DIATOM-BASED WATER QUALITY ASSESSMENT IN STREAMS INFLUENCE BY URBAN POLLUTION: EFFECTS OF NATURAL AND TWO SELECTED ARTIFICIAL SUBSTRATES, SÃO CARLOS-SP, BRAZIL

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ABSTRACT

Braz. J. Aquat. Sci. Technol. 15(1): 54-63. ISSN 1983-9057. Diatoms are good indicators of water quality in lotic systems. The purpose of this study was to provide information about the effects of natural and artificial substrates on diatom-based water quality assessment. Two artificial substrates (bricks and glass) were placed at 6 sampling sites and left for a month during summer base-flow period (2008). Water quality variables were measured at the beginning and end of the experiment. The IndVal method was used to find indicator species and species assemblages characterizing the three substrates. Species richness, diversity and equitability differed among sampling sites, tending to be higher in relatively unpolluted compared to polluted sites. The relatively less polluted upstream sites were characterized by such species as *Aulacoseira ambigua*, *Aulacoseira granulata*, *Cymbopleura naviculiformis*, *Eunotia bilunaris*, *Fragilaria capucina* and *Gomphonema angustatum*. On the other hand, the highly polluted downstream sites were characterized by *Gomphonema parvulum*, *Nitzschia palea, Pinnularia amazonica* and *Synedra ulna*. Species diversity and richness differed between the substrates, tending to be high on natural compared artificial substrate. Indicator species analysis showed that common diatom species were not restricted to single substrate. However, some species tended to prefer certain substrates as suggested by their highest indicator values in these preferred substrates. Specificity was generally high for natural compared to artificial substrates. Among the artificial substrates, more species tended to prefer glasses compared to bricks. Pollution tolerant species, *N. palea*, *G. parvulum* and *Achnanthidium minutissimum*, were highly associated with artificial substrates. Substrate differences may affect the interpretation of water quality results because the absence of a particular species on a given site is can be mistaken for the effects of the perturbations under study. The use of natural substrate is recommended compared to artificial substrate given the advantages of the former compared to the latter.

Keywords: artificial substrate; indicator values; species diversity; streams; water quality assessment

INTRODUCTION

Multiple factors prevailing at different temporal and spatial scales play an important role in structuring benthic diatom communities in lotic systems (Potapova & Charles 2002; Moura *et al.,* 2007), with local environmental conditions playing a more important role compared to broad-scale climatic, vegetational and geographical factors (Pan *et al.,* 1996). Each particular species requires different structural, physical and chemical characteristics intrinsic to its habitat. Whenever these characteristics are subject to variations, niche composition is affected. Species vary in their sensitivity and those more resistant to environmental changes, caused either by natural fluctuations or human activities, may be favoured by selection (Rocha, 1992). Changes in any of these factors need not necessarily bring about the death of some algal species so long as the changes remain within the limits of tolerance of the species. On the contrary, these changes will inhibit the multiplication of some of the species originally present, and encourage that of others, so that primarily the association, that is the percentage composition and not the flora as such, will be changed (Pan *et al.,* 1996).

Diatom assemblages in lotic ecosystems provide a direct, holistic and integrated measure of the integrity of these systems. Two major approaches to the use of diatoms for assessment of the ecological integrity of lotic systems are generally used worldwide. The first approach involves direct sampling of natural substrate – the favourite being epiliton, while the second approach involves sampling of artificial substrate placed in water – the favourite being glass (Round, 1991).

Direct sampling of natural substrate has no a priori recommendations and has several advantages (Descy & Coste, 1991; Leclercq & Manquet, 1987). However, direct sampling has the following disadvantages: 1) in silted lowland rivers stones are often thickly coated with silt that modifies or eliminate the eplithic flora; 2) it is not always easy to sample concrete and bad rock; 3) in

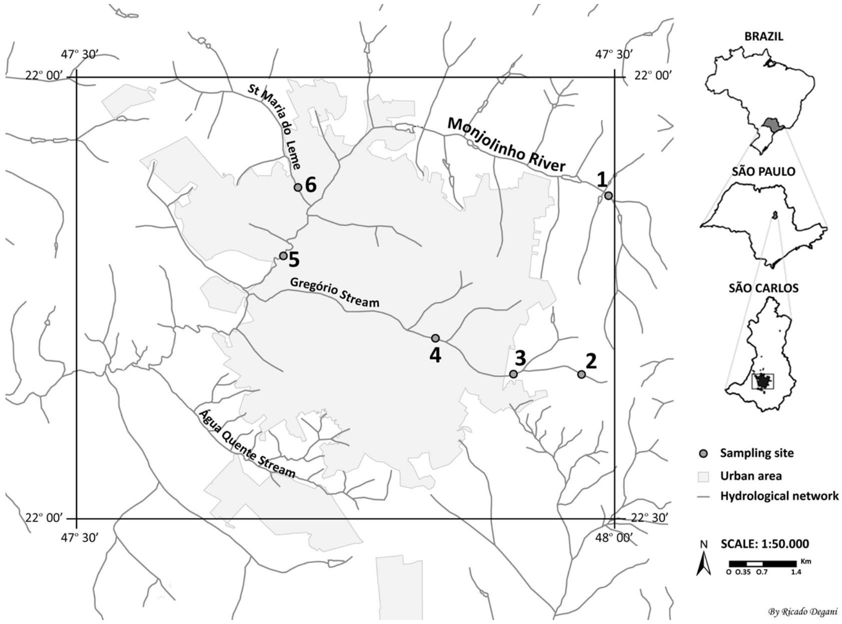

Figure 1 - The location of the sampling sites in the study area.

torrential upper stretches sampling can be difficult and often the flora is depleted; and 4) shading by surrounding vegetation reduces species richness and biomass (Round, 1991). Diversity and productivity of diatoms vary from one rock type to another depending on the nature of the physical and chemical properties of the rock. Large stones are expected to have stable communities, whilst small ones may be so moved during periods of high flow that the flora diversity and richness is reduced (Marker & Willoughby, 1988). A careful consideration of these factors during sample collection and subsequent data interpretation is, therefore, necessary as ignoring them is likely to lead to biased results.

The challenges posed by direct sampling in the assessment of biotic integrity of lotic systems can be circumvented by use of artificial substrate, traditionally favourable being glass slides and in some case bricks are also used. The artificial substrate has the advantage that flora can be observed directly, substratum is standard at all sampling sites and time of exposure can be controlled (Round, 1991). However, the disadvantages are overwhelming (Descy & Coste, 1991): 1) require apparatus to be fixed in the river and there are often loses; 2) there is need to experiment to obtain the optimum time of exposure and often 4 or even 8 weeks is necessary, preventing a rapid estimation of water quality such as can be obtained within hours of sampling the epiliton directly; 4) the flora is an artificial assemblage selected by smooth slide and perhaps by differences due to positioning of slides in relation to the currents; 5) the smooth surface often results in sloughing of the community; and 6) random sampling is not allowed.

Studies on the periphytic community are scarce in the study area. Our comprehension of the role of environmental factors in shaping these communities and its subsequent effects on diatom based water quality is thus still in its infancy. The main purpose of this study was to describe diatom species associations in relation to environmental variables in the system using natural and artificial substrates. The study of periphytic community on artificial substrate (glass) carried out in other systems (Arroio Sampaio and its tributaries (Oliveira *et al.,* 2001)) showed that diatom groups were more representative of the community in this system considering species richness and abundance; hence we employed the same technique in the study area. Specifically the study aimed at: 1) comparing the development of benthic diatom communities on two artificial substrates (glass or bricks) relative to natural substrate; 2) assessing the suitability of artificial substrates compared to natural substrates for diatom-based water quality assessment; and 3) assessing the importance of environmental variables in structuring benthic diatom communities.

MATERIALS AND METHODS

Study area

The area under study is shown in Figure 1. Headwaters of the study system (Monjolinho River and its tributaries, Gregório and Maria Madalena) fall within mainly agricultural area. From agricultural area, the streams pass through urban area of the city of São Carlos, which covers a total area of 1143.9 km^2. The area is characterized by rugged topography and an average annual temperature around 19.5 °C, with mean monthly maximum around 21.9 °C recorded in January and February, and the mean monthly minimum around 15.9 °C recorded in July.

In 2008, the population of São Carlos was estimated at 218 080 inhabitants (IBGE, 2008). Now, the expansion of the city does not meet the technical standards that go with it in terms of sewage treatment, collection of garbage, urban drainage and other aspects. Streams in the study area, therefore, receive untreated or partially treated effluent from various domestic and industrial sources as well as other diffuse sources as they pass through the city. This results in stream health deterioration, loss of the remaining primary vegetation and eutrophication, among other problems.

Six sites were established in three stream systems; 3 sites in the relatively less impacted headwaters to act as references, and 3 sites in the urban area. Sites 1 and 5 were located along Monjolinho River; sites 2, 3 and 4 were located along Gregório stream; site 6 was located along Maria Madalena stream. The rationale for selecting the sampling sites was to obtain a pollution gradient, from relatively unpolluted headwaters to highly polluted downstream sites.

Data collection

Diatom and water quality sampling was done during dry season when flow was stable (September to October 2008) at the 6 sites. Dry season was selected to avoid variable effects of rainy season, such as variations in water level and velocity, which affect diatom development, especially growth rate and relative abundance of different species (Duong *et al.,* 2006). At each site, dissolved oxygen (DO), electrical conductivity, temperature, pH, concentration of total dissolved solids (TDS), and turbidity were measured using a Horiba U-23 and W-23XD Water Quality Meter (Horiba Ltd, Japan). The depth and current velocity were relatively uniform among the sites (10-30 cm and 1.5-2.0 ms^{-1} respectively). The percentage riparian vegetation cover was estimated at each site. Altitude was determined using a GPS (Northport Systems, Inc. Toronto, Canada). Light intensity was measured using LI-193 Spherical Quantum Sensor (LI-COR Worldwide, Brazil).

Water samples for total nitrogen (TN) and total phosphorus (TP) analysis were also collected at each site using acid-cleaned polyethylene containers (Valderrama, 1981). Water samples for biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) were collected following Apha (1988). All the physical and chemical characteristics that vary with time were measured twice, on 16 September when the artificial substrates were placed at the different sites and on 21 October when the artificial substrate was sampled.

At each site 2 bricks measuring 10 x 21 cm (total area = 420 cm^2) and 4 rugose glass slides measuring 7 x 17 cm (total area = 714 cm^2), as artificial substrate for algal attachment, were immersed in the water column, parallel to the current at a depth of 20 to 30 cm below the surface. These substrates were left for 4 weeks, which is the recommended colonization time of periphyton (Round, 1991; Descy & Coste, 1991). On sampling, the material were carefully brought to the surface and thoroughly rinsed with filtered river water. Biofilms were collected by brushing the material with a toothbrush. The resulting suspensions from the replicates were pooled and sub-samples were used in the subsequent procedures.

Epipelic diatoms were sampled at the time of sampling of artificial substrates by pressing a Petri dish lid (area = 17 cm^2) into the top layer of silt/clay to a depth of 5-7 mm followed by sliding a spatula blade under the Petri dish to isolate the contents in the dish, which were then gently brought to the surfaces. The contents were then emptied into a labelled container. Samples from 6 locations in each sampling were pooled into a single sample; the total area sampled was 102 cm^2. Epipelic diatoms were sampled instead of the commonly used epilithic diatoms in water quality assessment because stones ware not present at some sites.

Laboratory analysis

Sub-samples of the diatom suspensions were cleaned of organic material using wet combustion with concentrated sulphuric acid and mounted in Naphrax (Northern Biological supplies Ltd. UK. RI = 1.74) following Biggs & Kilroy (2000). Three replicate slides were prepared for each sample. A total of 500 – 600 frustules per sample (depending on the abundance of diatoms) were identified and counted using the phase contrast light microscope (1000 X). The diatoms were identified to species level based on studies by Mizuno 1964; Patrick & Reimer 1966a, b; Bourrelly 1981; Lobo *et al.,* 1996; John, 2000; Biggs & Kilroy 2000; Oliveira *et al.,* 2001; Lobo *et al.,* 2002; Lobo *et al.,* 2004; Bicudo & Menezes 2006; Salomoni *et al.,* 2006; Delgado *et al.,* 2007; Moura *et al.,* 2007; Soares *et al.,* 2007; Zalocar de Damitrovic *et al.,* 2007 and the website of the Phycology Section of The Academy of Natural Sciences of the USA (http://diatom.acnatsci.org).

The concentration of TN and TP in the water samples was determined following the method by Golterman *et al.* (1978) and Valderrama (1981) respectively. BOD_5 was determined by filling with water samples from all sites, to overflowing, airtight bottles of known size and incubating them at 25 ^{o}C for 5 days. DO was measured initially and after incubation following Winkler Method, the BOD_5 was computed from the difference between initial and final DO following Apha (1988). COD was determined by oxidation of organic matter by boiling water samples in mixture of chromic and sulphuric acids following Apha (1988).

Data analysis

Species richness (*S*), Shannon's diversity (*H´*) and equitability (*E*) indices calculated according to the Shannon (1946) were used as measures of community structure. The IndVal method (Dufrêne & Legendre 1997) was used to find indicator species and species assemblages characterizing the three substrates. This method combines a species' relative abundance with its relative frequency of occurrence in the various substrates. Indicator species are defined as the most characteristic species of each substrate, found mostly in a single substrate and present in the majority of those substrates. For each species *i* in each substrate type *j*, we computed the product of $\mathbf{A}_{ij}$, the mean abundance of species *i* in the substrate type *j* compared to all substrate studied (specificity), by $\mathbf{B}_{ij}$, the relative frequency of occurrence of species *i* in the substrate type *j* (fidelity), according to the formula modified from Dufrêne & Legendre (1997) as follows:

$$\mathbf{A}_{ij} = \mathbf{N}_{ij}/\mathbf{N}_i$$
$$\mathbf{B}_{ij} = \mathbf{NS}_{ij}/\mathbf{NS}_j$$
$$\mathbf{IndVal}_{ij} = \mathbf{A}_{ij} * \mathbf{B}_{ij} * 100$$

where $\mathbf{IndVal}_{ij}$ = Indicator Value of species *i* in substrate type *j*, $\mathbf{N}_{ij}$ = mean number of individuals of species *i* across substrate type *j*, $\mathbf{N}_i$ = sum of the mean number of individuals of species *i* over all substrates, $\mathbf{NS}_{ij}$ = the number of substrates *j* where species *i* is present, $\mathbf{NS}_j$ = the total number of substrates.

Multivariate data analyses were performed on the diatom data set to explore the main gradients of floristic variation and to detect and visualize similarities in diatom samples. Detrended correspondence analysis (DCA) was applied on diatom data set to determine the length of the gradient (Hill and Guach 1980). DCA revealed that the gradient was greater than 3 standard deviation units (3.9); therefore, unimodial ordination techniques would be more appropriate. Canonical correspondence analyses (CCA), was performed to relate diatom community structure to simultaneous effects of environmental variables and to explore the relationship among and between species and these predictor variables (ter Braak & Verdonschot, 1995). Preliminary CCA identified collinear variables and selected a subset on inspection of variance inflation factors (VIF< 20; ter Braak & Smilauer, 2002). Exploratory variables were subjected to step wise forward selection procedure in which the statistical significance of each variable was tested by the Monte Carlo permutation test (999 unrestricted permutations, p d" 0.05). CCA and DCA were performed using PAlaeontological STatistics (PAST) software version 1.95 (Hammer et al. 2009).

RESULTS

Physical and chemical characteristics of water

The values of physical and chemical variables measured in the study area during the study period are shown in Table 1. The water quality generally tended to deteriorate downstream as the streams passed through the urban area due to discharge of treated and untreated domestic and industrial effluent, as well as other diffuse sources of pollution from the city. The pH increased slightly down the agricultural to urban gradient being slightly acidic at upstream sites and slightly alkaline/ neutral at downstream sites. Temperature, conductivity, turbidity, TDS, stream width and light intensity tended to increase downstream while dissolve oxygen, altitude and percentage canopy cover tended to decrease downstream.

The concentration of TN and TP in the water samples showed a general tendency of increasing along agricultural to urban gradient (Table 1) due to domestic and industrial pollution. The levels BOD_5 and COD also followed a broadly similar pattern to that of nutrient levels tending to be generally low in agricultural sites and increasing downstream due to domestic and industrial pollution (Table 1).

Table 1 - The mean values of physical and chemical variables measured on all the sites at the placing and collection of artificial substrate.

Site	1	2	3	4	5	6
Temperature (°C)	18.3	20.9	20.6	21.2	20.39	24.8
Conductivity (µS cm^{-1})	45.0	20.0	53.0	89.0	30.0	715.0
TN (mg L^{-1})	0.65	0.18	0.24	1.29	0.93	38.32
TP (mg L^{-1})	0.01	0.01	0.01	0.16	0.02	2.97
DO (mg L^{-1})	7.3	8.2	7.6	6.9	7.2	1.9
BOD5 (mg L^{-1})	0.9	1.0	2.6	6.9	7.2	19.5
pH	6.6	6.4	6.3	6.8	6.8	7.2
Turbidity (NTU)	0.0	0.0	0.0	0.0	0.0	0.1
TDS (g L^{-1})	29.4	13.4	22.6	57.4	19.3	457.8
Altitude (m)	761.0	837.0	831.0	794.0	761.0	724.0
Canopy cover (%)	80.0	95.0	60.0	50.0	45.0	20.0
Light intensity (µmol s^{-1} m^{-2})	105.0	327.0	431.0	1500.0	649.0	1780.0
Mean width (m)	0.6	0.8	1.1	1.9	1.0	12.5

Table 2 - Species richness (*S*), diversity (*H'*) and evenness indices (*E*) for the sites and substrates sampled (E, epipelion; B, bricks; GL, glass).

Site	1			2			3			4			5			6	
Substrate	E	B	G	E	B	G	E	B	G	E	B	G	E	B	G	E	G
H'	3.21	2.42	2.81	3.15	2.72	2.96	3.09	2.92	2.85	2.68	2.10	2.27	2.39	1.94	2.12	1.70	1.52
E	0.89	0.77	0.84	0.90	0.89	0.85	0.92	0.86	0.90	0.93	0.91	0.82	0.75	0.66	0.85	0.93	0.81
S	63	34	40	47	23	45	48	39	27	31	11	23	30	26	20	25	19

Diatom distribution

A total of 112 diatom species belonging to 44 genera that are distributed among the families Achnanthidiaceae, Achnanthaceae, Bacillariaceae, Eunotiaceae, Cymbellaceae, Gomphonemataceae, Fragilariaceae, Melosiraceae, Naviculaceae, Rhoicospheniaceae, Rhopalodiaceae and Surirellaceae were recorded in all the diatom samples collected. Among the 112 species observed, 53 species (Table 3) were considered as mostly frequent in the study area (e” 10 cells/cm^2 occurrence and present in at least 2 samples).

Species richness, diversity and equitability tended to be higher in relatively unpolluted sites (1, 2, 3) compared to the polluted sites (4, 5 and 6) being highest at site 1 and lowest at site 4 (Table 2). Species diversity and richness tended to be high on glass compared to bricks. Species diversity and richness were generally high on natural substrate compared to artificial ones.

Indicator species analysis showed that common diatom species were not restricted to single substrate (Table 3). Indicator values can vary from 0% for a taxon that has the same occurrence and abundance in all the groups of substrates to 100% for a taxon that is confined to one group of substrate. However, some species tended to prefer certain substrates as indicated by their highest indicator values in these preferred substrates. Specificity was generally high for natural compared to artificial substrates. Among the artificial substrates, more species tended to prefer glasses compared to bricks.

Nitzschia palea (Kützing) Smith, and *Gomphonema parvulum* (Kützing) Cleve commonly reported to be resistant to organic and heavy metal pollution and have been frequently recorded in waters that are nutrient rich and poorly oxygenated (Round 1991; Biggs & Kilroy 2000; Potapova & Charles 2003; Duong *et al.,* 2006), were highly associated with artificial substrates. *Achnanthidium minutissimum*, also commonly considered a disturbance-tolerant species (Stevenson & Bahls 1999) was highly associated with the artificial substrate.

The results of CCA are presented in Figure 2. Only 5 variables, pH, TP, DO, BOD_5 and percentage canopy cover were used in the analysis. The first and second axes of CCA explained 22.6 % of the proportion of the diatom species variance; CCA axis 1, eigenvalue = 0.38 and axis 2, eigenvalue = 0.25. Monte Carlo unrestricted permutation test indicated that axis 1 (99 permutations) and axis 2 (99 permutations of axis 2 with axis 1 as a covariable) were statistically significant (*p* d” 0.05). CCA axis 1 and 2 roughly separated relatively

Figure 2 - Canonical correspondence analysis (CCA) diagram showing environmental variables and diatom species in the ordination space of the first and second axes. Environmental variables that had low correlations with ordination axes are not shown. Species codes correspond to those in Table 3, **E**, epipelion; **G**, glass and **B**, bricks.

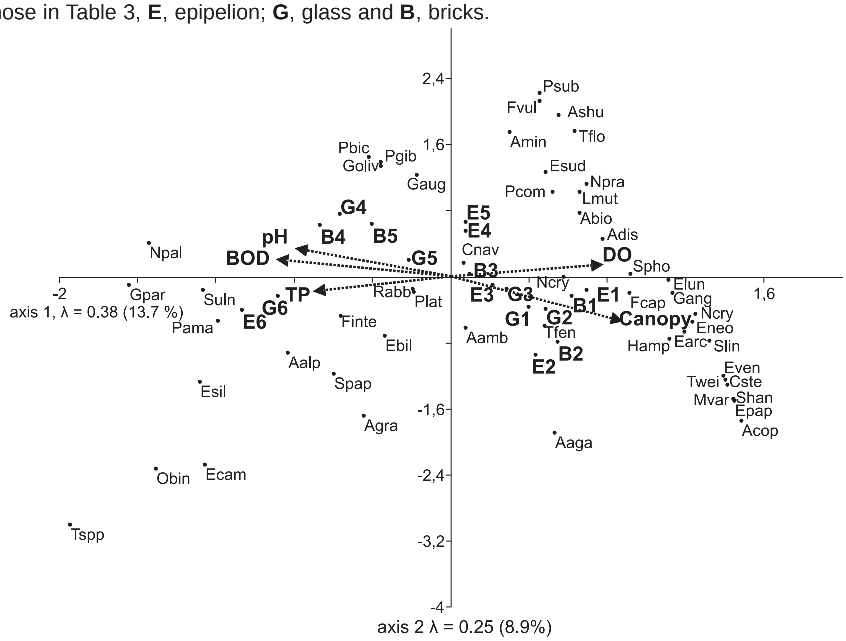

Table 3 - Indicator species and species assemblages characterizing the three substrates based on the most frequently occurring diatom. Species with the highest indicator values (%) in the different substrates are highlighted.

		Indicator values (%)		
Species	**Code**	**Epipelion**	**Brick**	**Glass**
Achnanthidium biosolettianum Grunow	Abio	**58.1**	13.9	28.0
Achnanthidium minutissimum (Kützing) Czarnecki	Amin	7.7	33.0	**59.3**
Amphora copulate (Kützing) Schoeman and Archibald	Acop	**59.1**	7.6	0.0
Encyonopsis schubartii (Hustedt) Krammer	Ashu	0.0	28.4	**38.3**
Aulacoseira agassizii (Ostenf) Simonsen	Aaga	15.4	**65.2**	19.4
Aulacoseira alpigena (Grunow) Krammer	Aalp	33.9	**36.7**	29.4
Aulacoseira ambigua (Grunow) Simonsen	Aamb	21.6	21.5	**56.9**
Aulacoseira distans (Ehrenberg) Simonsen	Adis	26.4	26.6	**47.0**
Aulacoseira granulate (Ehrenberg) Simonsen	Agra	**60.8**	23.8	15.4
Cyclotella pseudostelligera Hustedt	Cpse	**53.3**	0.0	13.4
Cyclotella stelligera (Cleve and Grunow) Van Heurck	Cste	**52.5**	0.0	14.2
Cymbopleura naviculiformis (Auerswald) Krammer	Cnav	**52.4**	16.4	31.2
Encyonema neomesianum Krammer	Eneo	28.5	**50.5**	20.9
Encyonema silesiacum (Bleisch) Mann	Esil	**45.0**	24.9	30.1
Eunotia arcus Ehrenburg	Earc	19.3	**46.8**	33.9
Eunotia bilunaris (Ehrenberg) Mills	Ebil	**42.3**	17.4	40.4
Eunotia camelus Ehrernberg	Ecam	24.3	16.8	**58.8**
Eunotia lunaris (Ehrenberg) Grunow	Elun	14.9	**52.5**	32.6
Eunotia papilla (Ehrenberg) Hustedt	Epap	**36.1**	19.1	11.4
Eunotia sudetica Müller	Esud	**56.0**	0.0	10.6
Eunotoa veneris (Kützing) de Toni	Even	14.6	**74.0**	11.3
Fragilaria capucina Desmaziéres	Fcap	5.2	**54.2**	40.6
Fragilaria intermedia Grunow	Finte	18.6	**50.8**	30.6
Frustulia vulgaris (Thwaites) De Toni	Fvul	**50.8**	**32.4**	16.9
Gomphonema angustatum (Kützing) Rabenhorst	Gang	17.2	**44.2**	38.6
Gomphonema augur (Ehrenberg) Lange-Bertalot	Gaug	4.0	37.9	**58.1**
Gomphonema olivaceum (Lyngbye) Kützing	Goliv	4.9	43.9	**51.2**
Gomphonema parvulum (Kützing.) Cleve	Gpar	21.3	32.8	**45.9**
Hantzschia amphioxys (Ehrenberg) Grunow	Hamp	**58.6**	0.0	8.1
Luticola mutica (Kützing) Mann	Lmut	**51.7**	0.0	15.0
Melosira varians Agardh	Mvar	**54.4**	0.0	12.2
Navicula cryptocephala (Grunow) Cleve,	Ncry	41.3	15.4	**43.4**
Navicula cryptotenella Lange-Bertalot	Ncry	8.9	37.5	**53.6**
Nitzschia palea (Kützing) Smith	Npal	30.2	32.9	**36.9**
Nupela praecipua (Reichardt) Reichardt	Npra	**90.8**	6.5	2.7
Oxyheis binalis	Obin	39.1	14.5	**46.4**
Pinnularia amazonica Metzeltin and Lange-Bertalot	Pama	13.5	17.4	**69.1**
Pinnularia biceps Patrick and Reimer	Pbic	0.0	43.1	**56.9**
Pinnularia gibba (Ehrenberg) Grunow	Pgib	**41.3**	40.3	18.4
Pinnularia lata (Brébisson) Smith	Plat	16.2	13.1	**70.7**
Pleurosigma compactum Greville	Pcom	**56.8**	18.3	24.8
Psammothidium subatomoides (Hustedt) Bukhtiyarova	Psub	0.0	0.0	**33.3**
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot,	Rabb	**73.2**	5.5	21.3
Sellaphora pupula (Kützing) Mereschkowsky	Spap	**80.0**	16.7	3.3
Stauroneis phoenicenteron (Ehrenberg) Hustedt	Spho	**69.4**	20.3	10.3
Stephanodiscus hantzschii Grunow	Shan	0.0	55.0	11.7
Surirella linearis Smith	Slin	0.0	**36.8**	29.9
Ulnaria ulna (Nitzsch) Compère	Suln	**78.7**	5.9	15.4
Thalassiosira spp	Tspp	0.0	28.2	**38.5**
Thalassiosira weissflogii (Grunow) Fryxell and Hasle	Twei	**33.3**	0.0	0.0
Total number of species with highest indicator values		**23**	**12**	**17**

less polluted sites (1, 2 and 3) on the right of the first axis from polluted sites (4, 5 and 6) at the left of the first axis. An exception to this was epipelion substrate for sites 4 and 5 that were placed in the former group instead of the expected later polluted later group. The first group of sites was associated with high DO, low BOD_5, high canopy cover (which was highly negatively correlated to temperature, light intensity and mean stream width), low turbidity, slightly acidic pH and low TP (which was highly positively correlated with TDS, TN, and conductivity) while the second group of sites was associated with low DO, high BOD_5, low canopy cover, high turbidity, slightly neutral pH and high TP.

The upstream, relatively less polluted, sites (1, 2 and 3) were characterized by such species as *Aulacoseira ambigua* (Grunow) Simonsen, *Aulacoseira granulata* (Ehrenberg) Simonsen, *Cymbopleura naviculiformis* (Auerswald) Krammer, *Eunotia bilunaris* (Ehrenberg) Mills, *Fragilaria capucina* Desmaziéres, and *Gomphonema angustatum* (Kützing) Rabenhorst. These species were highly positively associated with CCA axis 1. On the other hand, downstream, highly polluted, sites (4, 5 and 6) were characterized by *G. parvulum*, *N. palea*, *Pinnularia amazonica* Metzeltin and Lange-Bertalot and *Ulnaria ulna* (Nitzsch) Compère (negatively related to CCA axis 1) which have been reported to be highly pollution tolerant (Round 1991; Biggs & Kilroy 2000; Potapova & Charles 2003; Duong *et al.,* 2006).

DISCUSSION

Based on organic pollution, eutrophication and other environmental variables, a gradient along the streams from agricultural to urban area characterised by oligotrophic, low organic pollution, high altitude and high percentage canopy cover upstream sites, and eutrophic, high organic pollution, low altitude and low percentage cover downstream sites were observed. This pollution gradient and environmental variables determine the overall benthic diatom communities. The ecological niche of a taxon is determined by a combination of these factors along with its competitive ability (Kelly & Whitton, 1995).

Diatom community structure closely followed the pollution increase gradient with species richness, diversity and equitability generally differing among sampling sites, tending to be higher in relatively unpolluted compared to polluted sites. As pollution increased, low pollution tolerant species such as *A. ambigua*, *A. granulate*, *C. naviculiformis*, *E. bilunaris*, *F. capucina*, and *G. angustatum* were replaced by pollution tolerant species such as *G. parvulum*, *N. palea*, and *S. ulna*. The tolerant species has been reported to be associated with waters of relatively high ionic strength and high conductivity, and is known to be resistant to organic and heavy metal pollution (Round, 1991; Biggs & Kilroy, 2000; Potapova & Charles, 2003; Duong *et al.,* 2006). These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated (Round, 1991). Lange-Bertalot (1979) stated that species are indicative of the upper limits of pollution that they can tolerate and not the lower limit. Thus, species that develop well in polluted zones (e.g. *G. parvulum*, *N. palea*, *P. amazonica* and *S. ulna* in this case) may also occur in clean water. Their value as indicators is their presence in polluted water.

A comparison of artificial and natural substrate

The flora of artificial substrates is an artificial assemblage selected by physical and chemical properties of the substrate (e.g. texture, chemical composition) and perhaps positioning of substrate in relation to the currents. The smooth surface of glass slides, for example, often results in sloughing of the community (Descy & Coste, 1991); the species found on the glass substrate were mostly tightly attached ones.

Each species has specific microhabitat requirements (Round, 1991) and these requirements in most cases are not met by artificial substrate limiting the number of species that can grow on these substrates. This affects the interpretation of water quality management results as the absence of a particular species on a given site is likely to be mistaken for the effects of the perturbations under study. For example, epipelion substrate for sites 4 and 5 were grouped together with relatively less polluted sites while artificial substrates from these sites were classified as polluted (Figure 2). The separation is due to differences in community structure between the artificial and natural substrate, which in turn is due to differences in physical and chemical properties of the substrate and has nothing to do with water quality as the substrates were sampled from the same sites.

Komarek & Sukacova (2004) have shown that diatom communities is indicative of more successional processes than water quality often characterized by introduced artificial substrates. They recommend leaving artificial substrate for a year before sampling to allow the diatom communities to progress from a colonization community to a stable community reflecting environmental conditions and typical of natural communities. This prevents rapid estimation of water quality, such as can be obtained within hours of direct sampling of epipelion.

Besides, use of artificial substrate requires apparatus to be fixed in the river and there are often losses (e.g. loss of bricks in this study at site 6), and random sampling is not possible (Round, 1991; Descy & Coste, 1991). This further complicates the use of artificial substrate for water quality management. We therefore recommend the sampling of natural substrate compared to artificial substrate provided the same microhabitat type is available at all the sites to be assessed.

Species diversity, richness and equitability were high on glass compared to bricks. More species had highest indicator values on glass substrate compared to bricks. This could be due to differences in physical and chemical attributes of the two substrates. This also affects the interpretation of water quality management results as the absence of a particular species on a given site is likely to be mistaken for the effects of the perturbations under study. From our results, we recommend that water quality assessment based on species diversity from artificial substrates should consider using glass instead of bricks. However, care should be taken in the use of rugose glass slides as smooth surface of glass slides often results in sloughing of the community (Descy & Coste, 1991), thus affecting water management results.

Relationship with environmental variables

Organic pollution, eutrophication, pH and percentage canopy cover were the major drives of diatom community structure in this study. Environmental monitoring studies in Southern Brazil (Lobo *et al.,* 1996; Oliveira 2001; Lobo *et al.,* 2004; Salomoni *et al.,* 2006) showed that diatom communities in lotic ecosystems are a result of the interaction of variables characterising the process of organic contamination as well as eutrophication. Lobo *et al.* (2004) classified benthic diatom species into 5 groups based on tolerance to organic pollution and eutrophication using TWINSPAN results leading to the development of the first Brazilian-based water quality index, Biological Index of Water Quality (BIWQ) trophic index. Eight of these species, *E. silesiacum*, *F. capucina*, *G. angustatum*, *M. varians*, *N. cryptotenella*, *N. palea*, *G. parvulum* and *A. minitissimum*, were collected in this study. The distribution of these species generally seemed to agree with their classification except for that of *F. capucina* that they classified as highly tolerant of eutrophication, but in this study, they seemed to be favoured in less polluted sites. However, this species has been described as having broad eutrophication tolerance ranges, occurring successfully from oligotrophic to eutrophic environments (Van Dam *et al.,* 1994).

Lobo *et al.* (2004) described *N. palea* as partially pollution tolerant. In this study, however, this species had high relative abundance in eutrophic sites 4, 5 and 6. Many studies describe *N. palea* and *G. parvulum* as cosmopolitan, high pollution tolerant species, especially eutrophication and organic pollution (e.g. Lange-Bertalot, 1979; Kobayasi & Mayama, 1989; Tapia, 2008) and are good indicators of polisaprobic conditions (Lobo *et al.,* 1996). The success of some *Nitzschia* species in eutrophic conditions has been attributed to obligate nitrogen heterotrophy (Hellebust & Lewin, 1977; Kilham *et al.,* 1986), which would overcome the problem associated with low N: P ratios. Features that may affect a taxon's performance in high pollution environments as observed at sites 4, 5 and 6 in this study also include ability to survive low concentrations of dissolved oxygen and avoidance of settled solids (Kelly & Whitton, 1995).

Lobo *et al.* (2002) have considered *G. parvulum,* the relatively highly abundant species at downstream eutrophic sites with high organic pollution as á-mesosaprobic. In streams located in the Municipal District of Mato Leitão (Brazil), Lobo *et al.* (1999) classified this species as belonging to both á-mesosaprobic and polysaprobic environments. In the same streams, Rodrigues & Lobo (2000) registered the occurrence of this species in moderately polluted, â-mesosaprobic waters. In a study carried out in the same area as the current investigation, Souza (2002) encountered this species in oligosaprobic environments. However, in other studies carried out in rivers, Japan Kobayasi & Mayama (1989) and Lobo *et al.* (1996) classified *G. parvulum* as highly tolerant to organic pollution in agreement with the results of the current study. Similarly, Kelly & Whitton (1995), working in UK rivers described this species as highly tolerant of eutrophication (indicative value = 3 and sensitivity value = 5) in their calculation of the Trophic Diatom index (TDI). Diverse mophotypes of *G. parvulum*, however, there is the probably of corresponding to different varieties (Morales & Jasiki 2002), which would explain the variety of responses attributed to this species.

Percentage canopy cover and pH were also found to be determining diatom community structures. Percentage canopy cover affects light intensity that is important for the process of photosynthesis (Round 1991; Pan *et al.,* 1996; Potapova & Charles 2002). The relationship between diatoms and pH is strong because pH exerts a direct physiological stress on diatoms (Gensemer, 1991), and also strongly influences other water chemistry variables (Stumm & Morgen, 1981).

CONCLUSION

Substrate differences may affect the interpretation of water quality results, because the absence of a particular species on a given site can be mistaken for the effects of the perturbations under study. Thus the use of a natural substrate is recommended compared to artificial substrate given the advantages of the former compared to the latter.

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