

## Use of diatoms for freshwater quality evaluation in Portugal.

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### ABSTRACT

Diatoms have been extensively used as water quality indicators in Europe for the last two decades. In Portugal, the use of diatoms is not common, except for research at a few universities.

Tychoplanktonic diatoms were sampled at 18 sites in the North-Central region of Portugal. Biological water quality using diatom indices was consistent with physical and chemical data.

The diatom indices (SPI and CEC) were better than the saprobic indices (SLA and LMI) because the former were more sensitive to differences between sampling sites, and were in greater accordance with chemical and physical parameters, especially pH, conductivity and organic content.

The aim of this work is to show that some of the diatom indices used in Europe, and particularly, in France, show quite promising results for routine application in Portugal.

Keywords: diatoms, indices, freshwater quality

### RESUMEN

*En las últimas dos décadas, las diatomeas han sido ampliamente utilizadas en Europa como indicadores de la calidad del agua. En Portugal, el uso de las diatomeas no es común con excepción de la investigación en las universidades.*

*En 18 estaciones de la región Norte de Portugal se muestrearon las diatomeas ticoloplancnéticas. Los resultados han mostrado que la calidad biológica del agua son consistentes con los datos físicos y químicos.*

*Los índices de diatomeas (SPI y CEC) dieron mejores resultados que los índices saprobios (SLA y LMI) ya que los primeros fueron más sensibles a las diferencias entre estaciones, y estuvieron más acordes con las variables físicas y químicas, especialmente pH, conductividad y contenido orgánico.*

*El objetivo de este trabajo es mostrar algunos de los índices de diatomeas utilizados en Europa, y en particular, en Francia, que muestran resultados prometedores para la aplicación rutinaria en Portugal.*

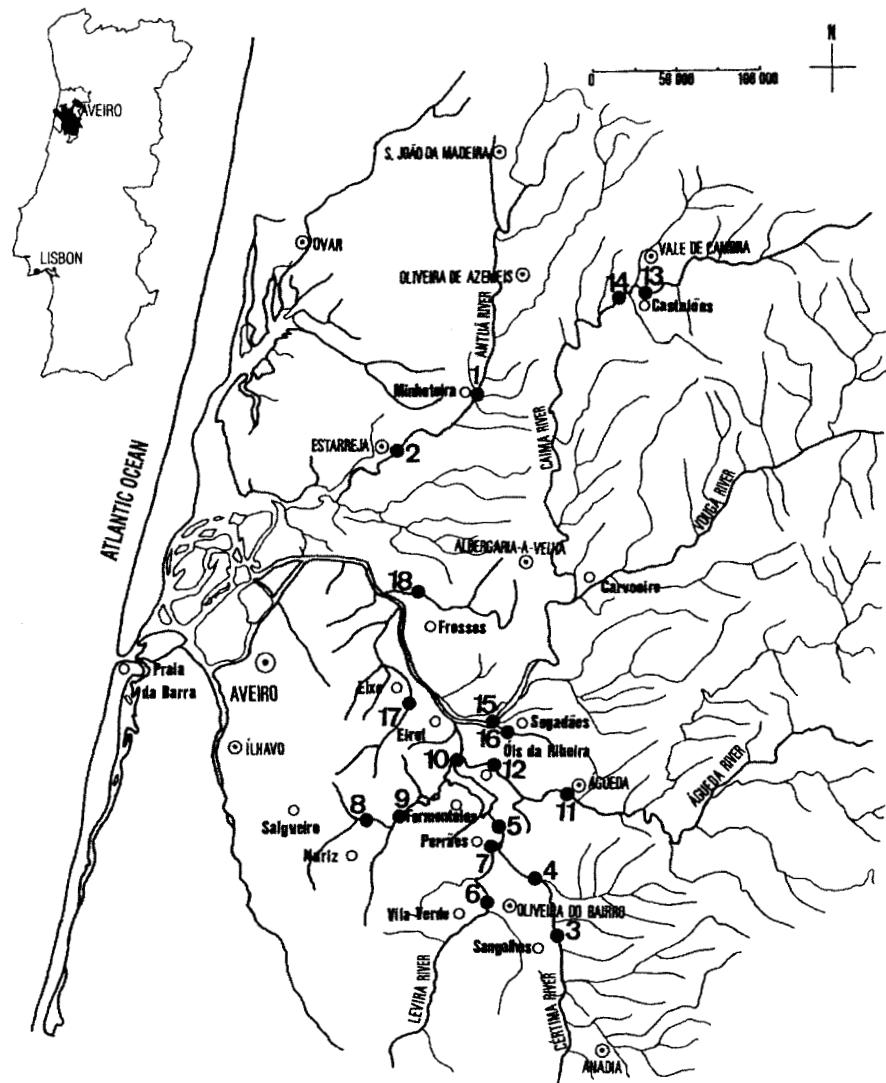
Palabras clave: diatomeas. índices calidad del agua

### INTRODUCTION

Diatoms have been used as freshwater quality indicators in the central region of Portugal during the 1980 and 1990 (Rino & Gil, 1987; Gil, 1988; Gil *et al.*, 1989-90; Almeida, 1998; Almeida & Gil, 2001). Several diatom indices prepared for use in other European countries, such as the SPI (Specific Polluosensitivity Index; 1984 in Coste, 1986), CEC (Commission for Economic Community Index; Descy & Coste, 1991), LMI (Leclercq & Maquet Index; Leclercq & Maquet, 1987) and SLA (Sladecek's Index; Sládecek, 1986) have been tested in this Portuguese region.

All of these indices have been used in other European countries, especially in France, Belgium and Luxemburg (Prygiel & Coste, 1995). The diatom index IBD (Indice Biologique Diatomees; Prygiel & Coste, 1998) has recently been tested in France for routine monitoring.

The use of tychoplanktonic diatoms for water quality evaluation is not very common and epilithic ones (i.e. attaching to stoney substrates) are usually preferred. Planktonic communities are transported with water currents, providing a quality indicator of the water coming from upstream. Organisms attaching to surfaces usually live in permanent contact with the surrounding



**Fig. 1.** Location of the 18 sampling sites in the Northern-Central region of Portugal. *Situación de las 18 estaciones de muestreo en la región Centro-Norte de Portugal.*

environment and are, thus, better indicators of the water quality of a site. In lowland slow-flowing rivers with sandy bottoms it is not always easy to find naturally-occurring stones. In these situations it is convenient to use tychoplankton as a biological water quality indicator (Almeida & Gil, 2001).

The main aim of this paper is to show that some of the existing diatom indices can be applied with success in Portugal.

## MATERIAL AND METHODS

Eighteen sites (Fig. 1) were sampled during one year. These sites were located in lowland slow-flowing waters, with sandy bottoms.

Water and biological samples were taken monthly. Several physical and chemical parameters were determined (Table 1).

Tychoplanktonic diatom samples were collected from the water column, and absolute counts

**Table 1.** Physical and chemical variables analysed, and methods used. *Variables físicas y químicas analizadas y métodos utilizados.*

Physical and Chemical parameters	Method and/or apparatus
Current velocity	" <i>in situ</i> " - current measurer - 203 DR
Water temperature	" <i>in situ</i> " - thermometer
pH, Conductivity	" <i>in situ</i> " - DSPH1
Total mineralization	Equations in Rodier (1984)
Dissolved Oxygen	" <i>in situ</i> " - WTW OXI 196
Oxygen saturation (%)	Truesdale formula
COD (Mn)	Oxidation by KMnO <sub>4</sub>
COD (Cr <sub>2</sub> O <sub>7</sub> )	Oxidation by K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>
BOD,	Winkler modified method
Alcalinity	Titration with H <sub>2</sub> SO <sub>4</sub>
Cl <sup>-</sup>	MOHR method
Ca <sup>2+</sup> , Mg <sup>2+</sup>	Titration by EDTA
Na <sup>+</sup> , K <sup>+</sup>	Flame Photometry - Jenway PFP7
N-(NO <sub>3</sub> <sup>-</sup> ), N-(NO <sub>2</sub> <sup>-</sup> ), N-(NH <sub>4</sub> <sup>+</sup> ), P-ICP(PO <sub>4</sub> <sup>3-</sup> ), SiO <sub>2</sub>	ICP
Al, Mn, Fe, Ni, Cu, Zn, Pb	

performed of about 400 valves, under the light microscope with a 100 x objective and numerical aperture of objective 1.32.

The following diatom indices were calculated: the SPI, CEC, SLA and LMI.

The SLA (Sládecek, 1986) and LMI (Leclercq & Maquet, 1987) indices are saprobic indices, i.e. for detection of organic pollution.

Derived from Sládecek's method, Leclercq and Maquet (1987) proposed other values for the "saprobic valencies" (s) and for the "indicator values" (v).

CEC and SPI indices intend to evaluate organic and also inorganic pollutions based on the sensitivity of each taxon, while taking into account the response of the whole diatom community.

The SPI index uses the same formulae as in Descy's method (Descy, 1979), varying the "specific sensitivities" (i<sup>i</sup>) and the "indicator values" (v<sup>i</sup>) (CEMAGREF 1984 in COSTE 1986). The number of taxa included in the SPI is about 2000.

The CEC index is based on a double entry table. Horizontally, it is composed of 8 groups of

taxa arranged from sensitive (group 1) to pollution-resistant (group 8). Vertically, there are 4 sub-groups of taxa, classified according to their postulated habitats, defined by alcalinity and mineralization.

The indicial value is obtained by interception of the median group and the "sub-group", defined as the group including 50% or more of taxa. This index varies between 0 (strong pollution) and 10 (no pollution).

Descy & Coste (1987-88) transformed indicial values, to vary between 1 and 20 for easier comparison.

They found indicial results were correlated (Pearson's correlation coefficient; Sokal & Rohlf, 1987) with physical and chemical variables.

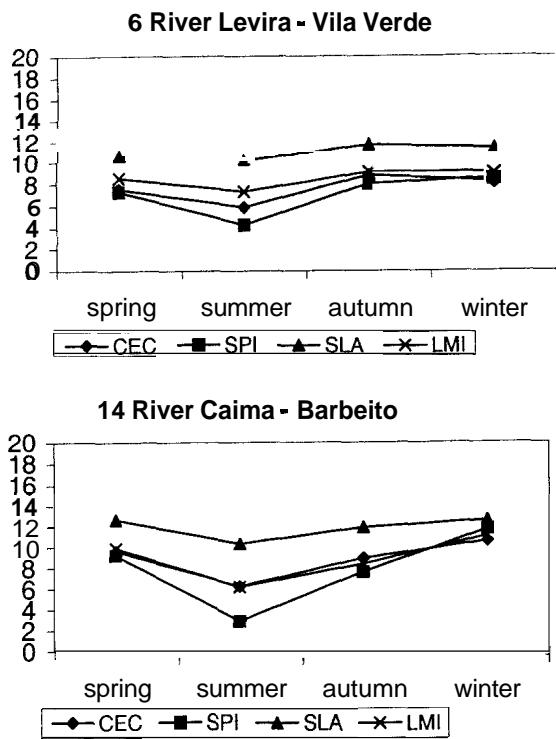
## RESULTS

Water quality as shown by physico-chemistry, at the 18 sites was worse during summer.

Sites 1 (river Antuã, Minhoteira), 2 (river Antuã, Estarreja), 3 (river Certima, Vale de Estevão), 6 (river Levira, Vila Verde) and 14 (river Caima, Barbeito) had the highest organic

**Table 2.** Variation of the environmental variables showing a significant correlation to diatom indices at the 18 sampling sites. Units are  $\mu\text{g}\cdot\text{l}^{-1}$  for Zn<sup>+2</sup> and Mn<sup>+2</sup> and mg·l<sup>-1</sup>. for the rest ("d.l." is detection limit). *Variación de las variables ambientales mostrando una correlación significativa con los índices de diatomeas en las 18 estaciones muestreadas. Las unidades son  $\mu\text{g}\cdot\text{l}^{-1}$  para el Zn<sup>+2</sup> y Mn<sup>+2</sup> y mg·l<sup>-1</sup> para el resto ("d.l." es el límite de detección).*

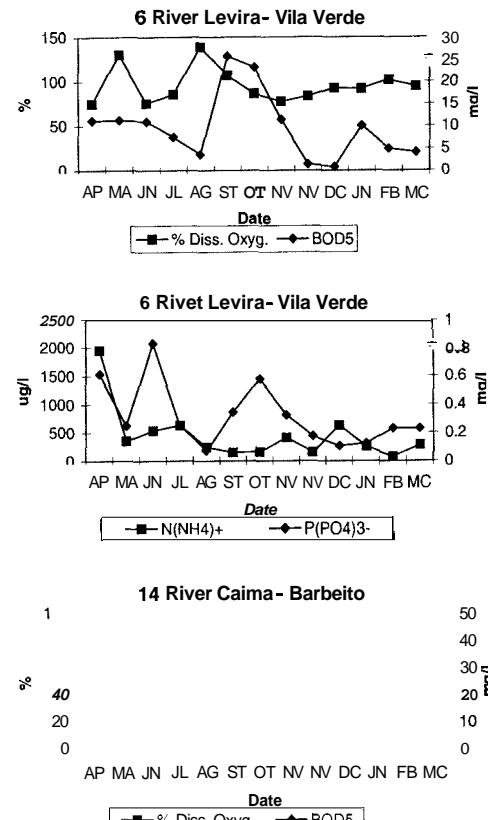
	Minimum	Maximum	Average
<b>pH</b>	5.7	8.5	6.8
<b>Tot. mineralization</b>	49	1068	298
<b>CBO,</b>	d.l.	62.7	5.9
<b>HCO<sub>3</sub><sup>-</sup></b>	4.9	481.9	92.8
<b>N-(NO<sub>3</sub><sup>-</sup>)</b>	d.l.	7.10	1.18
<b>N-(NO<sub>2</sub><sup>-</sup>)</b>	d.l.	0.290	0.038
<b>N-(NH<sub>4</sub><sup>+</sup>)</b>	0.028	4.610	0.728
<b>P-(PO<sub>4</sub><sup>3-</sup>)</b>	d.l.	4.932	0.304
<b>Mn</b>	3	4371	206.3
<b>Zn</b>	10	2203	131.3



**Fig. 2.** Seasonal variation of the SLA, LMI, CEC and SPI indices in river Levira - Vila Verde (6), and river Caima - Barbeito (14). *Variabilidad estacional de los indices SLA, LMI, CECy SPI en el rio Levira-Vila Verde (6) y rio Caima-Barbeito (14).*

**Table 4.** Correlations between biotic indices and environmental variables. (\*P<0.01; +P<0.05; n.s. - not significant). *Correlaciones entre los indices bídicos y las variables ambientales. (\*P<0.01; +P<0.05; n.s. - no significativo).*

	CEC	SPI	SLA	LMI
pH	-.7081*	-.7123*	-.7828*	-.6904*
Tot. min.	-.6487*	-.6169*	-.6262*	-.5226*
CBO,	-.3409*	-.4175*	-.2606*	-.3989*
HCO <sub>3</sub> <sup>-</sup>	-.6844*	-.6757*	-.7216*	-.6018*
N-(NO <sub>3</sub> )	-.3634*	-.2878*	-.2413+	-.3374*
N-(NO <sub>2</sub> )	-.6207*	-.5488*	-.4574*	-.5758*
N-(NH <sub>4</sub> <sup>+</sup> )	-.3169	-.3317*	-.2315+	-.2211+
P-(PO <sub>4</sub> <sup>3-</sup> )	-.6237*	-.5991*	-.4955*	-.5622*
Zn	-.2310+	-.2335+	n.s.	-.2504+
Mn	-.3905	-.4625*	-.4405*	-.4343*



**Fig. 3.** Monthly variation of some physical and chemical variables in river Levira - Vila Verde (6) and in river Caima - Barbeito (14). *Variabilidad mensual de algunas variables físicas y químicas en el rio Levira-Vila Verde (6) y rio Caima-Barbeito (14).*

**Table 3.** Temporal ranking of the 5 most abundant taxa at sites 6 and 14. Zero (0) corresponds to the most abundant taxon and 5 to the fifth most abundant taxon. *Rango de variación temporal de los cinco taxones más abundantes de las estaciones 6 y 14. Cero (0) corresponde al taxón masabundante y 5 al quinto más abundante.*

#### 6 River Levira - Vila Verde

Taxon	05/92	06/92	07/92	08/92	09/92	10/92	11/92	11/92	12/92	01/93	02/93	03/93	04/93	05/93
<i>Navicula gregaria</i> Donkin							5	3	5	3	0	3		
<i>Nitzschia linearis</i> (Agardh) W. Smith										2				
<i>Nitzschia palea</i> (Kutz.) W. Smith	0	0	0	0	0	0	0	1	0	1		3		3
<i>Nitzschia capitellata</i> Hustedt	1	5												
<i>Surirella angusta</i> Kutzing	2	3	1	1	1	1	1	0	5	0	1		0	0
<i>Nitzschia gracilis</i> Hantzsch	3	2		5	3	5			2		2			4
<i>Navicula aquaedurae</i> Lange-Bertalot	4	1	2	4	4	2	2	4		3			4	
<i>Navicula halophiloides</i> Hustedt	5									4				
<i>Cocconeis placentula</i> Ehrenberb		4								1				
<i>Gomphonema parvulum</i> Kutzing			3	2		4					0			5
<i>Navicula pupula</i> Kutzing			4				4							
<i>Nitzschia umbonata</i> (Ehrenb) L.-Bert.			5											
<i>Gomph. paw.</i> Kütz. var. <i>pawulius</i> L.-B. & Reich				3										
<i>Nitzschia paleacea</i> Grunow					2						5			
<i>Achnanthes lanceolata</i> (Brebisson) Grunow						5	3	3	2		4	1	1	1
<i>Navicula minima</i> Grunow							5					4	2	2
<i>Achnanthes minutissima</i> Kutzing								3						
<i>Navicula cryptocephala</i> Kutzing									4			5		
<i>Nitzschia graciliformis</i> L.-Bertalot & Simonsen											2			
<i>Navicula minuscula</i> Grunow												5		

#### 14 River Caima - Barbeito

Taxon	05/92	06/92	07/92	08/92	09/92	10/92	11/92	11/92	12/92	01/93	02/93	03/93	04/93	05/93
<i>Nitzschia palea</i> (Kutz.) W. Smith	1	1	0	0	0	0	2	0	1	1	1	1	2	

**Table 3.** (Continuation. *Continuación*)**14 River Caima - Barbeito**

TAXON	05/92	06/92	07/92	08/92	09/92	10/92	11/92	12/92	01/93	02/93	03/93	04/93	05/93
<i>Navicula minima</i> Grunow	0	0	2	2	2	1	0	1	0	0	0	0	0
<i>Diatoma mesodon</i> (Ehrenberg) Kiitzing													5
<i>Gomphonema parvulum</i> Kiitzing	3	2	1	1		2	3		5	3		3	1
<i>Fragilaria capucina</i> Desmazieres		5	3		3		4				5	2	2
<i>Navicula cryptocephala</i> Kutzing	5		5				5	5		4	3	4	5
<i>Pinnularia subcapitata</i> Gregory	2		4	4				2	4	2	4		
<i>Pinnularia gibba</i> Ehrenberg	4				3	5	5						
<i>Nitzschia dissipata</i> (Kutzing) Grunow		4									1	4	4
<i>Achnanthes minutissima</i> Kiitzing	3				4	3	1	3	3		2		1
<i>Nitzschia gracilis</i> Hantzsch				5									
<i>Navicula heimansii</i> Van Dam & Kooyman					1	4		4					
<i>Achn. subatomoides</i> (Hust)L.-B. & Arch.									2	5			

pollution, while sites 11 (river Agueda, Agueda), 12 (river Agueda, Óis da Ribeira) and 13 (river Caima, Vale de Cambra) showed the lowest.

Sampling of sites could be grouped into two, sites 1 to 10, with pH around 7; sites 11 to 18, with pH closer to 6.

Conductivity delimited three groups of sites, i.e. sites 3 to 10, plus site 17, with conductivity ranging between 463 and 834.5  $\mu\text{Scm}^{-1}$ ; sites 1, 2 and 18, with conductivity about 300  $\mu\text{Scm}^{-1}$ ; and sites 11 to 16, with very low conductivities, ranging between 78 and 197.5  $\mu\text{Scm}^{-1}$ .

Physical and chemical variables +h<sub>a</sub> significantly correlated with the diatom indices are shown in Table 2.

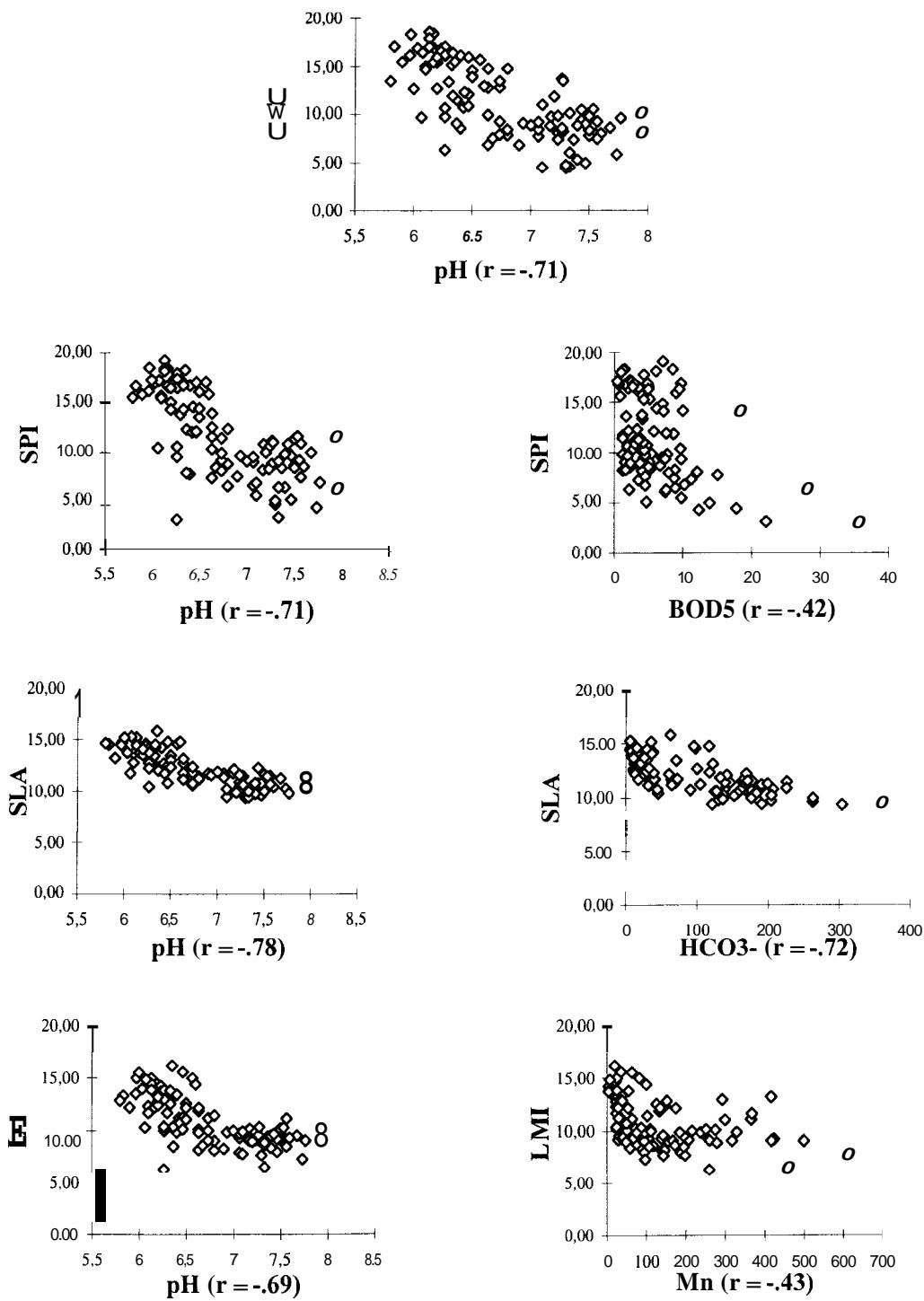
Dominant taxa are the most important for the calculation of indices. The most abundant taxon varied across the 18 sampling sites over the year of sampling. The most abundant taxa at

sites 6 (river Levira, Vila Verde) and 14 (river Caima, Barbeito) are shown on Table 3, taxa were numbered from 0 (most abundant) to 5 (least abundant).

*Nitzschia palea* (Kiitzing) W. Smith was the most abundant taxon at site 6 almost the whole year round. *Nitzschia capitellata* Hustedt, *Surirella angusta* Kiitzing, *Nitzschia gracilis* Hantzsch, and *Navicula aquaedurae* Lange-Bertalot were also abundant at this site.

At site 14, the dominant taxon was *Navicula minima* Grunow, except during summer, when *Nitzschia palea* (Kutzing) W. Smith was more abundant. The rest of taxa varied without a clear pattern.

The four indices changed with chemical variables (Figs. 2 & 3). During summer, indices showed lowest values, coinciding with lowest water quality as shown by chemical data. During



**Fig. 4.** Variation of indices and environmental variables. Units are mg/l - BOD5, HCO<sub>3</sub><sup>-</sup>; mg/l - Mn; "r" is Pearson's correlation coefficient. *Variabilidad de los valores de los índices y de las variables ambientales. Las unidades están en mg/l - BOD5, HCO<sub>3</sub><sup>-</sup>; mg/l - Mn; "r" es el coeficiente de correlación de Pearson.*

the rest of the sampling period water quality improved, but was still bad.

Variation in % O<sub>2</sub> saturation, biological oxygen demand (BOD<sub>5</sub>) and chemical oxygen demand (COD) also indicated deterioration of water quality during summer.

Good correlations were found between environmental variables and indicial values of the eighteen sites taken together (Table 4.) see Figure 4.

The saprobic indices LMI and SLA varied less than the indices CEC and SPI.

## DISCUSSION

Water quality evaluation using diatom indices was consistent with the physical and chemical determinations. The decrease in water quality during summer was very well represented by the diatom indices.

Negative correlations between indicial values and both organic pollution parameters (N, COD and BOD<sub>5</sub>) and with inorganic pollution ones (Zn and Mn) were found. Thus, diatom indices are considered adequate for gross pollution detection.

Most of the taxa found in Portuguese waters are listed in the indices. Diatom communities found in Central Portugal are similar to the communities found in other European regions, making them adequate for routine monitoring in these waters.

Although the four indices showed reasonable results, the CEC and SPI revealed better the differences in water quality than the saprobic indices SLA and LMI. The latter did not distinguish well different pollution situations. Descy & Coste (1991) and Prygiel & Coste (1995) concluded the same, attributing their low sensitivity to the small variation in the saprobic valencies of the diatoms (i.e. ranging from oligosaprobic to α-mesosaprobic). The use of the indices CEC and SPI is therefore recommended for routine water quality monitoring in Portugal. This test, however, should be extended to the rest of the country as well as to waters with different levels and types of pollution.

Because these indices need the identification of the diatoms to specific and subspecific levels,

they are difficult to non-specialists. Nevertheless, taxonomic preparation is possible in a few months and a large literature is now available. Thus, the taxonomic difficulty should not be, in our view, the reason for not using diatoms for water quality assessment.

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