D is far easier from a taxonomical point of view, while still retaining its effectiveness as an indicator of water quality.

We therefore evaluated water quality of the entire Guadalquivir River basin using the EPI-D index. This approach was possible even in those sites without chemical information since the effectiveness of EPI-D index had been proven through the satisfactory correlation between it and water chemistry. According to these results, 55% of the sites had either high or good water quality and thus met the Water Framework Directive's requirements. The majority of the sites with better quality were located at the rivers heads with but a few exceptions—e.g., when there were villages near those low-order channels.

The characterization of the flora corresponding to each water category constitutes the first step toward knowledge of the different diatom assemblages that can develop in this basin as a reflection of the environmental conditions. This study lays the foundations for future evaluations of ecological status through the use of diatoms according to the WFD requirements not only in the studied basin but also in other basins throughout the southern Iberian Peninsula.

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