Application of water quality biological indices using diatoms as bioindicators in the Gravataí river, RS, Brazil

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Received August 20, 2010 – Accepted January 11, 2011 – Distributed November 30, 2011
(With 3 figures)

Abstract

The Gravataí river situated in the metropolitan region of Porto Alegre has an area of approximately 2.020 km^2 and provides public water supply to about 500,000 inhabitants in 5 municipalities (latitude 29° 45'-30° 12' S; longitude 50° 27'-51° 12' W). The river basin has two regions with distinctive characteristics of occupation: the upper course shows intensive farming and the lower course presents urban and industrial uses. In this context, the aim of this study was to evaluate the water quality in the Gravataí River (RS, Brazil) by using physical, chemical and microbiological variables, and the water quality biological indices (WQBI) formulated for southern Brazilian rivers based on epilithic diatom communities as indicators. For comparison purposes, a local WQBI, called the Gravataí WQBI, was also used where species were given new saprobic values (*s*) and indicative values (*vi*) according to their occurrence and abundance in the river, using multivariate analytical techniques. The biological samples were taken every three months at six stations along the Gravataí River between September 2000 and August 2002. The results of the physical and chemical analyses of the water indicated a pollution gradient down the river, from the headwaters to the mouth, detected mainly by considering a significant decrease in the concentration of dissolved oxygen and turbidity, as well as a significant increase in BOD_5, total nitrogen, ortho-phosphate and thermotolerant coliforms. Comparing the results obtained, differences were found regarding the predominant pollution levels as higher in the Gravataí WQBI, although both corroborated a tendency for the contamination gradient to increase from the headwaters to the mouth. Given the local anthropic changes, it is of great importance to continue the study of diatom species tolerance to organic pollution and eutrophication in different lotic systems of the region.

Keywords: epilithic diatoms, bioindicators, Water Quality Biotic Indices (WQBI), organic contamination, eutrophication.

Aplicação de índices biológicos da qualidade água utilizando diatomáceas como bioindicadoras no rio Gravataí, RS, Brazil

Resumo

O rio Gravataí situado na região metropolitana de Porto Alegre tem aproximadamente 2.020 km^2 e abastece aproximadamente 500.000 habitantes em cinco municípios (latitude 29° 45'-30° 12' S; longitude 50° 27'-51° 12' W). A bacia do rio tem duas regiões com características distintas de ocupação: o curso superior mostra atividades intensivas de agricultura, e o curso inferior apresenta usos industriais e urbanos. Neste contexto, este trabalho teve por objetivo avaliar a qualidade da água do rio Gravataí, RS, utilizando variáveis físicas, químicas e microbiológicas, e o Índice Biológico da Qualidade de Água (IBQA), formulado para rios sul-brasileiros, baseado na comunidade de diatomáceas epilíticas como indicadoras. Para fins de comparação, aplicou-se um IBQA regional denominado IBQA Gravataí, no qual houve uma nova classificação dos valores sapróbicos "s" e valores indicativos "vi", de acordo com a ocorrência e abundância das espécies no rio, a partir da utilização de técnicas analíticas multivariadas. As amostragens biológicas foram realizadas trimestralmente, em seis estações de amostragem ao longo do rio Gravataí, no período de setembro de 2000 a agosto de 2002. Os resultados das análises físicas da água indicaram um gradiente de poluição ao longo do rio, da nascente à foz, detectado basicamente pela diminuição significativa na concentração do oxigênio dissolvido e turbidez, bem como um aumento significativo na DBO_5, nitrogênio total, ortofosfato e coliformes termotolerantes.

Comparando os resultados da aplicação do IBQA, verificou-se que houve diferenças quanto à predominância de níveis de poluição, sendo mais elevados no IBQA Gravataí, embora tenha sido corroborado em ambos a tendência no aumento do gradiente de contaminação da nascente à foz. Em função das alterações antrópicas locais, é de extrema importância dar continuidade ao estudo das tolerâncias das espécies de diatomáceas à poluição orgânica e eutrofização em diferentes sistemas lóticos da região.

Palavras-chave: diatomáceas epilíticas, bioindicação, Índices Biológicos da Qualidade da Água (IBQA), contaminação orgânica, eutrofização.

1. Introduction

Agricultural activities and high population densities expose most of southern Brazilian hydrographical basins to heavy and increasing environmental impacts, especially pollution by organic residues (Salomoni et al., 2006). This situation has led to the development of biological methods to evaluate contamination levels in running waters. From this point of view, different biological communities have been used for assessing and monitoring freshwater quality. Among them, epilithic diatoms are recognized worldwide as indicators of organic pollution and eutrophication (Kelly and Whitton, 1995; Ector anb Rimet, 2005).

The variation in communities´ epilithic diatoms is expressed spatially and temporally, based on the climate, geological settings, water chemistry and geomorphological conditions (Stevenson and Pan, 1999).

In Brazil, few related studies on algae as bioindicators have been carried out in the southern region. Lobo et al. (2002, 2004, 2010); Hermany et al. (2006) and Salomoni et al. (2006), performed quantitative analysis with oxidizable material, while Schneck et al. (2007) and Salomoni and Torgan (2008), with sedimentation chambers considering the living cells only.

Among the biological methods developed to indicate the trophic level of running waters, the trophic diatom index (TDI) proposed by Kelly and Whitton (1995), and reviewed by Kelly (1998), has been widely used in the European Community, especially after the publication of the Municipal Sewage Treatment Plants policy in 1991 (EUROPEAN COMMUNITY, 1991). The TDI uses the equation of the weighted average of Zelinka and Marvan (1961) to interpret the structure of epilithic diatom biocenoses in terms of nutrient concentrations in rivers, mainly phosphate.

Compared to the use of trophic indices in the European Community, the Water Quality Biological Index (WQBI) proposed by Lobo et al. (2004), incorporates an integrated response of the epilithic diatom community to eutrophication processes and organic pollution in south Brazilian rivers. In Latin America, Gomes and Licursi (2001) published the use of a regional index to evaluate water quality of rivers and streams of the Argentine Pampa plains, called the Pampeano diatoms Index (PDI). Two hundred and ten species were classified according to their tolerance to organic pollution and eutrophication, considering their responses to the concentrations of phosphate, ammonia nitrogen and biochemical oxygen demand. Unlike WQBI, which uses the epilithic diatom community, the PDI is based on the sensitivity of epipelic diatom biocenoses, integrating the effects of organic enrichment and eutrophication.

In Central America, Michels-Estrada (2003) investigating the ecology of benthic diatom communities in many rivers and streams of Costa Rica, highlighted the urgent need to establish a baseline of information about the ecology of aquatic ecosystems in the tropics, aiming to develop efficient methodologies for monitoring water quality.

Hence, before testing, applying and comparing the indices we consider, among the epilithic diatoms in the Gravataí River, there are species that might be considered indicators and which occurrence and abundance can be used in the assessment of water quality. We also consider if the water quality biological index is based on the diatoms indicative values and developed for the subtropical temperate Brazilian Rivers, it is a suitable tool to assess the water quality of the Gravataí River.

Taking this into account, the current study was designed to compare the application of two biotic indices to the assessment of water quality in the Gravataí River, RS, Brazil, the Water Quality Biological Index (WQBI) proposed by Lobo et al. (2004) and the WQBI for the Gravataí River, derived from new saprobic and indicator values of the diatom species determined in this study.

2. Material and Methods

2.1. Study area

The hydrographic basin of the Gravataí River is located in the northeastern region of the state of Rio Grande do Sul in southern Brazil. It extends between Porto Alegre and the Jacuí River delta to the west, and the lagoon zones of the Atlantic coast to the east, between longitudes 50° 27’-51° 12’ W. To the north, it meets the hydrographic basin of the Sinos River, and to the south the swamps and streams that drain into the Patos Lagoon, between latitudes 29° 45’-30° 12’ S (Brasil, 1985).

The basin, which has an area of 2,020 km^2, includes completely or partially the towns of Santo Antônio da Patrulha, Taquara, Glorinha, Gravataí, Alvorada, Viamão, Cachoerinha, Canoas, and Porto Alegre. Situated in the region of Porto Alegre, Rio Grande do Sul state, it is responsible for the public supply of water to about five hundred thousand inhabitants in five towns. About two-thirds of the water in this basin is drained into Banhado Grande and the rest into the river itself. The central area of the basin known as Banhado Grande consists of an ecosystem of wetlands, marshy forest and flooded fields, with an important role in the hydrodynamics of this water source and remarkable biological diversity (FZB, 1976). Banhado Grande, which works as a groundwater outflow regulator, originally occupied an area of 450 km^2, but is now reduced to 50 km^2, water usage for the irrigation of rice cultures (Leite et al., 1992/1994).

The river basin has two regions with distinctive characteristics of occupation: the upper course shows intensive farming, and the lower course presents urban and industrial uses. In fact, the industries located in the lower reach of the Gravataí sub-basin generate a gross organic load of approximately 2.516 ton/year of BOD_5, and despite the treatment implemented in the industrial plants the remaining load discharged still contains 1,100 ton/year of BOD_5. Additionally, the domestic waste load generated is 19,524 ton/year of BOD_5 (FEPAM, 1998).

The hydrographical basins of Gravataí river has a relevant social, economical and cultural importance. The industrial cluster has grown considerably over the past years as has the urban population as well. This fast growth generated an increase of pollutants dumped in some spots of the river, as a result of farming irrigation, water supply for animals, industrial and domestic sewage, solid residue, urban draining, rural diffusing sources, atmospheric pollutants precipitation, boosted by low water flow.

2.2. Environmental variables

The water samples were collected over 2 years from September 2000 to August 2002 at three-month intervals to include the four seasons of the year at six sampling stations set upstream and downstream from the main polluting sources in the Gravataí River basin (Figure 1). The following variables were measured according to APHA (2005): water and air temperature, electrical conductivity, turbidity, pH,

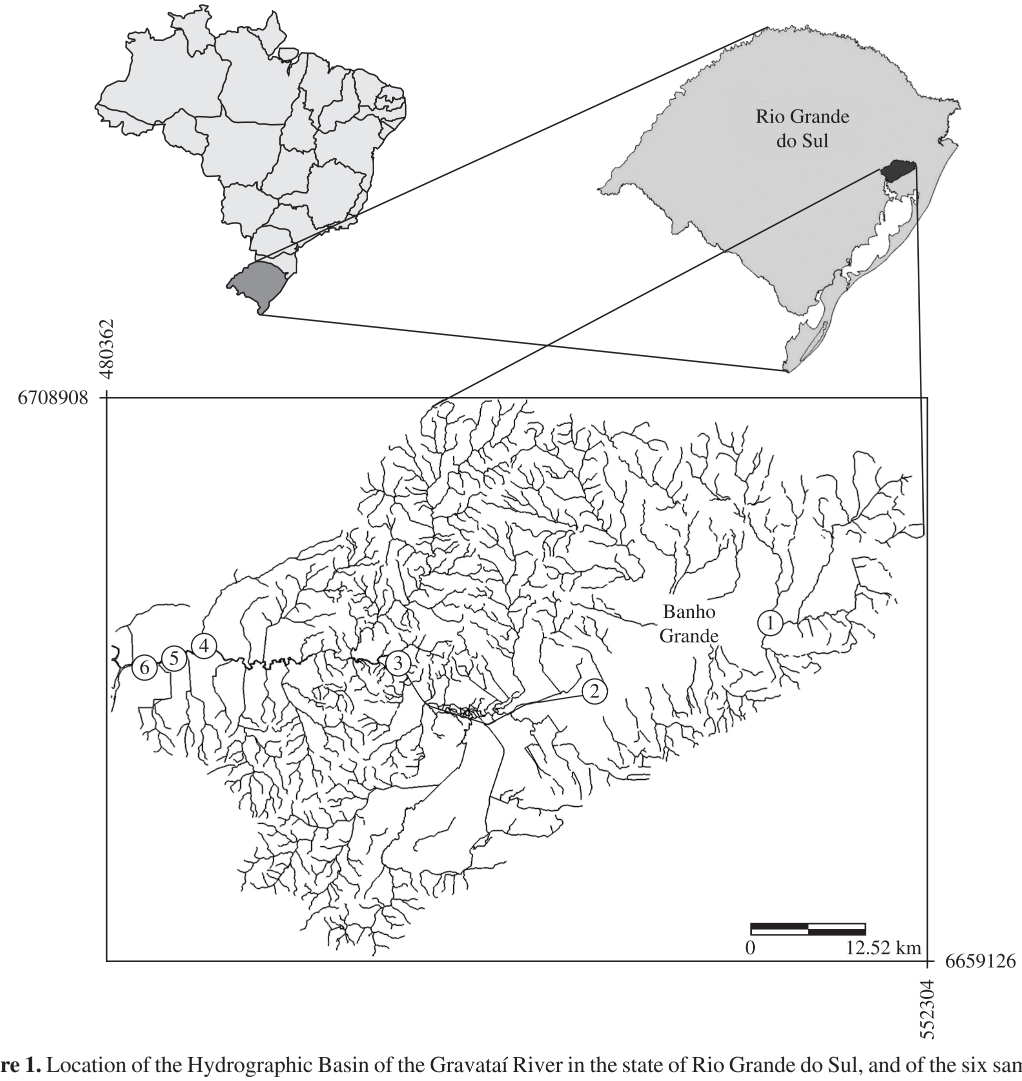

Figure 1. Location of the Hydrographic Basin of the Gravataí River in the state of Rio Grande do Sul, and of the six sampling stations along the Gravataí River. S1 = Chico Lomã Stream district of Santo Antônio da Patrulha; S2 = Juca Barcelos farm, district of Glorinha; S3 = Passo dos Negros, district of city Gravataí; S4 = Pumping station, city of Porto Alegre/Canoas; S5 = Areia Stream, city of Porto Alegre/Canoas; S6 = Gravataí River mouth, city of Porto Alegre.

dissolved oxygen concentration (DOC), biochemical oxygen demand after 5 days (BOD_5), chemical oxygen demand (COD), ammonium concentration, total organic nitrogen, dissolved orthophosphate, total phosphorus, chloride, and thermotolerant coliforms. Data were supplied by the Rio Grande do Sul State Environment Foundation (FEPAM) and the Porto Alegre Municipal Department of Water and Sanitation (DMAE).

2.3. Sampling of epilithic diatoms

Simultaneous to the collection of water samples, the epilithic diatoms were sampled from rock substrates (basaltic cobbles) previously suspended below the sub-surface of the river (depth of 20 cm), using polystyrene floats as supports, installed at six sampling stations along the river (Salomoni et al., 2006, 2007). The supports and substrates were placed near the river banks, avoiding shaded spots. The stones were exposed in the river for four weeks, as recommended by Lobo and Buselato-Toniolli (1985) and sampling was performed every three months. The samples for quantitative analysis were taken by scrubbing each stone with a toothbrush, over a surface area of 25 cm^2. At each sampling station, three rocks were scrubbed (75 cm^2) and washed with distilled water yielding a compound sample of 150 mL fixed with formaldehyde 4%. After this procedure, the compound sample was well mixed and an aliquot of 40 mL was separated for analysis. This material was oxidized to clear the diatoms with potassium permanganate and concentrated hydrochloric acid. An aliquot (1 mL) of the cleared sample was deposited and mounted on permanent slides for further observation, using the resin Naphrax^{TM}. Algae were counted using procedures described by Kobayasi and Mayama (1982). In each sample, at least 400 valves were counted in transects by the method proposed by Bate and Newall (1998).

To identify taxa to specific and infra-specific levels, epilithic diatoms were observed and photographed using an optical binocular microscope at 1000x magnification. The samples, catalogued as HAS 103699 to 103813, were deposited in the Prof. Dr. Alarich R. H. Schultz Herbarium (HAS) of the Museum of Natural Sciences at the Zoobotanical Foundation of Rio Grande do Sul (MCN/FZB).

To identify the species, the following taxonomical sources were used: Krammer and Lange-Bertalot (1986, 1988, 1991a,b), Metzeltin and Lange-Bertalot (1998, 2002, 2007), Krammer (2000), Rumrich et al. (2000) and Metzeltin et al. (2005). To determine abundant and dominant species, the criterion suggested by Lobo and Leighton (1986) was applied.

2.4. Biological index (WQBI)

From the quantitative analysis of the epilithic diatom communities at the six sampling stations, the Water Quality Biological Index (WQBI) was calculated, as proposed by Lobo et al. (2004) (Equation 1).

$$\mathrm{WQBI} = \frac{\sum(\mathrm{s}\ .\ \mathrm{h}\ .\ \mathrm{vi})}{\sum(\mathrm{h}\ .\ \mathrm{vi})} \tag{1}$$

where *s* is the saprobic value of a given species, according to the classification proposed by Lobo et al. (2002); *h* percentage of abundance of the species in the sample and *vi* the indicative value of tolerance to eutrophication of diatom species, according to Lobo et al. (2004). Table 1 shows the relation between the Water Quality Biological Index (WQBI) values and the water quality.

Using the empirical saprobic and indicative values determined in this study for each of the abundant diatom species, the WQBI was recalculated. This modified index was denominated the Gravataí WQBI. To calculate the WQBI for a given station and time, the saprobic value of "*s*" = 1 and indicative value of "*vi*" = 1 were ascribed for abundant species not included in the diatoms list of the regional WQBI.

2.5. Data processing

The species and sampling stations were grouped by TWINSPAN (Two-way Indicator Species Analysis) according to Hill (1979). Using a matrix of relative abundance of species vs. samples, the indicator species were assembled by TWINSPAN, as described by Dufrêne and Legendre (1997) resulting in three groups of species. Secondly, Canonical Correspondence Analysis (CCA), a direct multivariate analysis of gradient, developed by Ter Braak (1986), was used to reveal the main gradients of the variation in species composition corresponding to the degree of organic pollution and eutrophication. The analyses were run with PC-ORD version 4.0 of *MjM Software Design* for Windows (Mccune and Mefford, 1999). The first canonical axes were tested for significance using the Monte Carlo Test (999 permutations) at the 5% level.

3. Results

3.1. Environmental variables

Physical and chemical results showed a decrease in the concentration of dissolved oxygen (5.6 ± 2.1 to 3.4 ± 2.3 $mg.L^{-1}$), an increase of BOD_5 (1.4 ± 0.8 to 6.8 ± 4.4 $mg.L^{-1}$), a decrease in turbidity (72.7 ± 43.6 to 37.8 ± 16.3 NTU), an increase in total nitrogen (1.1 ± 0.8 to 5.3 ± 3.0 $mg.L^{-1}$), and ortho-phosphate (0.2 ± 0.2 to 1.3 ± 1.1 $mg.L^{-1}$ PO_4), from station S1 to S6. A rising number of thermotolerant coliforms were also observed along the river (312 ± 249 to 175.120 ± 186.047 MPN/100 mL). Hence, an evident pollution gradient down the river,

Table 1. Relation between the Water Quality Biological Index (WQBI) and the water quality class (Lobo et al., 2004).

WQBI	Level of pollution
≤1.00	Null
1.01-1.47	Low
1.48-2.10	Moderate
2.11-2.80	High
2.81-4.00	Very high

Table 2. Mean (n = 8) and standard deviation (sd) of limnological variables from September 2000 to August 2002 at the six sampling locations of Gravataí River (S1 to S6). Data Source: Rio Grande do Sul Environmental Foundation (FEPAM) and Porto Alegre Municipal Department of Water and Sanitation (DMAE).

Sampling stations	**S1**		**S2**		**S3**		**S4**		**S5**		**S6**	
	mean	**sd**	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**	**mean**	**sd**	**Mean**	**sd**
Temperature (°C)	**22.5**	4.92	**20.81**	4.22	**2 23.00**	5 5.05	**22.34**	4.87	**22.75**	4.95	**21.76**	5.01
Depth (m)					**5.44**	1 1.14	**4.28**	1.11	**4.69**	1.34	**4.ghf 4.69**	0.61
Secchi depth (m)					**0.27**	0 0.10	**0.22**	0.10	**0.22**	0.10	**0.26**	0.92
Turbidity (TNU)	**72.70**	43.60	**55.57**	31.83	**7 71.58**	3 30.33	**66.26**	20.79	**67.06**	26.53	**37.76**	16.28
Conductivity (mS.cm^{-1})	**73.12**	36.44	**52.84**	17.03	**4 4 9.81**	1 16.20	**104.09**	45.45	**124.98**	56.21	**139.09**	66.21
Chlorides (mg.L^{-1} Cl)	**8.04**	4.07	**5.85**	1.68	**7 7.13**	2.71	**11.25**	5.28	**11.91**	5.70	**13.38**	4.51
pH	**6.22**	1.36	**6.30**	0.35	**6.37**	0 0.41	**6.40**	0.45	**6.45**	0.43	**7.02**	0.27
DO (mg.L^{-1} O_2)	**5.65**	2.13	**4.74**	1.77	**4.70**	2 2.01	**2.50**	1.53	**2.00**	1.23	**3.45**	2.32
BOD (mg.L^{-1} O_2)	**1.45**	0.80	**1.20**	0.52	**1.36**	0. 0.95	**3.64**	1.71	**5.43**	3.23	**6.81**	4.38
COD (mg.L^{-1} O_2)	**35.70**	13.13	**32.28**	9.95	**3 38.74**	9. 9.78	**40.87**	10.63	**42.39**	11.34	**38.84**	9.16
Ammonium (mg.L^{-1} N)	**0.30**	0.14	**0.15**	0.07	**0.36**	0. 0.53	**2.16**	1.99	**2.87**	2.78	**3.37**	2.70
Organic nitrogen (mg.L^{-1} N)	**1.08**	0.65	**0.65**	0.19	**0.89**	0. 0.35	**1.49**	1.06	**2.40**	2.53	**1.78**	1.01
Total nitrogen (mg.L^{-1} N)	**1.12**	0.75	**0.77**	0.26	**1.27**	0. 0.84	**3.82**	2.77	**4.97**	4.64	**5.27**	3.01
Orthophosphate (mg.L^{-1} PO_4O)	**0.06**	0.04	**0.03**	0.02	**0.05**	0. 0.08	**0.26**	0.21	**0.30**	0.25	**0.82**	0.61
Total phosphate (mg.L^{-1} TP)	**0.21**	0.19	**0.17**	0.23	**0.16**	0. 0.14	**0.51**	0.35	**0.62**	0.41	**1.32**	1.14
Thermotolerant coliforms (MPN100 mL^{-1})	**313**	248	**271**	207	**859**	1 10001	**49304**	59381	**95175**	99623	**175120**	186048

from the headwaters to the mouth was detected, based on physical, chemical and microbiological variables (Table 2).

3.2. Indicator species analysis

From the analysis of the indicator species three main groups were identified. The species *Eolimna subminuscula, Luticola goeppertiana, L. mutica, Nitzschia palea*, *Sellaphora pupula*, and *S. seminulum* were classified in Group A. Group B consisted of the species *Achnanthidium minutissimum*, *Brachysira vitrea*, *Cyclotella meneghiniana*, *Diadesmis contenta*, *Encyonema minutum*, *Eunotia bilunaris*, *E. bilunaris var. mucophila*, *Frustulia saxonica*, *F. crassinervia*, *Gomphonema gracile*, *Lemnicola hungarica*, *Navicula cryptocephala*, *N. cryptotenella*, *N. radiosa*, *N. rostellata*, *Nitzschia amphibia*, *N. clausii*, *N. palea* var. *tenuirostris*, *Pinnularia braunii*, *P. divergentissima*, *P. microstauron*, *Surirella angusta*, and *Ulnaria ulna*. Finally, the species *Nupela impexiformis*, *Cocconeis placentula, Encyonema mesianum, Eolimna minima*, *Eunotia pectinalis*, *Gomphonema clevei*, and *G. parvulum* were classified in Group C. These TWINSPAN groups and their associated river sites formed the base for canonical ordination.

The canonical correspondence analysis (Figure 2) explained 30.2% of the total variability of data in its first two axes, which showed eigenvalues of 0.414 and 0.308, respectively (Table 3). The species/environment correlation coefficients obtained for axes 1 (r = 0.913) and 2 (r = 0.837), indicated a strong correlation between the distribution of species and the environmental variables used in the ordination. The Monte Carlo permutation test revealed that the ordination of these axes was statistically

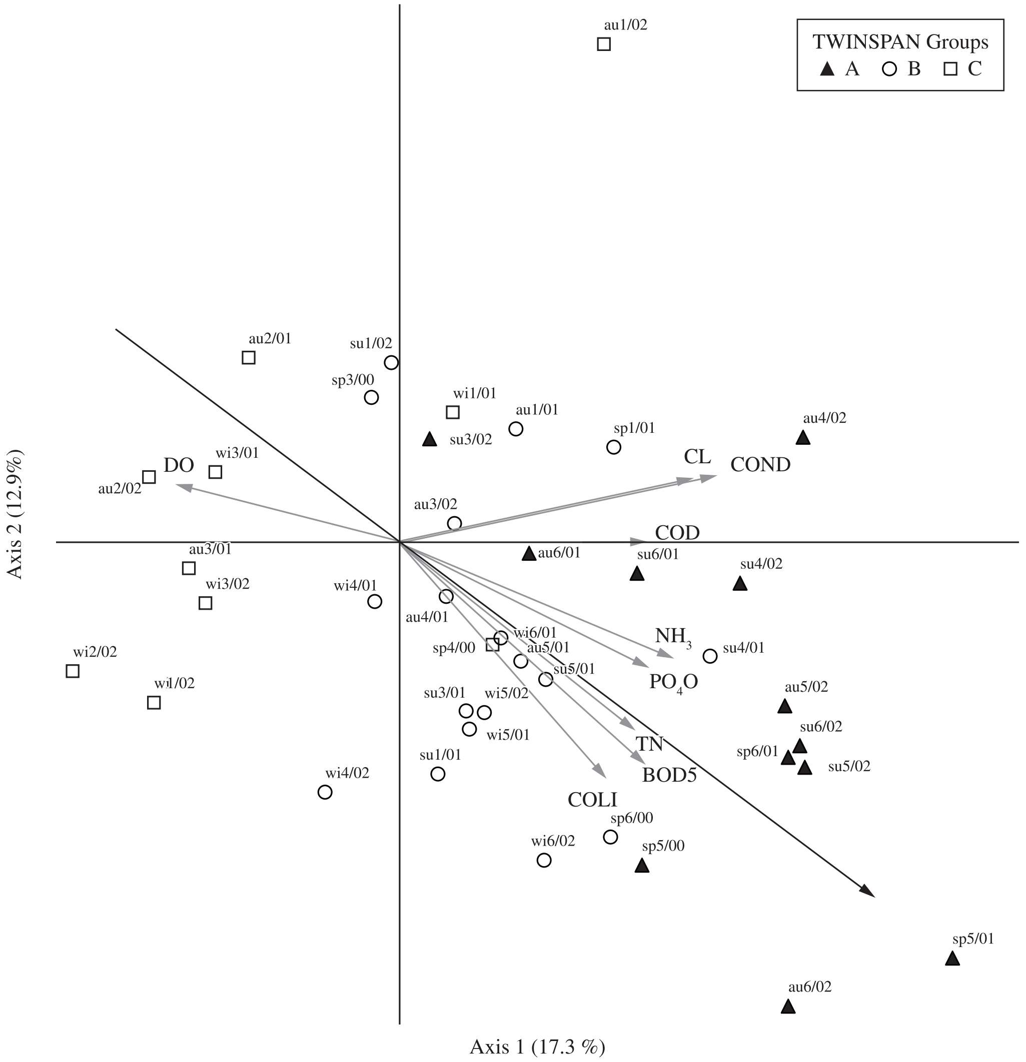

Figure 2. CCA biplot showing the ordination of 40 sampling sites based on TWINSPAN groups of diatom species composition. (CL: chlorides; COLI: thermotolerant coliforms; COND: conductivity; BOD_5: biochemical oxygen demand after five days; COD: chemical oxygen demand; NH3: ammonium; TN: total nitrogen; DO: dissolved oxygen; PO_4O: orthophosphate). The long arrow indicates pollution gradient.

significant ($p < 0.01$), indicating a high probability that these eigenvalues were not the result of chance.

The CCA results revealed that axis 1 explained 17.3% of the variability highlighting the chloride environmental variable (0.417), associated with its positive side. In the intra-set correlations (Table 4), conductivity, chloride, BOD_5, COD, ammonium, total nitrogen, orthophosphate, and thermotolerant coliforms were highlighted as variables positively correlated to axis 1, showing a clear gradient of organic contamination and eutrophication.

For axis 2 (12.9% of explained variation), the canonical coefficient showed a high correlation with BOD_5, associated with the negative end of the axis, where the intra-set correlation ratified this variable as the most significant ($r = -0.594$), showing an evident gradient of organic pollution.

As observed in Figure 2, the distribution of the three groups of TWINSPAN indicator species on the CCA chart revealed an evident sequence along the gradient of organic contamination and eutrophication. Group C showed the lowest tolerance to pollution, followed by Group B with an intermediate tolerance and Group A with the highest tolerance to pollution.

These groups show peculiar environmental conditions throughout the detected pollution gradient. From Group C of sampling stations, characterized from Oligosaprobic to b-mesosaprobic, based on the average values of BOD_5 ($1.32 \pm 0,66$ mg.L^{-1} O_2) and phosphate ($0.19 \pm 0,21$ mg.L^{-1} PO_4), respectively. Group B of sampling stations characterized from b-mesosaprobic to Polisaprobic, based on the average values of phosphate (0.33 ± 0.24 mg.L^{-1} PO_4) and ammoniacal nitrogen (1.26 ± 1.26 mg.L^{-1} N), respectively. Finally, Group A of sampling stations, characterized as Polisaprobic, based on the average values of phosphate (0.97 ± 0.77 mg.L^{-1} PO_4) and ammoniacal nitrogen (3.12 ± 2.74 mg.L^{-1} N), respectively.

Table 3. Result of CCA analysis applied to environmental descriptors (physical and chemical water variables) and relative abundance of epilithic diatoms.

Statistics	Axis 1	Axis 2
Eigenvalues (λ)	0.414	0.308
Percentage of explained variance (%)	17.3	12.9
Accumulated variance (%)	17.3	30.1
Pearson correlation (species-environment)	0.913	0.837
Monte Carlo Test for eigenvalues: P	0.010*	0.004*

*Significant ordination.

Taking this into account, the species belonging to Group C were assigned, operationally, the saprobic value "*s*" = 1 and the indicative value "*vi*" = 1, corresponding to oligosaprobic/mesosaprobic levels and low tolerance to eutrophication, respectively. Similarly, the saprobic value "*s*" = 2.5 and the indicative value "*vi*" = 3, representing mesosaprobic levels and medium tolerance, were assigned to the characteristic species of Group B. Finally, the species characteristic of Group A were assigned the empirical saprobic value "*s*" = 4 and indicative value "*vi*" = 5, for polysaprobic levels and high tolerance to eutrophication, respectively.

Gravataí WQBI results obtained for station 1, 2 and 3 ranged from 1.26 and 3.73, representing "Low" to "Very High" pollution conditions (Figure 3a). A single value (5.9%) corresponding to "Low" condition, 35.2% to "Moderate/High" and 58.8% to "Very High" condition. For stations 4, 5, and 6, the values ranged from 3.07 and 3.97 corresponding to 100% of "Very High" pollution level (Figures 3b).

The values obtained for stations 1, 2, and 3 varied between 2.29 and 3.08 representing "High to Very High" pollution, most of the results (88.2%) being at the level "High" (Figure 3c). As for the values at stations 4, 5, and 6, they varied from 2.55 to 3.90, characterizing similarly the pollution as "High and Very High" (Figure 3d). In general these values were higher than those recorded at stations 1, 2, and 3 as 91.3% of the results were concentrated in the level "Very High", differently from the upper and middle stretches, where 11.8% of the values were concentrated in this level. As in the case of the Gravataí

Table 4. Canonical coefficients and intra-set correlations between 10 limnological variables and the first two axes.

Variables	Canonical coefficient		Correlation coefficient intraset	
	Axis 1	Axis 2	Axis 1	Axis 2
Conductivity	0.231	0.194	0.816	0.158
Chlorides	0.417	0.379	0.887	0.173
Dissolved oxygen	–0.053	0.059	–0.623	0.144
BOD_5	–0.067	–0.594	0.686	–0.567
COD	0.092	0.330	0.685	0.008
Ammonium	–0.012	–0.210	0.757	–0.301
Total nitrogen	–0.079	–0.154	0.653	–0.482
Orthophosphate	0.219	0.369	0.689	–0.318
Total phosphate	–0.192	0.103	0.077	0.046
Thermotolerant coliforms	0.050	–0.143	0.580	–0.608

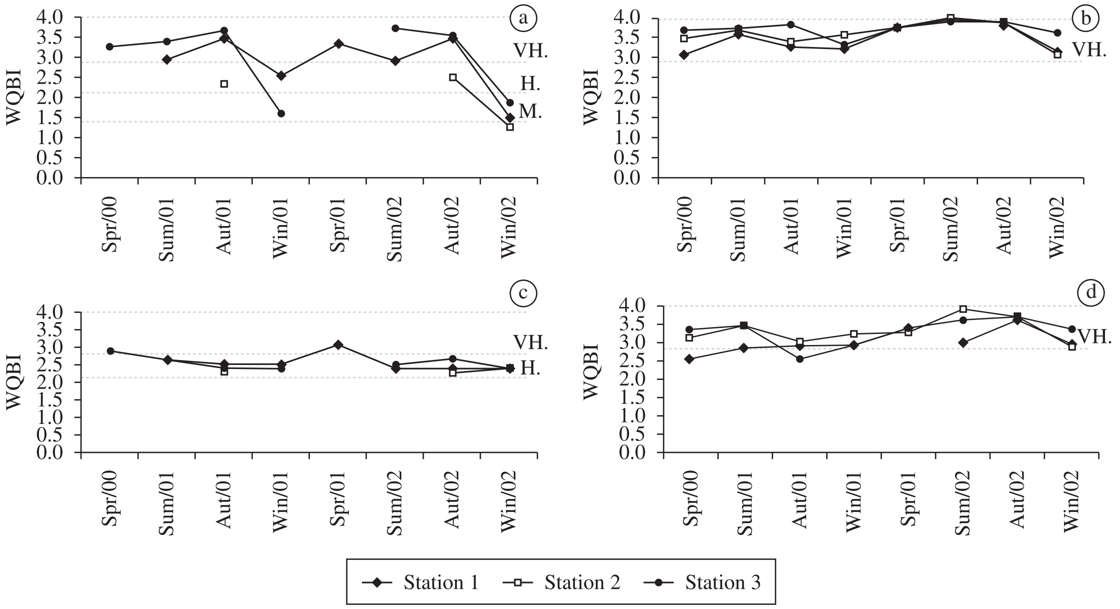

Figure 3. Variation of water quality biological index from September 2000 to August 2002. a) Gravataí WQBI at stations S1, S2 and S3; b) Gravataí WQBI at stations S4, S5 and S6; c) Regional WQBI at S1, S2, and S3; d) Regional WQBI at stations S4,S5 and S6 (M: moderate; H: high; VH: Very High).

WQBI, the spatial variations clearly reflected a gradient of rising organic pollution and eutrophication from the upper to lower reaches.

Comparing the results generated by the Gravataí WQBI and the regional WQBI, there were some differences with regarding to the exact pollution levels, but both indices corroborate the tendency for contamination to increase from the headwaters to the mouth. In fact, the Spearman correlation coefficient between the two WQBI's was highly significant ($r = 0.745$ $p < 0.0001$).

4. Discussion

For the regional WQBI at stations 1, 2, and 3, the predominant level was "High", with 88.2% of the samples, the Gravataí index level "High" reached only 17.6%, while the level "Very High" reached 58.8%. At stations 4, 5, and 6, the regional index was predominantly "Very High", with 91.3% of the total, while the Gravataí index was "Very High" in 100% of the samples. It was found therefore, in the case of the Gravataí WQBI, that an adaptation of diatom species to the local conditions occurred, which was detected by the multivariate analyses, resulting in a recalibration of saprobic and indicative values, relative to the regional WQBI, reflecting the local environmental impact predominant in the basin.

Thus, the differences that result from the use of the Gravataí or the regional WQBI arising from the different attribution of saprobic and indicative values to the species should be explained by the local environmental characteristics in the Gravataí River, reflecting on the tolerances of the species to water contamination.

There is, for example, the case of *Gomphonema parvulum* sensu lato which was the most abundant species in the upper stretches of the Gravataí River, representing 37.0, 78.0, and 48.0% of the total relative abundance at sampling stations 1, 2 and 3, respectively, a condition that led to the classification of this taxon in Group C of indicator species, characterizing oligotrophic/mesotrophic environments. Hence, the saprobic and indicative values for this species were $s = 1$ and $vi = 1$ (Table 5), quite different from those assigned by Lobo et al. (2004) to this taxon: 2.5 and 4, respectively, characterizing mesoprobic and eutrophic environments. Thus, depending on the relative abundance of this taxon in any given sample, there may be significant differences between the level of pollution estimated when the saprobic and indicative values obtained in this study and those of Lobo et al. (2004) are adopted.

In fact, there is much controversy in the literature regarding the classification of this species in relation to its tolerance to contamination. Kobayasi and Mayama (1989) and Lobo et al., (1995) classified *G. parvulum* as highly tolerant to organic pollution in Japanese rivers, and Kelly and Whitton (1995), evaluating the water quality in English rivers assigned to this species indicative and saprobic values corresponding to highly eutrophic environments.

Similarly, Rodrigues and Lobo (2000) registered the occurrence of this species in moderately polluted, b-mesosaprobic waters, in lotic systems of the drainage basin of the Sampaio Stream, RS, Brazil. Meanwhile, in lotic systems of the town of São Carlos, SP, Brazil, Souza (2002) recorded this species in high abundance in oligotrophic and mesotrophic environments, corroborating the current observation. Morales and Jasinski (2002)

Table 5. Diatom species in the Gravataí River community. a) saprobic values (s) and indicative values (vi) for the Gravataí WQBI. b) saprobic values (s) and indicative values (vi) for the regional WQBI (Lobo et al., 2004).

Species	Gravataí WQBI		Regional WQBI		Twinspan groups
	s	*vi*	*s*	*vi*	
Achnanthidium minutisssimum (Kützing) Czar Necki	2.5	3	2.5	3	B
Brachysira vitrea (Grun.) Ross in Hartley	2.5	3	1	1	B
Cocconeis placentula Ehrenberg	1	1	2.5	3	C
Cyclotella meneghiniana Kützing	2.5	3	2.5	3	B
Diadesmis contenta (Grunow) D.G. Mann	2.5	3	2.5	3	B
Encyonema minutum (Hilse in Rab.) D.G. Mann comb. nov.	2.5	3	1	1	B
Encyonema mesianum (Cholnoky) Mann	1	1	1	1	C
Eolimna minima Grunow	1	1	1	4	C
Eolimna subminuscula Manguin	4	5	4	1	A
Eunotia bilunaris (Ehrenberg) Mills	2.5	3	1	1	B
Eunotia bilunaris (Ehrenberg) Mills var. *mucophila* Lange-Bertalot & Nörpel	2.5	3	1	1	B
Eunotia pectinalis (Dillwyn) Rabenhorst	1	1	1	1	C
Frustulia saxonica Rabenhorst	2.5	3	1	1	B
Frustulia crassinervia (Bréb. In W. Sm.) Lange-Bertalot & krammer	2.5	3	1	1	B
Gomphonema clevei Frickei	1	1	4	3	C
Gomphonema gracile Ehrenberg	2.5	3	2.5	1	B
Gomphonema parvulum (kützing) Kützing	1	1	2.5	4	C
Lemnicola hungarica (Grunow) Round & Basson	2.5	3	1	1	B
Luticola goeppertiana (Bleisch in Rab.) D.G. Mann	4	5	4	1	A
Luticola mutica (Kützing) D.G. Mann	4	5	1	1	A
Navicula cryptocephala Kützing	2.5	3	2.5	1	B
Navicula cryptotenella Kützing	2.5	3	4	3	B
Navicula radiosa Kützing	2.5	3	1	1	B
Navicula rostellata Kützing	2.5	3	2.5	4	B
Nitzschia amphibia Grunow	2.5	3	4	2	B
Nitzschia clausii Hantzsch	2.5	3	1	1	B
Nitzschia palea (Kützing) W. Smith	4	5	4	3	A
N. palea (Kützing) W. Smith var. *tenuirostris* Grunow	2.5	3	1	1	B
Nupela impexiformis (Lange-Bertalot) Lange-Bertalot	1	1	1	1	C
Pinnularia divergentissima (Grunow) Cleve	2.5	3	1	1	B
Pinnularia microstauron (Ehrenberg) Cleve	2.5	3	1	1	B
Sellaphora pupula (Kützing) Mereschkowsky	4	5	4	1	A
Sellaphora seminulum (Grunow) D.G. Mann	4	5	2.5	5	A
Surirella angusta Kützing	2.5	3	2.5	1	B
Ulnaria ulna (Nitzsch) Ehrenberg	2.5	3	4	1	B

point out that many morphotypes of *G. parvulum* can be observed and such represent distinct varieties. As shown by Salomoni et al. (2006), diatom morphology can vary as a result of both genetic variability and also variation in the ecology of the species, which may result in the formation of ecotypes, a situation that could explain the variety of responses attributed to the same species. Clearly, a more detailed study of the ecology and physiology of morphotypes of *G. parvulum* is necessary to clarify this issue.

Another point of disagreement between the quality indices arises from the fact that many species that were abundant in the Gravataí River, such as *Brachysira vitrea*, *Encyonema minutum*, *Eunotia bilunaris*, *E. bilunaris* var. *mucophila*, *Frustulia saxonica*, *F. crassinervia*, *Lemnicola hungarica*, *Navicula radiosa*, *Nitzschia clausii*, *N. palea* var. *tenuirostris*, *Pinnularia braunii*, *P. divergentissima*, and *P. microstauron*, were included in the Group B indicator species, characterizing b-mesosprobic to polisaprobic environments. Therefore, these species were assigned the saprobic and indicative values "*s*" = 2.5 and "*vi*" = 3 (Table 5), different from those given by Lobo et al. (2004), 1 and 1, respectively, characterizing oligosaprobic and oligotrophic environments. This situation arises because in the regional system these species were not abundant and, therefore, did not form part of the list of indicator species. Hence, operationally they were classified as having saprobic values "*s*" = 1 and indicative values "*vi*" = 1, for the purpose of calculating the biotic index.

A variation in the composition and the abundance of selected species as indicators of the degree of pollution can be incorporated into biotic indices, which combine the ecological information with environmental information through specific indicative rates or values assigned to species derived from multivariate analysis. Indices generated based on extensive databases obtained for a given region and tested for different types of rivers, can be a useful tool to find out not only the river classification, but also their parts with regards to the water quality (Prygiel, 2002).

The comparison between the Gravataí index with the regional WQBI showed similarities. This comparison also seemed to be a suitable model when executed. However, it is important to highlight that many abundant and indicators species to the WQBI Gravataí are not listed in the regional WQBI. We hope that with the WQBI Gravataí data shown in this study, we may contribute to the calibration of the regional index based on the ecological tolerance of the species that are not yet part of the regional WQBI.

Comparing the results obtained by using the Gravataí WQBI with those from the regional WQBI, differences were found with regards to the predominant pollution levels, which were higher in the Gravataí index. However, both indices corroborate the tendency of contamination to increase from the headwaters to the mouth. These results corroborate the hypothesis of Tundisi et al. (2002) that due to the complexity of environmental diagnosis - based on the physical and chemical characteristics of the water with the biological communities, conservation and recovery of water resources might demand the creation of a differentiated database for each hydrological basin.

By taking into account these results, we hope more studies concerning the development of a WQBI may be created even in others river in the region.

We also hope that more water with the biological communities, conservation and recovery of water resources may require the creation of more studies concerning the development of a WQBI for other regional rivers.

Acknowledgements – The authors would like to express their thanks to the Rio Grande do Sul Research Aid Foundation (FAPERGS) for financing the project and to the Museum of Natural Sciences of the Zoobotanical Foundation of Rio Grande do Sul (MCN/FZB-RS) for the use of facilities. To CAPES Foundation and to the Brazilian National Research Council (CNPq) for the scholarship to the first author. To Prof. Dr. Pedro Américo Cabral Senna (in memoriam) for his excellent suggestions at the start of the project. To technicians of the "Departamento Municipal de Água e Esgoto (DMAE) who made the results of the analyses available. Furthermore, we express gratitude to Tim Roberts for reviewing the English text.

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