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The diatom assemblages as indicators of field and laboratory conditions in lotic systems: conservation and water quality management in São Carlos-SP catchment, Brazil

(Assembléias de diatomáceas como indicadores de condições de campo e de laboratório em sistemas lóticos: conservação e gestão da qualidade da água na captação de São Carlos-SP, Brasil)

TAURAI BERE

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais, do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, como parte dos requisitos para obtenção de grau de Doutor em Ciências (Ciências Biológicas), Área de concentração em Ecologia e Recursos Naturais.

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Taurai Bere

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Tese apresentada à Universidade Federal de São Carlos, como parte dos requisitos para obtenção do título de Doutor em Ciências.

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BANCA EXAMINADORA

Presidente _____
Prof. Dr. José Galízia Tundisi
(Orientador)

1° Examinador _____
Profa. Dra. Odete Rocha
PPGERN/UFSCar

2° Examinador _____
Profa. Dra. Takako M. Tundisi
PPGERN/UFSCar

3° Examinador _____
Prof. Dr. Eduardo Alexis Lobo Alcayaga
Universidade Santa Cruz do Sul/Santa Cruz do Sul-RS

4° Examinador _____
Prof. Dr. Luiz Antonio Martinelli
CENA/USP/Piracicaba-SP

Dedicated to my wife Miriam Elizabeth Bere, my son Makomborero Joel Bere, parents and relatives and all those who love nature

"Few objects are more beautiful than the minute siliceous cases of the diatomaceae: were these created that they might be examined and admired under the higher powers of the microscope?"

Charles Darwin

The origin of Species, 1872

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CONTENTS

ABSTRACT

Periphyton communities (especially diatoms) constitute a system rich in information for environmental monitoring, placing them among important indicators of ecological conditions in lotic systems. In this thesis, field studies and laboratory experiments were conducted to elucidate the confounding effects of substrate selection, eutrophication, organic pollution, ionic strength, land-use patterns, and heavy metal pollution on benthic diatom communities. Characteristics of benthic diatom communities in relation to pollution level and type were analysed through general criteria (chlorophyll *a*, dry weight, ash-free dry weight, and cell densities in the case of laboratory experiments) and specific criteria (indicator value method, multivariate techniques and diatom-base indices).

For field studies, epilithic, epiphytic, epipsammic and epipelic diatom communities and those growing on bricks and glasses and water quality were assessed. A gradient of decreasing water quality was observed from the agricultural/forested area to the urban area. Diatom community structure closely reflected this gradient. Upstream sites with good water quality were characterized by such species as *Aulacoseira alpigena, Cymbopleura naviculiformis, Eunotia bilunaris, E. intermedia* and *Stauroneis phoenicenteron.* Urban sites with medium to bad water quality were characterised by such species as *Frustulia rhomboids, Nitzschia linearis, Cyclotella pseudostelligera, Pinnularia gibba* and *Achnanthidium minutissimum.* Downstream urban sites with very bad water quality were characterised by such species as *Luticola geoppertiana, Nitzschia palea, Sellaphora pupula, Planotidium lanceolatum* and *Fallacia monoculata.* Common diatom species were not restricted to a single substrate, though preference was generally high for natural (especially macrophytes) compared to artificial substrates. The results of diatom-based multivariate water quality assessment based on different substrates were shown to be interchangeable. Variance in diatom data was partitioned between two sets of exploratory variables, i.e. ionic strength (26.9%), other variables, particularly eutrophication and organic pollution (23.0%), shared variance (11.3%) and unexplained variance (38.8%). Finally, 17 indices developed in other regions proved useful in providing an indication of the quality of the investigated waters.

For laboratory experiments, effects of cadmium, chromium III and lead on natural periphyton community sampled from the Monjolinho River were studied. Hormesis was demonstrated with a Cd EC_{50} of 0.077 $mg.L^{-1}$ being recorded. High metal accumulation

capacity (total and intracellular) by periphyton was demonstrated depending on metal concentration and exposure duration. Pb and Cr III were shown to decrease the toxicity effects of Cd on periphyton communities suggesting antagonism. Finally, combined effects of frequency, duration, recovery period, chemical type and timing of pulses with elevated Cd, Cr III and Pb concentrations on periphyton communities were assessed. The closer the frequency and duration of the pulse is to a continuous exposure, the greater the effects of the contaminant on aquatic life. The higher the frequency of short duration pulses the more likely they are to produce effects similar to that of long duration exposures. Light was shown to have a potential role in modulating the effects of metal toxicants on aquatic life. Shifts in species composition (development of more resistant species like *A. minutissimum* and reduction of sensitive ones like *Navicula viridula, Navicula cryptocephala,* and *Eunotia bilunaris*), decreases in species richness and diversity and morphological alterations (deformities) of diatom cells with increasing metal concentration and exposure duration and different exposure scenarios were observed.

RESUMO

Comunidades perifíticas (especialmente diatomáceas) constituem um sistema rico em informações para o monitoramento ambiental, colocando-as entre os indicadores mais importantes das condições ecológicas em sistemas lóticos. Nesta tese, os estudos de campo e experimentos de laboratório foram realizados para esclarecer os confusos efeitos da seleção do substrato, eutrofização, poluição orgânica, força iônica, padrões de uso do solo e poluição por metais pesados nas comunidades bentônicas de diatomáceas. Características das comunidades bentônicas de diatomáceas em relação ao nível e tipo de poluição foram analisadas através de critérios gerais (clorofila *a*, peso seco, peso seco de cinza e densidade celular no caso dos experimentos de laboratório) e critérios específicos (método de valor de indicador, técnicas de análise multivariada e índices baseados nas diatomáceas).

Para estudos de campo, as comunidades de diatomáceas epilíticas, epífitas, epipsâmicas e epipelicas, além das que crescem em tijolos e vidros, foram avaliadas, assim como a qualidade da água. Um gradiente decrescente da qualidade da água foi observado a partir da área agrícola/florestal até a área urbana. A estrutura da comunidade de diatomáceas refletiu este gradiente. Pontos em áreas de nascentes, com boa qualidade da água, foram caracterizados por espécies como *Eunotia bilunaris*, *E. intermedia*, *Aulacoseira alpigena, Cymbopleura naviculiformis* e *Stauroneis phoenicenteron*. Pontos em áreas urbanas, com média à baixa qualidade da água foram caracterizados por espécies como *Frustulia rhomboids, Nitzschia linearis, Cyclotella pseudostelligera, Pinnularia gibba* e *Achnanthidium minutissimum* e os pontos em áreas urbanas próximo à jusante, com baixa qualidade da água, por espécies como *Luticola geoppertiana, Nitzschia palea, Sellaphora pupula, Planotidium lanceolatum* e *Fallacia monoculata*. Espécies comuns de diatomáceas não foram restritas em um único substrato, embora a preferência fosse geralmente alta para natural (especialmente macrófitas) em comparação com substratos artificiais. Os resultados da análise multivariada da qualidade da água baseados em diatomáceas amostradas em diferentes substratos demonstraram ser intercambiáveis. Variância nos dados das diatomáceas foi dividida entre dois conjuntos de variáveis exploratórias, ou seja, força iônica (26,9%), outras variáveis como, eutrofização e poluição orgânica (23,0%), variância compartilhada (11,3%) e variação não explicada (38,8%). Finalmente, 17 índices desenvolvidos em outras regiões provaram ser úteis para fornecer uma indicação da qualidade das águas estudadas.

Para os experimentos de laboratório, os efeitos do cádmio, cromo III e chumbo sobre as comunidades perifíticas naturais amostradas no rio Monjolinho, foram estudados. *Hormese* foi demonstrado com um EC_{50} de 0,077 mgL^{-1} Cd registrado. Boa capacidade de acumulação de metal (total e intracelular) pelo perifíton foi demonstrada, dependendo da concentração do metal e duração da exposição. Pb e Cr III diminuíram os efeitos da toxicidade de Cd em comunidades perifíticas sugerindo antagonismo. Finalmente, os efeitos combinados de freqüência, duração, período de recuperação, tipo de produto químico e tempo de pulsos com elevadas concentrações de Cd, Cr III e Pb em comunidades perifíticas foram avaliados. Quanto mais a freqüência e a duração do pulso se aproximam de uma exposição contínua, maiores serão os efeitos dos contaminantes sobre a vida aquática. Quanto maior a freqüência de pulsos de curta duração, mais provável é a produção de efeitos semelhantes aos das exposições de longa duração. A luminosidade mostrou ter um papel importante na modulação dos efeitos de toxicidade de metais sobre a vida aquática. Mudanças na composição de espécies (desenvolvimento de espécies mais resistentes como *A. minutissimum* e redução das espécies mais sensíveis, como *Navicula viridula*, *Navicula cryptocephala* e *E. bilunaris*), diminuição da riqueza e diversidade, alterações morfológicas (deformidade) das células de diatomáceas, aumento da concentração de metais, duração de exposição e diferentes cenários de exposição foram observados.

GENERAL INTRODUCTION

High population growth, industrial and agricultural development heavily exposes most lotic systems to pollution due to associated waste discharge. This has captured public interest because of the consequent loss of biodiversity, deterioration of water quality, eutrophication, health problems, pest plants and animals, and other problems. Man probably cannot have his cake and eat it too; he cannot maintain highly structured and diverse ecosystems resistant to change and at the same time maximize their recycling and energy-use efficiency.

The fundamental feature of lotic systems is that activities or disturbances at one location affect processes and organisms downstream. They are characterised by interactions among physical, chemical and biological processes, which reach a higher degree of complexity downstream (WEHR and DESCY, 1998). Lotic systems are also characterised by longitudinal differences in the time scales of chemical and biological processes rendering it difficult to design polices and assess the results of management actions (TUNDISI and MATSUMURA-TUNDISI, 2008). Characterising and managing dynamic environmental conditions in heterogeneous systems such as these, therefore, requires innovative approaches (TUNDISI and MATSUMURA-TUNDISI, 2008).

Two threads of basic approaches to the assessment of water quality deterioration in lotic systems run through the literature. The first approach involves the assessment of physico-chemical variables of lotic systems in order to gain some insight into their water quality. The second approach involves use of biological methods (biomonitoring), the theory behind which is to provide a direct measure of ecological integrity by using the response of biota to environmental changes (KARR, 1991). Biotic indices has gained momentum in their usage as alternative to chemical analyses because the latter provide, at best, a fragmented overview of the state of lotic systems as sporadic or periodic sampling cannot reflect fluxes of effluent discharge. In contrast, biotic indices give a time-integrated indication of the water quality components (TAYLOR et al., 2007).

Many biotic indices have been developed worldwide among which diatom-based indices bear special relevance to the present study. Diatoms have the following advantages: methods are cost effective; data is comparable; techniques are rapid and accurate; they lie at the base of aquatic food; they have a short life cycle; they are rich in species composition; and have a wide distribution (ROUND, 1993).

Numerous studies focusing on the application of standardized methods based on diatom assemblages for water quality assessment have been carried out, especially in the Northern Hemisphere, in particular in European countries (e.g. ROUND, 1993, KELLY and WHITTON, 1995; PRYGIEL et al., 1999). However, there is evidence that diatom metrics or indices developed in temperate regions are less successful when applied to tropical regions due to floristic differences among regions (PAN et al., 1996; TAYLOR et al., 2007) and environmental differences that modify species responses to water-quality characteristics (PAN et al., 1996, POTAPOVA and CHARLES, 2005). For this cause, ecological assessment methods should be river type specific (TUNDISI and MATSUMURA-TUNDISI, 2008).

In Brazil, intensive floristic or taxonomic studies on freshwater diatoms are yet incipient. Knowledge of the ecological status of diatom communities in aquatic system is essential before applying or adapting biotic indices. Some diatom-based water quality monitoring practices have been carried out in Brazil (e.g. LOBO et al., 2002, 2004; SALOMONI et al., 2006). However, the studies are concentrated or restricted mainly to the southern part of the country (TUNDISI and MATSUMURA-TUNDISI, 2008), and very little was done in other lotic systems. For this course, there is lack of capacity in the form of data, which hampers full understanding and the subsequent management of the lotic systems in other regions. This underlines a clear vision to fill this information gap by studying ecological responses of benthic diatom communities to different environmental variables.

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OBJECTIVES

1. To summarise the basic concepts associated with benthic diatom-based biological monitoring.
2. To assess the effects of substrate on benthic diatom community structure and subsequently diatom-based multivariate water quality assessment.
3. To elucidate the confounding effects of eutrophication, organic pollution and ionic strength and conductivity on diatom communities.
4. To assess the response of stream diatom assemblages to changes in water quality associated to land-use, i.e., from agricultural and forest to the urban areas.
5. To assess the applicability of different benthic diatom-based indices for assessment of lotic systems developed in different regions to the study area.
6. To assess the effects of five Cd concentrations on tropical periphyton community growth as well as Cd toxicity to diatom assemblages in laboratory mesocosm experiments.
7. To characterize periphyton growth under interactive effects of dissolved Cd, Cr III and Pb as well as Cd, Cr III and Pb mixtures toxicity to diatom assemblages in laboratory mesocosm experiments.
8. To investigate ecotoxicological effects of intermittent heavy metal exposures on periphyton communities with the overarching aim of discussing toxicity in the context of increased realism in the exposure scenarios.

OUTLINE OF THE THESIS

This thesis shows the results of studies conducted on periphytic diatom communities form São Carlos-SP hydrological system. Firstly, a summary of the basic concepts associated with biological monitoring using benthic diatoms was given with examples from work carried out in Brazil being given where possible (Chapter 1; published in *Brazilian Journal of Biology,* 70: 493-502). Secondly, *in situ* studies aimed at assessing benthic diatom community structure and composition in relation to environmental conditions along agricultural to urban pollution gradient were conducted (Chapter 2, 3 and 4; published in the following journals: Chapter 2, *Water Air Soil Pollution,* 216: 391-409; Chapter 3, *Hydrobiologia,* 261: 261-276; Chapter 4, *Water SA,* 37: 93-102). Thirdly, applicability of different benthic diatom-based indices for assessment of lotic systems developed in different regions to the study area was assessed (Chapter 5; published in *Hydrobiologia* (in press, DOI 10.1007/s10750-011-0772-7)). Fourthly, laboratory-based mesocosm experiments were conducted to examine the effects of dissolved concentrations of Cd, Cr III and Pb on tropical periphyton community growth and development, as well as metal toxicity to diatom assemblages (Chapter 6 and 7). The seven chapters of the manuscript are presented as a compilation of papers already published, in press or in preparation.

MATERIALS AND METHODS

Study area

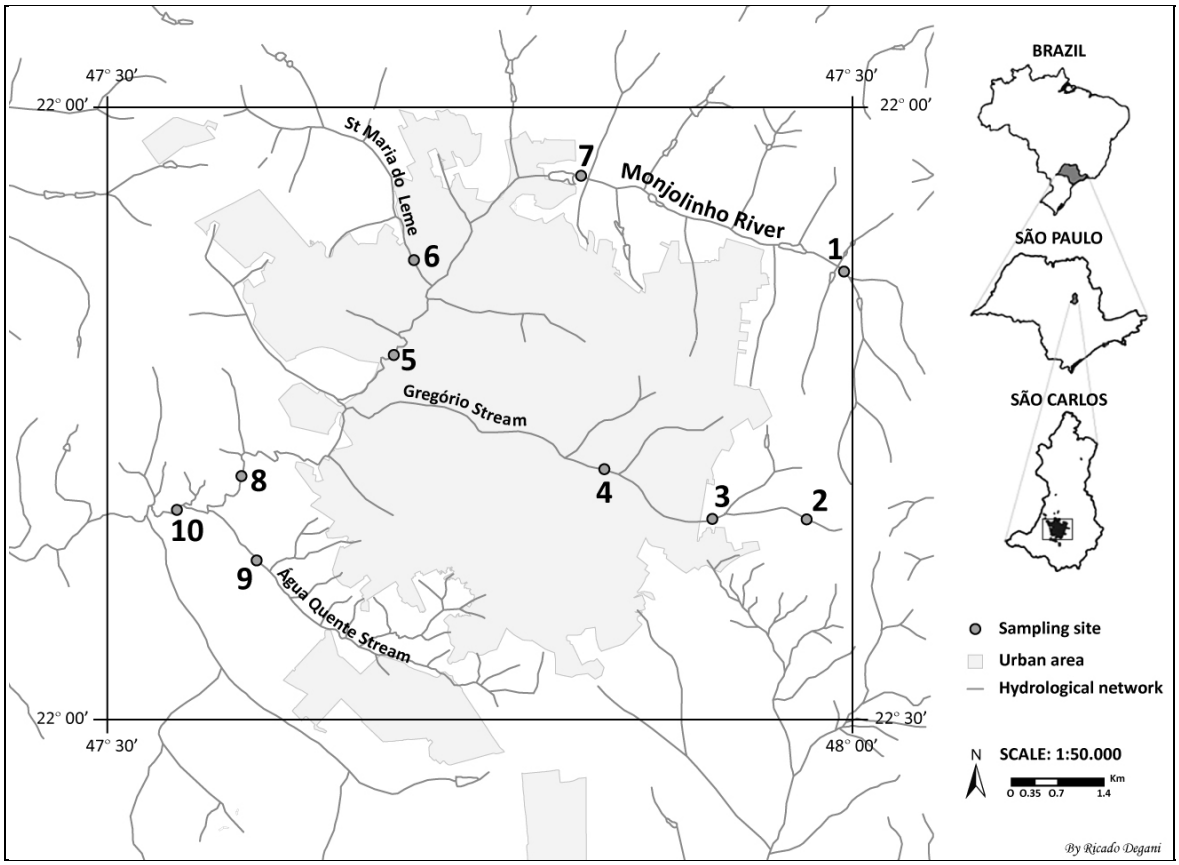
The area under study is shown in Figure 1. The Monjolinho River basin is approximately 43.3 km long and is part of the basin of the Jacaré-Guaçú, one of the major tributaries of the Tietê River located in central-eastern part of the state of São Paulo, with a length of approximately 1.100km. The basin covers part of the municipalities of Analândia, Brotas, Ibaté, Itirapina and São Carlos. Headwaters of Monjolinho River and the tributaries studied fall mainly within agricultural area. From agricultural area, the streams run through the urban area of the city of São Carlos, which covers a total area of 1143.9 km^2 and has about 400 industries that discharge their industrial effluent directly into Monjolinho River and its tributaries affecting the ecological health and integrity of these ecosystems (BARRETO, 1999).

In addition to this, the expansion of the city of São Carlos does not meet the technical standards such as sewage treatment, collection of garbage, urban drainage and so on. Streams in the study area, therefore, receive untreated or semi-treated effluent from various domestic and industrial sources as well as other diffuse sources as they pass through the city. This disorderly growth of the city resulted in stream health deterioration, metal pollution, loss of the remaining primary vegetation and eutrophication among other problems.

According to the Köppen system, the climate of the study area is classified as type Cwa, i.e. mesothermal humid subtropical dry winter, with average temperature of the coldest month (July) of below 18 º C and mean monthly maximum of above 22 º C recorded in January and February. The area is characterised by a distinct dry (May-September) and rainy season (October to April). The average annual rainfall varies between 1200 and 1550 mm (SÈ, 1992).

Table 1: Location of sampling sites.

Site	Latitude	Longitude	Altitude
1	22°00'03.55"S	47°50'17.23"E	761m
2	22°02'03.85"S	47°50'39.74"E	837m
3	22°01'59.40"S	47°51'43.43"E	831m
4	22°01'35.57"S	47°52'31.43"E	774m
5	22°00'36.16"S	47°54'22.24"E	794m
6	21°59'46.01"S	47°54'09.10"E	745m
7	21°59'09.99"S	47°52'38.85"E	761m
8	21°01'24.21"S	47°55'26.76"E	724m
9	21°02'03.91"S	47°56'22.82"E	630m
10	21°02'07.57"S	47°57'26.99"E	627m



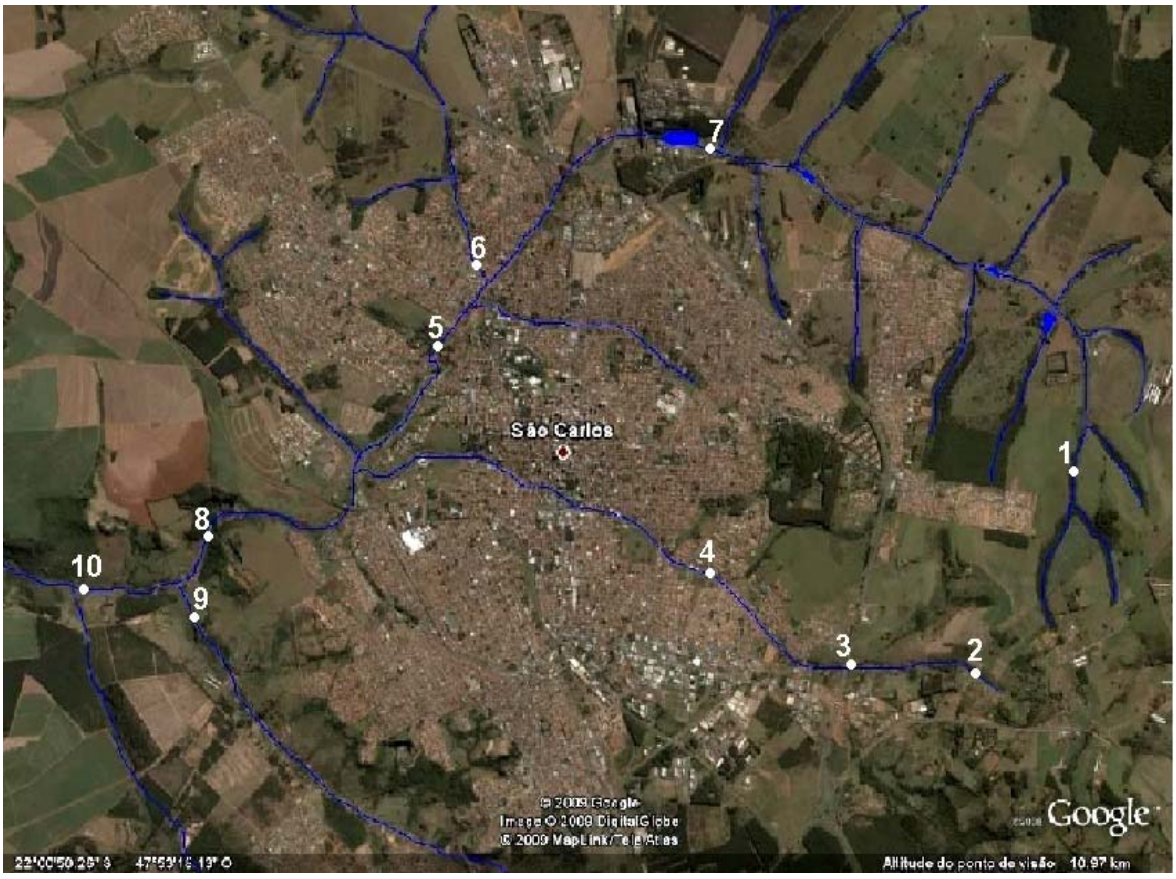


Figure 1: The location of the sampling sites in the study area.

Plate 1: Photos of the sampling sites. The sites are arranged in general order of increasing pollution.

Study design

Ten sites were established along Monjolinho River and its tributaries: four sites (1, 2, 3 and 7) in the relatively less impacted agricultural and forested headwaters to act as reference sites; 3 sites (4, 5 and 6) in the moderately polluted urban area; and 3 sites (8, 9 and 10) in highly polluted downstream area after the urban area (Table 1, Figure 1, Plate 1). The rational for choosing the sampling sites was to obtain a pollution gradient of all the stream systems from relatively unpolluted agricultural headwaters to highly polluted urban downstream sites.

Diatom and water sampling was done during dry season when flow was stable. Four samples were collected, two in September and October 2008 and 2 in May and June 2009. Sampling was done during the dry season to avoid variable effects of the rain season such as great variations in water level and velocity, floods and inundations. These variations affect diatom development, especially growth rate and relative abundance of different species (e.g. ROUND, 1991; BIGGS and KILROY, 2000). In addition, micropollutant alterations are best estimated during the dry season i.e. under extreme conditions, typically exhibiting the lowest discharge and highest pollutant concentrations (MORIN et al., 2008).

Physical and chemical variables

Field measurements

At each site, dissolved oxygen (DO), electrical conductivity, temperature, pH and concentration of total dissolved solids (TDS) were measured using a Horiba U-23 and W-23XD Water Quality Meter (Horiba Ltd, Japan). Depth and current velocity were measured at each site with an FP 201 global flow probe (Global Water Instrumentation Inc. Alaska, USA). The percentage riparian vegetation cover was visually estimated at each site. Light intensity at the air-water interface was measured using LI-193 Spherical Quantum Sensor (LI-COR Worldwide, Brazil). Altitude was determined at each site using a GPS (Northport Systems, Inc. Toronto, Canada).

The percentage embeddedness was also estimated along each stretch and rated on a 0–5 scale following PLATTS et al. (1983). The following physical substrate characteristics were visually estimated following USGS NAWQA protocol (FITZPATRICK et al., 1998): percentage of silt-clay size particles, sand size particles, gravel size particles, cobble size particles, boulder size particles; ratio of silt-gravel size particles, gravel-cobble size particles, and cobble-boulder size particles. Collection of

sediments for metal and nutrient analysis and of water samples for metals, total nitrogen (TN), total phosphorus (TP), biological oxygen demand (BOD_5), chemical oxygen demand (COD) and fluoride, chloride, nitrite, nitrate, phosphate, sulphate, sodium, ammonium, potassium, calcium and magnesium ions took place at each site following standard methods (APHA 1988).

Laboratory analyses

The concentration of TN and TP in the water samples was determined following the method by GOLTERMAN et al. (1978) and VALDERRAMA (1981) respectively. TN and TP levels in sediments were determined following the micro-kjeldahl method (APHA, 1988) and the ignition method (ANDERSEN, 1976) respectively. BOD_5 and COD were determined following standard methods (APHA, 1988). Water and sediment samples were processed following standard methods (APHA, 1988) and analyzed using Flame Atomic Absorption Spectrometry Analytical Methods (Varian Australia Pty Ltd, Victoria, Australia) for aluminium (Al), cadmium (Cd), lead (Pb), zinc (Zn), chromium (Cr III), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and nickel (Ni). The concentrations of fluoride, chloride, nitrite, nitrate, phosphate, sulphate, sodium, ammonium, potassium, calcium and magnesium ions were analysed by isocratic ion analysis using suppressed conductivity detection ion chromatography method using Dionex DX-80 Ion Analyzer (DX-80) (DIONEX CORPORATION, 2001).

BOD_5, nitrate, phosphate, DO (percent saturation), temperature, pH, turbidity and TDS were used for calculation of a water quality index (WQI) after BROWN et al. (1970) as follows:

$$WQI = \frac{\sum_{i=1}^{n} Wi \times Qi}{\sum Wi}$$

where, Wi is the weight and Qi is the quality score of variable i.

Diatom sampling

Natural substrate sampling

At each site, epilithic, epiphytic, epipelic and epipsammic diatoms were sampled separately. Epilithic diatoms were sampled by brushing stones with a toothbrush. Prior to sampling of epilithic surfaces, all substrata were gently shaken in stream water to remove any loosely attached sediments and non-epilithic diatoms. At least five pebble-to-cobble sized stones were randomly collected at each sampling site and brushed, and the resulting

diatom suspensions were pooled to form a single sample, which was then put in a labelled plastic bottle.

Epiphytic diatoms were sampled from different species of submerged and emergent macrophytes (e.g. *Rumex crispus, Alternanthera philoxeroides, Ludwigia spp, Rhynchathera spp, Ageratum spp, Hedychium coronarium, Eleocharis spp, Heteronthera spp, Polygonum spectable* and *Brachiaria arrecta*) at each site depending on the availability of the macrophytes. The macrophyte's whole stalk comprising of stalk and leaves was carefully removed from the stream. Periphyton was then removed from the macrophytes by brushing with a toothbrush adding distilled water. The resulting diatom suspensions from all the submerged macrophytes sampled were pooled to form a single sample, which was then put in a labelled plastic bottle.

Epipelic and epipsammic diatoms were sampled by pressing Petri dish lid into the top layer of sand or 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. Five samples at each site were collected and pooled into a single sample.

Artificial substrate placing and sampling

For field experiments, at each site, two bricks and a plastic rack fitted with 7 separate and vertical glass substrates (6 X 15 cm) were immersed in the water column parallel to the current at a depth of 20 to 30 cm below the surface and secured accordingly (Figure 2). The artificial substrate was left for 4 weeks, which is the recommended colonization time of periphyton (ROUND, 1991). Biofilms were collected by brushing material with a toothbrush. The resulting suspensions from the replicates were pooled.

For laboratory mesocosm experiments, four plastic racks, each fitted with 10 separate and vertical glass substrates (6 X 15 cm) were immersed at site 7 (Figure 1) parallel to the current 20 to 30 cm below the water surface. The racks were secured accordingly and left for 4 weeks prior to sampling. On sampling, biofilms colonizing the glass substrates were brushed with a toothbrush into culture medium. The biofilms from all the glass substrates were pooled into one sample of approximately 2 L. This biofilm suspension was immediately transported to the laboratory in cooler box (4 ^{o}C).

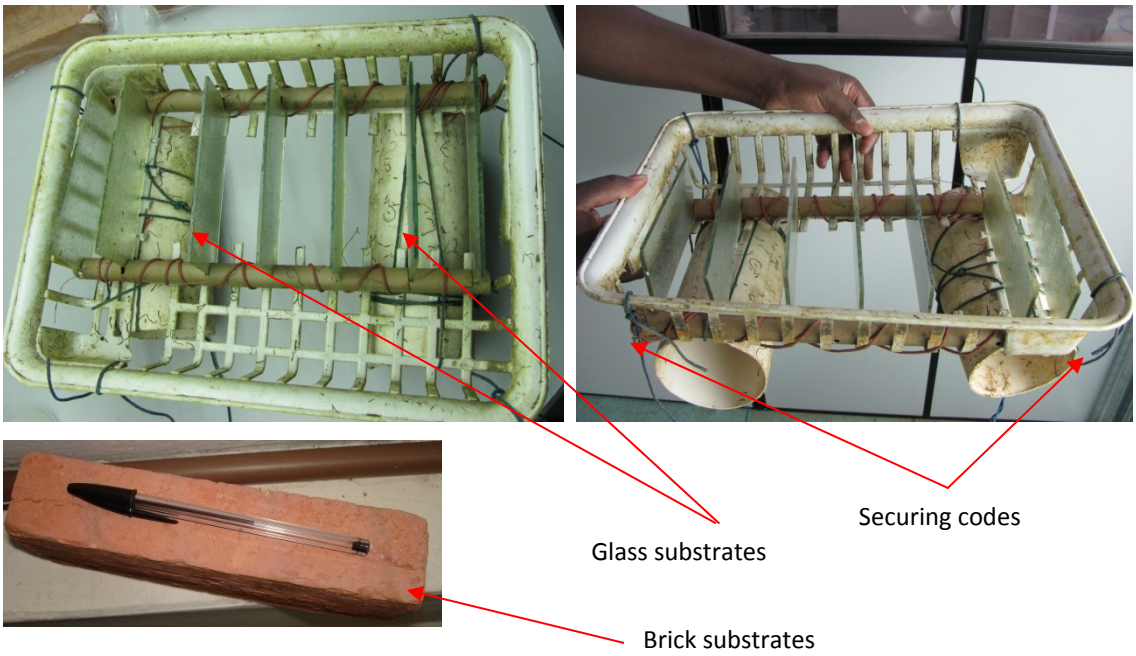


Figure 2: Artificial substrates used for benthic diatom sampling.

Diatom sample cleaning, mounting and identification

Sub-samples of the diatom suspensions were cleaned of organic material using wet combustion with concentrated sulphuric acid and mounted in Naphrax (in toluene) fixative (Northern Biological supplies Ltd. UK. RI = 1.74) as following BIGGS and KILROY (2000). Three replicate slides were prepared for each sample. Between 550 and 600 valves per sample (depending on the abundance of diatoms) were identified and counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type - 020-519.503 LB30T, Germany). The diatoms were identified to species level based on studies by LOBO et al. (2002), METZELTIN et al. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007).

Laboratory experiments

Closed experimental systems (EUs) were set up to allow the exposure of natural periphyton communities to Cd, Cr and Pb under controlled conditions. Each EU consisted of three half-polyvinyl chloride (PVC) tubes 50 cm long with a radius of 5 cm as artificial streams with a capacity of 2.8 L each. The three streams were connected in parallel to a 30 L tank (Figure 3). All systems were filled with diluted (4x) modified Woods Hole culture medium (Table 2). A pump (Boyu bomba submersa SP-0100-600/h, SP-Brazil) allowed continuous circulation of the water through each system at a rate of 10 ± 0.25 ml

s^{-1}, corresponding to a velocity of 0.2 cm s^{-1}. Each stream was fitted with 6 clean glass substrates (6 X 15 cm) in a slightly slanting position for periphyton colonisation. Subsamples of periphyton suspension from the field reference environment were introduced into EUs, the EUs were equilibrated over night, and then the desired concentrations of Cd, Cr III or Pb were obtained by addition of aliquots of appropriate stock solutions to different systems. Depending on the objective, biofilms were collected after a certain colonization period and analysed for algal cell densities, diatom taxonomic composition, chlorophyll *a*, dry weight (DW), ash-free dry mass AFDM, and total and non-exchangeable metals.

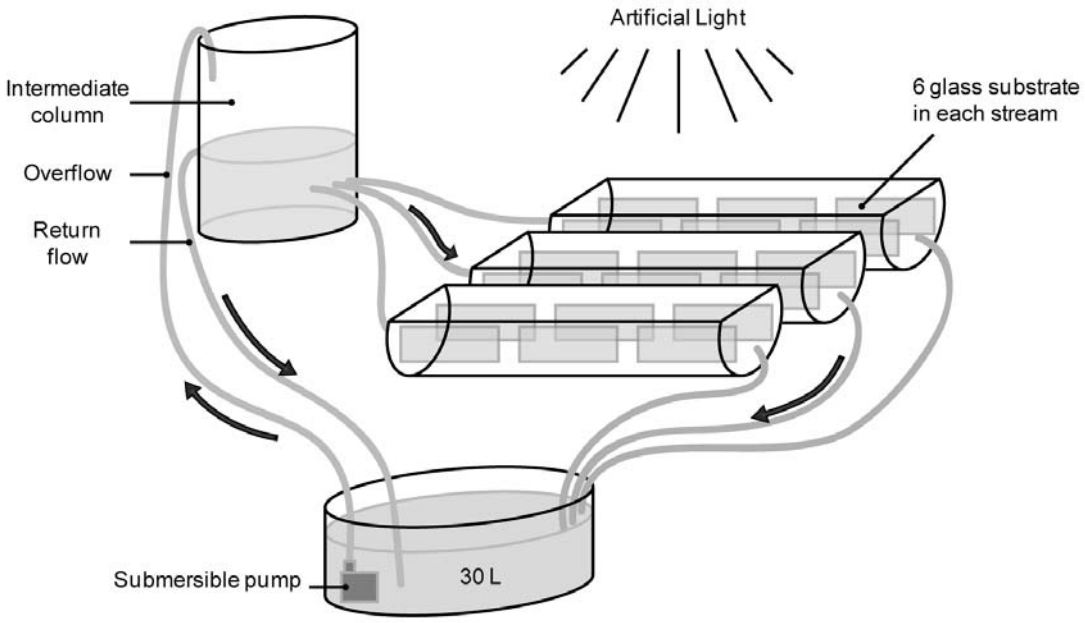


Figure 3: Schematic representation of a closed experimental system, consisting of three artificial streams (50 cm length, 5 cm radius), each containing 6-glass substrata (6 X 15 cm). Arrows indicate flow direction, (by: Ricardo M. Degani).

Table 2: Composition of the Woods Hole culture medium, without EDTA, supplemented with silica and diluted 4x used in the laboratory experimental systems.

Nutrient	Final concentration(mg L^{-1})
$CaCl_2.2H_2O$	9
$MgSO_4.7H_2O$	9.25
$NaHCO_3$	3.15
$NaNO_3$	21.25
K_2HPO_4	2.17
$FeCl_3.6H_2O$	1.05
H_3BO_3	0.25
$MnCl_2.4H_2O$	45×10^{-3}
$ZnSO_4.7H_2O$	5.5×10^{-3}
$CuSO_4.5H_2O$	2.5×10^{-3}
$CoCl_2.6H_2O$	2.5×10^{-3}
$Na_2MoO_4.2H_2O$	1.5×10^{-3}
Thiamine	25×10^{-3}
Biotin	125×10^{-6}
B12 vitamin	125×10^{-6}
$Na_2SiO_3.9H_2O$	71

For algal cell densities analysis and diatom taxonomic identification, 20 ml of the sample was fixed with 4% (final concentration) formalin. Cells in 100 μL subsample were counted in a Nageotte counting chamber at X400 with cell densities expressed as living algal cells per unit area (cells.cm^{-2}). For diatom identification to species level, sub-samples of the suspensions were cleaned, mounted and valves counted as described above. Another fraction (20 ml) was used for chlorophyll *a* analysis. The samples were filtered onto Whatman GF/C filters. Chlorophyll *a* from the filters was measured spectrophotometrically (at 665 nm and 750 nm) following extraction in boiling 80% ethanol (5 min) and steeping at 4°C in the dark (24 h). A phaeopigment correction was obtained by acidification NUSCH (1980). Another fraction (20 mL) was filtered through pre-combusted GF/C filters and dried at 60 °C for 48 h to determine DW. After final weighing, samples were ashed at 500 ^{o}C for 1 hr and weighed again to obtain AFDM.

Growth rates inferred from AFDM measurement data were calculated for the exponential phase and were expressed as micrograms of AFDM per unit area of glass substrate per day following the formula:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i}$$

where μ_{i-j} is the average growth rate between time *i* to *j*; X_i and X_j are the AFDM at time *i* and *j* respectively and t_i and t_j are the time (days) of i^{th} and j^{th} AFDM measurement respectively after beginning the exposure. From these growth rates, the percent inhibition (stimulation) of each treatment was calculated following the formula:

$$I_\mu = \frac{\mu_c - \mu_T}{\mu_c}$$

where I_μ is the percent inhibition of growth rate; μ_c is the mean growth rate in the control and μ_T is the mean growth rate in each treatment. From these results, the EC_{50} was then determined from linear regression equation.

Another fraction (20 mL) was used to determine the total amount of metal accumulated in biofilm as described in below. To measure intracellular metal (non-exchangeable) content in biofilm, another fraction (20 mL) of sample was washed with EDTA 4 mM at pH = 8, for 10 min to remove metal adsorbed onto the surface of algal cells and most of inorganic complexes embedded in the biofilm (SOLDO et al., 2005).

The resultant sample was then analysed for metal as described in below. Adsorbed metal level was calculated as the difference between the metal content before and after washing with EDTA. Concentration factors (CFs) of the biofilm for metal were calculated according to FOSTER (1976) by dividing concentrations of metal in biofilms (total and non-exchangeable fractions) by those in water column.

Samples for total and non-exchangeable metal analysis were filtered through a tarred metal free paper (0.45 μm membrane, Millipore) to obtain the dry weight after drying at 60 °C for 48h. Dried biofilm samples were first digested with nitric acid following method 3050B (Environmental Protection Agency-USA). The digestates were diluted with ultra pure water (Millipore, Simpakkr1, Simplicity 185, SP-Brazil) to 100 ml (final volume). Metal concentrations in biofilm (washed with EDTA or not) were measured by inductively coupled plasma mass spectrometry (ICP-MS), (Analytical Instrument Recycle, Inc, USA).

Data analysis

Physico-chemical variables and diatom community structure

Environmental variables that were not normally distributed (Shapiro-Wilk, $p \leq 0.05$) or had no equal variance (Levene's test, $p \leq 0.05$) were transformed accordingly. Two-way Analysis of Variance (Two-way ANOVA) was used to compare means of environmental variables among sampling periods, among sites or among treatments.

Cluster analysis and principal component analyses (PCA) were performed on benthic diatom community data to show the main differences and similarities in communities. Multivariate data analyses were performed on the diatom data set to indicate the main gradients of floristic variation and to detect and visualize similarities in diatom samples. Preliminary detrended correspondence analyses (DCA) were applied on diatom data set to determine the length of the gradient. Canonical correspondence analysis (CCA) was used to investigate relationships between predictor variables and benthic diatom communities from different sites (TER BRAAK and VERDONSCHOT, 1995). Partial CCA (BORCARD et al., 1992) was used to separate and examine the relative importance of different sets of exploratory variables on the diatom community composition. CCA, PCA and DCA were performed using CANOCO version 4.5 (TER BRAAK and ŠMILAUER, 2002).

For laboratory experiments, variations in physico-chemical characteristics of the water, diatom community structure (species richness, diversity, cell densities and relative

abundance), chlorophyll *a*, DW, AFDM and frequency of morphological deformities with treatments and duration of exposure were examined by means of a repeated measures analysis of variance (RM-ANOVA; STATISTICA software package, Release 7, Stat Soft. Inc., USA) considering treatment as fixed factor among objects, and time a fixed factor within objects.

Indicator species

The IndVal method (DUFRÊNE and LEGENDRE, 1997) was used to find indicator species and species assemblages characterizing different substrates and sites. For each species *i* in each substrate/site group *j*, we computed the product of A_{ij}, the mean abundance of species *i* in the sites of group *j* compared to all groups in the study, by B_{ij}, the relative frequency of occurrence of species *i* in the sites of group *j*, following (DUFRÊNE and LEGENDRE, 1997) as follows:

$$\mathbf{A_{ij} = Nindividuals_{ij}/Nindividuals_{i.}}$$

$$\mathbf{B_{ij} = Nsites_{ij}/Nsites_{.j}}$$

$$\mathbf{IndVal_{ij} = A_{ij} * B_{ij} * 100}$$

where **IndVal** is the Indicator Value of species *i* in site cluster *j*, Nindividualsij is the mean number of individuals of species *i* across sites of group *j*, $Nindividuals_{i.}$ is the sum of the mean numbers of individuals of species *i* over all groups, $Nsites_{ij}$ is the number of sites in cluster *j* where species *i* is present, and $Nsites_{.j}$ is the total number of sites in that cluster. B_{ij} is maximum when species *i* is present in all objects of cluster *j*.

Diatom-based indices of water quality assessment

The diatom species counts were entered into the diatom database and index calculation tool, OMNIDIA version 5.3 (LECOINTE et al., 1993), and the following indices were calculated and tested: the Artois-Picardie Diatom Index or APDI (PRYGIEL et al., 1996); the Eutrophication/Pollution Index or EPI (DELL'UOMO, 1996); the Biological Diatom Index or BDI (LENOIR and COSTE, 1996); Schiefele and Schreiner's index or SHE (SCHIEFELE and SCHREINER, 1991); the Saprobic Index or SI (ROTT et al., 1997); the Trophic Index or TI (ROTT et al., 1999); the Watanabe index or WAT (WATANABE et al., 1986); the Specific Pollution sensitivity Index or SPI (COSTE in CEMAGREF, 1982); the Sládeček's index or SLA (SLÁDEČEK, 1986); Descy's pollution Index or DES (DESCY, 1979); Leclercq or IDSE (LECLERQ and MAQUET, 1987); the Generic Diatom Index or GDI (COSTE and AYPHASSORHO, 1991); the

Commission of Economical Community Index or CEC (DESCY and COSTE, 1991); the Trophic Diatom Index or TDI (KELLY and WHITTON, 1995); the Pampean Diatom Index or PDI (GÓMEZ and LICUIRSI, 2001) and the Biological Index of Water Quality trophic index or BIWQ (LOBO et al., 2004).

Pearson's correlation analysis was used to determine the relationship between the calculated index scores and measured physical and chemical water quality data. Forward stepwise multiple regression analysis was performed on the data to determine the indices that gave the best reflection of general water quality.

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CHAPTER 1

Biological monitoring of lotic ecosystems: a review of the role of benthic diatoms[1]

Abstract: Increasing anthropogenic influence on lotic environments because of civilization has captured public interest because of the consequent problems associated with deterioration of water quality. Various biological monitoring methods that provide a direct measure of ecological integrity by using the response of biota to environmental changes have been developed to monitor the ecological status of lotic environments. Diatoms have been used extensively in this regard and this review attempts to summarise the basic concepts associated with biological monitoring using benthic diatoms. Where possible, examples from work carried out in Brazil are used.

Key words: biological monitoring, lotic environment, benthic diatoms

Biomonitoramento dos ecossistemas lóticos: revisão do papel das diatomáceas bentónicas

***Resumo*:** Aumento da influência antropogênica sobre ambientes lóticos como resultado da civilização conquistou o interesse público por causa de problemas relacionados com a conseqüente deterioração da qualidade da água. Vários métodos de biomonitoramento que fornecem uma medida direta da integridade ecológica usando a resposta da biota a alterações ambientais têm sido desenvolvidos para monitorar o estado ecológico de ecossistemas lóticos. Diatomáceas têm sido amplamente utilizadas e esta revisão tenta resumir os conceitos básicos associados ao monitoramento biológico utilizando diatomáceas bentônicas. Sempre que possíveis, exemplos de trabalhos realizados no Brasil serão utilizados.

Palavras-chave: biomonitoramento, ecossistemas lótico, diatomáceas bentônicas,

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1.1 Introduction

Lotic environments are fundamental components of the regional and global biogeochemical cycles, acting as both transport pathways and sites of elemental transformations and storage and they save as sources of drinking water, fisheries resources, irrigation supplies, and waste removal systems. They are characterised by interactions among physical, chemical and biological processes, which reach a higher degree of complexity downstream (WEHR and DESCY, 1998).

The fundamental feature of lotic systems is that activities or disturbances at one location affect processes and organisms downstream, complicating the management of these systems. The systems are also characterised by longitudinal differences in the time scales of chemical and biological processes rendering it difficult to design polices and assess the results of management actions (TUNDISI and MATSUMURA-TUNDISI, 2008). Characterising and managing dynamic environmental conditions in heterogeneous systems such as these, therefore, requires innovative approaches, with the management process striking a balance between human needs and ecological integrity (EDWARDS, 1995; TUNDISI and MATSUMURA-TUNDISI, 2008). Ecological principles are now playing an important role in the management of these systems.

High population densities and multiplicity of industrial and agricultural activities expose most hydrographic basins to heavy and rising environmental impacts especially to pollution by domestic and industrial waste residues (SALOMONI et al., 2006). This increasing anthropogenic influence on lotic environments that parallels civilization has captured public interest because of the consequent deterioration of water quality, health problems, pest plants and animals, and other problems (SALVIA et al*.,* 1999; BERE, 2007). The purpose of this review is to summarise the basic concepts associated with biological monitoring using benthic diatoms. Where possible, examples from work carried out in Brazil are used.

1.2 Approaches to monitoring the ecological status of lotic system

Two threads of basic approaches to the assessment of water quality deterioration in lotic systems run through the literature. The first approach involves the assessment of physical and chemical variables of lotic systems in order to gain some insight into their water quality. In most cases, this method allows only instantaneous measurements, therefore restricting the knowledge of water conditions to the period when the measurements were taken. The chemistry at any given time is a snapshot of the water

quality at the time of sampling ignoring temporal variation of water quality variables that is usually high in lotic environments (ROCHA, 1992). Sophisticated chemical analytical methods have been developed but still they cannot identify and quantify pertinent compounds, especially synthetic organic compounds that are highly toxic (AIDAR and SIGAND, 1993).

The second approach involves use of biological methods (biological monitoring), the theory behind which is to provide a direct measure of ecological integrity by using the response of biota to environmental changes (KARR, 1991). This allows long-term environmental effects to be detected because of the capacity of reflecting conditions that are not present at the time of sample and analysis. The key to use of the aquatic biota as reliable indicators of the changes in lotic environmental conditions is deciphering the integrated environmental information in species rich assemblages (PAN et al., 1996).

This biological monitoring has gained momentum in aquatic health management programmes due to several shortcomings in standard physical and chemical methods described above. Physical and chemical methods are, however, complimentary to biological methods, contributing to the correct assessment of the quality of running waters (LOBO et al*.,* 2004). Since the biological response is to the integrated physical and chemical environment to which the organism has been exposed for some time, it is not surprising that the physical and chemical indicators often do not correlate with biological indices (ROUND, 1991).

1.3 Biotic indices

The patterns of biota inhabiting lotic systems are responsive to the nature of the physical and chemical characteristics of these systems (KARR, 1991). The integrity of biota inhabiting the lotic ecosystems thus provides a direct, holistic and integrated measure of the integrity of the systems. To this effect, therefore, the ultimate monitor of aquatic systems is the aquatic life itself (JOHN, 2000). It is on this basis that biotic indices enjoy widespread use in the assessment of ecological status of lotic ecosystems.

Several indices of biotic integrity have been developed worldwide to assess the health status of the lotic systems. These indices make use of the niche requirements and habitat preferences of the individual species (autecology), a population (synecology) or higher taxonomic groupings to infer environmental conditions in an ecosystem (STOERMER and SMOL, 1999). Long-term data gathered about the tolerances of a species are used to compile an index that can, in turn, be used to deduce environmental

conditions from the species composition by taking into account the specific tolerances of the species in the community surveyed (PATRICK, 1986; DE LA REY et al., 2008). These indices can be constructed to measure specific pollutants or general environmental conditions.

Many indices have been developed using fish, macroinvertebrates, zooplankton and phytoplankton and especially benthic diatoms. A comparison of three indices of water quality (chemical, zoological and botanical assessment using diatoms) concluded that the later gave the most precise data (LECLERCQ and MAQUET, 1987). However, in some cases aquatic macroinvertebrates have been demonstrated to be superior to diatoms as biological indicators, with a capacity to reflect on sedimentation in the riverbed that cannot be reflected by diatoms (SCHOEMAN and HAWORTH, 1986; KATOH, 1992; PRYGIEL and COSTE, 1993). In the cases of intermediate ranges of pollution where the diatoms and macroinvertebrates are less sensitive, chemical analysis have been shown to be more efficient (LECLERCQ and MAQUET, 1987; SCHOEMAN and HAWORTH, 1986).

1.4 Distribution of diatoms in lotic systems

A fundamental part of lotic ecosystems is the periphyton community assemblages whose diversity increases as anthropogenic influences on the system increases (ARCHIBALD, 1972; LOBO and KOBAYASI, 1990; ROUND, 1991). This is consistent with the intermediate disturbance hypothesis (CONNELL, 1978), which states that the highest diversity is maintained at intermediate levels of pollution. These assemblages have important implications for ecosystem processes in lotic environments. Firstly, they are an integral part of the energy cycle in nearly all lotic ecosystems providing much of the food thus maintaining higher trophic levels i.e. they are important for establishment of ecological balance (ROCHA, 1993).

Secondly, they purify waters by absorbing many impurities such as nutrients and heavy metals and are sites of the breakdown of bacterial and other organic matter contamination. Thirdly, they respond rapidly to degradation of water quality, often changing in both taxonomic composition and biomass where even slight contamination occurs (ROCHA, 1993; PATRICK and HENDRICKSON, 1993; BIGGS and KILROY, 2000; DOUNG et al., 2007). They also play an important role in global cycling of silica and carbon (MANN, 1999). The maintenance of proper community structure and functioning of periphyton assemblages in lotic systems in the face of encroaching human

development and climate change, among other threats is, therefore, important in river health management.

A major part of these periphyton assemblages is made up of diatoms which are various microscopic one-celled or colonial members of the algal division or phylum Bacillariophyta, of the class Bacillariophyceae, having cell walls of silica consisting of two interlocking symmetrical valves. They are universally distributed in all types of aquatic environment with others being endemic to specific regions (POTAPOVA and CHARLES, 2003). They multiply rapidly, maintaining a dynamic population of varying size depending on the prevailing environmental conditions. Diatoms are the most species rich group of algae with tens of thousands of species (MANN, 1999). ROUND (1991) states that there are currently over 260 genera of living diatoms with over 100 000 species.

Multiple factors prevailing at different temporal and spatial scales play an important role in structuring benthic diatom communities in lotic systems (POTAPOVA and 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). Our comprehension of the role of temporary factors in shaping global communities is, however, still in its infancy (PASSY, 2007).

Some of the factors most often found to be important in shaping the distribution patterns of benthic diatoms in lotic systems are water chemistry (particularly pH, ionic strength and nutrient concentrations), substrate, current velocity, light (degree of shading) grazing, temperature (which also correlates strongly with latitude and altitude) (ROUND, 1991; PAN et al., 1996; POTAPOVA and CHARLES, 2002; NECCHI-JÚNIOR, 2003). Most of these factors depend strongly on climate, geology, topography, land-use and other landscape characteristics, and therefore diatom communities are similar within ecological regions defined by these characteristics (PAN et al., 1996). Short-term differences in community composition are also driven by immigration of cells, differences in growth rate between populations and loss processes such as death, emigration and sloughing.

Thus, growth and development of benthic diatoms in streams is an outcome of complex interaction between hydrological, chemical and biotic factors (Figure 1.1). Local "proximate" variables, like discharge regime, are controlled by regional "ultimate" factors like geology, topography or climate operating at spatial scales of catchments or even ecoregions. In addition, human activities act to change both proximate and ultimate

variables in an increasing rate, leading towards variously impacted biological communities, e.g. algal communities with increased amount and biomass of nuisance species, or in general, impoverished biological communities (SONNEMAN et al., 2001).

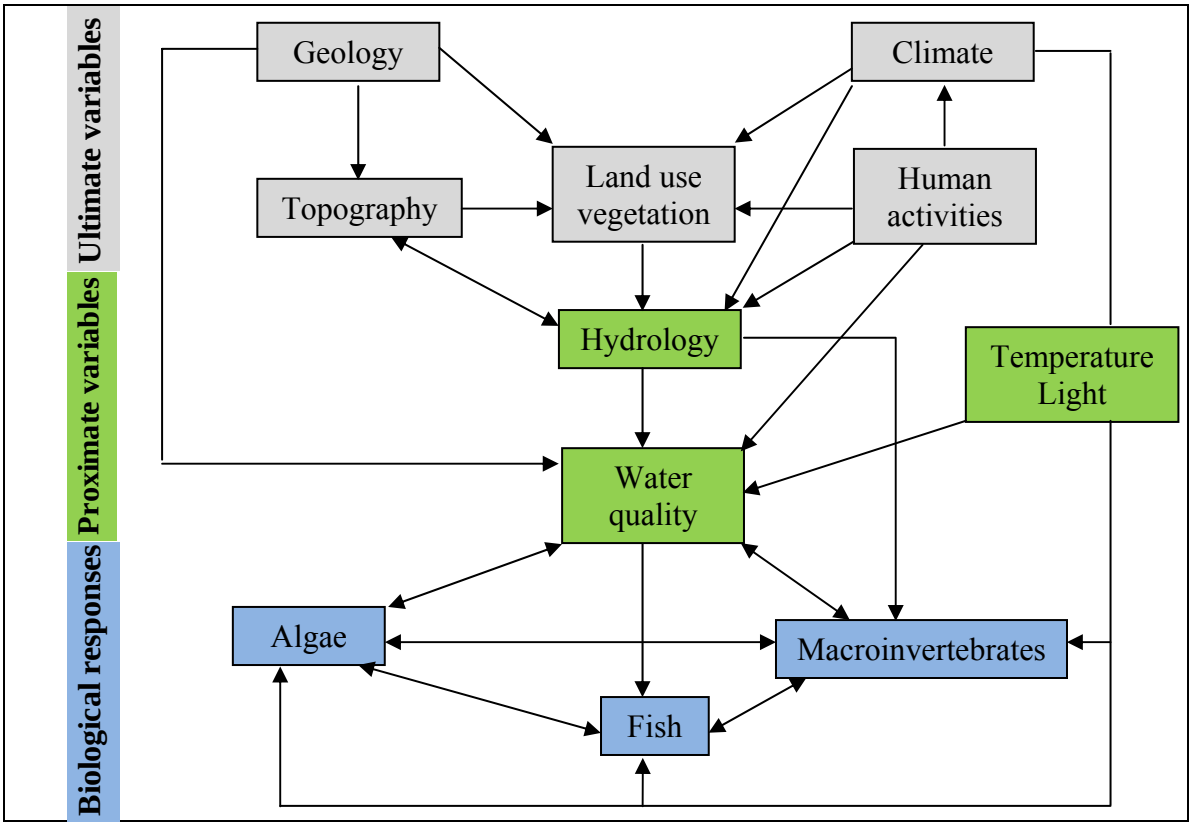

Figure 1.1: Interaction of biological, proximate and ultimate factors (Modified from BIGGS, 1996).

Changes in any of the factors described above, however, 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).

Diatoms are 'sub-cosmopolitan', i.e. they occur anywhere in the world if certain environmental conditions are fulfilled (PAN et al., 1996). This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a specific site that determine this distribution. Coupled with this also is the recent

discovery of the ubiquity-biogeography transition, where organisms smaller than about 1 mm occur worldwide wherever their required habitats are realised (FINLAY, 2005).

A key issue in understanding the patterns of diatom distribution is understanding the extent to which they are constrained by geographical factors that limit species dispersal vs. the extent to which they are limited only by the ability of the species to grow under a specific combination of environmental factors (POTAPOVA and CHARLES, 2002). Is a species not in a particular habitat only because it was not dispersed there or because, though it might have reached the habitat, the environmental conditions were unsuitable for it to survive and compete? Conversely, is a species in a specific location only because it can compete better under those particular environmental conditions, or might it be there simply because it reached a site and its potential competitor did not? Factors influencing dispersal should be clearly understood.

1.5 Diatoms and biological monitoring of lotic systems

The relation between diatoms and environmental variables are robust and quantifiable making diatoms appropriate quantitative indicators of ecological conditions in lotic systems (PAN et al., 1996; OLIVEIRA et al., 2001). The relationship has been found to be even stronger than those from lentic environments (ROUND, 1991). Each particular species relate very closely to other species and requires different structural, physical and chemical characteristics intrinsic to its habitat. Whenever these characteristics are subject to variations, the composition of the niche is affected; species vary in their sensitivity and those more resistant to environmental changes either caused by natural fluctuations or by human activities may be favoured by selection (ROCHA, 1992).

Understanding the relation of geographical and environmental factors to diatom distribution is important to the process of developing diatom-based water quality indicators. Differences in diatom species composition among geographical areas, caused not only by environmental variation, but also by historical processes of species dispersal and colonisation, add difficulty in applying uniform methods for water quality assessment using diatoms (POTAPOVA and CHARLES, 2002). Reliable diatom metrics development requires careful investigation of data sets in terms of the major environmental gradients underlying species composition.

Considering the complexity of factors affecting species composition of benthic diatoms, it is advisable that diatom-based river metrics should first be developed for

limited geographical areas with the most uniform environmental variables possible (POTAPOVA and CHARLES, 2002). This still needs to be done very carefully factoring in natural variation. Even if communities are developed in very similar environments, there is considerable variation (30-35 %) in the number of species and percent of dominant species attributed to unaccounted-for natural factors and this can be easily mistaken for the effects of the perturbation under study (PATRICK and HENDRICKSON, 1993). Unaccounted-for natural variations and probably simply random fluctuations are important factors affecting community structures that have not always been allowed for when evaluating differences between communities leading to erroneous interpretations. Correlations among environmental factors that determine diatom community structures should be taken into account for the best outcome in biological monitoring studies, because these internal correlations of environmental factors in a data set can lead to incorrect conclusions about species environmental requirements.

Despite all this, however, diatoms represent outstanding bio-indicators for different degrees of pollution. They provide excellent indicators of water quality. ROUND (1993) lists numerous reasons why diatoms are useful tools of biological monitoring, amongst which are the following: methods are cost effective, data is comparable, and techniques are rapid and accurate. Benthic diatoms have also been dimmed excellent organisms for biological monitoring because they lie at the base of aquatic food webs and are among the first organisms to respond to environmental change (LAVOIE et al., 2008). They also have a short life cycle allowing rapid respond to environmental stress especially eutrophication providing detailed information on nutrient changes (SONNEMAN et al., 2001; LOBO et al., 2004; BILLINGER et al., 2006; RESH, 2007). A strong correlation between diatom community composition and total nitrogen and total phosphorus has been demonstrated (WINTER and DUTHIE, 2000). Relationships between benthic stream diatom community composition and total phosphorus are strongest at low to medium concentrations (<0.085 $mg.L^{-1}$, PAN et al., 1996; <0.1 $mg.L^{-1}$, WINTER and DUTHIE, 2000).

Nutrient concentrations, particularly phosphorus, increases with urban development, associated with storm water run-off and are caused by other catchment activities such as agriculture (SONNEMAN et al., 2001). Studies of streams draining urban centres with mixed storm water and sewage impacts have shown declines in diatom species richness associated with high loads of organic pollution (LOBO et al., 1995).

Changes in water quality associated with urbanization – judged small to moderate using chemical measurements – are associated with profound changes in biotic community composition of diatoms (SONNEMAN et al., 2001).

Many field experimental methods have also been designed worldwide to assess the ecological integrity of lotic systems using diatoms. Different methods of manipulating nutrients, for example, have been developed (e.g. PETERSON and STEVENSON, 1989; PRYGIEL et al., 1999). The objective of all these methods is to supply varied levels of nutrients to monitor the subsequent growth and productivity (biovolume or biomass assessment) of diatoms, with those species responding to nutrient addition being considered to be growing sub-optimally in the natural habitats. Another technique involves the transfer of colonised substrate (stones, glass etc.) from on site (e.g. polluted) to another site (e.g. non-polluted) followed by monitoring of changes in flora.

Some investigations has also been carried out on the effects of pH, conductivity and trace metals on diatoms (e.g.; PAN et al., 1996; OLIVEIRA et al., 2001; SONNEMAN et al., 2001; POTAPOVA and CHARLES, 2002, 2003; LOBO et al., 2004; BILLINGER et al., 2006; SALOMONI et al., 2006). Monitoring the changes in pH and the ionic composition is carried out by simple observation of shifts in the dominant taxa or by inferring ion concentrations or conductivity, using reported optima and some numerical procedures e.g. weighted averaging. The quantification of species responses to concentrations of major ions in fresh water would significantly enhance this (POTAPOVA and CHARLES, 2002).

1.6 Brief history of the use of diatoms in biological monitoring

The assessment of water quality conditions in freshwater habitats using benthic diatoms dates back to as early as the beginning of the 1900 century when KOLKWITZ and MARSSON (1908) first attempted to use diatoms as indicators of pollution in aquatic environments. Their study showed that water conditions determined algal communities in aquatic environments but made no efforts to define the various habitats in which the diatoms grow. They developed firstly the saprobic system – Saprobity index- that was modified by LIEBMANN (1951) and PANTLE and BUCK (1951) which gives values to the species present in the system under consideration in relation to levels of pollution. Their research forms the basis of most subsequent studies on the use of diatoms for biological monitoring.

PATRICK (1953) created a biological indicator system, based on changes in energy flow; the system classified water as clean, polluted, very polluted and typical. SLÃDECKOVA and SLÁDECÉK (1963) developed a biological indicator system based on periphyton believing that these organisms, being independent of water movement because they are able to adhere to a variety of substrates, might adapt to environments changes more easily than phytoplankton. PALMER (1969) created a system that uses algae as biological indicators of pollution. He suggested numbers for each genus of algae as an indication of pollution level – based on extensive study.

The use of indices to assess water quality was also attempted by ZELINKA and MARVAN (1961) and SLÀDECEK (1973, 1986), but DESCY'S work (1979) is given most credit. These studies revealed the need for detailed studies on the tolerance of individual species by actual sampling and relationship to chemical data, rather than relying on statements in literature. LECLERCQ and MAQUET'S (1987) work provide the most complete comparison of chemical invertebrate and diatom indices. KOBAYASI and MAYAMA (1989), working in the rivers of Tokyo, classified diatoms along gradients of organic pollution in to the following categories: i) more tolerant; ii) less tolerant and iii) more sensitive to pollution.

At present, there is a lot of recorded or published data available on diatom ecology and their application to biological monitoring. They are used, for example, in Europe (SLADECEK, 1973; DESCY, 1979; LANGE-BERTALOT, 1979; KELLY and WHITTON, 1995; KELLY et al., 1998; PRYGIEL et al., 1999), North America (LOWE and PAN, 1996; PAN et al., 1996), Central America (SILVA-BENAVIDES, 1996*a*,*b*; MICHELS-ESTRADA, 2003), South America (LOEZ and TOPALIAN, 1999; GÓMEZ and LICURSI, 2001; LOBO et al., 2002, 2004) Australia (CHESSMAN et al*.,* 1999; JOHN, 2000), Asia (WATANABE et al., 1988; KOBAYASI and MAYAMA, 1989) and Africa (TEYLOR et al., 2007; DE LA REY et al., 2008). Some of the studies are focused on inferring past hydrochemical characteristics of lakes, while others are designed to monitor present-day conditions in lotic ecosystems.

These attempts have had varying degrees of success. ROUND (1991) noted three features that have confused the use of diatoms in biological monitoring of lotic systems. Firstly, there are semantic problems in which language and translation and writing in languages other than one's own has led to confusion that manifests itself in proliferation of terminology that is mere variations of expressing the same thing. Secondly, there is an almost total lack of appreciation of microhabitats with their characteristic floras. Thirdly,

there is excessive searching for and counting of numbers of cells (valves) of species, resulting in lengthy lists of diatoms. This is often compounded by dubious academically rigorous pseudo-mathematical manipulations that are not in any way cost effective and as FRYER, (1987) puts it; numbers are increasingly being used as a substitute for real explanations.

Whereas mathematical analyses, whether models or indices, are important in calling attention to differences between populations, elucidation of the causes of observed patterns of diatom assemblages require other supportive data. Knowledge of the autoecology of constituent species needs to be incorporated in making statements concerning pollution levels in various areas. In many instances, similarity indices, ordination techniques and so on are used, but usually not discussed in any detail and sometimes based on inadequate sampling and identification.

Many people have stated that they count 200, 400, or 1,000 specimens without giving the rational for terminating the count. Statement about diatom communities based on small counts may be inaccurate. One should determine how many specimens to be counted by the use of the asymptote curve or the log normal or some other statistical method to be sure that the community is well represented and that the dominance of the count is characteristic of the community (PATRICK, 1986). An important conclusion is that the structure of diatom assemblages is more often characterised by the present of one or a few, very abundant species and a rather limited number of rare species (ROUND, 1991), so it is not necessary to have some of the high counts recorded in some of the literature.

1.7 Natural and artificial substrate sampling

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 epilithon, while the second approach involves sampling of artificial substrate placed in water – the mostly used being glass (ROUND, 1991). Direct sampling of stone has no *a priori* recommendations and has the following advantages: 1) ability to find stones in most reaches of the river; 2) stability of the substratum and flora and its presence at all times of the year; 3) growth is not constant throughout the year but this affects only the total biomass and not the overall occurrence of indicator species; 4) recovery of flora after denudation by floods is rapid; 5) flora of the rock surface integrates the effects of variation in water quality over time; and 6)

sampling is easy and there are large number of cells per cm^2 and removal is easy (DESCY and COSTE, 1991; LECLERCQ and MANQUET, 1987).

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 torrential upper stretches sampling can be difficult and often the flora is depleted; and 4) shading by surrounding vegetation reduces species richness and biomass. Overly, however, the epilithon does provide an excellent sample of diatom species at most points in a river and is an excellent community for monitoring changes in the environment (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 is reduced (MARKER and 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 use of artificial substrate (glass slides, bricks, cotton threads e.t.c) have the advantage that the flora can be observed directly, the substratum is standard at all sampling sites and the time for exposure can be controlled (ROUND, 1991). The disadvantages are, however, overwhelming: 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 epilithon directly; 4) the floral 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 (DESCY and COSTE, 1991).

1.8 Diatoms and biological monitoring in Brazil

In Brazil, pioneer studies on phytoplankton in lotic systems were carried out in the Amazonian region rivers (DICKIE, 1881). First studies on the use of aquatic biota, particularly the phytoplankton, for monitoring of the ecological status of lotic systems were carried out in the catchment areas of São Paulo City by a French researcher, Henric Charles Potel, between 1907 and 1910 (ROCHA, 1992). This work remained unpublished until translated to Portuguese and published by BRANCO (1964) and formed the basis of

most subsequent studies. Although they were based on empirical and qualitative data, these subsequent studies are extremely important since they constitute the only records of studies on river basins, reservoirs and sources of water supply at that time (ROCHA, 1992). Limited data could be gathered at that time due to inadequate infrastructure, lack of skilled personnel and poor policies or strategies for environmental management (ROCHA, 1992).

Other studies followed up on this work based on quantitative data. All these studies confirmed diatoms as excellent indicators of environmental conditions in lotic system (e.g. LOBO et al., 2004, 2006; HERMANY et al., 2006; SCHNECK et al., 2007; SALOMONI et al., 2008). In South America in general, GÓMEZ and LICUIRSI (2001) published a regional water quality evaluation index, for rivers and streams in the Pampas of Argentina, Pampean Diatom Index (PDI) based on the sensitivity of epipelic diatoms to organic enrichment and eutrophication.

In most cases, however, the assessment of the ecological conditions of lotic systems was determined by foreign methods (e.g. LANGE-BERTALOT, 1979; WATANABE et al., 1988; KOBAYASI and MAYAMA, 1989), because no information on pollution tolerant diatoms in Brazilian rivers was present. This direct adoption of these indices can lead to erroneous interpretation of water quality because there are limited overlaps in species composition between two regions, or at least some ecological characteristics of the key taxa vary among the regions (PAN et al., 1996). The occurrence of endemic diatoms further complicates the situation necessitating the compilation of a diatom index unique to the region (TAYLOR et al*.,* 2007). This underlines a clear vision to develop a biological monitoring protocol that is unique to Brazil.

This prompted the first attempts to classify diatoms in terms of tolerance of species to organic pollution in rivers in southern Brazil by LOBO et al., (1996). Subsequently, LOBO et al., (2002) determined the tolerance to organic pollution of diatom species in different conditions of pollution of lotic systems of Guaiba, RS, and they came up with 3 groups of diatoms: Group A (species more tolerant to pollution), Group B (species tolerant to pollution) and Group C (species less tolerant to pollution), each of which were assigned the following saprobic values 4; 2.5 and 1 respectively.

Based on this information LOBO et al., (2002) developed the first saprobic system in the country, which uses epilithic diatoms for water quality assessment in southern Brazil. This study was completed by LOBO et al., (2004) leading to the formation of Biological Index of Water Quality (BIWQ) trophic index. This was the first index to be

published in rivers of Brazil and it incorporate the effects of organic contamination from the classification described in LOBO et al. (2002), and eutrophication from the values obtained using techniques of multivariate analysis.

Recent studies of environmental monitoring, using the community of diatoms algae in water systems of Guaíba, RS, have demonstrated clear evidence of eutrophication (OLIVEIRA et al., 2001; LOBO et al., 2002, 2004, 2006; SALOMONI et al., 2006). However, the studies are concentrated or restricted mainly to the southern part of the country (TUNDISI, 2008), and very little, was done in other lotic systems. For this course, there is lack of capacity in the form of data, which hampers full understanding and the subsequent management of the lotic systems in other regions of the country and lotic environments in general.

1.9 Conclusion

Diatoms have an important role in the biological monitoring of lotic ecosystems. Building on the existing data from the past studies, especially in the southern part of Brazil, there is a promising future in using diatoms for characterization and monitoring of ecological conditions in other parts of Brazil. Resources should be channelled towards tackling challenges associated with diatom-based biological monitoring, principally taxonomic studies, training of skilled labour and acquiring and maintaining the necessary infrastructure. Other bioindicators should also be taken into consideration, i.e. fish, macrophytes, macroinvertebrates and other groups of algae, and supplemented by physical and chemical methods. There is need to adopt new paradigms in environmental monitoring such as a shift from an anthropocentric to an ecosystem-cantered holistic approach, with man viewed as an element of the ecosystem and the principle consumer of its services. Environmental monitoring boards, responsible for setting targets for required ecological health or integrity of lotic systems, should rely on biological monitoring to assess progress towards achieving these goals. There is need for establishment of networks of competence at national, regional and global levels to improve biological monitoring through research and innovative practices that are ecologically oriented. This should be followed by fostering two-way interactions between scientists, on the one hand and the general public and decision makers, on the other.

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CHAPTER 2

The effects of substrate type on diatom-based multivariate water quality assessment in a tropical river (Monjolinho), São Carlos-SP, Brazil[2]

Abstract: Diatoms are good indicators of water quality in lotic systems. Unlike in the temperate region, the effect of substrate on diatom-based water quality assessment in tropical lotic systems is not fully understood. The purpose of this study was to assess the effect of substrate on diatom-based multivariate water quality assessment. Epilithic, epiphytic, epipsammic and epipelic diatom communities and water quality sampling was done 4 times at 10 sites during the dry season (2008 and 2009). Artificial substrates (brick and glasses) were also placed at the sampling sites during this period and sampled after one month. Cluster analysis was performed to show the main differences and similarities in community composition among substrates sampled and among sampling sites. The IndVal method was used to identify indicator species characterizing different substrates. CCAs were performed to relate the structure of diatom communities from different substrates to predictor variables. A gradient of increasing metal and organic pollution, eutrophication and ionic strength was observed from the agricultural/forested area to the urban area. Diatom community structure closely reflected this gradient, with communities from polluted sites (8, 9 and 10) being different from other communities. Polluted sites were associated with such species as *Nitzschia palea, Planothidium lanceolatum*, *Achnanthes exigua*, *Cyclotella hyaline*, *C. meneghiniana*, *Gomphonema parvulum*, *Fallacia monoculata*, *Luticola goeppertiana*, *Pinnularia microstauron*, *P. subcapitata* and *Sellaphora pupula*. Indicator species analysis showed that common diatom species were not restricted to a single substrate, though preference was generally high for natural (especially macrophytes) compared to artificial substrates. Six CCAs corresponding to six substrates performed to relate diatom community structure to simultaneous effects of predictor variables explained ~ 50 % of the diatom species variance in all cases and roughly separated highly polluted sites from the rest of the sites. This indicates that the results of diatom-based multivariate water quality assessment based on different substrates may be interchangeable. Only one substrate has to be collected at each site for water quality assessment surveys, thus, avoiding unnecessary

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expensive and time-consuming over sampling. Given the limitations of artificial substrates, sampling of natural substrates is highly recommended.

Key words: benthic diatoms, substrate, water quality assessment

Os efeitos do tipo de substrato na avaliação multivariada da qualidade da água de um rio tropical (Monjolinho, São Carlos-SP, Brasil) usando-se as diatomáceas como indicadores

Resumo: As diatomáceas são bons indicadores da qualidade da água em sistemas lóticos. Ao contrário das regiões temperadas, o efeito do substrato na avaliação da qualidade da água de sistemas lóticos tropicais baseando-se nas características das algas diatomáceas presentes não é totalmente compreendido. O objetivo deste estudo foi avaliar o efeito do substrato sobre a avaliação multivariada da qualidade da água baseado-nos diatomácea. Para isso, comunidades de diatomáceas epilíticas, epífitas, epipsammicas e epipelicas e amostragem da qualidade foram feito quatro vezes em 10 pontos na estação seca (2008 e 2009). Substratos artificiais (tijolos e vidros) também foram colocados nos pontos de amostragem durante este período e verificados após um mês. A análise de agrupamento foi realizada para avaliar as principais diferenças e semelhanças na composição da comunidade entre os substratos amostrados nos diferentes pontos de amostragem. O método IndVal foi utilizado para identificar espécies indicadoras que caracterizam diferentes substratos. ACCs foram realizadas para correlacionar a estrutura das comunidades de diatomáceas de diferentes substratos com as variáveis de prognóstico. Gradientes crescentes de metais, poluição orgânica, eutrofização e da força iônica foram observados desde a área agrícola/florestal até a área urbana. A estrutura das diatomáceas reflete este gradiente, sendo as comunidades nos pontos mais poluídos (8, 9 e 10) diferente das demais. Pontos poluídos foram associados com a presença de espécies tais como *Nitzschia palea, Planothidium lanceolatum, Achnanthes exigua, Cyclotella hyaline, C. meneghiniana, Gomphonema parvulum, Fallacia monoculata, Luticola goeppertiana, Pinnularia microstauron, P. subcapitata* e *Sellaphora pupula.* A análise de espécies indicadoras mostrou que diatomáceas comuns não foram restritas a um único tipo de substrato, embora a preferência fosse geralmente alta para os naturais (especialmente macrófitas) em comparação aos artificiais. Seis ACCs, correspondentes a seis substratos, realizadas para relacionar a estrutura da comunidade de diatomáceas com os efeitos das

variáveis prognósticas explicaram aproximatamente 50% da variância de espécies diatomáceas em todos os casos e grosseiramente separadas as pontos altamente poluídas do resto dos pontos. Isso mostra que os resultados da avaliação multivariada da qualidade da água baseando-se nas algas diatomáceas amostradas em diferentes substratos podem ser intercambiáveis. Apenas um substrato deve ser amostrado em cada local para levantamentos de avaliação da qualidade da água, evitando-se assim procedimentos desnecessários, caros e demorados de amostragem. Devido às limitações dos substratos artificiais, a amostragem de substratos naturais é altamente recomendável.

Palavras-chave: diatomáceas bentônicas, substrato, avaliação da qualidade da água,

2.1 Introduction

Diatoms are good indicators of water quality in lotic systems with worldwide application (e.g. LOWE and PAN, 1996; KELLY et al., 1998; PRYGIEL et al., 1999; STEVENSON and PAN, 1999). They are used in Europe (KELLY et al., 1998; PRYGIEL et al., 1999), North America (STEVENSON and PAN, 1999; LOWE and PAN, 1996), South America (LOBO et al., 1996; LOEZ and TOPALIAN, 1999), Australia (CHESSMAN et al., 1999; JOHN, 2000), Asia (LOBO et al., 1995; ROTHFRITZ et al., 1997) and Africa (SCHOEMAN, 1979; GASSE et al., 1995). Some of the studies are focused on inferring past hydro-chemical characteristics of lakes, while others are designed to monitor present-day conditions in lotic ecosystems.

These works had varying degrees of success. ROUND (1991) noted three features that have confounded the use of diatoms in biological monitoring of lotic systems, among which is an almost total lack of appreciation of substrates with their distinct characteristic floras. Species composition and abundance of benthic diatom communities sampled at the same site but from different substrates (e.g., sand, rock surface, submerged or emergent macrophytes) often differ substantially because species are better adapted to one substrate than other substrates (ROUND, 1991; PATRICK and HENDRICKSON, 1993; POTAPOVA and CHARLES, 2005; FISHER and DUNBAR, 2007). As set forth by HUTCHINSON (1993), each species has a hyper-volume, which is a multi-dimensional niche, and each parameter has a range of values within which the species can live and reproduce. Thus, differences in substrates can potentially confound responses of diatom assemblages to stress associated with human activities. This is particularly important in

large-scale water quality assessments carried out in diverse landscapes where a single substrate may not be present at all sampling sites (POTAPOVA and CHARLES, 2005). 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.

Previous diatom-based water quality assessment studies offers seemingly contrasting results with some emphasizing the importance of substrates (e.g., LOWE and PAN, 1996; KELLY et al., 1998). On the other hand, other studies have not found significant between-substrate differences in diatom assemblages possibly because the effects of other environmental variables were overriding (JÜTTNER et al., 1996; ROTT et al., 1998; KITNER and POULÍ-ČKOVÁ, 2003; SOININE and ELORANTA, 2004). The studies where the diatom communities were found to differ considerably among substrates were conducted at small scales (one or few lakes or rivers), with other factors being relatively unimportant, while substrate effect is less noticeable when other environmental characteristics vary a lot, as in large-scale studies (POTAPOVA and CHARLES, 2005).

In some cases, to circumvent the problems caused by sampling of natural substrates, artificial substrates can be used. The artificial substrate (e.g. glass slides) 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 and COSTE, 1991; ROUND, 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 epilithon directly; 3) the floral is an artificial assemblage selected by smooth slide and perhaps by differences due to positioning of slides in relation to the currents; 4) the smooth surface often results in sloughing of the community; and 5) the system does not allow for sampling at random, but only at pre-selected sites.

Most of the studies on the effects of substrates on benthic communities have been carried out in temperate regions while few studies have been carried out tropical regions. Direct adoption of the research finds from temperate region studies to tropical regions can lead to erroneous interpretation of water quality because there are limited overlaps in species composition between two regions, or at least some ecological characteristics of the key taxa vary among the regions (PAN et al., 1996). The occurrence of endemic diatoms further complicates the situation necessitating the compilation of a diatom index

unique to the region (TAYLOR et al., 2007). For this cause, ecological assessment methods should be river type specific and express the ecological running water quality as a deviation from the respective reference conditions (EUROPEAN COMMISSION, 2003). Consequently, special research effort needs to be made in order to define the reference sites for river type and local research must be undertaken to identify the constrains of each water body type (PINTO et al., 2005).

Four analytical approaches are used in diatom-based water quality assessment: biotic indices, multivariate analysis, diversity indices and species abundance analysis (LOBO et al., 1995). Several studies have cautioned against the use of diversity indices in biological monitoring of aquatic systems (e.g. ROUND, 1991; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003; DUONG et al., 2006, 2007; DE LA REY et al., 2008). The use of multivariate analysis in diatom-based water quality analysis has gained momentum in recent years. Various direct, indirect (ordination) and classification multivariate techniques are used in diatom-based water quality assessments. In view of the above issues, the present study was designed to assess the effects of substrates on benthic diatom community structure and subsequently diatom-based multivariate water quality assessment.

2.2 Materials and Methods

2.2.1 Study area and study design

The area under study was located in southern part of Brazil (Figure 2.1). Headwaters of Monjolinho and the tributaries studied fall within mainly agricultural area. From agricultural area, the streams run through urban area of the city of São Carlos, which covers a total area of 1143.9 km^2. The area is characterized by undulating terrain and an average annual temperature of around 19.5 °C, with mean monthly maximum of around 21.9 °C recorded in January and February and the mean monthly minimum of around 15.9 °C recorded in July.

In 2008, the population of São Carlos was estimated at 218 080 inhabitants by Instituto Brasileiro de Geografia e Estatística (IBGE). 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 so on (DUPAS et al., 2006). Streams in the study area, therefore, receive untreated or semi-treated effluent from various domestic and industrial sources as well as other diffuse sources as they pass through the city (DUPAS et al.,

2006). This disorderly growth of the city resulted in stream health deterioration, loss of the remaining primary vegetation and eutrophication among other problems.

Ten sites were established along Monjolinho river and its tributaries: four sites (1, 2, 3 and 7) in the relatively less impacted agricultural and forested headwaters to act as reference sites; 3 sites (4, 5 and 6) in the moderately polluted urban area; and 3 sites (8, 9 and 10) in highly polluted downstream area after the urban area (Figure 2.1). The rational for choosing the sampling sites was to obtain a pollution gradient of all the stream systems from relatively unpolluted agricultural headwaters to highly polluted urban downstream sites.

Substrate assessment, diatom and water quality sampling were done during dry seasons (autumn and winter) when flow was stable. Four samplings were carried out, two in September and October 2008 and 2 in May and June 2009. Sampling was done during dry season to avoid variable effects of rainy season like great variations in water level and velocity, floods and inundations, which affect diatom development, especially growth rate and relative abundance of different species (ROUND, 1991; PATRICK and HENDRICKSON, 1993; DUONG et al., 2006).

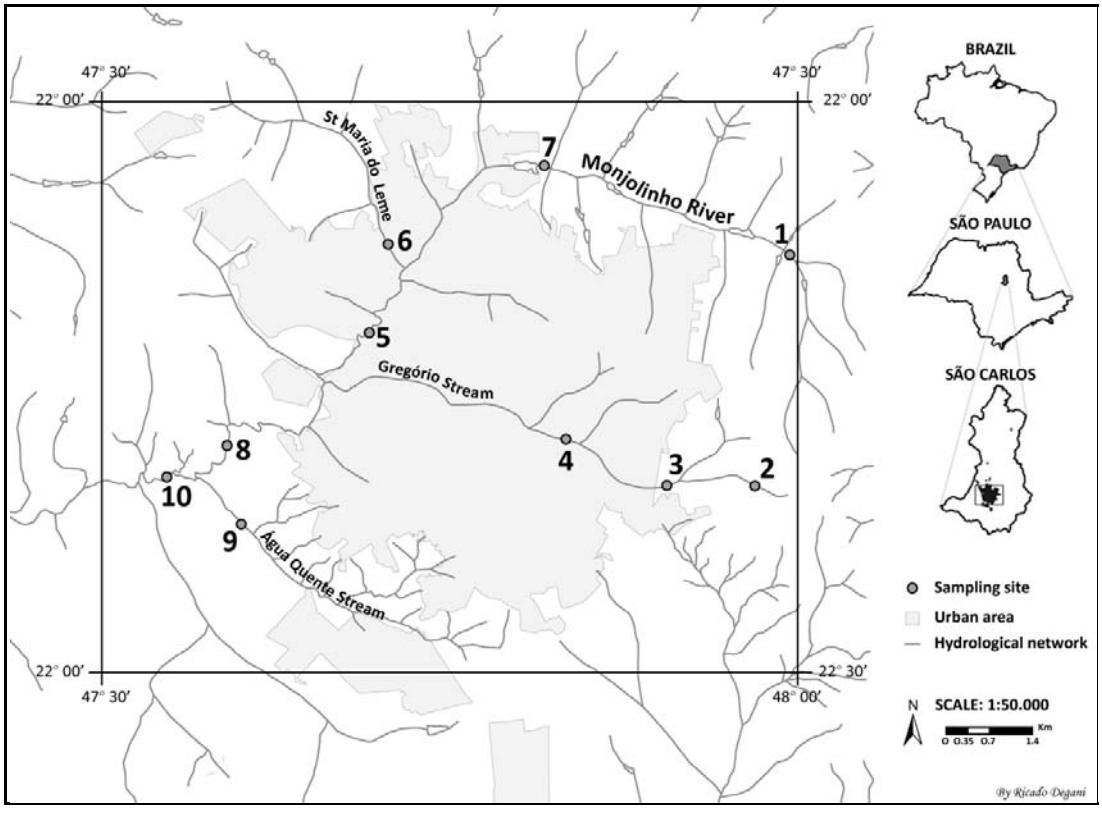

Figure 2.1: The location of the sampling sites in the study area.

2.2.3 Environmental variables

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). Depth and current velocity were measured at each site with an FP 201 global flow probe (Global Water Instrumentation Inc. Alaska, USA). The percentage riparian vegetation cover was visually estimated at each site. Altitude was determined at each site using a GPS (Northport Systems, Inc. Toronto, Canada). The percentage embeddedness was also visually estimated along each stretch and rated on a 0-5 scale following PLATTS et al. (1983).

The following physical substrate characteristics were visually estimated following USGS NAWQA protocol (FITZPATRICK et al., 1998): percentage of silt-clay size particles, sand size particles, gravel size particles, cobble size particles, boulder size particles; ratio of silt-gravel size particles, gravel-cobble size particles, and cobble-boulder size particles.

Water samples for metals, ions, total nitrogen (TN), total phosphorus (TP), biological oxygen demand (BOD_5) and chemical oxygen demand (COD) analysis were collected at each site into acid-cleaned polyethylene containers (APHA, 1988). In the laboratory, the concentrations of TN and TP, BOD_5 and COD in the water samples were determined following standard methods APHA (1988). The following metals were analysed in water samples using Flame Atomic Absorption Spectrometry Analytical Methods (Varian Australia Pty Ltd, Victoria, Australia): cadmium (Cd), lead (Pb), Zinc (Zn), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe) manganese (Mn) and nickel, (Ni). The concentrations of fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were analysed by isocratic ion analysis using suppressed conductivity detection ion chromatography method using Dionex DX-80 Ion Analyzer (DX-80) (DIONEX CORPORATION, 2001).

2.2.4 Biological elements

Substrates present at each sampling site were classified into four categories; (1) stones – epilithic, (2) macrophytes (submerged/emergent) – epiphytic, (3) sand – epipsammic and (4) silt/clay – epipelic, based on USGS NAWQA protocol

(FITZPATRICK et al., 1998). At each site, epilithic, epiphytic, epipelic and epipsammic diatoms were sampled separately, avoiding mixing as much as possible.

Epilithic diatoms were sampled by brushing stones with a toothbrush. Prior to sampling, the stones were gently shaken in stream to remove any loosely attached sediments and non-epilithic diatoms. At least five pebble-to-cobble sized stones were randomly collected along each sampling stretch, brushed and the resulting diatom suspensions were pooled to form a single sample that was then put in a labelled plastic bottle. Epiphytic diatoms were sampled from deferent species of submerged and emergent macrophytes (e.g. *Rumex crispus, Alternanthera philoxeroides, Ludwigia spp, Rhynchathera spp, Ageratum spp, Hedychium coronarium, Eleocharis spp, Heteronthera spp, Polygonum spectable* and *Brachiaria arrecta*) at each site depending on the availability of the macrophytes. The macrophyte's whole stalk comprising of stalk and leaves was carefully removed from the stream. Periphyton was then removed from the macrophytes by brushing with a toothbrush adding distilled water. The resulting diatom suspensions from all the submerged macrophytes sampled were pooled to form a single sample, which was then put in a labelled plastic bottle. Epipelic and epipsammic diatoms were sampled by pressing a Petri dish lid into the top layer of sand or 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 that were then gently brought to the surfaces. The contents were then empted into a labelled container. Samples from five locations in each sampling reach were pooled into a single sample.

At each site, two bricks and four rough glass slides mounted on a rack (artificial substrates) were immersed in the water column, parallel to the current at a depth of 20 to 30 cm below the surface. The artificial substrates were secured by means of ropes and pegs. This first batch of artificial substrates was placed at all sites in September 2008 and sampled in October 2008. The second batch was placed at all sites in May 2009 and sampled in June 2009. In all the cases, the artificial substrate was left for 4 weeks, which is the recommended colonization time of periphyton (ROUND, 1991; DESCY and COSTE, 1991; KELLY et al., 1998). On sampling, the artificial substrates were carefully brought to the surface and thoroughly rinsed with filtered river water. Biofilms were collected by brushing material with a toothbrush. The resulting suspensions from the replicates were pooled.

In the laboratory, 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 and KILROY, 2000). Three replicate slides were prepared for each sample. A total of 250 – 600 valves per sample (based on counting efficiency determination method by PAPPAS and STOERMER (1996)) were identified and counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type - 020-519.503 LB30T, Germany). The mean and standard deviations of counting efficiencies of diatom communities calculated according to PAPPAS and STOERMER (1996) on different substrates were as follows: macrophytes, 82.5 ±11.4 %; sand, 86.1 ±7.6 %; stones, 83.6 ±18.5 %; silt/clay, 82.9 ±14.2 %; bricks, 76.4 ±15.3% and glass, 78.0 ±17.1 %. The diatoms were identified to species level based on studies by LOBO et al. (2002), METZELTIN et al. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007).

2.2.5 Data analysis

A pooled data set, consisting of diatoms sampled during four sampling periods, was used to investigate the effects of substrate type on diatom communities and spatial trends in the composition of diatom communities. Cluster analysis with unweighted pair-group average and Euclidian distance was performed based on pooled benthic diatom community data to show the main differences and similarities in community composition among the 6 substrates sampled and among the 10 sites sampled.

The available environmental data consisted of 45 environmental variables (Table 2.1). Environmental variables that were not normally distributed (Shapiro-Wilk, $p \leq 0.05$) or had no equal variance (Levene's test, $p \leq 0.05$) were transformed as follows: a) log transformation for BOD_5, COD, conductivity, TDS, TN, TP, NH_4^+, SO_4^{2-}, Na^+, K^+, Mg^{2+}, F^-, % silt-clay, % gravel, % cobble, % boldders, Cu and Fe; b) $\log(\chi + 1)$ transformation for PO_4^{3-}, % gravel-cobble, and % cobble-boldders and; c) arcsine transformation for DO, turbidity, NO_2^-, % sand, % silt-gravel, Cr, Co, Pb and Zn.

Two-way Analysis of Variance (Two-way ANOVA) was used to compare means of transformed environmental variables among the four sampling periods and among the three sites categories (section 2.2.2). No significant difference was observed in means of environmental variables among the four sampling periods (ANOVA, $p > 0.05$). This is expected since all sampling was confined to stable base flow period when variations in water chemistry are low compared to the rain season. Therefore, the mean environmental variables of the four sampling periods at each site were used for statistical analysis. Metal

levels at a given site are likely to have additive effects even at chronic concentration (GUASCH et al., 2009). A measure of total metal concentration in water, the cumulative criterion unit (CCU; CLEMENTS et al., 2000) which has already been used to analyse the response of different organisms to metals in streams (CLEMENTS et al., 2000; GUASCH et al., 2009) was therefore calculated and used as a representative of all metals.

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. Preliminary detrended correspondence analysis (DCA) was applied on diatom data set to determine the length of the gradient. This DCA revealed that the gradient was greater than three standard deviation units (3.9) justifying the use of unimodial ordination techniques (TER BRAAK and VERDONSCHOT, 1995). Thus, canonical correspondence analysis (CCA) was used to investigate relationships between water-quality variables and benthic diatom communities from different substrates.

A series of CCAs were run with one selected environmental variable at a time for each of the six substrates sampled. To select the environmental variables for use in these CCAs, Principal Components Analysis (PCA) based on correlation matrices (i.e., centred and standardized PCA that resulted in equal weighting of all taxa) was performed on environmental data from all the four sampling periods to determine important environmental gradients along which sampling sites vary. Most of the variables were highly positively correlated with each other and with the first and second axes (Figure 2.2). Six variables pH, Cr, BOD_5, Cl^-, COD and DO were selected based on inspection of their loadings with respect to first and second axes. A loading of 0.2, arbitrary and chosen for convenience, was used as a cut-off point.

The strengths of relationships between algal assemblages and environmental variables in the CCAs were assessed using the ratios of the first and second eigenvalues ($\lambda 1/\lambda 2$). In this case, the first axis (constrained) corresponds to the direction of greatest variability of the data set that can be explained by the environmental variable (TER BRAAK and PRENTICE, 1988), while the second axis is the first "residual" axis (PALMER, 2008). This ratio measures the strength of the constraining variable with respect to the first unconstrained gradient in the assemblage composition data. Large numbers indicate strong responses of algal assemblages to the environmental variable (TER BRAAK and PRENTICE 1988). The strength of relationship is considered very high if $\lambda 1/\lambda 2 > 1$, moderately high if $0.5 < \lambda 1/\lambda 2 < 1$, and weak if $\lambda 1/\lambda 2 < 0.5$. The total number of CCAs was 36 (6 substrates × 6 constraining variables). Kruskal-Wallis, with

Mann-Whitney pairwise comparisons and Bonferroni correction tests was used to compare λ1/ λ2 ratios among groups of CCAs.

Other six CCAs corresponding to six substrates sampled were performed to relate diatom community structure to simultaneous effects of predictor variables (TER BRAAK and VERDONSCHOT, 1995) and to determine whether algal communities on different substrates responded to the same environmental gradients. Preliminary CCAs in each case identified collinear variables and selected a subset on inspection of variance inflation factors (VIF< 20; TER BRAAK and PRENTICE, 1988). Monte Carlo permutation tests (999 unrestricted permutations, $p \leq 0.05$) were used to test the significance of the axis and hence determine if the selected environmental variables could explain nearly as much variation in the diatom data as all the 44 environmental variables combined.

The IndVal method (DUFRÊNE and LEGENDRE, 1997) was used to identify indicator species and species assemblages characterizing different 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. Kruskal-Wallis, with Mann-Whitney pairwise comparisons and Bonferroni correction tests was used to compare indicator values of species on different substrates.

Kruskal-Wallis with Mann-Whitney pairwise comparisons and Bonferroni correction, Levene's test, Shapiro-Wilk, Two-way ANOVA CCA, PCA and DCA were performed using PAlaeontological STatistics (PAST) software version 1.95 (HAMMER et al., 2009).

2.3 Results

2.3.1 Physical and chemical variables

The values of physical and chemical variables measured in the study area during the study period are shown in Table 2.1. The water quality generally tended to deteriorate downstream as the streams pass 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. However, the difference in pH among the three site categories (section 2.2.2) was not statistically significant (ANOVA, $p > 0.05$). Temperature increased downstream, but as in the case of pH, the increase was not significant (ANOVA, $p > 0.05$). On the other hand,

conductivity, BOD_5, COD, TDS, turbidity, TN, TP, most of metals and embeddedness increased significantly downstream (ANOVA, $p < 0.05$) while percentage of fine particles, DO and percentage riparian vegetation cover decreased significantly downstream (ANOVA, $p < 0.05$).

The concentrations of all the ions in water increased significantly downstream (ANOVA, $p < 0.05$) (Table 2.1). Chloride and alkaline earth metals especially Na^+ and Ca^{2+} were the dominant ions in the study area, while PO_4^{3-} and F^- were low at upstream sites and high at highly polluted downstream sites. As noted before, no significant differences were observed in means of environmental variables among the four sampling periods (ANOVA, $p > 0.05$). This is expected since all sampling was confined to stable base flow period when variations in water chemistry are low compared to the rain season.

From the PCA performed on environmental data from all the four sampling periods to determine important environmental gradients along which sampling sites vary, most of the variables were highly positively correlated with each other and with the first and second axes that accounted for 64 and 7 % variation respectively (Figure 2.2). DO and canopy cover were negatively related to the first axis. Sites 8, 9 and 10 were positively related to the first axis while the rest of the sites were generally negatively associated with this axis.

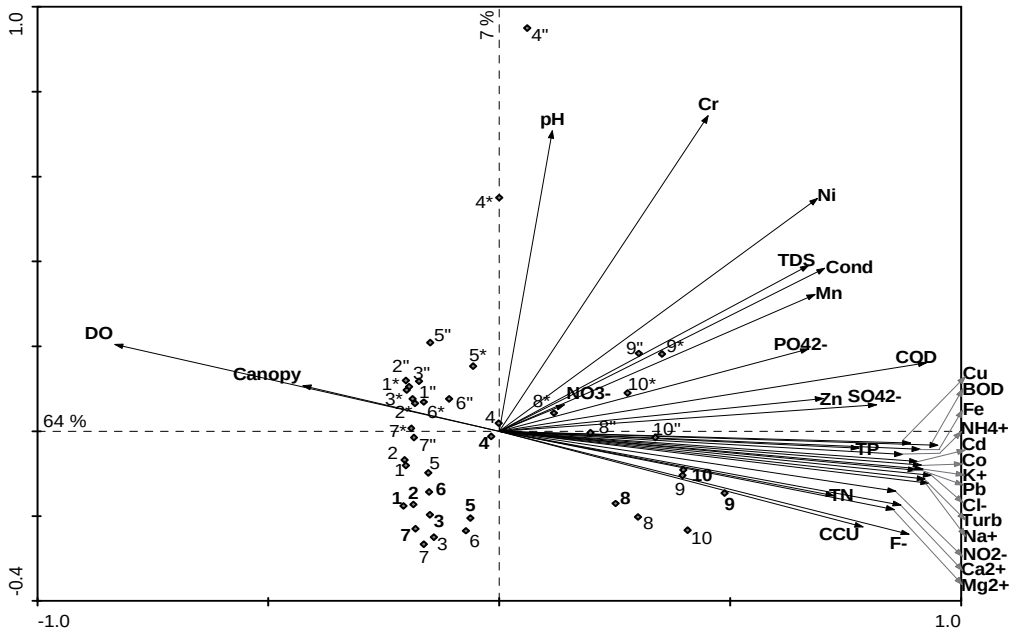

Figure 2.2: Principal Component Analysis (PCA) based on physical and chemical variables data from the 4 samplings; September 2008 (normal), October 2008 (bold), May 2009 (“) and June 2009 (*).

Table 2.1: The mean values of physical and chemical variables measured at 10 sites during 4 sampling periods. The sites are arranged in order of general increase in pollution based on physical and chemical variables and dotted line separates 'reference' sites from the rest of the sites.

Site	1	2	3	7	4	5	6	8	9	10
Temperature (^{o}C)	18.3 ±1.1	20.9 ±1.6	20.6 ±1.7	20.4 ±1.5	24.0 ±2.6	21.2 ±1.7	21.2 ±1.1	24.8 ±3.0	23.0 ±1.9	21.3 ±2.2
Altitude (m)	761	837	831	761	774	794	745	724	630	627
Canopy cover (%)	80	95	60	45	20	50	4	20	50	5
BOD_5 (mg.L^{-1})	0.9 ±0.2	1.0 ±1.2	2.6 ±0.2	1.2 ±2.3	6.9 ±0.2	1.6 ±0.6	7.2 ±1.1	19.5 ±1.1	24.5 ±1.2	26.2 ±1.3
COD (mg.L^{-1})	3.7 ±0.8	4.2 ± 1.3	12.3 ±4.2	4.8 ±0.7	83.3 ±14.5	6.3 ±0.5	11.7 ±1.5	54.0 ±19.9	103.0 ±3.2	103.0 ±22.7
DO (mg.L^{-1})	7.3 ±2.3	8.2 ±1.0	7.6 ±0.6	7.2 ±1.5	6.8 ±2.1	6.9 ±1.0	7.6 ±1.3	1.9 ±2.8	2.1 ±1.0	0.4 ±1.2
Conductivity (μS.cm^{-1})	45.0 ±7.5	20.0 ±10.5	53.0 ±9.5	30.0 ±4.0	28.0 ±7.7	89.0 ±8.9	103.0 ±6.4	715.0 ±22.3	322.0 ±191.3	283.0 ±201.7
pH	6.6 ±0.8	6.4 ±1.0	6.3 ±1.0	6.8 ±1.0	6.7 ±0.6	6.8 ±0.9	7.2 ±0.4	7.2 ±0.5	7.2 ±0.4	7.1 ±0.4
TDS (g.L^{-1})	29.4 ±0.5	13.4 ±1.2	22.6 ±3.2	19.3 ±0.2	18.1 ±1.4	57.4 ±3.4	66.5 ±2.9	457.8 ±27.1	206.1 ±11.1	182.0 ±34.1
Turbidity (NTU)	5.1 ±1.2	4.2 ±1.1	4.7 ±1.3	7.3 ±1.6	19.5 ±4.4	11.1 ±2.9	13.2 ±3.4	45.3 ±10.7	53.2 ±12.1	60.4 ±15.4
TN (mg.L^{-1})	0.65 ±0.3	0.18 ±0.3	0.24 ±1.4	0.93 ±0.7	1.72 ±0.5	1.29 ±0.4	1.41 ±0.5	38.32 ±8.3	14.87 ±4.1	10.17 ±2.9
TP (mg.L^{-1})	0.01 ±0.0	0.01 ±0.0	0.01 ±0.1	0.02 ±0.0	0.03 ±0.0	0.16 ±0.2	0.06 ±0.1	2.97 ±1.0	1.12 ±0.2	0.75 ±0.2
Nitrite (μg.L^{-1})	<10	<10	<10	<10	423.9 ±84.8	42.4 ±50.3	24.3 ±48.6	884.9 ±105.8	2036.6 ±391.9	3164.9 ±641.7
Nitrate (μg.L^{-1})	51.9 ±22.3	195.6 ±33.1	470.0 ±38.5	98.2 ±8.8	714.8 ±232.3	819.6 ±667.2	745.0 ±595.3	1141.2 ±1025.4	176.5 ±142.5	441.1 ±273.2
Ammonium (μg.L^{-1})	11.8 ±3.6	116.1 ±23.5	11.9 ±3.7	15.0 ±3.1	858.9 ±175.5	418.8 ±401.9	141.4 ±128.2	1361.6 ±783.1	4610.8 ±2482.1	3310.5 ±703.0
Phosphate (μg.L^{-1})	15.0 ±17.8	<2	2.4 ±2.9	<2	136.2 ±49.3	26.7 ±14.3	7.7 ±9.1	21.4 ±5.0	132.5 ±51.8	190.8 ±131.2
Sulphate (mg.L^{-1})	0.1 ±0.1	1.5 ±1.0	1.1 ±0.3	0.3 ±0.1	8.3 ±1.8	4.9 ±3.1	3.1 ±1.3	3.7 ±0.9	15.1 ±3.9	9.5 ±3.9
Fluoride (μg.L^{-1})	38.3 ±12.1	45.8 ±14.7	67.5 ±40.5	43.7 ±14.8	88.8 ±28.4	95.7 ±66.5	124.7 ±88.3	287.5 ±87.3	305.4 ±93.2	262.9 ±104.3
Chloride (mg.L^{-1})	2.0 ±0.4	4.9 ±1.0	4.1 ±1.7	2.8 ±0.6	15.5 ±5.9	7.3 ±4.8	6.8 ±4.8	19.8 ±8.3	21.7 ±4.2	30.0 ±7.0
Sodium (mg.L^{-1})	2.0 ±0.8	2.3 ±0.6	2.4 ±1.2	2.2 ±0.6	7.5 ±1.5	4.1 ±2.3	4.1 ±2.6	12.4 ±6.5	15.3 ±2.9	19.7 ±4.2
Potassium (mg.L^{-1})	1.0 ±0.2	0.6 ±0.2	1.0 ±0.3	0.7 ±0.1	2.3 ±0.6	1.3 ±0.9	0.9 ±0.4	2.4 ±1.0	4.3 ±1.2	3.8 ±0.8
Magnesium (mg.L^{-1})	0.7 ±0.1	0.6 ±0.1	0.8 ±0.2	0.7 ±0.1	1.2 ±0.3	1.2 ±0.6	1.0 ±0.5	1.7 ±0.3	2.5 ±0.7	1.6 ±0.3
Calcium (mg.L^{-1})	1.4 ±0.3	1.9 ±0.4	1.3 ±0.2	2.4 ±0.5	3.9 ±1.7	5.7 ±3.5	4.5 ±2.1	7.4 ±1.5	11.9 ±2.8	8.2 ±1.6
Depth (m)	0.2 ±0.02	0.3 ±0.08	0.4 ±0.10	0.4 ±0.01	0.2 ±0.05	0.4 ±0.08	0.3 ±0.05	0.5 ±0.07	0.3 ±0.04	0.3 ±0.01
Velocity (m.s^{-1})	2.5 ±1.3	2.8 ±1.4	2.6 ±1.3	2.9 ±1.1	2.2 ±1.1	2.7 ±1.2	1.4 ±0.6	3.5 ±1.8	2.4 ±0.9	2.34 ±1.0
Embeddedness	0	0	1	1	2	3	1	5	1	4
Silt-Clay (%)	95	95	10	50	30	15	10	5	10	10
Sand (%)	2	0	90	80	60	50	90	10	90	10
Gravel (%)	0	2	3	5	40	5	5	5	3	15
Cobble (%)	0	2	4	5	10	20	5	5	5	50
Boulders (%)	0	3	0	2	0	5	2	10	0	60
Silt-gravel (%)	95	97	95	85	85	80	83	35	95	40
Gravel-Cobble (%)	0	2	5	10	10	15	10	50	5	50
Cobble-Bolder (%)	0	1	0	5	5	10	7	60	0	70
Cr (mg.L^{-1})	0.01 ±0.001	0.01 ±0.001	0.01 ±0.002	0.00 ±0.001	0.01 ±.003	0.02 ±0.001	0.01 ±0.002	0.03 ±0.001	0.03 ±0.002	0.03 ±0.002
Cu (mg.L^{-1})	0.004 ±0.001	0.004 ±0.001	0.005 ±0.001	0.004 ±0.000	0.006 ±0.000	0.004 ±0.001	0.009 ±0.003	0.0225 ±0.002	0.021 ±0.002	0.024 ±0.002
Mn (mg.L^{-1})	0.01 ±0.01	0.03 ±0.01	0.04 ±0.00	0.02 ±0.01	0.06 ±0.04	0.03 ±0.02	0.01 ±0.01	0.04 ±0.02	0.20 ±0.02	0.07 ±0.01
Fe (mg.L^{-1})	3.04 ±1.2	0.35 ±0.11	0.50 ±0.01	0.43 ±0.04	0.26 ±0.01	0.48 ±0.06	0.29 ±0.03	0.45 ±0.06	1.02 ±0.11	0.79 ±0.16
Co (mg.L^{-1})	0.01 ±0.001	< 0.01	0.02 ±0.002	< 0.01	0.02 ±0.003	0.01 ±0.001	0.02 ±0.001	0.02 ±0.004	0.02 ±0.005	0.02 ±0.003
Ni (mg.L^{-1})	0.01 ± 0.001	0.01 ± 0.002	0.01 ±0.001	< 0.01	0.01 ±0.003	0.01 ±0.007	0.02 ±0.005	0.02 ±0.004	0.02 ±0.009	0.03 ±0.0008
Cd (mg.L^{-1})	< 0.001	< 0.001	0.001 ±0.001	< 0.001	0.001 ±0.001	0.002 ±0.001	0.002 ±0.001	0.002 ±0.002	0.005 ±0.002	0.004 ±0.001
Pb (mg.L^{-1})	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02 ±0.005	0.01 ±0.002	0.02 ±0.004	0.01 ±0.005	0.02 ±0.1
Zn (mg.L^{-1})	0.02 ±0.002	0.01 ±0.001	0.03 ±0.007	0.02 ±0.001	0.04 ±0.007	0.01 ±0.009	0.02 ±0.001	0.23 ±0.01	0.02 ±0.01	0.03 ±0.009
Al (mg.L^{-1})	< 0.01	< 0.01	< 0.01	< 0.01	< d	0.21 ±0.1	< 0.01	< 0.01	0.51 ±0.3	< 0.01
CCU	5.2 ±1.4	3.35 ±0.8	7.7 ±2.7	0.85 ±1.1	9.86 ±3.4	14.44 ±4.2	13.74 ±1.4	17.66 ±2.9	32.95 ±5.5	27.48 ±4.4

2.3.2 Community analysis

A total of 208 diatom species belonging to 63 genera that are distributed among the families Achnanthidiaceae, Achnanthaceae, Bacillariaceae, Eunotiaceae, Cymbellaceae, Gomphonemataceae, Fragilariaceae, Melosiraceae, Naviculaceae, Rhoicospheniaceae, Rhopalodiaceae and Surirellaceae were recorded. Of the 208 species observed, 71 species were considered the most frequent in the study area (≥ 5% occurrence and present in at least 2 substrates from all sampling sites, POTAPOVA and CHARLES, 2005; Table 2.2). These 71 species made up 88.6 % of the overall diatom community.

Based on Cluster analysis carried out to show the differences and similarities in community composition among the 6 substrates sampled and among the 10 sites sampled, 3 major groups of sites were observed (Figure 2.3). The separation can be attributed to pollution; group 1 and 2 were characterized by diatom communities growing on natural and artificial substrates from highly polluted sites 8, 9 and 10. Diatom communities growing on all natural substrates from site 6 were also included in group 2 whilest communities from this site growing on artificial substrates were placed in group 3. Group 3 was characterized by diatom communities growing on natural and artificial substrates from moderately and less polluted sites 1, 2, 3, 4, 5 and 7. Sites previously designated as reference and moderately polluted (Section 2.2.2) were not clearly separate. Benthic diatom communities from different substrates sampled on the same site were generally similar as they were grouped close to each other in most cases.

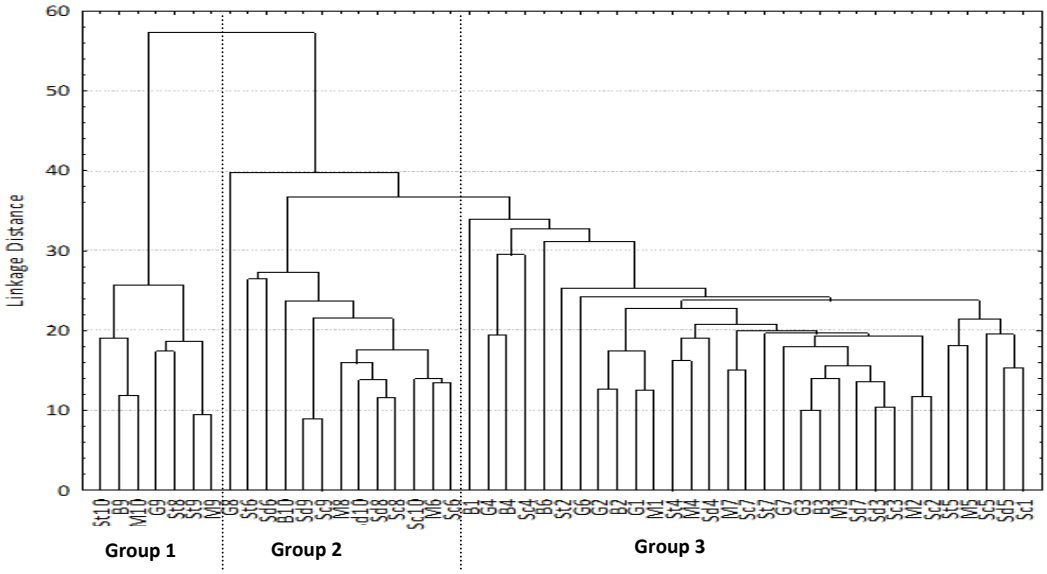

Figure 2.3: A cluster analysis based on pooled benthic diatom community data sampled at 10 sampling sites from different substrates during the 4 samplings. Some natural substrates were not available on all the sampling sites, while there were loses of some artificial substrates at some site. Sd = sand, St = stones, Sc = silt-clay, M = macrophytes, B = bricks and G = glass.

2.3.3 Species distribution

Indicator species analysis showed that common diatom species were not restricted to a single substrate. Indicator values ranged from 21.1 to 65.9 % in this study (Table 2.2). 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. For example, species such as *Pleurosigma compactum* Greville, *Cyclotella stelligera* (Cleve & Grunow) Van Heurck, *Hantzschia amphioxys* (Ehrenberg) Grunow and *Thalassiosira weissflogii* (Grunow) Fryxell & Hasle had highest indicator values on silt/clay substrate. Species such as *Planothidium dubium* (Grunow) Round & Bukhtiyarova, *Sellaphora pupula* (Kützing) Mereschkowsky, *Surirella angusta* Kützing and *Pinnularia braunii* (Grunow) Cleve had highest indicator values on sand. Species such as *Gomphonema augur* (Ehrenberg) Lange-Bertalot, *Pinnularia microstauron* Patrick and Reimer and *Meridion anceps* (Ehrenberg) Williams had highest indicator values on macrophytes. Species such as *Surirella robusta* Enrenburg, *Surirella linearis* Smith and *Fallacia monoculata* (Hustedt) Mann had highest indicator values on stones. On the other hand, species such as *Planothidium lanceolatum (*Brébisson) Grunow, *Nitzschia scalaris* (Kütz) Grunow, *Gomphonema turris* Ehrenberg and *Luticola goeppertiana* (Bleisch) Mann had highest indicator values on artificial substrates. Preference was generally high for natural compared to artificial substrates though the difference was not statistically significant (Kruskal-Wallis, $p > 0.05$). More species tended to prefer macrophytes substrate compared to other substrates.

2.3.4 Community structure on different substrates in relation to environmental gradients

The strengths of relationships between algal communities and selected environmental variables ($\lambda 1/ \lambda 2$ ratios) were not significantly different (Kruskal-Wallis, $p > 0.05$) among the substrates (Figure 2.4). However, $\lambda 1/ \lambda 2$ ratios were highest on bricks and macrophytes and lowest on silt-clay substrate. The strength of relationships between benthic diatom communities and selected environmental variables (section 2.2.5) was generally high for BOD_5, DO, COD and Cl^-, variables that were highly associated with the first PCA axis (Figure 2.2). The relationship was week for pH and Cr, variables that were associated with the second PCA axis.

Table 2.2: The most frequently occurring diatom taxa with the highest indicator values (%) in the different substrates sampled.

		Substrate type					
Species	Code	Macrophytes	Sand	Silt/Clay	Stones	Bricks	Glass
Achnanthes exigua Grunow	Aexi						39.4
Achnanthidium biosolettianum Grunow	Abia	36.0					
Achnanthidium minutissimum (Kützing) Czarnecki	Amin		23.3				
Amphora copulate (Kützing) Schoeman & Archibald	Acop	45.5					
Aulacoseira agassizii (Hustedt) Simonsen	Aaga	30.0					
Aulacoseira alpigena (Grunow) Krammer	Aalp			29.6			
Aulacoseira ambigua (Grunow) Simonsen	Aamb				38.6		
Aulacoseira distans (Ehrenberg) Simonsen	Adis		23.6				
Aulacoseira granulate (Ehrenberg) Simonsen	Agra		34.3				
Caloneis hyaline Hustedt	Chya				36.2		
Craticula cuspidata (Kützing) Mann	Ccus		38.3				
Cyclotella meneghiniana Kützing	Cmen			41.4			
Cyclotella pseudostelligera Hustedt	Cpse			34.5			
Cyclotella spp	Cspp		31.8				
Cyclotella stelligera (Cleve & Grunow) Van Heurck	Cste	39.6					
Cymbopleura naviculiformis (Auerswald) Krammer	Cnav	29.9	26.7				
Diatoma spp	Dspp						
Encyonema neomesianum Krammer	Eneo			24.7			
Encyonema silesiacum (Bleisch) Mann	Esil	31.5					
Eunotia intermedia (Krasske & Hustedt) Nörpel & Lange-Bertalot	Eint					29.2	
Eunotia papillo(Ehrenberg) Hustedt	Epop	36.4					
Eunotia pectinalis (Kützing) Rabh	Epec					26.3	
Eunotia sudetica Müller	Esud	37.8					
Fallacia monoculata (Hustedt) Mann	Fmon				55.4		
Fragilaria capucina Desmaziéres	Fcap					40.5	
Fragilaria intermedia Grunow	Fint		26.3				
Frustulia rhomboides (Rabenhorst) De Toni	Frho						32.0
Frustulia saxonica Rabenhorst	Fsax						24.8
Frustulia vulgaris (Thwaites) De Toni	Fvul					33.1	
Gomphonema accuminatum Ehrenberg	Gacc				24.6		
Gomphonema angustatum (Kützing) Rabenhorst	Gang					36.5	
Gomphonema augur (Ehrenberg) Lange-Bertalot	Gaug			60.7			
Gomphonema gracile Ehrenberg	Ggra						30.9
Gomphonema olivaceum (Lyngbye) Kützing	Goli					28.0	
Gomphonema parvulum (Kützing) Cleve	Gpar						19.2
Gomphonema turris Ehrenberg	Gtur						48.5
Hantzschia amphioxys (Ehrenberg) Grunow	Hamp	37.4					
Luticola goeppertiana (Bleisch) Mann	Lgeo					43.9	
Melosira varians Agardh	Mvar				21.2		
Meridion anceps (Ehrenberg) Williams	Manc			36.8			
Meridion constrictum Ralfs	Mcon		16.7				
Navicula cryptocephala (Grunow) Cleve	Ncry	21.7					
Navicula cryptotenella Lange-Bertalot	Ncrt	21.1					
Navicula oblonga (Kützing) Kützing	Nobl		22.9				
Navicula radiosa Kützing	Nrad			23.9			
Navicula rostellata Kützing	Nros				42.9		
Neidium affine (Ehrenberg) Pfitzer	Naff	24.0					
Neidium ampliatum (Ehrenberg) Krammer	Namp		33.2				
Nitzschia linearis (Agardh) Smith	Nlin	26.4					
Nitzschia palea (Kützing) Smith	Npal				25.8		
Nitzschia scalaris (Kütz) Grunow	Nsca						50.8
Nupela praecipua (Reichardt) Reichardt	Npra	35.6					
Pinnularia braunii (Grunow) Cleve	Pbra		39.9				
Pinnularia divergens Krammer	Pdiv						35.3
Pinnularia gibba (Ehrenberg) Grunow	Pgib	24.1					
Planothidium lanceolatum (Brébisson) Grunow	Plan					45.9	
Pinnularia lata (Brébisson) Smith	Plat						41.5
Pinnularia microstauron Patrick and Reimer	Pmic			41.3			
Pinnularia subcapitata Gregory	Psub				24.2		
Planothidium dubium (Grunow) Round & Bukhtiyarova	Pdub		46.5				
Pleurosigma compactum Greville	Pcom	50.8					
Psammothidium subatomoides (Hustect) Bukhtiyarova and Round	Psub			24.6			
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	Rabb			25.3			
Sellaphora pupula (Kützing) Mereschkowsky	Spup		41.0				
Stauroneis phoenicenteron Ehrenberg	Spho	32.6					
Surirella angusta Kützing	Sang		34.8				
Surirella linearis Smith	Slin				43.8		
Surirella ovata Kützing	Sova	58.3					
Surirella robusta Enrenburg	Srob				65.9		
Ulnaria ulna (Nitzsch) Compère	Suln				27.9		
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	Twei	38.0					
Total		**19**	**14**	**10**	**11**	**8**	**9**

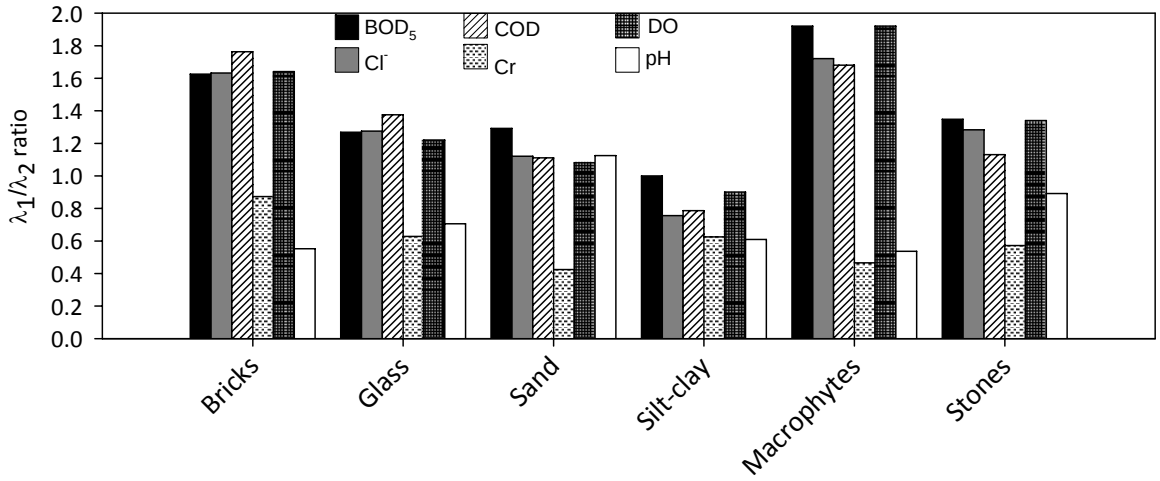

Figure 2.4: Strengths of relationships between benthic diatom communities from different substrates and BOD_5, COD, Cr, DO and pH expressed as the ratio of 1st and 2nd eigenvalues (λ1/ λ 2) in CCAs carried out with single constraining variables.

The six CCAs corresponding to six substrates sampled performed to relate diatom community structure to simultaneous effects of predictor variables explained approximately 50 % of the diatom species variance in all cases. Monte Carlo unrestricted permutation test indicated that axis 1 (999 permutations) and axis 2 (999 permutations of axis 2 with axis 1 as a covariable) were statistically significant ($p \leq 0.05$) in all cases. CCAs for natural substrates clearly separated highly polluted sites (8, 9 and 10) from the rest of the sites, though in case of silt-clay substrate and macrophytes, site 6 was grouped together with highly polluted sites. CCAs for artificial substrates also roughly separated highly polluted sites from the rest of the sites, though in the case of glass substrate, the separation was not very clear. In all the CCAs, as in the case of cluster analysis results (section 2.3.2), sites previously designated as reference and moderately polluted (Section 2.2.2) were difficult to separate. This is expected since human activities, in one way or the other, have altered most of the lotic systems around urban areas in Brazil, with pristine or reference sites almost nonexistent (RÖRIG et al., 2007).

In these CCAs, sampling sites were associated to different environmental variables and the species associated to different sites differed from on substrate to another. In CCA based on communities growing on sand, sampling sites were more associated to BOD_5, DO, metals represented by CCU, and percentage fine particles compared to other variables (Figure 2.5a). Highly polluted sites in this CCA were associated with such species as *Nitzschia palea* (Kützing) Smith, *P. lanceolatum*,

Caloneis hyaline Hustedt, *Cyclotella meneghiniana* Kützing, *C. stelligera*, *Cyclotella* spp, *F. monoculata*, *Fragilaria intermedia* Grunow and *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot.

In CCA based on communities growing on silt-clay, sampling sites were more associated to BOD_5, COD, pH, turbidity, and percentage fine particles compared to other variables. In this case, site six was grouped together with highly polluted sites 8, 9 and 10 (Figure 2.5b). Highly polluted sites in this CCA were associated with such species as *N. palea*, *C. meneghiniana*, *Cyclotella pseudostelligera* Hustedt, *P. microstauron* and *S. pupula*. In CCA based on communities growing on stones, sampling sites were more associated to BOD_5, pH, and percentage fine particles and gravel compared to other variables (Figure 2.5c). Highly polluted sites in this CCA were associated with such species as *N. palea*, *C. hyaline*, *F. monoculata*, *L. goeppertiana*, *Pinnularia subcapitata* Gregory, *Gomphonema parvulum* (Kützing) Cleve, *Gomphonema accuminatum* Ehrenberg, *Nupela praecipua* (Reichardt) Reichardt and *Achnanthes exigua* Grunow. In CCA based on communities growing on macrophytes, sampling sites were more associated to BOD_5, conductivity, pH, and percentage riparian vegetation cover compared to other variables (Figure 2.5d). Highly polluted sites in this CCA were associated with such species as *N. palea*, *F. monoculata*, *L. goeppertiana*, *G. parvulum*, *A. exigua*, *P. lanceolatum*, *N. praecipua* and *Craticula cuspidata* (Kützing) Mann.

In CCA based on communities growing on bricks, sampling sites were more associated to BOD_5, COD, conductivity and percentage riparian vegetation cover (Figure 2.5e). Highly polluted sites in this CCA were associated with such species as *N. palea* (Kützing) Smith, *F. monoculata*, *L. goeppertiana*, *P. subcapitata*, *A. exigua*, *P. lanceolatum*, *C. hyaline* and *C. meneghiniana*. In CCA based on communities growing on glass, sampling sites were more associated to BOD_5, Mg^{2+} and PO_4^{3-} (Figure 2.5f). Highly polluted sites in this CCA were associated with such species as *N. palea*, *P. subcapitata*, *P. lanceolatum*, *S. pupula* and *G. parvulum*.

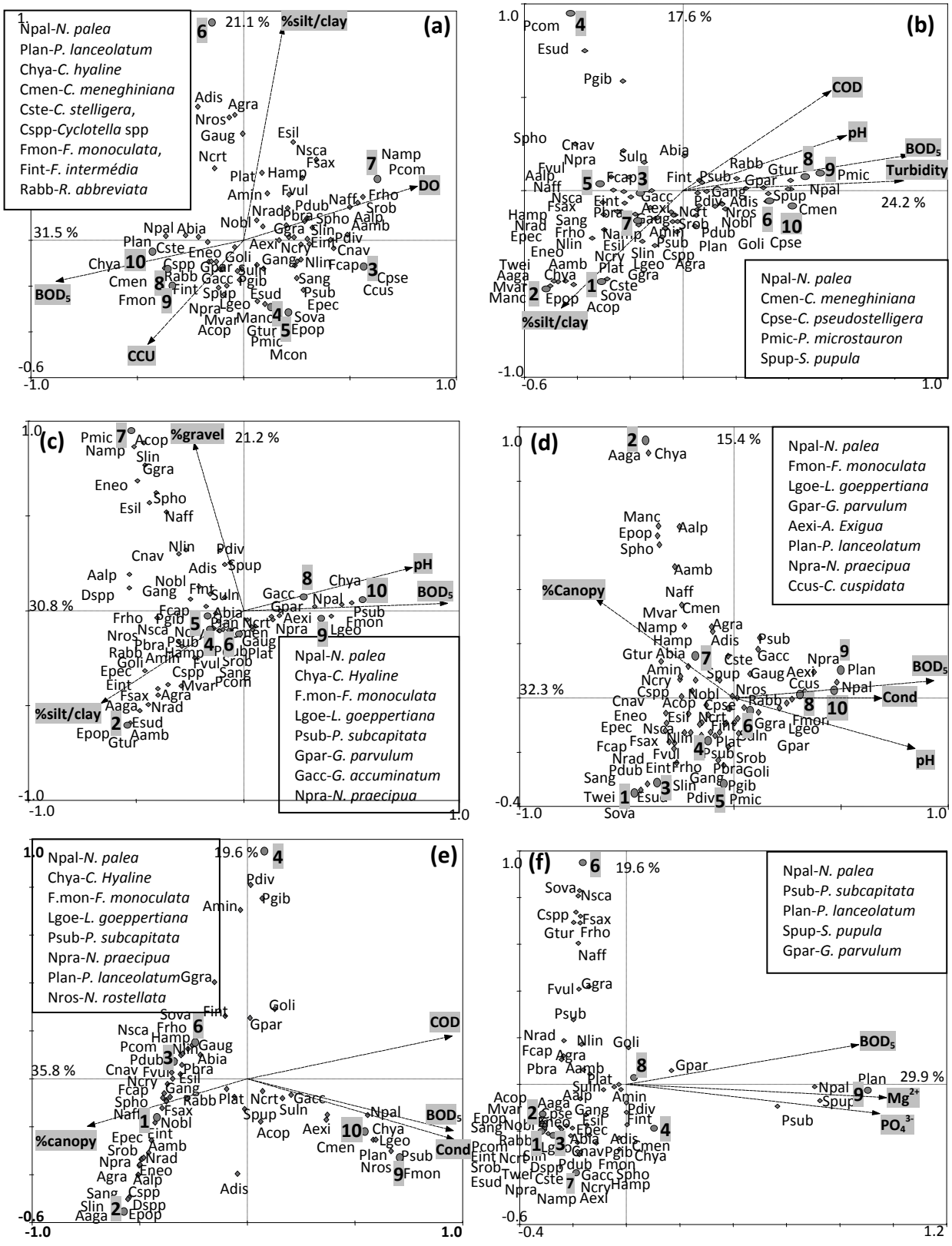

Figure 2.5: Canonical correspondence analysis (CCA) diagrams showing most frequently occurring diatom taxa on **(a)** sand,**(b)** silt-clay, **(c)** stones, **(d)** macrophytes, **(e)** bricks and **(f)** glass in relation to selected environmental variables in the ordination space of the 1st and 2nd axes. Taxa codes for species associated with highly polluted sites are indicated while the rest correspond to those in Table 2.2. Cond = conductivity.

2.4 Discussion

2.4.1 Suitability of substrates for multivariate-based water quality assessment techniques

Based on cluster analysis, benthic diatom communities from different substrates sampled on the same site were generally similar as they were grouped close to each other in most cases. The power of statistical tests depends on the magnitude of the effect under consideration. An existing effect may not be detected if it is subtle (POTAPOVA and CHARLES, 2005). This could be the reason why between-substrate differences in benthic diatom communities were not detected in this study. It could also be that roles of other factors such as differences in hydrology and chemical history among sites become more important than the role of substrates (SOININEN and ELORANTA, 2004). In this study, the effects of pollution seemed to be more important in determining benthic diatom community structure compared to the effects of substrate.

However, more species tended to prefer macrophytes substrate compared to other substrates as indicated by their highest indicator values in this substrate thought the preference was not significant (Table 2.2). Traditionally stones have been the preferred substrate for sampling during water quality assessment exercises using benthic diatoms (DESCY and COSTE, 1991; ROUND, 1991; PAN et al., 1996; BIGGS and KILROY 2000; DUONG et al., 2006; LOWE and PAN, 1996; KELLY et al., 1998). Values of some diatom indices indeed varied with substrate type in Finnish rivers, being highest, indicating best water quality, for samples collected from macrophytes, slightly low for samples from stones, and lowest for samples from soft-sediment samples (ELORANTA and ANDERSSON, 1998). However, the statistical significance of these differences was not established. Studies by PORTER et al. (1993), LOWE and PAN (1996) and KELLY et al. (1998) noted that there is no consensus concerning the comparison between water quality assessment based on epiphytic and epilithic communities while SOININEN and ELORANTA (2004) have collected evidence that cautions against it.

Thought no indices were used in this study, based on CCAs performed with on variable at a time, the strength of relationship between benthic diatom communities and chosen water quality variables ($\lambda 1/ \lambda 2$ ratios) did not differ significantly among substrates. As noted earlier on, this similarity in the relationships of diatom communities on different substrates to water quality variables can be attributed to the overriding effects of chemistry and other (unquantified) factors that masked the influence of substrate. This is supported by JÜTTNER et al. (1996) and ROTHFRITZ et al. (1997) who found that

epiphytic and epilithic diatom assemblages in Nepalese streams varied similarly along water chemistry gradients because substrate influence was negligible compared to these factors. However, the strength of relationship between diatom community structure and water quality variables was generally low on silt-clay compared to other substrates. This is expected because algae on fine-grained sediments are more influenced by sediment-bound chemicals, and are affected only weakly by water column chemistry (WETZEL, 1983; KELLY et al., 1998).

All the six CCAs carried out to investigate the simultaneous effects of environmental variables on communities growing on different substrates roughly separated highly polluted sites 8, 9 and 10 from the rest of the sites, except in the case of glass substrate where the separation was poor. Benthic diatom-based water quality inference models developed from data sets representing different substrates by POTAPOVA and CHARLES (2005) did not differ significantly in their ability to infer water chemistry. PAN et al. (1996) compared performance of inference models based on diatom assemblages from erosional and depositional habitats in Appalachian streams. The predictive powers of their WA inference models based on pH and TP were approximately the same for both habitats. The ability of the CCAs performed using four natural and 2 artificial substrates to separate highly polluted sites from the rest of the sites in this study indicates that all are equally useful for water quality assessment. Other techniques based on species autecologies, such as metrics or indices, are, in fact, simplified inference models. Therefore, they should provide similar accuracy of water-quality assessment regardless of the substrates from which they were collected (POTAPOVA et al., 2004).

Since all the CCAs produced similar results and diatom communities were also similar among substrates in this study, results of multivariate-based water quality assessment based on different substrates appear interchangeable. In the light of these results, only one sample has to be collected at each site for water quality assessment surveys. The sample can come from whatever substrate is available. This suggestion is supported by other studies in which values of trophic and saprobic diatom indices did not differ whether they were derived from epilithon, epipelon, or epiphyton (ROTT et al., 1998; KITNER and POULÍ-ČKOVÁ, 2003).

While sampling standard substrates is a desirable way to eliminate the possible influence of substrate, a single preferred substrate type may not be available at all sites as in the case of some of the sites sampled where no stone could be encountered. In such cases, any single available habitat should be sampled at each site, maintaining uniformity

as much as possible. Thus, resources should be invested in collecting single samples from as many sites as possible, rather than in sampling multiple substrates from fewer sites. The choice of substrate sampled should not affect accuracy of multivariate-based water quality assessments.

2.4.2 Comparison on natural and artificial substrates

Generally, more species had highest indicator values on natural substrate compared to artificial substrate, though the differences were not statistically significant. In addition, CCA carried out to investigate the simultaneous effects of environmental variables on communities growing on glass substrate poorly separated highly polluted sites compared to the rest of the other substrates. 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 species found on the glass substrate were mostly those with a tight attachment habit. This is likely to affect 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. However, in this study, artificial substrates were equally related to selected environmental variables (Figure 2.4) as in the case of natural substrates. In situations where it is difficult to encounter one substrate among sampling sites and variation in community structure are expected as other studies have demonstrated (e.g., LOWE and PAN, 1996; KELLY et al., 1998), the use of artificial substrate can be an alternative option with the advantage that substratum is standard at all sampling sites and time of exposure can be controlled (ROUND, 1991).

However, KOMÁREK and SUKACOVÁ (2004) have shown that introduced artificial substrates are often characterized by diatom communities indicative of more successional processes than water quality. 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 natural substrates. Besides, use of artificial substrate requires apparatus to be fixed in the river and there are often losses, as in this case, and random sampling is not possible (ROUND, 1991; DESCY and COSTE, 1991). This further complicates the use of artificial substrate for water quality

management. Sampling of natural substrates is thus highly recommended compared to artificial substrates.

2.4.3 Community structure in relationship to environmental variables

From the PCA results (Figure 2.2), most of the variables were highly positively correlated with each other and with the first axis with DO and canopy cover being negatively related to this axis. Highly polluted sites 8, 9 and 10 were positively related to the first axis while the rest of the sites were generally negatively associated with this axis. Thus, a gradient of increasing metal and organic pollution, eutrophication, ionic strength and other variables was observed from the agricultural/forested area to the urban area. The upstream sites were oligotrophic, with low levels of organic pollution, and had a high percentage canopy cover. The downstream sites were hypereutrophic, with higher levels of organic pollution, and had a low percentage canopy cover. Diatom community structure closely reflected this gradient of increasing pollution, with communities from highly polluted sites being different from other communities (Figure 2.3). Thus, the effects of metal and organic pollution, eutrophication, ionic strength and other variables were integrated into overall resultant benthic diatom communities making it difficult in this study to separate the effects of these variables on diatom communities.

Different species of diatoms responded differently to metal and organic pollution and eutrophication, ionic strength and other environmental variables because of differences in tolerance developed. As pollution increased, low or moderate pollution tolerant species were replaced by high pollution tolerant species such as *P. lanceolatum*, *A. exigua*, *C. hyaline*, *C. meneghiniana*, *C. stelligera*, *C. pseudostelligera*, *Cyclotella* spp, *G. accuminatum*, *G. augur*, *G. parvulum*, *F. monoculata*, *F. intermedia*, *L. goeppertiana*, *C. cuspidata N. palea, N. praecipua*, *P. microstauron*, *P. subcapitata*, *R. abbreviata* and *S. pupula*.

This group of species is known to be resistant to organic and heavy metal pollution (ROUND, 1991; VAN DAM et al., 1994; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003; DUONG et al., 2006). These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated with high electrical conductivity (ROUND, 1991; VAN DAM et al., 1994, BERE and TUNDISI 2009, 2010). Numerous studies conducted on lotic benthic diatoms sampled in various countries have shown the influence of organic pollution, eutrophication, and dissolved oxygen in structuring of diatom communities (ROUND, 1991; VAN DAM et al., 1994;

KELLY and WHITTON, 1995; KOBAYASI and MAYAMA, 1998; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003; LOBO et al., 1996, 2004). Environmental monitoring studies in Southern Brazil (e.g. OLIVEIRA et al., 2001; LOBO et al., 1996, 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.

C. meneghiniana, C. stelligera and *C. pseudostelligera* have been recorded in low and moderate dissolved oxygen levels and organically polluted environments (VAN DAM et al., 1994) with *C. pseudostelligera* being more sensitive to pollution compared to the other two (KOBAYASI and MAYAMA, 1998; LOBO et al., 1996). *L. goeppertiana, A. exigua* and *P. lanceolatum* are tolerant of organic pollution, eutrophication and low concentrations of DO (KOBAYASI and MAYAMA, 1998; LOBO et al., 1996, 2002; VAN DAM et al., 1994). *F. monoculata* has been described as characteristic of high organic pollution and eutrophic environments (Van Dam et al. 1994). *S. pupula* has also been described as characteristic of high organic pollution and eutrophic environments (LANGE-BERTALOT, 1979; VAN DAM et al., 1994; KOBAYASI and MAYAMA, 1998). *P. microstauron* is tolerant of eutrophication and organic pollution (VAN DAM et al., 1994).

Many studies describe *N. palea* (whose relative abundance was high at highly polluted sites) as cosmopolitan, high pollution tolerant species, especially to eutrophication (e.g. LANGE-BERTALOT, 1979; KOBAYASI and MAYAMA, 1989; LOBO et al., 1996). The success of this species in eutrophic conditions has been attributed to obligate nitrogen heterotrophy, which is common in some *Nitzschia* species (KILHAM et al., 1986). This in addition, would help them overcome the problem associated with low N: P ratios. This species as well as *G. parvulum* recorded at highly polluted site with high chloride levels in this study, often become dominant in streams with maintenance water depending on treated sewage rich in chlorine (FUKUSHIMA et al., 1994). DICKSON et al. (1977) suggested that many chlorine-tolerant organisms are also tolerant of other types of stressed environment. This could also apply to diatoms though the mechanisms are not yet fully understood. *N. palea* and G. parvulum have also been shown to be tolerant to metal pollution (GOLD et al., 2003; MORIN et al., 2008; DUONG et al., 2010).

Studies carried out in rivers of Japan by KOBAYASI and MAYAMA (1986) classified *G. parvulum* (whose relative abundance was high at highly polluted sites) as

highly tolerant to eutrophication, which is in agreement with the results of the current study. Similarly, KELLY and WHITTON (1995), working in rivers of UK described this species as highly tolerant to eutrophication (indicative value = 3 and sensitivity value = 5) in their calculation of the Trophic Diatom index (TDI). This has also been described as an indicator of high organic pollution, low concentrations of DO and eutrophication (VAN DAM et al., 1994; LOBO et al., 2002). *G. augur* has been classified as an indicator of eutrophic environments (GUZKOWSKI and GASSE, 1990), but according to LOBO et al. (2002), this species is sensitive to organic pollution.

Working on epipelic diatoms in the streams of Argentina in a similar environment as in this study, LICURSI and GÓMEZ (2001) associated *R. abbreviata* with levels 0 to II of their Pampean Diatom Index (IDP) i.e. unpolluted to moderately polluted respectively. This species has also been frequently reported in rivers in Japan where it is classified as sensitive to pollution (KOBAYASI and MAYAMA, 1989; ASAI and WATANABE, 1995).

The ecological preferences of species associated with highly polluted sites in this study are in agreement with the above discussion. LANGE-BERTALOT (1979) stated that species are indicative of the upper limits of pollution that they can tolerate and not the lower limit. Thus, the species that develop well in polluted zones may also occur in fairly clean water. Their value as indicators is their presence in polluted water.

2.4.4 Conclusion

Only one substrate has to be collected at each site for multivariate-based water quality assessment surveys, thus, avoiding unnecessary expensive and time-consuming over sampling which retards data processing leading to delays in getting the desired results for water quality management. Given the limitations of artificial substrates, sampling on natural substrates is highly recommended. Certain species like *P. lanceolatum*, *A. exigua*, *C. hyaline*, *C. meneghiniana*, *C. stelligera*, *C. pseudostelligera*, *Cyclotella* spp, *G. accuminatum*, *G. augur*, *G. parvulum*, *F. monoculata*, *F. intermedia*, *L. goeppertiana*, *C. cuspidata N. palea, N. praecipua*, *P. microstauron*, *P. subcapitata*, *R. abbreviata* and *S. pupula* associated with the gradient extremes for metal and organic pollution, eutrophication and ionic strength may be used in future studies as potential indicator species for these variable changes. These species can be subjected to further experiments to confirm their status of indicator species. The information gained through this study augments previous works on the use of diatoms for water quality assessment in

streams in other regions and is a stepping-stone towards development of diatom-based biological monitoring protocols for tropical streams.

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CHAPTER 3

Influence of ionic strength and conductivity on benthic diatom communities in a tropical river (Monjolinho), São Carlos-SP, Brazil[3]

Abstract: Benthic diatoms are important indicators of ecological conditions in lotic systems. The objective of this study was to elucidate the confounding effects of eutrophication, organic pollution and ionic strength and conductivity on benthic diatom communities. Benthic diatoms and water quality sampling was done at 10 sites during summer base flow period (2008 and 2009). Detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were used to determine environmental gradients along which species vary with respect to ionic strength and conductivity and other environmental variables. Using variance partitioning, we assessed the individual importance of a set of environmental variables (eutrophication and organic pollution) versus ionic strength and conductivity on diatom community structure. The effects of ionic strength and conductivity and organic pollution, eutrophication and other environmental variables were integrated into overall resultant benthic diatom communities. Through partial CCA, we partitioned the variance in diatom data between two sets of exploratory variables, i.e. ionic strength and conductivity (26.9%); other variables, particularly eutrophication and organic pollution (23.0%); shared variance (11.3%) and unexplained variance (38.8%). Due to the interaction of the effects of ionic strength and conductivity and other variables in this study, laboratory experiments must be performed to confirm the observed effects of ionic strength and conductivity.

Keywords: ionic strength, conductivity, benthic diatom communities, tropical streams

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Influência da força iônica e da condutividade nas comunidades bentônicas de diatomáceas em um rio tropical (Monjolinho, São Carlos-SP, Brasil)

Resumo: Diatomáceas bentônicas são importantes indicadores das condições ecológicas em sistemas lóticos. O objetivo deste estudo foi elucidar os efeitos conjuntos de eutrofização, poluição orgânica, força iônica e condutividade nas comunidades de diatomáceas bentônicas. Amostragens de diatomáceas bentônicas e da qualidade da água foram feitas em 10 pontos do rio Monjolinho durante o período de seca (2008 e 2009). Análises de correspondência retificada (ACR) e de correspondência canônica (ACC) foram utilizadas para determinar os gradientes ambientais ao longo dos quais as espécies variam com relação à força iônica e condutividade e outras variáveis ambientais. Ao utilizar o particionamento de variância, foi avaliada a importância individual de um conjunto de variáveis ambientais (eutrofização e poluição orgânica) versus força iônica e condutividade na estrutura da comunidade de diatomáceas. Os efeitos da força iônica, da condutividade, da poluição orgânica, da eutrofização e de outras variáveis ambientais foram integrados nas comunidades resultantes de diatomáceas bentônicas. Mediante ACC parcial, dividiu-se a variância dos dados de diatomáceas em conjuntos de variáveis exploratórias, ou seja, força iônica e condutividade (26,9%), eutrofização e poluição orgânica (23,0%); variância compartilhada (11,3%) e variação não explicada (38,8%). Devido à interação dos efeitos da força iônica e condutividade com outras variáveis nesse estudo, experimentos de laboratório devem ser realizados para confirmar os efeitos observados de força iônica e condutividade.

Palavras-chave: força iônica, condutividade, comunidades bentônicas de diatomáceas, riachos tropicais.

3.1 Introduction

Multiple factors prevailing at different temporal and spatial scales play an important role in structuring benthic diatom communities in lotic systems (POTAPOVA and CHARLES, 2002), with local factors playing a more important role compared to broad-scale climatic, vegetational and geographical factors (PAN et al., 1996). Some of the factors most often found to be important in shaping the distribution patterns of benthic diatoms in lotic systems are water chemistry (particularly pH, ionic strength and nutrient concentrations), substrate, current velocity, light (degree of shading) grazing, temperature (which also correlates strongly with latitude and altitude) (PATRICK and REIMER, 1966; ROUND, 1991; PAN et al., 1996; POTAPOVA and CHARLES, 2002). Most of these factors depend strongly on climate, geology, topography, land-use and other landscape characteristics, and therefore, diatom communities are similar within ecological regions defined by these characteristics (PAN et al., 1996).

Ionic composition and strength and conductivity are amongst the important factors that structure diatom communities in lotic systems (PAN et al., 1996; POTAPOVA and CHARLES, 2002). Continental waters vary greatly in their mineral, ionic strength (salinity) and composition, mainly because of the variability in lithology, climate, vegetation and anthropogenic factors (POTAPOVA and CHARLES, 2003). Soil erosion, irrigation, or the direct input of industrial, municipal or agricultural wastes into rivers often increases total mineral content, or concentration of individual ions in river water affecting conductivity (LELAND, 1995; CARPENTER and WAITE, 2000). These chemical changes in turn may affect the physiological response and thus, the species composition of the biota of lotic ecosystems, including diatoms.

Diatoms have been used for aquatic ecosystem assessment around the world. Biological monitoring of lotic ecosystems using diatoms can be more precise than a 1-time measurement of water chemistry because they integrate stressor effects over time (STEVENSON, 2006). Diatoms most often have been used to diagnose levels of stressors, such as organic contamination, lake acidification, climate change and nutrient concentrations. Diatoms are also good monitors of levels of ions in streams because of their range of response to ionic content and composition. Their use would be enhanced significantly if species responses to the concentration of major ions in fresh waters were better quantified (POTAPOVA and CHARLES, 2003). Monitoring the changes in the ionic composition could be carried out by simple observation of shifts in the dominant

taxa or by inferring ion concentrations or conductivity, using reported optima and some numerical procedures, e.g. weighted averaging (POTAPOVA and CHARLES, 2003).

Despite their ecological importance, practical usefulness and previous studies by taxonomists and ecologists elsewhere, current knowledge of diatom autecology is incomplete in the study area, gleaned from studies that are not specifically designed to determine the environmental requirements of common species. Few studies, especially in tropical systems, have been carried out relating diatoms communities in lotic systems draining urban areas that are subject to pollution (from industrial discharge, wastewater treatment plants and other sources) to ionic strength and conductivity. In these environments, the simultaneous occurrence of high ionic strength and conductivity with eutrophication and organic pollution may confound the specific effects of ionic strength and conductivity on diatom communities (BOISSON and PERRODIA, 2006). The main aim of the study was to elucidate the confounding effects of eutrophication, organic pollution and ionic strength and conductivity on diatom communities. Specifically, the study aimed at (1) describing the pollution gradient and the corresponding diatom communities and (2) investigating the relative contribution of different types of pollution, eutrophication and organic pollution versus ionic strength and conductivity to diatom species composition using variance partitioning multivariate analysis.

3.2 Materials and methods

3.2.1 Study area and study design

The study area is shown in Figure 3.1. Headwaters of Monjolinho and the tributaries studied 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 1,143.9 km^2. The area is characterised by rugged topography and an average annual temperature of around 19.5 °C, with mean monthly maximum of around 21.9 °C recorded in January and February and the mean monthly minimum of around 15.9 °C recorded in July.

In 2008, the population of São Carlos was estimated at 218,080 inhabitants by Instituto Brasileiro de Geografia e Estatística). 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 so on. Streams in the study area, therefore, receive untreated or semi-treated effluent from various domestic and industrial sources as well as other diffuse sources as they pass through the city. This disorderly growth of the city results in

stream health deterioration, loss of the remaining primary vegetation organic pollution and eutrophication amongst other problems.

Ten sites were established along Monjolinho river and its tributaries: 4 sites (1, 2, 3 and 7) in the relatively less impacted agricultural and forested headwaters to act as reference sites, 3 sites (4, 5 and 6) in the moderately polluted urban area, and 3 sites (8, 9 and 10) in highly polluted downstream area after the urban area (Figure 3.1). The rational for choosing the sampling sites was to obtain a pollution gradient of all the stream systems from relatively unpolluted agricultural headwaters to highly polluted urban downstream sites.

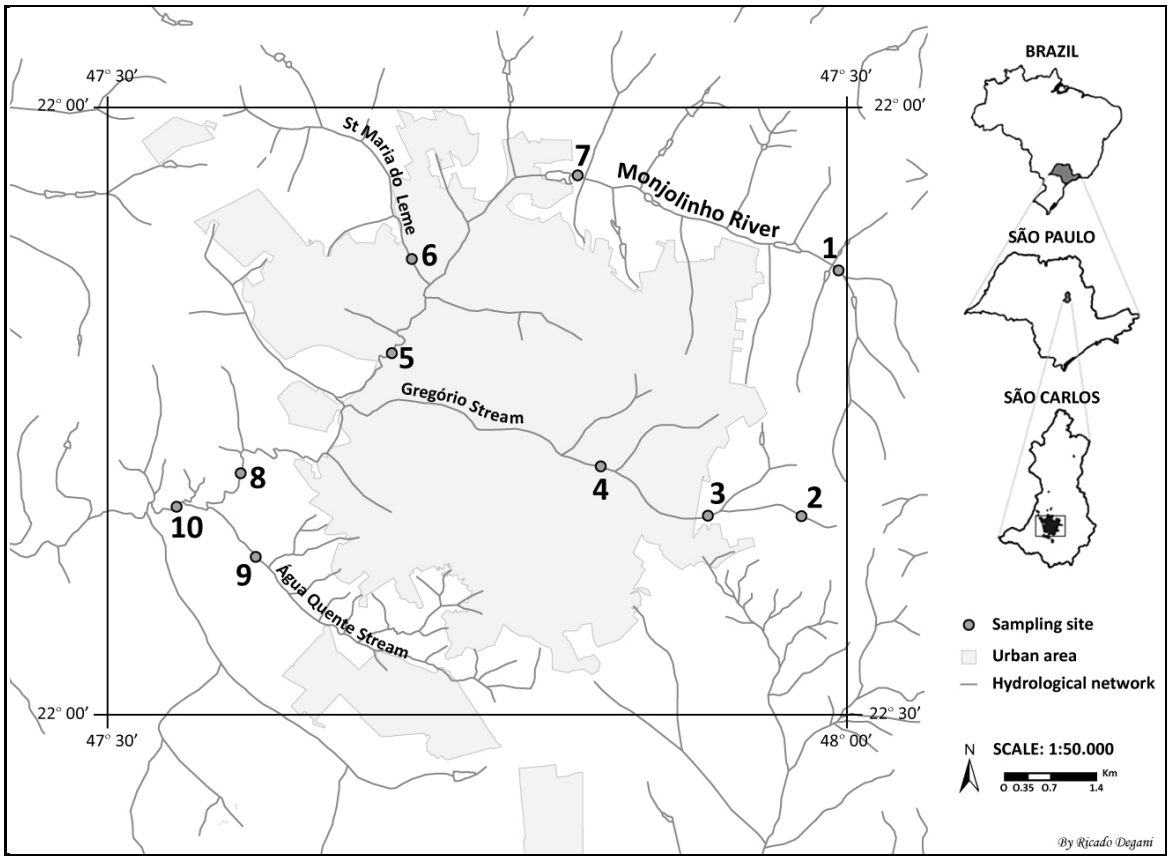

Figure 3.1: The location of the study area and sampling sites.

3.2.2 Diatom and water quality data sampling

Diatom and water quality sampling was done during summer season when flow was stable at 10 sites. Four samples were collected, one in September, one in October 2008, one in May, and one in July 2009. Dry season was selected to avoid variable effects of rainy season. These variations include large variations in water level and velocity, floods and inundations, which affect diatom development, especially growth rate and relative

abundance. At each site, dissolved oxygen (DO), electrical conductivity, temperature and pH, were measured using a Horiba U-23 and W-23XD Water Quality Metre (Horiba Ltd., Japan) during the four sampling periods. Depth and current velocity were measured at each site with an FP 201 global flow probe (Global Water Instrumentation Inc., AK, USA). Water samples for ions, total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD_5) analysis were collected at each site into acid-cleaned polyethylene containers (APHA, 1988).

At each site, epilithic, epiphytic, epipelic and epipsammic diatoms were sampled separately. Epilithic diatoms were sampled by brushing stones with a toothbrush. Prior to sampling of epilithic surfaces, all substrata were gently shaken in stream water to remove any loosely attached sediments and non-epilithic diatoms. At least five pebble-to-cobble sized stones were randomly collected at each sampling site and brushed, and the resulting diatom suspensions were pooled to form a single sample, which was then put in a labelled plastic bottle. Epiphytic diatoms were sampled from different species of submerged and emergent macrophytes (e.g. *Rumex crispus, Alternanthera philoxeroides, Ludwigia spp, Rhynchathera spp, Ageratum spp, Hedychium coronarium, Eleocharis spp, Heteronthera spp, Polygonum spectable* and *Brachiaria arrecta*) at each site depending on the availability of the macrophytes. The macrophyte's whole stalk comprising of stalk and leaves was carefully removed from the stream. Periphyton was then removed from the macrophytes by brushing with a toothbrush adding distilled water. The resulting diatom suspensions from all the submerged macrophytes sampled were pooled to form a single sample, which was then put in a labelled plastic bottle. Epipelic and epipsammic diatoms were sampled by pressing Petri dish lid into the top layer of sand or 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. Five samples at each site were collected and pooled into a single sample.

3.2.3 Laboratory analysis

The concentrations of TN and TP and BOD_5 in the water samples were determined following standard methods (APHA, 1988). The concentrations of fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), sodium (Na^+), ammonium ($NH4^+$), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions were analysed by isocratic ion analysis using suppressed conductivity

detection ion chromatography method using Dionex DX-80 Ion Analyser (DX-80) (DIONEX CORPORATION, 2001). Ion concentrations were expressed as mg L^{-1} or µg L^{-1} whilst the proportions of anions and cations were expressed as percent equivalents of each ion of the sum of all anions or cations (% eq).

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 and KILROY (2000). Three replicate slides were prepared for each sample. A total of 250 – 600 valves per sample (based on counting efficiency determination method by PAPPAS and STOERMER (1996)) were identified and counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type - 020-519.503 LB30T, Germany). The mean and standard deviations of counting efficiencies of diatom communities calculated according to PAPPAS and STOERMER (1996) on different substrates were as follows: macrophytes, 82.5 ±11.4 %; sand, 86.1 ±7.6 %; stones, 83.6 ±18.5 % and silt/clay, 82.9 ±14.2 %. The diatoms were identified to species level based on studies by LOBO et al 2002, METZELTIN ET AL. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007).

3.2.4 Data analysis

Cluster analysis with weighted pair-group average and Euclidian distance was performed based on benthic diatom community data to show the main differences and similarities in community composition amongst the four sampling periods, amongst the four substrates and amongst the 10 sites sampled. From this cluster analysis (Figure 3.2), diatom communities from different habitats sampled at the same site were generally grouped together. This similarity in diatom communities on different substrates can be attributed to the overriding effects of chemistry and other (unquantified) factors that masked the influence of substrate (JÜTTNER et al., 1996; ROTHFRITZ et al., 1997). Therefore, a pooled data set, consisting of epilithic, epiphytic, epipelic and epipsammic diatoms sampled at each sampling site was used to investigate spatial and temporal trends in the composition of diatom communities in relation to ionic strength and conductivity and other variables.

The original diatom data set consisted of 208 diatom species. Diatom counts from each site were expressed as relative abundances. Input for numerical analysis included the diatom taxa that were present in a minimum of two samples and had a relative abundance

of ≥ 1% in at least one sample. This was done in order to eliminate the effects of rare species. Of the 208 diatom taxa recorded in the 10 sites during the four sampling periods, 71 met this criterion.

The available environmental data consisted of 32 environmental variables which included 11 ion concentrations and their proportions, the monovalent-divalent cations ratios (M: D) as well as other physical and chemical variables (i.e. temperature, DO, BOD_5, TN, TP, depth and velocity) measured during the four sampling periods (Table 3.1). The M: D ratio was chosen because it has been shown to be an important factor in structuring benthic diatom communities (POTAPOVA and CHARLES, 2003). Two-way analysis of variance (two-way ANOVA) was used to compare means of transformed environmental variables (section 2.2.5) amongst the four sampling periods and amongst the three site categories (reference sites (1, 2, 3 and 7), moderately polluted sites (4, 5 and 6), and highly polluted sites (8, 9 and 10)). The relationship between conductivity and ion concentrations was identified from a Pearson's correlation matrix.

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. Preliminary detrended correspondence analysis (DCA) was applied on diatom data set to determine the length of the gradient. This DCA revealed that the gradient was greater than three standard deviation units (4.2) justifying the use of unimodial ordination techniques (TER BRAAK and VERDONSCHOT, 1995). Thus, canonical correspondence analyses (CCA), with only one environmental variable at a time were performed.

A total of 32 CCAs corresponded to 32 tested variables (11 for ion concentrations and 11 for proportions of ions, 1 for M: D and nine for other environmental variables (i.e. temperature, DO, BOD_5, TN, TP, depth and velocity; Table 3.1) were performed. The significance of the effect of each variable was evaluated using Monte Carlo permutation tests with 999 unrestricted permutations. The strengths of relationships between algal assemblages and environmental variables were assessed using the ratios of the first and second eigenvalues $\lambda 1/ \lambda 2$). This ratio measures the strength of the constraining variable with respect to the first unconstrained gradient in the assemblage composition data. Large numbers indicate strong responses of algal assemblages to the environmental variable (TER BRAAK & PRENTICE, 1988). The strength of relationship is considered very high if $\lambda 1/ \lambda 2 > 1$, moderately high if $0.5 < \lambda 1/ \lambda 2 < 1$, and weak if $\lambda 1/ \lambda 2 < 0.5$.

Partial CCA (BORCARD et al., 1992) was then used to separate and examine the relative importance of two sets of exploratory variables on the diatom community composition. We were interested in separating ionic strength and conductivity from all the other variables (i.e. temperature, DO, BOD_5, TN, TP, depth and velocity) and then testing whether these two different groups were redundant to each other, or they each explained unique aspects of species composition. The variance partitioning was conducted according to the following steps: (1) CCA of the species matrix constrained by ionic strength and conductivity matrix; (2) CCA of the species matrix constrained by other variables matrix; (3) partial CCA of species matrix constrained by ionic strength and conductivity matrix and using the matrix of other variables as covariables variables; (4) partial CCA of species matrix constrained by other variables matrix and using ionic strength and conductivity matrix as covariables variables.

Another CCA was performed to relate diatom community structure to simultaneous effects of predictor variables and to explore the relationship amongst and between species and predictor variables. Preliminary CCA identified collinear variables and selected a subset on inspection of variance inflation factors (VIF< 20; TER BRAAK and ŠMILAUER, 2002). Monte Carlo permutation tests (999 unrestricted permutations, $p \leq 0.05$) were used to test the significance of the axis and hence determine if the selected environmental variables could explain nearly as much variation in the diatom data as all the 32 environmental variables combined.

Two-way ANOVA, cluster analysis, CCA and DCA were performed using PAlaeontological STatistics (PAST) software version 1.95 (HAMMER et al., 2009).

3.3 Results

3.3.1 Physical and chemical variables

The values of physical and chemical variables measured in the study area during the study period are shown in Table 3.1. Water quality in general, 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. However, the difference in pH amongst the three site categories was not statistically significant (ANOVA, $p > 0.05$). Temperature increased downstream but as in the case of pH, the increase was not significant (ANOVA, $p > 0.05$). On the other hand, BOD_5, TN

and TP, conductivity increased significantly downstream (ANOVA $p < 0.05$) whilst DO decreased significantly downstream (ANOVA $p < 0.05$). Conductivity varied from 20.0 μScm^{-1}, corresponding to waters poor in electrolytes, at the upstream reference sites to 715.0 μScm^{-1} at downstream highly polluted sites.

The concentrations of all the ions in water increased significantly (ANOVA, $p > 0.05$) downstream along the agricultural to urban gradient due to urban pollution (Table 3.1). The concentrations were generally lowest at the reference sites and highest at downstream highly polluted sites. Chloride and alkaline earth metals especially Na^+ and Ca^{2+} were the dominant ions in the study area, whilst PO_4^{3-} and F^- were low at upstream sites and high at highly polluted, low DO urban downstream sites. The ratio of Ca^{2+} to Mg^{2+} tended to increase downstream along the agricultural to urban gradient. The ions, in order of decreasing prevalence, were as follows: $Cl^- > Na^+ > Ca^{2+} > SO_4^{2-} > K^+ > Mg^{2+} > NH4^+ > NO_3^- > NO_2^- > F^- > PO_4^{3-}$. There was no clear relationship between conductivity and dominant ions. Correlations between conductivity and F^- and NO_3^- were high indicating that highest values of conductivity were because of the increased concentration of these ions (Table 3.2).

Table 3.1: The mean values of physical and chemical variables measured at all the sites during the four sampling periods. The sites are arranged in order of general increase in pollution based on physical and chemical variables and dotted line separates 'reference' sites from the rest of the sites.

Site	1	2	3	7	4	5	6	8	9	10
Temperature (^{o}C)	18.3 ±1.1	20.9 ±1.6	20.6 ±1.7	20.4 ±1.5	24.0 ±2.6	21.2 ±1.7	21.2 ±1.1	24.8 ±3.0	23.0 ±1.9	21.3 ±2.2
Conductivity (μS.cm^{-1})	45.0 ±7.5	20.0 ±10.5	53.0 ±9.5	30.0 ±4.0	28.0 ±7.7	89.0 ±8.9	103.0 ±6.4	715.0 ±22.3	322.0 ±191.3	283.0 ±201.7
DO (mg.L^{-1})	7.3 ±2.3	8.2 ±1.0	7.6 ±0.6	7.2 ±1.5	6.8 ±2.1	6.9 ±1.0	7.6 ±1.3	1.9 ±2.8	2.1 ±1.0	0.4 ±1.2
BOD_5 (mg.L^{-1})	0.9 ±0.2	1.0 ±1.2	2.6 ±0.2	1.2 ±2.3	6.9 ±0.2	1.6 ±0.6	7.2 ±1.1	19.5 ±1.1	24.5 ±1.2	26.2 ±1.3
TN (mg.L^{-1})	0.65 ±0.3	0.18 ±0.3	0.24 ±1.4	0.93 ±0.7	1.72 ±0.5	1.29 ±0.4	1.41 ±0.5	38.32 ±8.3	14.87 ±4.1	10.17 ±2.9
TP (mg.L^{-1})	0.01 ±0.0	0.01 ±0.0	0.01 ±0.1	0.02 ±0.0	0.03 ±0.0	0.16 ±0.2	0.06 ±0.1	2.97 ±1.0	1.12 ±0.2	0.75 ±0.2
pH	6.6 ±0.8	6.4 ±1.0	6.3 ±1.0	6.8 ±1.0	6.7 ±0.6	6.8 ±0.9	7.2 ±0.4	7.2 ±0.5	7.2 ±0.4	7.1 ±0.4
Velocity (m.s^{-1})	2.5 ±1.3	2.8 ±1.4	2.6 ±1.3	2.9 ±1.1	2.2 ±1.1	2.7 ±1.2	1.4 ±0.6	3.5 ±1.8	2.4 ±0.9	2.34 ±1.0
Depth (m)	0.2 ±0.02	0.3 ±0.08	0.4 ±0.10	0.4 ±0.01	0.2 ±0.05	0.4 ±0.08	0.3 ±0.05	0.5 ±0.07	0.3 ±0.04	0.3 ±0.01
Nitrite (µg.L^{-1})	<10	<10	<10	<10	423.9 ±84.8	42.4 ±50.3	24.3 ±48.6	884.9 ±105.8	2036.6 ±391.9	3164.9 ±641.7
Nitrate (μg.L^{-1})	51.9 ±22.3	195.6 ±33.1	470.0 ±38.5	98.2 ±8.8	714.8 ±232.3	819.6 ±667.2	745.0 ±595.3	1141.2 ±1025.4	176.5 ±142.5	441.1 ±273.2
Ammonium (μg.L^{-1})	11.8 ±3.6	116.1 ±23.5	11.9 ±3.7	15.0 ±3.1	858.9 ±175.5	418.8 ±401.9	141.4 ±128.2	1361.6 ±783.1	4610.8 ±2482.1	3310.5 ±703.0
Phosphate (μg.L^{-1})	15.0 ±17.8	<2	2.4 ±2.9	<2	136.2 ±49.3	26.7 ±14.3	7.7 ±9.1	21.4 ±5.0	132.5 ±51.8	190.8 ±131.2
Sulphate (mg.L^{-1})	0.1 ±0.1	1.5 ±1.0	1.1 ±0.3	0.3 ±0.1	8.3 ±1.8	4.9 ±3.1	3.1 ±1.3	3.7 ±0.9	15.1 ±3.9	9.5 ±3.9
Fluoride (μg.L^{-1})	38.3 ±12.1	45.8 ±14.7	67.5 ±40.5	43.7 ±14.8	88.8 ±28.4	95.7 ±66.5	124.7 ±88.3	287.5 ±87.3	305.4 ±93.2	262.9 ±104.3
Chloride (mg.L^{-1})	2.0 ±0.4	4.9 ±1.0	4.1 ±1.7	2.8 ±0.6	15.5 ±5.9	7.3 ±4.8	6.8 ±4.8	19.8 ±8.3	21.7 ±4.2	30.0 ±7.0
Sodium (mg L^{-1})	2.0 ±0.8	2.3 ±0.6	2.4 ±1.2	2.2 ±0.6	7.5 ±1.5	4.1 ±2.3	4.1 ±2.6	12.4 ±6.5	15.3 ±2.9	19.7 ±4.2
Potassium (mg L^{-1})	1.0 ±0.2	0.6 ±0.2	1.0 ±0.3	0.7 ±0.1	2.3 ±0.6	1.3 ±0.9	0.9 ±0.4	2.4 ±1.0	4.3 ±1.2	3.8 ±0.8
Magnesium (mg.L^{-1})	0.7 ±0.1	0.6 ±0.1	0.8 ±0.2	0.7 ±0.1	1.2 ±0.3	1.2 ±0.6	1.0 ±0.5	1.7 ±0.3	2.5 ±0.7	1.6 ±0.3
Calcium (mg.L^{-1})	1.4 ±0.3	1.9 ±0.4	1.3 ±0.2	2.4 ±0.5	3.9 ±1.7	5.7 ±3.5	4.5 ±2.1	7.4 ±1.5	11.9 ±2.8	8.2 ±1.6
Fluoride (% eq)	27.09 ±2.4	36.20 ±5.3	30.90 ±4.1	40.00 ±101	29.40 ±2.2	25.61 ±3.3	27.88 ±6.6	34.07 ±1.4	27.17 ±5.5	35.47 ±6.9
Chloride (% eq)	14.52 ±3.2	19.40 ±3.2	16.56 ±5.4	21.44 ±4.2	15.76 ±3.7	13.73 ±8.2	14.94 ±3.8	18.26 ±2.2	14.56 ±2.9	19.01 ±4.4
Nitrite (% eq)	0.00	0.00	0.00	0.00	0.09 ±0.01	0.00	0.00	0.04 ±0.01	1.04 ±0.3	1.64 ±0.6
Nitrate (% eq)	0.15 ±0.1	0.45 ±0.1	2.12 ±1.1	0.71 ±0.3	1.42 ±0.2	0.80 ±0.4	0.39 ±0.1	1.98 ±0.2	0.14 ±0.1	0.35 ±0.1
Phosphate (% eq)	0.00	0.00	0.03 ±0.01	0.12 ±0.01	0.02 ±0.01	0.00	0.00	0.04 ±0.02	0.13 ±0.01	0.24 ±0.01
Sulphate (% eq)	0.50 ±0.1	6.60 ±1.1	3.60 ±0.5	6.05 ±2.1	7.70 ±1.1	7.67 ±1.4	1.40 ±0.7	3.21 ±1.0	8.48 ±3.2	3.46 ±0.5
Sodium (% eq)	16.61 ±2.3	15.24 ±3.2	13.31 ±5.3	13.27 ±2.7	12.98 ±4.4	14.53 ±2.1	16.20 ±3.3	15.01 ±5.8	15.72 ±4.9	19.90 ±6.2
Ammonium (% eq)	0.15 ±0.1	0.81 ±0.3	0.17 ±0.1	1.93 ±0.6	2.09 ±1.1	0.32 ±0.1	0.16 ±0.1	1.94 ±0.6	8.59 ±2.2	4.28 ±2.9
Potassium (% eq)	7.76 ±2.2	2.57 ±2.0	6.29 ±1.1	2.55 ±0.8	2.55 ±0.9	2.97 ±.07	3.77 ±1.5	2.10 ±1.0	3.07 ±0.5	2.23 ±1.3
Magnesium (% eq)	15.71 ±3.3	6.42 ±2.2	13.62 ±3.8	4.01 ±1.5	6.89 ±1.9	9.89 ±1.9	11.93 ±2.1	6.42 ±1.8	5.62 ±1.4	3.27 ±1.1
Calcium (% eq)	17.52 ±5.8	12.32 ±5.2	13.39 ±6.1	9.93 ±3.9	21.10 ±5.9	24.49 ±8.8	23.32 ±10.2	16.94 ±4.5	15.47 ±5.9	10.14 ±4.4
M: D	0.74 ±0.2	0.99 ±0.3	0.73 ±0.4	1.27 ±0.1	0.63 ±0.5	0.52 ±0.1	0.57 ±0.2	0.82 ±0.6	1.30 ±0.5	1.97 ±1.1

Table 3.2: Correlation coefficients of conductivity and ion concentrations in the study area. Significant correlations ($p < 0.01$) are highlighted.

	F^-	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
F^-											
Cl^-	**0.73**										
NO_2^-	0.59	**0.78**									
NO_3^-	0.54	0.38	-0.07								
PO_4^{3-}	0.55	**0.90**	**0.95**	0.05							
SO_4^{2-}	**0.78**	**0.77**	0.61	0.15	0.62						
Na^+	**0.78**	**0.96**	**0.91**	0.24	**0.95**	**0.77**					
NH_4^+	**0.79**	**0.75**	**0.80**	-0.02	**0.74**	**0.92**	**0.84**				
K^+	**0.80**	**0.88**	**0.79**	0.15	**0.81**	**0.93**	**0.91**	**0.95**			
Mg^{2+}	**0.93**	**0.77**	0.62	0.37	0.60	**0.93**	**0.80**	**0.90**	**0.92**		
Ca^{2+}	**0.93**	**0.81**	**0.66**	0.42	0.64	**0.92**	**0.84**	**0.87**	**0.90**	**0.98**	
Conductivity	**0.79**	0.51	0.31	**0.73**	0.32	0.31	0.51	0.39	0.42	0.59	0.58

3.3.2 Community analysis

A total of 208 diatom species belonging to 63 genera were recorded in all the diatom samples collected. Of the 208 species observed, 71 species were considered the most dominant in the study area (Table 3.3). These 71 species made up 88.6% of the overall diatom community.

Based on cluster analysis carried out to show the main differences and similarities in community composition amongst the four sampling periods, amongst the four substrates and amongst the 10 sites sampled, two major groups of sites were observed (Figure 3.2). The grouping reflected a change in community composition down the agricultural to urban pollution gradient. The first group was characterised by diatom communities from highly polluted sites 8, 9 and 10. Some diatom communities from site 4 were also included in this group. The second group was characterised by diatom communities from moderately and less polluted sites 1, 2, 3, 5 and 7 with some diatom communities from site 4 being also included in this group. Sites previously designated as reference and moderately polluted (Section 3.2.1) were not clearly separate. Benthic diatom communities from different substrates sampled on the same site were generally similar as they were grouped close to each other in most cases.

Table 3.3: The distribution of most abundant diatom species. * = 0-10 %, ** = 10-30 %, *** = >30 %).

Species	Code	1	2	3	4	5	6	7	8	9	10
Achnanthes exigua Grunow	Aexi	*	*	*	*	*	*	*			
Achnanthidium biasolettianum (Grunow) Lange-Bertalot	Abia	*	*	*	*	*	*	*			
Achnanthidium minutissimum (Kützing) Czarnecki	Amin	*	*	*	*	*	*	*			
Amphora copulata (Kützing) Schoeman and Archibald	Acop	*	*	*	*	*	*	*			
Aulacoseira agassizii (Hustedt) Simonsen	Aaga		*	*	*	*	*	*			
Aulacoseira alpigena (Grunow) Krammer	Aalp		*	*	*	*	*	*			
Aulacoseira ambigua (Grunow) Simonsen	Aamb	*	*	*		*	*	*			
Aulacoseira distans (Ehrenberg) Simonsen	Adis		*	*		*	*	*	*		
Aulacoseira granulata (Ehrenberg) Simonsen	Agra	*	*	*	*	*	*	*			
Caloneis hyaline Hustedt	Chya	*	*					*			
Craticula cuspidata (Kützing) Mann	Ccus	*		*					*		
Cyclotella meneghiniana Kützing	Cmen		*		*	*	*	*	*	*	*
Cyclotella pseudostelligera Hustedt	Cpse		*	*		*	*	*	*		
Cyclotella spp.	Cspp	*	*	*	*	*	*	*	*	*	*
Cyclotella stelligera (Cleve and Grunow) Van Heurck	Cste	*					*	*			
Cymbopleura naviculiformis (Auerswald) Krammer	Cnav	*	*	*	*	*	*	*			
Diadesmis contenta (Grunow) Mann	Dcon	*	*	*	*	*	*	*	*		*
Diadesmis dissimilis Moser, Lange-Bertalot & Metzeltin	Ddes	*		*			*				
Diatoma spp	Dspp	*	*	*				*			
Diatoma vulgare Bory	Dvul	*	*	*	*	*	*	*			
Encyonema neomesianum Krammer	Eneo	*	*	*	*	*	*	*			
Encyonema silesiacum (Bleisch) Mann	Esil	*	*	*	*	*	*	*	*		
Eunotia bilunaris (Ehrenberg) Mills	Ebil	*	*	**	*	*	*	*			
Eunotia camelus Ehrenberg	Ecam	*	*	*	*	*	*	*			
Eunotia intermedia (Hustedt) Nörpel and Lange-Bertalot	Eint	**	*	*	*	*	*	*			
Eunotia monodon Ehrenberg	Emon	*	*	*		*	*	*			
Eunotia papillo (Ehrenberg) Hustedt	Epop	*	*			*	*	*			
Eunotia pectinalis (Kützing) Rabh	Epec	**	*	*	*	*	*	*			
Eunotia rabenhorstii Cleve & Grunow	Erab	*		*			*	*			
Eunotia sudetica Müller	Esud		*	*	*	*		*			
Fallacia monoculata (Hust) Mann	Fmon				*	*	*	*	*	*	*
Fragilaria capucina Desmazières	Fcap	*	*	*	*	*	*	*			
Fragilaria intermedia Grunow	Fint	*	*	*	*	*	*	*			*
Frustulia rhomboides (Rabenhorst) De Toni	Frho	*	*	*	*	*	**	*			
Frustulia saxonica Rabenhorst	Fsax	*	*	*	*	*	*	*			
Frustulia vulgaris (Twaithes) de Toni	Fvul	*	*	*	*	*	*	*			
Gomphonema accuminatum Ehrenberg	Gacc	*	*	*	*	*	*	*	*		
Gomphonema angustatum (Kützing) Rabenhorst	Gang	*	*	*	*	*	*	*		*	*
Gomphonema augur (Ehrenberg) Lange-Bertalot	Gaug	*	*	*	*	*	*	*			
Gomphonema gracile Ehrenberg	Ggra	*	*	*	*	*	*	*			
Gomphonema olivaceum (Hornemann) Brébisson	Goli	*	*	*	*	*	*	*			
Gomphonema parvulum (Kützing) Kützing	Gpar	*	*	*	*	*	*	*	*	**	*
Gomphonema turris Ehrenberg	Gtur	*	*			*		*			
Hantzschia amphioxys (Ehrenberg) Grunow	Hamp	*	*	*	*	*	*	*			
Luticola goeppertiana (Bleisch) Mann	Lgeo	*	*	*	*	*	*	*	*	*	**
Melosira varians Agardh	Mvar		*	*	*	*	*	*			
Meridion anceps (Ehrenberg) Williams	Manc	*	*	*	*	*		*			
Navicula cryptocephala (Grunow) Cleve	Ncry	*	*	*	*	*	*	*			*
Navicula cryptotenella Lange-Bertalot	Ncrt	*	*	*	*	*	*	*	*		
Navicula oblonga Kützing	Nobl	*	*	*	*	*	*	*			
Navicula radiosa Kützing	Nrad	*	*	*	*	*	*	*			
Navicula rostellata Kützing	Nros			*	*	*	*	*			
Neidium affine (Ehrenberg) Pfitzer	Naff	*	*	*	*	*	*	*			
Neidium ampliatum (Ehrenberg) Krammer	Namp						*	*			
Nitzschia linearis (Agardh) Smith	Nlin	*	*	*	*	*	*	*		*	
Nitzschia palea (Kützing) Smith	Npal		*	*	**	*	**	*	***	**	***
Nitzschia recta Hantzsch ex Rabenhorst	Nrec	*	*	*	*	*	*	*	*	*	*
Nitzschia scalaris (Kütz) Grunow	Nsca	*		*	*	*	*	*			
Nupela praecipua (Reichardt) Reichardt.	Npra	*	*	*	*	**	*	*	*	**	*
Orthoseira dentroteres (Ehrenberg) Crawford	Oden	*	*	*							
Pinnularia braunii (Grunow) Cleve	Pbra	*		*	*	*	*	*	*	*	*
Pinnularia divergens Krammer	Pdiv	*		*	*	*	*	*			
Pinnularia gibba Ehrenberg	Pgib	*	*	*	*	*	*	*	*	*	*
Pinnularia lata (Brébisson) Rabenhorst	Plat	*		*	*	*	*	*			
Pinnularia legumen Ehrenberg	Pleg			*	*		*	*			
Pinnularia microstauron (Ehrenberg) Cleve	Pmic	*			*	*	*	*	*		
Pinnularia subcapitata Gregory	Psub				*	*	*	*	*	*	*
Placoneis clementis (Grunow) Cox	Pcle		*	*	*	*	*	*			
Planothidium dubium (Grunow) Round and Bukhtiyarova	Pdub	*	*	*	*	*	*	*			
Planothidium lanceolatum (Brébisson) Grunow	Plan	*	*	*	*	*	*	*	*	*	*
Planotidium heteroideum	Phet		*	*	*	*	*	*			
Pleurosigma compactum Greville	Pcom				*		*	*			
Psammothidium subatomoides (Hustedt) Bukhtiyarova	Psuba	*	*	*	*	*	*	*		*	*
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	Rabb	*	*	*	*	*	*	*	*	*	*
Sellaphora pupula (Kützing) Mereschkowsky	Spup	*	*	*	*	*	*	*	*	*	*
Stauroneis phoenicenteron (Nitzsch) Ehrenberg	Spho	*	*	*	*	*	*	*			
Surirella angusta Kützing	Sang	*		*	*	*	*	*			
Surirella linearis Smith	Slin	*	*	*	*	*	*	*			
Surirella ovata Kutzing	Sova	*		*		*	*				
Surirella robusta Enrenburg	Srob	*		*	*		*	*			
Thalassiosira weissflogii (Grunow) Fryxell and Hasle	Twei	*	*	*							
Ulnaria ulna (Nitzsch) Compère	Uuln	*	*	*	*	*	*	*	*		*

Based on CCAs carried out using individual variables (Table 3.4), the following ions were significantly (Monte Carlo permutation test, $p \leq 0.05$) associated with changes in diatom communities (in order of their decreasing importance, based on the ratio of the first to second axes eigenvalues): Cl^-, K^+, Na^+, Mg^{2+}, proportion of NH_4^+, proportion of K^+, proportion of Mg^{2+}, F^-, NH_4^+, proportion of NO_2^-, PO_4^{3-}, SO_4^{2-}, NO_2^- and proportion of Ca^{2+}. Thus, ion species associated with eutrophication (NH_4^+, NO_2^-, PO_4^{3-} and proportions of NO_2^-, NH_4^+ and PO_4^{3-}) were amongst the ions associated with changes in diatom communities. Eigenvalues of the first axis in all the analyses ranged from 0.06 to 0.51 with a low to moderate ratio of the first to the second eigenvalue. Apart from ion concentrations and proportions, TP, TN, BOD_5 and DO (in order of their decreasing importance, based on the ratio of the first to second axes eigenvalues) were also significantly (Monte Carlo permutation test, $p \leq 0.05$) associated with changes in diatom communities (Table 3.4).

From the partial CCA results, the first run of the data analysis (i.e. ionic strength and conductivity as exploratory variables) showed that Mg^{2+} and Ca^{2+} and proportions of NH_4^+, NO_2^-, Cl^-, Na^+, Mg^{2+} and Ca^{2+} were statistically significant (Monte Carlo permutation test, $p \leq 0.05$). In the second run (i.e. other variables as exploratory variables) BOD_5, TP and TN turned out to be statistically significant (Monte Carlo permutation test, $p \leq 0.05$). Ionic strength and conductivity alone accounted for 26.9% of explained variation. Other variables represented 23.0% of the explained variation. The results showed that 11.3% of the diatom data variation was shared by ionic strength and conductivity and other variables. Finally, the unexplained variation corresponded to 38.8%.

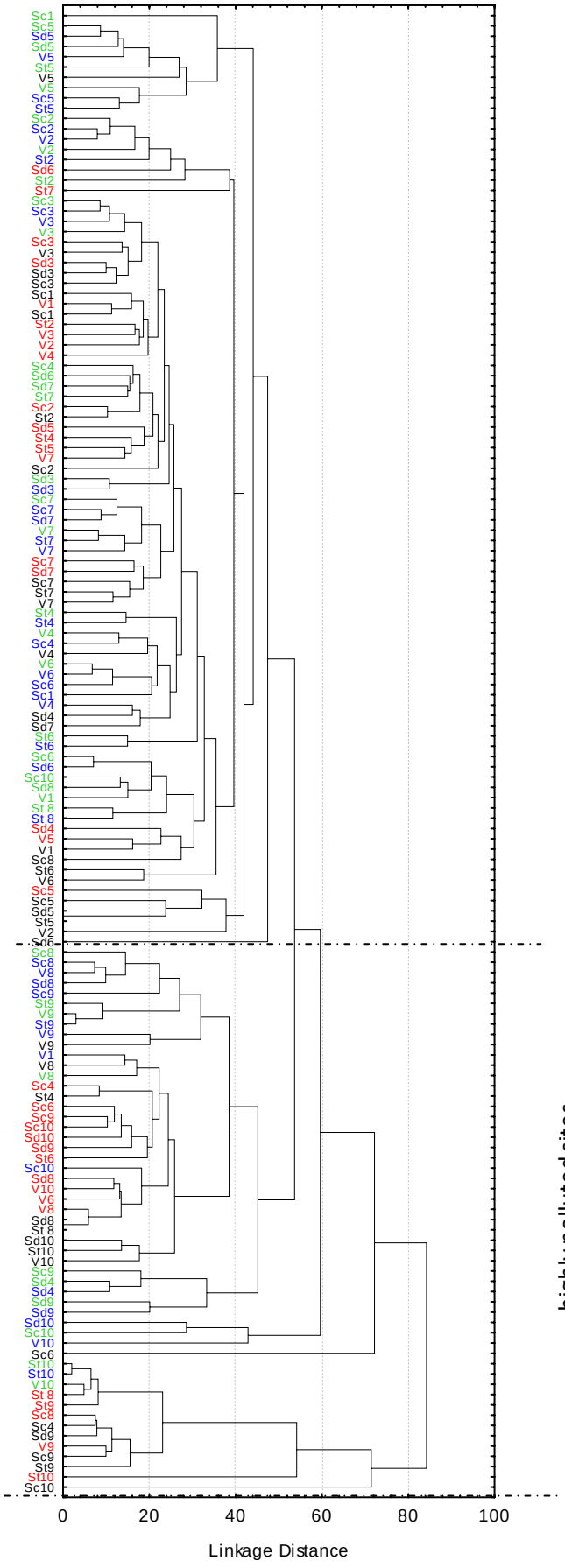

Figure 2: A cluster analysis based on benthic diatom community data sampled at 10 sampling sites from different substrates during the four samplings. Some substrates were not available on all the sampling sites. Sc = epipelic, St = epilithic, Sd = epipsammic, V = epiphytic, blue = Sept 2008, green = Nov 2008, red = May 2009, black = July2009.

Table 3.4: Results of CCAs showing the significance (Monte Carlo 999 unrestricted permutations $p < 0.05$) of the effects and strength of each variable on benthic diatom community composition. Significant variables are highlighted. λ_1 and λ_2 = first and second eigenvalues respectively.

Variable	λ_1	λ_2	λ_1/λ_2	p
Chloride (mg L^{-1})	**0.46**	**0.49**	**0.95**	**0.00**
Potassium (mg L^{-1})	**0.45**	**0.49**	**0.93**	**0.00**
Sodium (mg L^{-1})	**0.44**	**0.49**	**0.89**	**0.00**
Magnesium (mg L^{-1})	**0.43**	**0.49**	**0.89**	**0.00**
TP (mg L^{-1})	**0.40**	**0.49**	**0.83**	**0.00**
Ammonium (%eq)	**0.41**	**0.49**	**0.82**	**0.00**
Potassium (%eq)	**0.51**	**0.62**	**0.82**	**0.02**
TN (mg L^{-1})	**0.40**	**0.49**	**0.81**	**0.00**
BOD_5	**0.41**	**0.51**	**0.79**	**0.00**
Magnesium (%eq)	**0.38**	**0.50**	**0.76**	**0.02**
Fluoride (µg/L)	**0.36**	**0.48**	**0.74**	**0.00**
Ammonium (µg L^{-1})	**0.37**	**0.50**	**0.74**	**0.01**
Nitrite (%eq)	**0.37**	**0.50**	**0.74**	**0.00**
Phosphate (µg L^{-1})	**0.37**	**0.51**	**0.72**	**0.02**
Sulphate (mg L^{-1})	**0.35**	**0.50**	**0.71**	**0.00**
Nitrite (µg L^{-1})	**0.35**	**0.50**	**0.70**	**0.00**
DO (mg L^{-1})	**0.34**	**0.50**	**0.68**	**0.00**
Calcium (%eq)	**0.24**	**0.48**	**0.50**	**0.03**
M: D	0.21	0.49	0.42	0.07
Temperature (^oC)	0.19	0.51	0.37	0.14
Fluoride (%eq)	0.26	0.71	0.37	0.77
Depth (m)	0.18	0.53	0.34	0.21
Chloride (%eq)	0.16	0.50	0.33	0.27
Nitrate (µg L^{-1})	0.18	0.56	0.32	0.16
Sodium (%eq)	0.15	0.53	0.28	0.32
Phosphate (%eq)	0.16	0.55	0.28	0.30
Calcium (mg L^{-1})	0.16	0.57	0.28	0.27
Velocity (m s^{-1})	0.16	0.57	0.28	0.00
Conductivity (µScm^{-1})	0.14	0.52	0.27	0.30
Sulphate (%eq)	0.14	0.58	0.23	0.45
Nitrate (%eq)	0.13	0.58	0.22	0.48
pH	0.06	0.59	0.11	0.97

The CCA conducted to explore the simultaneous effects of all the variables (i.e. ionic strength and conductivity other variables) on diatom communities explained the following proportion of the diatom species variance: CCA axis 1 = 24.1% and axis 2 = 15.7%. Monte Carlo unrestricted permutation test showed that axis 1 (999 permutations) and axis 2 (999 permutations of axis 2 with axis 1 as a covariable) were statistically significant ($p \leq 0.05$). BOD_5 and DO were highly positively and negatively respectively associated with the first axis whilst pH was highly negatively associated with the second axis. Mg^{2+}, NH_4^+ and Ca^{2+} were also positively associated with the first axis (Figure 3.3).

The CCA separated highly polluted sites 8, 9 and 10 from the rest of the sites based on all the four sampling periods as in the case of cluster analysis. These sites were associated with high Mg^{2+}, Ca^{2+} and NH_4^+ ions as well as high BOD_5. Diatom species associated with these sites included *Nitzschia palea* (Kützing) Smith, *Gomphonema parvulum* (Kützing) Kützing, *Planothidium lanceolatum (Brébisson)* Grunow, *Caloneis hyaline* Hustedt, *Fallacia monoculata* (Hust) Mann, *Sellaphora pupula* (Kützing) Mereschkowsky, *Cyclotella meneghiniana* Kützing, *Luticola goeppertiana* (Bleisch) Mann, *Pinnularia subcapitata* Gregory, *Navicula rostellata* Kützing, and *Nupela praecipua* (Reichardt) Reichardt. In September and October 2008, highly polluted sites 8, 9 and 10 had generally high abundances of such species as *N. palea*, *G. parvulum, S. pupula* and *N. praecipua. N. palea* formed around 85 % of the species found at site 10. On the other hand, in May and July 2009, these sites had generally high abundance of such species as *N. palea*, *P. lanceolatum*, *F. monoculata*, *P. subcapitata C. hyaline* and *L. goeppertiana*. In addition to high abundance of *N. palea*, *L. goeppertiana* and F. *monoculata* had high abundances at these sites.

The rest of the species, mostly of the genus *Neidium, Eunotia, Aulacoseira, Surirella* and *Pinnularia,* that are characteristic of moderately and less polluted environments, were associated with the other sites. These diatom species are characteristic of less polluted waters low in alkaline cations (Ca^{2+}, Mg^{2+}) and other ions in general. As in the case of cluster analysis (Figure 3.2), sites previously designated as reference and moderately polluted (Section 3.2.1) were not clearly separate. This is expected since human activities, in one way or the other, have altered most of the lotic systems around urban areas in Brazil, with pristine or reference sites almost nonexistent (RÖRIG et al., 2007).

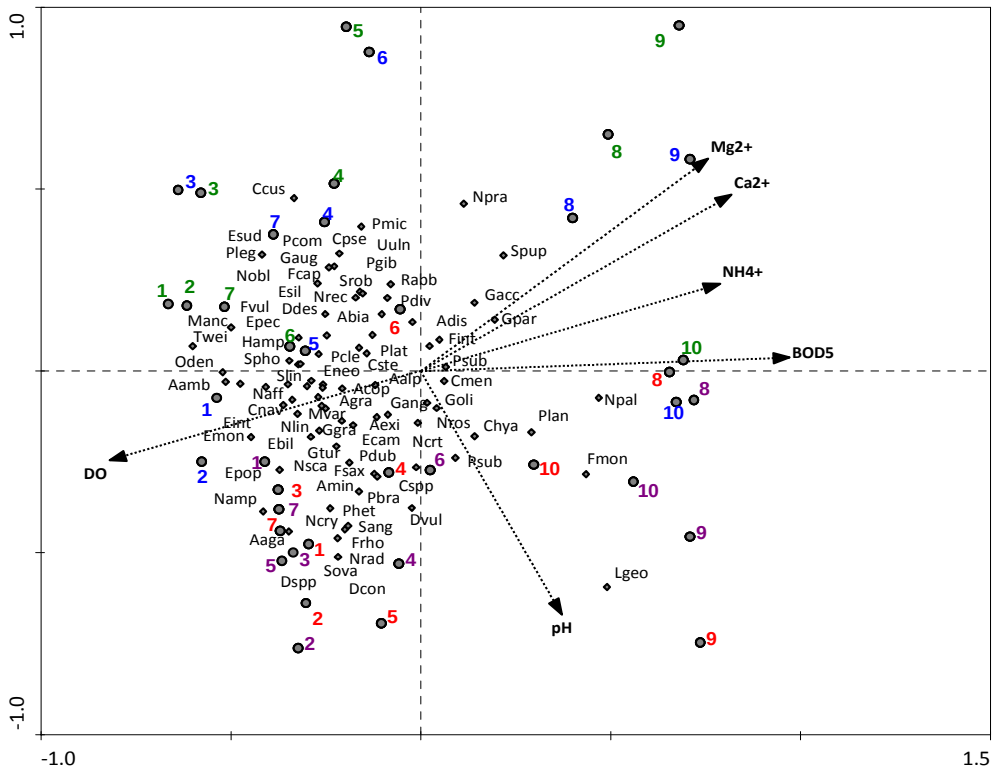

Figure 3.3: Canonical correspondence analysis (CCA) diagram showing simultaneous effects of ion concentrations and other variables on most frequently occur diatom taxa in the ordination space of the 1st and 2nd axes. Taxa codes correspond to those in Table 3.3. Sites in blue = Sept 2008, green = Nov 2008, red = May 2009, purple = July 2009.

3.4 Discussion

Based on ionic strength and conductivity, eutrophication and organic pollution, a gradient along the streams from agricultural to urban area was observed. Upstream sites were characterised by low ionic strength, conductivity, low nutrients and organic pollution levels. On the other hand, downstream sites were characterised by high ionic strength, conductivity, high nutrients and organic pollution levels. Diatom community structure closely followed this gradient, with the effects of ionic strength and conductivity and organic pollution, eutrophication and other environmental variables being integrated into overall resultant benthic diatom communities. Different diatom species in a community respond differently to ionic strength and composition, conductivity, eutrophication, organic pollution, and other variables because of differences in tolerance developed. Therefore the composition of diatom communities at different locations, or at different points in time, provide useful information about the environmental conditions. Thus, diatom communities were used in this study to differentiate the specific effects of ionic strength and conductivity from eutrophication and organic pollution.

3.4.1 Pollution gradient and the corresponding diatom communities

As pollution increased, low or moderate pollution tolerant species were replaced by high pollution tolerant species such as *P. lanceolatum*, *C. hyaline*, *C. meneghiniana*, *parvulum*, *F. monoculata*, *L. goeppertiana*, *N. palea, N. praecipua*, *N. rostellata*, *P. subcapitata* and *S. pupula*. This group of species is known to be resistant to organic pollution and high ionic strength and conductivity (DICKSON et al., 1977; ROUND, 1991; FUKUSHIMA et al., 1994; VAN DAM et al., 1994; LOBO et al. 1996; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003). These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated with high electrical conductivity (KILHAM et al., 1986; LANGE-BERTALOT, 1979; ROUND, 1991; VAN DAM et al., 1994; KOBAYASI and MAYAMA, 1998; LOBO et al., 2002; BERE and TUNDISI, 2009).

Environmental monitoring studies in Southern Brazil (e.g. OLIVEIRA et al., 2001; LOBO et al., 2002, 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 pollution as well as eutrophication. The importance of eutrophication and organic pollution in structuring benthic diatom has also been noted by other studies (e.g. SLÁDECÉK, 1986; KELLY and WHITTON, 1995; PONADER et al., 2007; LAVOIE et al., 2008). These two phenomena (organic pollution and eutrophication) can hardly be separated in nature (GÓMEZ and LICUIRSI, 2001) and can affect diatom species composition and abundances via different mechanisms (ROTT et al., 1998). However, in this study, organic pollution and eutrophication followed the same gradient making it difficult to separate the effects of these two phenomena. Causal relationships between these two phenomena can be determined through experimental work.

3.4.2 Effects of ionic strength and conductivity

Partial CCA results showed that ionic strength and conductivity alone accounted for 26.9% of explained variation in diatom data (slightly more than that of other variables i.e. 23.0%) demonstrating the importance of these variables in structuring benthic diatom communities. This is in agreement with previous field studies carried out in lotic and lentic systems (e.g. ROUND, 1991; GASSE et al., 1995, LELAND, 1995; WINTER and DUTHIE, 2000; POTAPOVA and CHARLES, 2003; BERE and TUNDISI, 2009).

Laboratory data also provided additional evidence that concentration of specific ions influences growth of diatoms (e.g. FRITZ, 1991, 1993).

The headwater communities were dominated by *Neidium, Eunotia, Aulacoseira,* and *Pinnularia,* species whose relative abundance declined downstream and were replaced by pollution-tolerant species from the genera *Navicula*, *Nitzschia*, and *Gomphonema* with *N. palea* constituting around 85% of the species found at highly polluted site 10. From the results of partial CCA, CCAs carried out using individual variables and CCA conducted to explore the simultaneous effects of all the variables, Cl^-, K^+, Na^+, Ca^{2+}, Mg^{2+}, NH_4^+, PO_4^{3-}, F^-, SO_4^{2-} and NO_2^- ions and proportions of NH_4^+, K^+, Mg^{2+}, Ca^{2+} and NO_2^- ions were found to be significantly associated with changes in diatom communities. Thus, ion species associated with eutrophication (NH_4^+, NO_2^-, PO_4^{3-} and proportions of NO_2^- and NH_4^+) were amongst the ions associated with changes in diatom communities. According to GÓMEZ and LICURSI (2001), NH_4^+ and PO_4^{3-} have a relationship with eutrophication and are considered useful indicators of this phenomenon in the Pampean streams, Argentina.

Recent studies of environmental monitoring, using diatom communities in hydrological systems of Guaíba-RS, Brazil, have demonstrated the importance of eutrophication in structuring benthic diatom communities (OLIVEIRA et al., 2001; LOBO et al., 2002, 2004, 2006 SALOMONI et al., 2006). Nutrient enrichment may expand the high end of a taxon's salinity tolerance range (SAROS and FRITZ, 2000). A review of the literature carried out by SAROS and FRITZ (2000) suggests that in saline environments, salinity and anion composition may influence nutrient availability to primary producers, as well as nutrient requirements and uptake by diatoms. Thus, we propose that shifts in diatom species composition along gradients of ionic concentration and composition may be driven in part by nutrients, and hence the process of eutrophication.

Besides the ionic indicators of eutrophication, other ions also played an important role in structuring benthic diatom communities in this study. PEARSALL (1932) and PATRICK and REIMER (1966) pointed out the great difference between diatom communities in calcareous and calcium-poor rivers. In this study, most of the species (especially from the genus *Eunotia*, *Pinnularia* and *Aulacoseira*) tended to favour sites with low concentrations of Ca^{2+} and Mg^{2+} (Figure 3.3). Calcium affects diatom motility and adhesion to surfaces (COHN and DISPARTI, 1994), but exact physiological

mechanisms responsible for the higher or lower affinity of diatoms to calcium (or the other alkaline cations) are still not known.

The proportion of SO_4^{2-} also played an important role in structuring benthic diatom communities in this study, being highly negatively associated with the second axis of CCA (Figure 3.3). PORTAPOVA and CHARLES (2003) recorded high variation in diatom communities associated with elevated sulphate concentration, which they attributed to a combined response of diatoms to the concentration of SO_4^{2-}, water pH and total mineral content. Sulphate may also affect nutrient uptake by diatoms, as this ion is a competitive inhibitor of molybdate (MoO_4^{2-}) assimilation. Molybdenum (Mo) is an essential trace element, as it is a component of enzymes involved in nitrogen fixation and assimilatory nitrate reduction (SAROS and FRITZ, 2000).

Chloride ions had the highest prevalence in the study area (Table 3.1) and were associated with changes in diatom communities (Table 3.4). Studies carried out using individual diatom species (VIDEAU et al., 1980) have demonstrated the negative effects of chlorine on diatom growth and development. The negative influence of chlorination on the production, biomass and community composition of periphytic algae has also been studied from both non-chlorinated (NAPOLITANO et al., 1994) and chlorinated environments (BOSTON et al., 1991). DIONISIO-SESE and MIYACHI (1992) showed that chloride ions could be toxic to some freshwater algae because they inhibit the activity of carbonic anhydrase, an enzyme responsible for the hydration of carbon dioxide during photosynthesis.

G. parvulum and *N. palea*, dominant species at highly polluted sites with high chloride levels in this study, often become dominant in streams with treated sewage effluent rich in chlorine as maintenance water (FUKUSHIMA et al., 1994). FUKUSHIMA and KANADA (1999) showed that during periods when the chlorine concentration in the discharge sewage was low, algae communities tended to recover. DICKSON et al. (1977) suggested that many chlorine-tolerant organisms are also tolerant of other types of stressed environment. This could also apply to diatoms though the mechanisms are not yet fully understood.

3.4.3 Interaction of ionic strength and conductivity and other variables

From the partial CCA results, 11.3% of the diatom data variation was shared by ionic strength and conductivity and other variables, particularly eutrophication and organic pollution. These other variables explained 23.0% of variation in diatom data also

emphasising the importance of these variables in structuring benthic diatom communities in this study. Numerous studies conducted on lotic benthic diatoms sampled in various countries have shown the influence of organic pollution (SLÁDECÉK, 1986; SALOMONI et al., 2006), eutrophication (KELLY and WHITTON, 1995; PONADER et al., 2007; LAVOIE et al., 2008), and dissolved oxygen (ROUND, 1991; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003) in structuring of diatom communities.

As is often the case in multivariate analysis of species data, a large part (38.8%) of the variation remained unexplained in partial CCA. Such uncertainty is probably associated with factors such as grazing pressure, competition for resources among species and river hydrology and hydraulic characteristics that might have provided further explanatory information about the diatom communities. Therefore, in order to fully comprehend diatom communities in tropical lotic systems, these factors should be taken into consideration. Another factor that may contribute to the high percentage of unexplained variance is the presence of many species with broad tolerances for the environmental gradient (centre of the plot, Figure 3.3) whose contribution for the CCA is practically null.

Due to the interactive nature of ionic strength and conductivity and other variables, validity of diatoms as indicators of ionic strength and conductivity is difficult to determine *in situ*. For example, even if sites show clear differences in ionic strength and conductivity in this study, variation in other environmental factors (mainly eutrophication and organic pollution) of natural and/or anthropogenic origin, which also explained important amount of variation in diatom data (23.0%), was inevitable amongst the sites. Combined effects of these factors may have a significant impact on the effects of ionic strength and conductivity on diatom communities. Environmental monitoring studies in Southern Brazil (e.g. OLIVEIRA et al., 2001; LOBO et al., 2002, 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. However, the interaction of organic pollution and eutrophication with ionic strength was not thoroughly studied.

In this study, we have partitioned the variance in diatom data between two sets of exploratory, i.e. ionic strength and conductivity and other variables. However, due to the small differences in the variances explained by ionic strength and conductivity and other variables and the high variance shared between the two sets of exploratory variables, little

more can be done than speculating the controlling factors on diatom communities. Causal relationships between factors cannot be determined without experimentation. Laboratory experiments, conducted under controlled conditions must, therefore, be performed to confirm the effects of ionic strength and conductivity reported in this field study. We propose that mesocosms experiments (e.g. FUKUSHIMA and KANADA, 1999; HERBST and BLINN, 1998; DEFEW et al., 2002) should be used as model ecosystems under controlled conditions to separate the effects of ionic strength and conductivity from other variables. Observations can then be extrapolated to predict natural systems.

3.5 Conclusion

We have partitioned the variance in diatom data between two sets of exploratory, i.e. ionic strength and conductivity and other variables in this study. Due to the interaction of the effects of ionic strength and conductivity and other variables in this study, laboratory experiments must be performed to confirm the reported effects of ionic strength and conductivity. Certain taxa associated with high ionic strength and conductivity, eutrophication and organic pollution (e.g. *P. lanceolatum*, *C. hyaline*, *C. meneghiniana*, *parvulum*, *F. monoculata*, *L. goeppertiana*, *N. palea, N. praecipua*, *N. rostellata*, *P. subcapitata* and *S. pupula*) may be used in future studies as potential indicator species for changes in these variables. These species can be subjected to further experiments to confirm their status of indicator species. The autecological data presented in this study improves our understanding of how diatoms are distributed in Brazilian water habitats with respect to ionic strength and composition. This is useful for the purposes of effective water quality assessment and general assessment of ecological conditions of lotic systems and contributes to further understanding of the ecology of diatoms.

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CHAPTER 4

Influence of land-use patterns on benthic diatom communities and water quality in the tropical Monjolinho hydrological basin, São Carlos-SP, Brazil[4]

Abstract: The objective of this study was to determine the effects of land-use patterns on both diatom community composition and water quality in tropical streams during the dry season. Benthic diatom collections and water quality sampling were made four times at 10 sites. A suite of environmental variables that varied with human land-use pattern was assessed to find the combination of variables that best explained patterns of diatom community composition. Canonical Correspondence Analysis CCA was used to determine environmental gradients along which species are distributed. Upstream, forested, agricultural sites, with good water quality (BOD_5 = 0.9 to 2.6 mg L^{-1}, DO = 6.8 to 8.2 mg L^{-1}, phosphate = >2 to 4.7 µg L^{-1}), were characterized by *Thalassiosira weissflogii, Orthoseira dentroteres, Meridion anceps, Melosira varians, Diatoma spp, Diadesmis contenta, Eunotia papillo, E. bilunaris, E. intermedia, E. sudetica, Aulacoseira alpigena, A. ambigua, Cymbopleura naviculiformis* and *Stauroneis phoenicenteron.* Urban sites, with medium to bad water quality (BOD_5 = ~7 mg L^{-1}, DO = ~7 mg L^{-1}, phosphate = 12.6 to 83.1 µg L^{-1}), were characterised by *Diadesmis dissimilis, Frustulia rhomboids, Nitzschia scalaris, Nitzschia linearis, Cyclotella pseudostelligera, Neidium ampliatum, N. affine, Encyonema silesiacum, E. neomesianum, Aulacoseira granulata, Navicula cryptotenella, Pinnularia legumen, P. gibba, P. divergens, Surirella linearis, S. robusta,* and *Achnanthidium minutissimum.* Downstream urban sites, with very bad water quality (BOD_5 = 19.5 to 26.2 mg L^{-1}, DO = 0.4 to 1.9 mg L^{-1}, phosphate = 142.5 to 248.7 µg L^{-1}), were characterised by *Gomphonema parvulum, G. accuminatum, Nitzschia palea, Nupela praecipua, Sellaphora pupula, Planotidium lanceolatum, Fallacia monoculata* and *Pinnularia subcapitata.* Diatom communities demonstrated potential of acting as indicators of changes in water quality due to changes in catchment land-use patterns.

Key words: land-use, benthic diatoms, environmental gradient, agricultural, forests, urban.

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Influência dos padrões de uso do solo nas comunidades de diatomáceas bentônicas e na qualidade da água da bacia hidrológica tropical do rio Monjolinho, São Carlos-SP, Brasil

Resumo: O objetivo deste estudo foi determinar os efeitos dos padrões de uso do solo na composição da comunidade de diatomáceas e na qualidade da água de córregos tropicais durante a estação seca. Amostragens de diatomáceas bentônicas e avaliação da qualidade da água foram feitas quatro vezes, em 10 pontos de coleta. Um conjunto de variáveis ambientais, que dependem do padrão do uso do solo pelo homem, foi avaliado para determinar a combinação de variáveis que melhor explica os padrões de composições das comunidades de diatomáceas. Análise de correspondência canônica (ACC) foi utilizada para determinar os gradientes ambientais ao longo dos quais as espécies são distribuídas. Pontos de coleta nas nascentes florestadas e agrícolas, com boa qualidade da água (DBO_5 = 0,9 para 2,6 $mg.L^{-1}$, OD = 6,8-8,2 $mg.L^{-1}$, fosfato = > 2.0 - 4,7 $mg.L^{-1}$), foram caracterizados por *Thalassiosira weissflogii, Orthoseira dentroteres, Meridion anceps, Melosira varians, Diatoma spp, Diadesmis contenta, Eunotia papillo, E. bilunaris, E. intermedia, E. sudetica, Aulacoseira alpigena, A. ambigua, Cymbopleura naviculiformis* e *Stauroneis phoenicenteron*. Pontos de coleta na área urbana, como média à baixa qualidade da água (DBO_5 = ~ 7 $mg.L^{-1}$, DO = ~ 7 $mg.L^{-1}$, fosfato = 12,6 - 83,1 $mg.L^{-1}$), foram caracterizados por *Diadesmis dissimilis, Frustulia rhomboids, Nitzschia scalaris, Nitzschia linearis, Cyclotella pseudostelligera Neidium ampliatum, N. affine, Encyonema silesiacum, E. neomesianum, Aulacoseira granulata, Navicula cryptotenella, Pinnularia legumen, P. gibba, P. divergens, Surirella linearis, S. robusta,* e *Achnanthidium minutissimum*. Pontos de coleta junto à área urbana, com muito baixa qualidade da água (DBO_5 = 19,5-26,2 $mg.L^{-1}$, DO = 0,4 - 1,9 $mg.L^{-1}$, fosfato = 142,5 - 248,7 $mg.L^{-1}$), foram caracterizados por *Gomphonema parvulum, G. accuminatum, Nitzschia palea, Nupela praecipua, Sellaphora pupula, Planotidium lanceolatum, Fallacia monoculata* e *Pinnularia subcapitata.* Comunidades de diatomáceas demonstraram potencial para atuar como indicadores de mudanças na qualidade da água devido às mudanças nos padrões de captação de uso do solo.

Palavras-chave: uso do solo, diatomáceas bentônicas, gradiente ambiental, agricultura, florestas urbanas.

4.1 Introduction

Land-use; a function of cultural and settlement patterns, economic factors, environmental characteristics (ROBBINS et al., 1983; BLACK et al., 1998); and water resources (hence biotic communities supported by these water resources) are unequivocally linked. For example, results from predominantly agricultural watersheds with some forests and urban development showed that land-use had a distinct overall and seasonal effect on water quality (OSBORNE and WILEY, 1988). Studies in Pinelands of New Jersey also showed substantial land-use effects on natural water quality because of agricultural development, urban densities, and domestic wastewater flow (ZAMPELLA, 1994). Nutrient concentration, particularly phosphorus, increases with urban development due to storm water runoff (OSBORNE and WILLEY, 1988; WELCH et al., 1998). Increase in nutrient concentration is also a result of other catchment activities, such as runoff from agriculture or sewage treatment plants.

Changes in water quality of lotic systems because of surrounding land-use patterns affects the resulting biotic communities as the patterns of this biota are responsive to the nature of the prevailing physical and chemical conditions. The integrity of biota inhabiting lotic ecosystems thus provides a direct, holistic and integrated measure of the ecological conditions of a system as a whole (KARR, 1991) and the effects of land-use patterns. The relationship between diatoms and water physical and chemical variables is robust and quantifiable, making diatoms appropriate quantitative indicators of ecological conditions in lotic systems (PAN et al., 1996; OLIVEIRA et al., 2001; ZAMPELLA et al., 2007).

Growth and development of benthic diatoms in streams is an outcome of complex interaction between hydrological, chemical and biotic factors. Local factors like hydrology, light, temperature and water chemistry are controlled by regional factors such as geology, topography or climate operating at spatial scales of catchments as well as ecoregions (BIGGS, 1990). In addition, human land-use activities act to change both local and regional variables in an increasing rate affecting the resultant biotic communities. WINTER and DUTHIE (2000) showed that the diatom community composition along an urban-rural gradient was correlated with total phosphorus and total nitrogen.

Many studies have related diatom community composition to environmental variation mainly in the temperate regions (e.g. BIGGS, 1990, 1995; DESCY and COSTE, 1991; ROCHA, 1992; PAN et al., 1996; LOBO et al., 1998; ROTT et al., 1998;

CHESSMAN et al.,1999; LOEZ and TOPALIAN, 1999; STEVENSON and PAN, 1999; POTAPOVA and CHARLES, 2003, 2005; DE LA REY et al., 2008). However, only few of them have specifically focused on the effect of land-use patterns in tropical systems, in particular north of Brazil. Understanding the relationship between land-use patterns, water quality and diatom composition in streams provides a useful starting point for establishing stream water quality control regulations, conservation goals, ecological restoration efforts, and necessary research hypotheses for management of Brazilian and tropical lotic systems in general.

The objective of this study was to assess the response of stream diatom assemblages to changes in water quality associated to land-use, i.e., from agricultural and forest to the urban areas. A suite of environmental variables that varied with human land-use pattern was assessed to find the combination of variables that best explained patterns of diatom community composition.

4.2 Materials and Methods

4.2.1 Study area and study design

The study area is shown in Figure 4.1. Headwaters of the study streams fall mainly within an agricultural area. Apart from agricultural practices in the headwaters, the streams flow through an urban area of the city of São Carlos that covers a total area of 1143.9 km^2. The area has rugged topography and an average annual temperature of around 19.5 °C, with mean monthly maximum of around 21.9 °C recorded in January and February and the mean monthly minimum of around 15.9 °C recorded in July.

In 2008, the population of São Carlos was estimated to be 218080 inhabitants by Instituto Brasileiro de Geografia e Estatística (IBGE). The current expansion of the city does not meet the technical standards for sewage treatment, garbage collection and urban drainage. Streams in the study area, therefore, receive untreated or semi-treated effluent from various domestic and industrial sources as well as other diffuse sources as they flow through the city. The city also expanded without considering environmental, geological and topographical factors, leading to deforestation, erosion, and siltation. This unplanned growth, typical for many Brazilian cities and most developing country cities, results in many problems including stream health deterioration, loss of primary vegetation, and eutrophication (DUPAS et al., 2006; RÖRIG et al., 2007).

Ten sites were established along Monjolinho River and its tributaries: four sites (1, 2, 3 and 7) in the relatively less impacted agricultural and forested headwaters; three

sites (4, 5 and 6) in the moderately polluted urban area; and 3 sites (8, 9 and 10) in highly polluted downstream area after the urban area (Figure 4.1). The rational for choosing the sampling sites was to cover a pollution gradient of all the stream systems from relatively unpolluted agricultural headwaters to highly polluted urban downstream sites. Most of the area above upstream sites 1, 2, 3 and 7 was mainly agricultural and characterized by mature deciduous riparian forest strips of about 5 to 50 m wide with a mixture of pastures, homesteads and farmland beyond the riparian strip (Table 4.1). The area also had a low density of buildings, and much of the road network was unpaved. The area above sites (4, 5, 6, 8, 9 and 10) was mainly urban with more residences along the streams, a mostly paved road network and reduced riparian strips dominated by grass. In some cases, the grass along the stream edges is continuously mown. Some sections of the streams are canalized. Land-use patterns were interpreted from Google Earth Satellite Image System, June 2005 (Figure 4.1) using ANDERSON et al. (1976) classification with classes being combined to form three broad categories (forest, agriculture, and urban).

Table 4.1: Estimated land-use patterns of catchments above the 10 sampling sites used in the study.

Site	1	2	3	4	5	6	7	8	9	10
Forest Area (km^2)	0.5	0.3	1.8	3.1	0.4	0.6	1.7	0.9	3.4	0.1
Agricultural Area (km^2)	1.3	0.2	5.7	4.6	0.2	0.2	9.1	1.1	0.6	0.2
Urban Area (km^2)	0.1	0.1	1.3	7.7	8.6	10.5	5.8	5.6	10.3	0.1

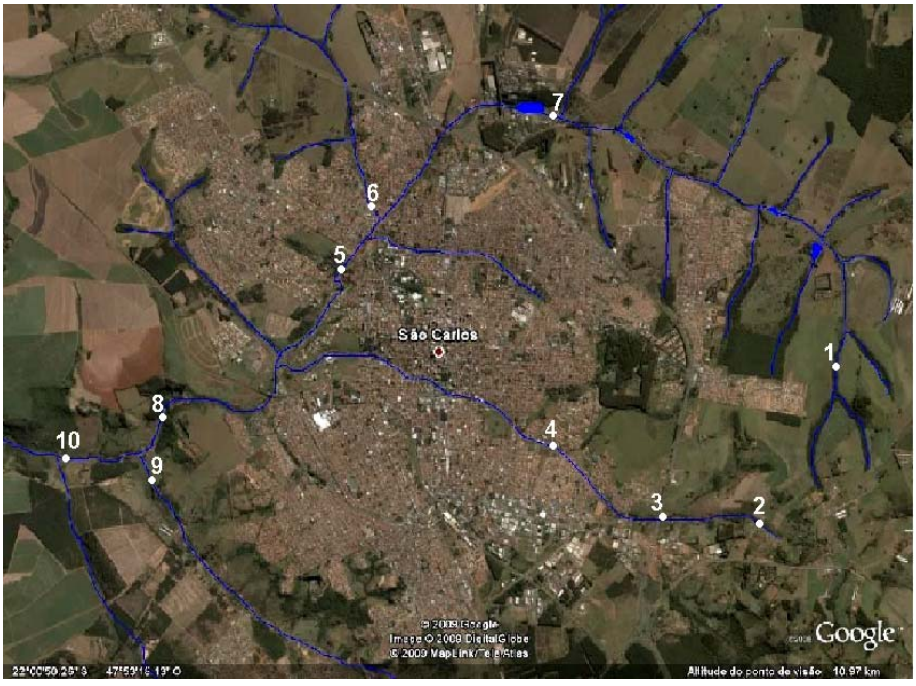

Figure 4.1: The location of the sampling sites in the study area. forest area, pastures and farmland (agricultural area), land preparation for crop cultivation (agricultural area) when outside urban area, buildings and roads (urban area).

4.2.2 Water quality

Water quality sampling was done during dry season when flow was stable. Four sampling trips were undertaken, two in September and October 2008 and two in May and June 2009. Dry season was selected to avoid variable effects of rainy season like great variations in water level and velocity, floods and inundations, 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 measured at each station with an FP 201 global flow probe (Global Water Instrumentation Inc. Alaska, USA). The percentage of riparian vegetation cover was estimated at each site. The percentage embededness (the degree to which large particles are covered with fine particles) was also estimated along each stretch and rated on a 0–5 scale following PLATTS et al. (1983). Altitude was determined at each site 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), total phosphorus (TP), biological oxygen demand (BOD_5), nitrate and phosphate analysis were also collected at each site into acid-cleaned polyethylene bottles following APHA (1988).

In the laboratory, the concentrations of TN, TP BOD_5, nitrate and phosphate in the water samples were determined following standard methods (APHA, 1988). BOD_5, nitrate, phosphate, DO (percent saturation), temperature, pH, turbidity and TDS were used for calculation of a water quality index (WQI) after BROWN et al. (1970). This is a method of expressing water quality that offers a simple, stable and reproducible unit of measurement that respond to changes in the principle characteristics of water quality. In this system, the values are given numerical ranking in relation to selected control values for different parameters. The information is then used to compute the standardized distance from control values for each parameter to produce an index of water quality calculated as the weighted average of variables. The water quality index was calculated; with modification due to absence of fical coli form data, as follows:

$$WQI = \frac{\sum_{i=1}^{n} Wi \times Qi}{\sum Wi}$$

where, *Wi* is the weight and *Qi* is the quality score of available variable *i*. *WQI* is a number between 0 and 100, with 90 to100 = excellent, 70 to 90 = good, 50 to 70 = medium, 25 to 50 = bad and 0 to 25 = very bad (BROWN et al., 1970).

4.2.3 Diatom sampling

At each site, epilithic, epiphytic, epipelic and epipsammic diatoms were sampled separately. Epilithic diatoms were sampled by brushing stones with a toothbrush. Prior to sampling of epilithic surfaces, all substrata were gently shaken in stream water to remove any loosely attached sediments and non-epilithic diatoms. At least five pebble-to-cobble sized stones were randomly collected at each sampling site and brushed, and the resulting diatom suspensions were pooled to form a single sample, which was then put in a labelled plastic bottle. Epiphytic diatoms were sampled from different species of submerged and emergent macrophytes (e.g. *Rumex crispus, Alternanthera philoxeroides, Ludwigia spp, Rhynchathera spp, Ageratum spp, Hedychium coronarium, Eleocharis spp, Heteronthera spp, Polygonum spectable* and *Brachiaria arrecta*) at each site depending on the availability of the macrophytes. The macrophyte's whole stalk comprising of stalk and leaves was carefully removed from the stream. Periphyton was then removed from the macrophytes by brushing with a toothbrush adding distilled water. The resulting diatom suspensions from all the submerged macrophytes sampled were pooled to form a single sample, which was then put in a labelled plastic bottle. Epipelic and epipsammic diatoms were sampled by pressing Petri dish lid into the top layer of sand or 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. Five samples at each site were collected and pooled into a single sample.

In the laboratory, 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 and KILROY (2000). Three replicate slides were prepared for each sample. A total of 250 – 600 valves per sample (based on counting efficiency determination method by PAPPAS and STOERMER (1996)) were identified and counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type - 020-519.503 LB30T, Germany). The mean and standard deviations of counting efficiencies of diatom communities calculated according to PAPPAS and STOERMER (1996) on different

substrates were as follows: macrophytes, 82.5 ±11.4 %; sand, 86.1 ±7.6 %; stones, 83.6 ±18.5 % and silt/clay, 82.9 ±14.2 %. The diatoms were identified to species level based on studies by LOBO et al., 2002; METZELTIN ET AL. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007).

4.2.4 Data analysis

The distributions of turbidity, conductivity, TDS, width, and embededness were positively skewed, and were therefore ln(× + 1) transformed (ZAR, 1984). Two-way Analysis of Variance (Two-way ANOVA) was used to compare means of environmental variables among the four sampling periods and among the three site categories (section 4.2.1).

Multivariate data analyses were performed on the diatom data set to indicate the main gradients of floristic variation and to detect and visualize similarities in diatom samples. Preliminary detrended correspondence analysis (DCA) was applied on diatom data set to determine the length of the gradient. This DCA revealed that the gradient was greater than three standard deviation units (4.2) justifying the use of unimodial ordination techniques (TER BRAAK and VERDONSCHOT, 1995). Thus, Canonical Correspondence Analysis (CCA) was used to investigate relationships between predictor variables and benthic diatom communities from different sites. Preliminary CCA identified collinear variables and selected a subset on inspection of variance inflation factors (VIF< 20; TER BRAAK and VERDONSCHOT, 1995). Monte Carlo permutation tests (999 unrestricted permutations, $p \leq 0.05$) were used to test the significance of the axis and hence determine if the selected environmental variables could explain nearly as much variation in the diatom data as all the measured environmental variables combined. Input for the programme included the relative abundance of diatom taxa that were present in a minimum of two samples and had a relative abundance of ≥1% in at least one sample. All statistical analyses were performed using PAlaeontological STatistics (PAST) software version 1.90 (HAMMER et al., 2009).

4.3 Results

4.3.1 Water quality

The values of physical and chemical variables measured in the study area during the study period are shown in Table 4.2. The pH increased slightly down the agricultural to urban gradient being slightly acidic at upstream sites and slightly alkaline/neutral at downstream sites. However, the difference in pH among the three site categories (section 2.1) was not statistically significant (ANOVA, $p > 0.05$). Temperature increased downstream, but as in the case of pH, the increase was not significant (ANOVA, $p > 0.05$). On the other hand, conductivity, BOD_5, TDS, turbidity, light intensity, TN, TP, nitrate, phosphate and embeddedness increased significantly downstream (ANOVA, $p < 0.05$) while DO and percentage riparian vegetation cover decreased significantly downstream (ANOVA, $p < 0.05$). No significant differences were observed in mean environmental variables among the four sampling periods (ANOVA, $p > 0.05$). This is expected since all sampling was confined to stable base flow period when variations in water chemistry are low compared to the rainy season. Therefore, the mean environmental variables of the four sampling periods at each site were used for subsequent analysis.

The water quality generally tended to deteriorate downstream as the streams pass 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 (Figure 4.2). The mean water quality of sites 1, 2 and 3, situated in forested agricultural area, as well as site 7, was good (with excellent water quality at site 1 during September and October 2008). Site 4 exhibited bad water quality while sites 5 and 6 showed medium water quality. On the other hand, the water quality of sites 8, 9 and 10, situated after the urban area, was very bad.

4.3.2 Community analysis

A total of 208 diatom species belonging to 63 genera were recorded. Twenty-two genera accounted for 91.7 % of the overall diatom community. Of the 208 species observed, 71 species were retained for subsequent analysis (present in a minimum of two samples and had a relative abundance of ≥ 1% in at least one sample; Table 4.3). A pooled data set, consisting of diatoms sampled during four sampling periods and among the four substrates sampled was used to investigate spatial trends in the composition of diatom communities.

Table 4.2: The mean values of physical and chemical variables measured at 10 sites during 4 sampling periods. The sites are arranged in order of general increase in pollution based on physical and chemical variables and dotted line separates 'reference' sites from the rest of the sites.

Site	1	2	3	7	4	5	6	8	9	10
Temperature (^{o}C)	18.3 ±1.1	20.9 ±1.6	20.6 ±1.7	20.4 ±1.5	24.0 ±2.6	21.2 ±1.7	21.2 ±1.1	24.8 ±3.0	23.0 ±1.9	21.3 ±2.2
Altitude (m)	761	837	831	761	774	794	745	724	630	627
Canopy cover (%)	80	95	60	45	20	50	4	20	50	5
Light intensity (μmol s^{-1} m^{-2})	99.8 ±5.3	347.7 ±61.0	439.3 ±7.2	612.3 ±99.2	1806.0 ±286.0	1757.5 ±53.3	1357.7 ±129	2019.8 ±61.6	1556.3 ±88.0	2216.8 ±19.2
BOD_5 (mg.L^{-1})	0.9 ±0.2	1.0 ±1.2	2.6 ±0.2	1.2 ±2.3	6.9 ±0.2	1.6 ±0.6	7.2 ±1.1	19.5 ±1.1	24.5 ±1.2	26.2 ±1.3
DO (mg.L^{-1})	7.3 ±2.3	8.2 ±1.0	7.6 ±0.6	7.2 ±1.5	6.8 ±2.1	6.9 ±1.0	7.6 ±1.3	1.9 ±2.8	2.1 ±1.0	0.4 ±1.2
Conductivity (μS.cm^{-1})	45.0 ±7.5	20.0 ±10.5	53.0 ±9.5	30.0 ±4.0	28.0 ±7.7	89.0 ±8.9	103.0 ±6.4	715.0 ±22.3	322.0 ±191.3	283.0 ±201.7
pH	6.6 ±0.8	6.4 ±1.0	6.3 ±1.0	6.8 ±1.0	6.7 ±0.6	6.8 ±0.9	7.2 ±0.4	7.2 ±0.5	7.2 ±0.4	7.1 ±0.4
TDS (g.L^{-1})	29.4 ±0.5	13.4 ±1.2	22.6 ±3.2	19.3 ±0.2	18.1 ±1.4	57.4 ±3.4	66.5 ±2.9	457.8 ±27.1	206.1 ±11.1	182.0 ±34.1
Turbidity (NTU)	5.1 ±1.2	4.2 ±1.1	4.7 ±1.3	7.3 ±1.6	19.5 ±4.4	11.1 ±2.9	13.2 ±3.4	45.3 ±10.7	53.2 ±12.1	60.4 ±15.4
TN (mg.L^{-1})	0.65 ±0.3	0.18 ±0.3	0.24 ±1.4	0.93 ±0.7	1.72 ±0.5	1.29 ±0.4	1.41 ±0.5	38.32 ±8.3	14.87 ±4.1	10.17 ±2.9
TP (mg.L^{-1})	0.01 ±0.0	0.01 ±0.0	0.01 ±0.1	0.02 ±0.0	0.03 ±0.0	0.16 ±0.2	0.06 ±0.1	2.97 ±1.0	1.12 ±0.2	0.75 ±0.2
Nitrate (μg.L^{-1})	51.9 ±22.3	195.6 ±33.1	470.0 ±38.5	98.2 ±8.8	714.8 ±232.3	819.6 ±667.2	745.0 ±595.3	1141.2 ±1025.4	176.5 ±142.5	441.1 ±273.2
Depth (m)	0.2 ±0.02	0.3 ±0.08	0.4 ±0.10	0.4 ±0.01	0.2 ±0.05	0.4 ±0.08	0.3 ±0.05	0.5 ±0.07	0.3 ±0.04	0.3 ±0.01
Velocity (m.s^{-1})	2.5 ±1.3	2.8 ±1.4	2.6 ±1.3	2.9 ±1.1	2.2 ±1.1	2.7 ±1.2	1.4 ±0.6	3.5 ±1.8	2.4 ±0.9	2.34 ±1.0
Embeddedness	0	0	1	1	2	3	1	5	1	4
Stream order	2	1	2	2	3	3	3	3	3	4
Site	1	2	3	7	4	5	6	8	9	10
Temperature (^{o}C)	18.3 ±1.1	20.9 ±1.6	20.6 ±1.7	20.4 ±1.5	24.0 ±2.6	21.2 ±1.7	21.2 ±1.1	24.8 ±3.0	23.0 ±1.9	21.3 ±2.2

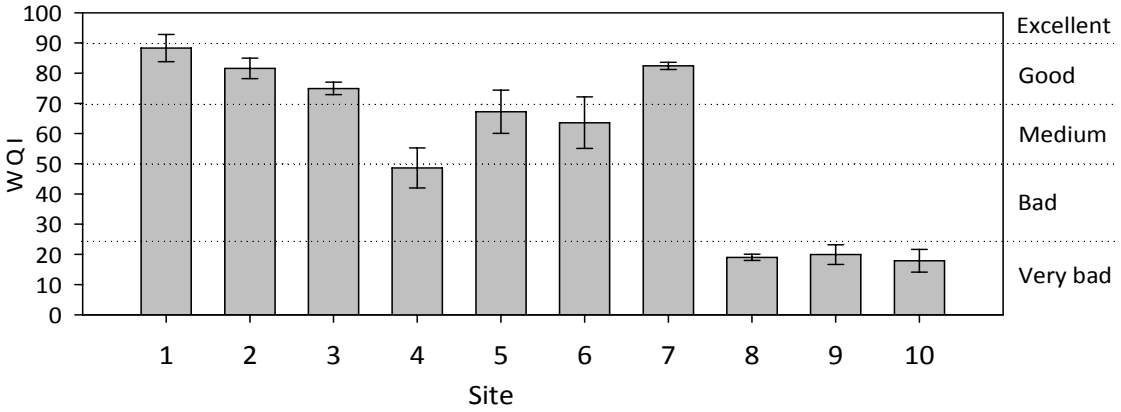

Figure 4.2: The mean values and standard deviations of water quality index (WQI) recorded at all sites.

The results of the Canonical Correspondence Analysis (CCA) are presented in Figure 4.3. The first four axes of the species-environment plot accounted for 68.7% of the total variance in the community due to measured environmental variables. Axis 1 and 2 explained 47.7% and 21.0% respectively of the diatom species variance. 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 ($p \leq 0.05$). BOD_5 and pH were positively associated with the first axis while DO and percentage canopy cover were negatively respectively associated with the first axis. pH was also positively associated with the second axis while stream velocity was negatively associated with the second axis.

CCA axis 1 and 2 separated the sites into three groups along an agricultural to urban gradient (human-induced increase in nutrients and organic pollution and decrease in DO and percentage canopy cover). The first group consisted of forested and agricultural sites with mean good water quality 1, 2 and 3 that were negatively associated with the first and second axis in the bottom left quadrant (Figure 4.3). These sites were associated with high canopy cover (which was negatively correlated to temperature, light intensity and mean stream width) and low BOD_5 (which was positively correlated with TDS, TN, TP, nitrate, phosphate, conductivity and depth). Diatom species characterising these sites include species such as *T. weissflogii, O. dentroteres, M. anceps, M. varians, Diatoma* spp*, D. contenta, E. papillo, E. bilunaris, E. intermedia, E. sudetica, A. alpigena, A. ambigua, C. naviculiformis* and *S. phoenicenteron.*

The second group consisted of bad to medium water quality urban sites 4, 5 and 6 that were negatively and positively associated with the first and second axis respectively in the upper left hand quadrant. Site 7 was also placed in this group despite its good water quality. Diatom species characterising these sites include species such as *D. dissimilis, F. rhomboids, S. robusta, N. scalaris, C. pseudostelligera, N. ampliatum, E. silesiacum, E. neomesianum, N. affine, N. linearis, A. granulata, N. cryptotenella, P. legumen, P. gibba, P. divergens, S. linearis* and *A. minutissimum.* The third group consisted of very bad water quality downstream sites 8, 9 and 10 positively associated with the first axis. These sites were associated with high nutrients and organic pollution, low percentage canopy cover and low DO. These sites were associated with such species as *N. palea*, *G. parvulum, S. pupula*, *N. praecipua, P. lanceolatum*, *F. monoculata*, *P. subcapitata* and *G. accuminatum*. These species have been reported to be pollution tolerant (BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003; DUONG et al., 2006).

Table 4.3: The distribution of most abundant diatom species. * = 0-10 %, ** = 10-30 %, *** = >30 %).

Species	Code	1	2	3	4	5	6	7	8	9	10
Achnanthes exigua Grunow	Aexi	*	*	*	*	*	*	*			
Achnanthidium biasolettianum (Grunow) Lange-Bertalot	Abia	*	*	*	*	*	*	*			
Achnanthidium minutissimum (Kützing) Czarnecki	Amin	*	*	*	*	*	*	*			
Amphora copulata (Kützing) Schoeman and Archibald	Acop	*	*	*	*	*	*	*			
Aulacoseira agassizii (Hustedt) Simonsen	Aaga		*	*	*	*	*	*			
Aulacoseira alpigena (Grunow) Krammer	Aalp		*	*	*	*	*	*			
Aulacoseira ambigua (Grunow) Simonsen	Aamb	*	*	*		*	*	*			
Aulacoseira distans (Ehrenberg) Simonsen	Adis		*	*		*	*	*	*		
Aulacoseira granulata (Ehrenberg) Simonsen	Agra	*	*	*	*	*	*	*			
Caloneis hyaline Hustedt	Chya	*	*					*			
Craticula cuspidata (Kützing) Mann	Ccus	*		*					*		
Cyclotella meneghiniana Kützing	Cmen		*		*	*	*	*	*	*	*
Cyclotella pseudostelligera Hustedt	Cpse		*	*		*	*	*	*		
Cyclotella spp.	Cspp	*	*	*	*	*	*	*	*	*	*
Cyclotella stelligera (Cleve and Grunow) Van Heurck	Cste	*					*	*			
Cymbopleura naviculiformis (Auerswald) Krammer	Cnav	*	*	*	*	*	*	*			
Diadesmis contenta (Grunow) Mann	Dcon	*	*	*	*	*	*	*	*		*
Diadesmis dissimilis Moser, Lange-Bertalot & Metzeltin	Ddes	*		*			*				
Diatoma spp	Dspp	*	*	*				*			
Diatoma vulgare Bory	Dvul	*	*	*	*	*	*	*			
Encyonema neomesianum Krammer	Eneo	*	*	*	*	*	*	*			
Encyonema silesiacum (Bleisch) Mann	Esil	*	*	*	*	*	*	*	*		
Eunotia bilunaris (Ehrenberg) Mills	Ebil	*	*	**	*	*	*	*			
Eunotia camelus Ehrenberg	Ecam	*	*	*	*	*	*	*			
Eunotia intermedia (Hustedt) Nörpel and Lange-Bertalot	Eint	**	*	*	*	*	*	*			
Eunotia monodon Ehrenberg	Emon	*	*	*		*	*	*			
Eunotia papillo (Ehrenberg) Hustedt	Epop	*	*			*	*	*			
Eunotia pectinalis (Kützing) Rabh	Epec	**	*	*	*	*	*	*			
Eunotia rabenhorstii Cleve & Grunow	Erab	*		*			*	*			
Eunotia sudetica Müller	Esud		*	*	*	*		*			
Fallacia monoculata (Hust) Mann	Fmon				*	*	*	*	*	*	*
Fragilaria capucina Desmazières	Fcap	*	*	*	*	*	*	*			
Fragilaria intermedia Grunow	Fint	*	*	*	*	*	*	*			*
Frustulia rhomboides (Rabenhorst) De Toni	Frho	*	*	*	*	*	**	*			
Frustulia saxonica Rabenhorst	Fsax	*	*	*	*	*	*	*			
Frustulia vulgaris (Twaithes) de Toni	Fvul	*	*	*	*	*	*	*			
Gomphonema accuminatum Ehrenberg	Gacc	*	*	*	*	*	*	*	*		
Gomphonema angustatum (Kützing) Rabenhorst	Gang	*	*	*	*	*	*	*		*	*
Gomphonema augur (Ehrenberg) Lange-Bertalot	Gaug	*	*	*	*	*	*	*			
Gomphonema gracile Ehrenberg	Ggra	*	*	*	*	*	*	*			
Gomphonema olivaceum (Hornemann) Brébisson	Goli	*	*	*	*	*	*	*			
Gomphonema parvulum (Kützing) Kützing	Gpar	*	*	*	*	*	*	*	*	**	*
Gomphonema turris Ehrenberg	Gtur	*	*			*		*			
Hantzschia amphioxys (Ehrenberg) Grunow	Hamp	*	*	*	*	*	*	*			
Luticola goeppertiana (Bleisch) Mann	Lgeo	*	*	*	*	*	*	*	*	*	**
Melosira varians Agardh	Mvar		*	*	*	*	*	*			
Meridion anceps (Ehrenberg) Williams	Manc	*	*	*	*	*		*			
Navicula cryptocephala (Grunow) Cleve	Ncry	*	*	*	*	*	*	*			*
Navicula cryptotenella Lange-Bertalot	Ncrt	*	*	*	*	*	*	*	*		
Navicula oblonga Kützing	Nobl	*	*	*	*	*	*	*			
Navicula radiosa Kützing	Nrad	*	*	*	*	*	*	*			
Navicula rostellata Kützing	Nros			*	*	*	*	*			
Neidium affine (Ehrenberg) Pfitzer	Naff	*	*	*	*	*	*	*			
Neidium ampliatum (Ehrenberg) Krammer	Namp						*	*			
Nitzschia linearis (Agardh) Smith	Nlin	*	*	*	*	*	*	*		*	
Nitzschia palea (Kützing) Smith	Npal		*	*	**	*	**	*	***	**	***
Nitzschia recta Hantzsch ex Rabenhorst	Nrec	*	*	*	*	*	*	*	*	*	*
Nitzschia scalaris (Kütz) Grunow	Nsca	*		*	*	*	*	*			
Nupela praecipua (Reichardt) Reichardt.	Npra	*	*	*	*	**	*	*	*	**	*
Orthoseira dentroteres (Ehrenberg) Crawford	Oden	*	*	*							
Pinnularia braunii (Grunow) Cleve	Pbra	*		*	*	*	*	*	*	*	*
Pinnularia divergens Krammer	Pdiv	*		*	*	*	*	*			
Pinnularia gibba Ehrenberg	Pgib	*	*	*	*	*	*	*	*	*	*
Pinnularia lata (Brébisson) Rabenhorst	Plat	*		*	*	*	*	*			
Pinnularia legumen Ehrenberg	Pleg			*	*		*	*			
Pinnularia microstauron (Ehrenberg) Cleve	Pmic	*			*	*	*	*	*		
Pinnularia subcapitata Gregory	Psub				*	*	*	*	*	*	*
Placoneis clementis (Grunow) Cox	Pcle		*	*	*	*	*	*			
Planothidium dubium (Grunow) Round and Bukhtiyarova	Pdub	*	*	*	*	*	*	*			
Planothidium lanceolatum (Brébisson) Grunow	Plan	*	*	*	*	*	*	*	*	*	*
Planotidium heteroideum	Phet		*	*	*	*	*	*			
Pleurosigma compactum Greville	Pcom				*		*	*			
Psammothidium subatomoides (Hustedt) Bukhtiyarova	Psuba	*	*	*	*	*	*	*		*	*
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	Rabb	*	*	*	*	*	*	*	*	*	*
Sellaphora pupula (Kützing) Mereschkowsky	Spup	*	*	*	*	*	*	*	*	*	*
Stauroneis phoenicenteron (Nitzsch) Ehrenberg	Spho	*	*	*	*	*	*	*			
Surirella angusta Kützing	Sang	*		*	*	*	*	*			
Surirella linearis Smith	Slin	*	*	*	*	*	*	*			
Surirella ovata Kutzing	Sova	*		*		*	*				
Surirella robusta Enrenburg	Srob	*		*	*		*	*			
Thalassiosira weissflogii (Grunow) Fryxell and Hasle	Twei	*	*	*							
Ulnaria ulna (Nitzsch) Compère	Uuln	*	*	*	*	*	*	*	*		*

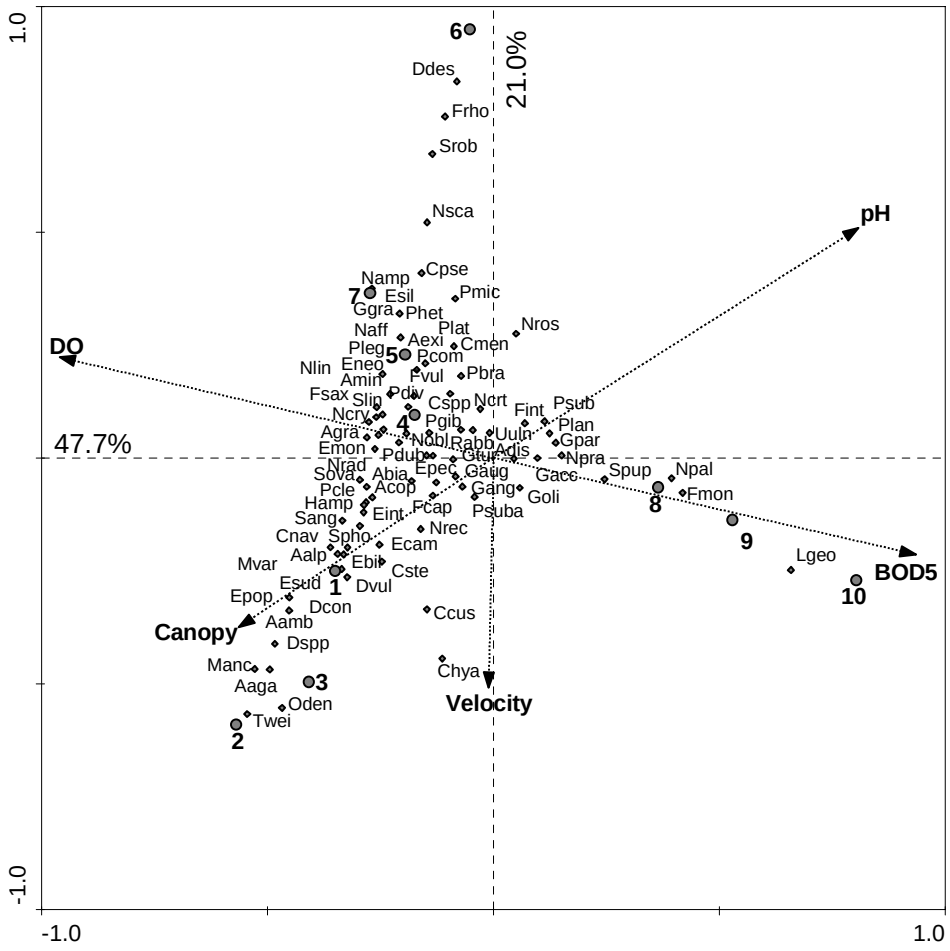

Figure 4.3: Ordination diagram based on canonical correspondence analysis (CCA) of most dominant diatom species composition in 10 sampling sites with respect to five environmental variables (percentage canopy cover, BOD5, stream velocity, pH and DO). Taxa codes correspond to those in Table 4.3.

4.4 Discussion

Diatom community structure showed a clear relationship with changes in water quality associated with changes in land-use pattern. Along forest and agricultural to urban land-use gradient (human-induced increasing nutrient and organic pollution gradient), low pollution tolerant species such as *T. weissflogii, O. dentroteres, M. anceps, M. varians, Diatoma spp, D. contenta, E. papilla, E. bilunaris, E. intermedia, E. sudetica, A. alpigena, A. ambigua, C. naviculiformis* and *S. phoenicenteron* were replaced by moderate pollution tolerant species such as *D. dissimilis, F. rhomboids, S. robusta, N. scalaris, C. pseudostelligera N. ampliatum, E. silesiacum, E. neomesianum, N. affine, N. linearis, A. granulata, N. cryptotenella, P. legumen, P. gibba, P. divergens, S. linearis* and *A. minutissimum.* The latter group of species was replaced by high pollution tolerant species such as *N. palea, G. parvulum, S. pupula, N. praecipua, P. lanceolatum, F.*

monoculata, P. subcapitata and *G. accuminatum* which are known to be resistant to organic and high ionic strength and conductivity (VAN DAM et al., 1994; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003). These species have also been frequently recorded in waters that are nutrient rich and poorly oxygenated with high electrical conductivity (VAN DAM et al., 1994; BERE and TUNDISI, 2009).

Researchers have reported different effects of land-use patterns on benthic diatom community structure, in terms of diversity and evenness. CUFFNEY et al. (1997) found that taxa richness of benthic-algal communities, with diatoms as a dominant component, did not show a strong relationship with land-use or water quality. HILL et al. (2001) concluded that diatom community structure has no predictable relationship with human-related watershed disturbances. However, CHESSMAN et al. (1999) associated an increase in genus richness with increasing alkalinity, conductivity hardness, and pH related to land-use pattern, and HILL et al. (2003) reported that diatom-species richness was positively correlated with total phosphorus and nitrogen also related to land-use pattern. This supports the results of the current study. Several other studies have also demonstrated the association of land-use with diatom community structure in support of the present study (e.g. MORGAN, 1987; PAN et al., 1996; KUTKA and RICHARDS, 1996; LELAND and PORTER, 2000; CARPENTER and WAITE, 2000; SALOMONI et al., 2006).

Perhaps the most relevant study to our study is that conducted by GÓMEZ and LICURSI (2001) on epipelic diatoms in the tropical streams of Argentina in an environment similar to this study. They classified 88 diatom species frequently found in the epipelion of Pampean streams and rivers based on ecological preferences according to water quality. Thirteen of these species were among the most frequently occurring species in our study (Table 4.3): *A. minutisimum*, *A. copulata*, *E. silesiacum*, *F. capucina*, *G. angustatum*, *G. parvulum*, *H. amphioxys*, *N. cryptocephala, N. linearis*, *N. palea, N. recta*, *S. phoenicenteron, P. gibba* and *S. pupula*. The distribution of all these species in relation to pollution was consistent with that described by GÓMEZ and LICURSI (2001).

The CCA results showed that BOD_5 (also positively correlated with TDS, TP, TN, phosphate, nitrate, conductivity, turbidity, temperature and embeddedness) was important in structuring benthic diatom communities in the study area. Environmental monitoring studies in Southern Brazil (e.g. OLIVEIRA et al., 2001; LOBO ET AL. 2002, 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. In this study, highly eutrophic and high organic pollution sites 8, 9 and 10 had high relative abundances of such species as *G. angustatum, G. parvulum, N. palea, N. praecipua* and *S. pupula*. *N. palea, G. parvulum, S. pupula, N. praecipua, P. lanceolatum, F. monoculata, P. subcapitata* and *G. accuminatum*. These species have been classified as typical for eutrophic and organically polluted environments (KELLY and WHITTON, 1995; VAN DAM et al., 1994). Numerous studies conducted on lotic benthic diatoms sampled in various countries have shown the influence of organic pollution (SLÁDECÉK, 1986; SALOMONI et al., 2006), eutrophication (KELLY and WHITTON, 1995; PONADER et al., 2007; LAVOIE et al., 2008), and dissolved oxygen (BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003) in structuring of diatom communities.

Canopy cover (which was negatively correlated to temperature, light intensity, mean stream width and turbidity and is highly dependent on land-use patters) was also found to be important in structuring benthic diatom communities in the study area. This is because of the importance of light for diatom photosynthesis (TER BRAAK and VAN DAM, 1989; BIGGS, 1990, 1995; PATRICK and HENDRICKSON, 1993; PAN et al., 1996; BIGGS and KILROY, 2000; CARPENTER and WAITE 2000; POTAPOVA and CHARLES, 2002, 2003, 2005). Diatom communities in forested agricultural sites were, thus, different from those from open urban sites.

However, stream velocity (not directly related to land-use pattern) was also found to be important in structuring diatom communities in the study area. Site 7 was grouped together with bad to medium water quality sites 4, 5 and 6 because of its low velocity, despite its good water quality. The importance of velocity in structuring benthic diatom communities has also been reported by other researchers (e.g. BIGGS, 1990, 1995; PATRICK and HENDRICKSON, 1993; PAN et al., 1996; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2005).

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CHAPTER 5

Applicability of diatom-based water quality assessment indices in streams around São Carlos-SP, Brazil [5]

Abstract: Diatom-based indices are increasingly becoming important tools for the assessment of ecological conditions in lotic systems. The applicability of regional and foreign diatom-based water quality assessment indices to streams around São Carlos-SP, Brazil is discussed. The relationship between measured water quality variables and diatom index scores was assessed. The indices, when compared to chemical analyses, proved useful in providing an indication of the quality of the investigated waters. Although all borrowed indices were applicable to the study area, because many widely distributed diatom species have similar environmental tolerances to those recorded for these species elsewhere, ecological requirements of some diatom species from Brazil need to be clarified and incorporated in a diatom-based water quality assessment protocol unique to the region.

Keywords: diatoms, indices, water quality, biomonitoring

Aplicabilidade dos índices de avaliação da qualidade da água baseados nas diatomáceas em córregos de São Carlos-SP, Brasil

Resumo: Os índices baseados nas diatomáceas estão se tornando cada vez mais, ferramentas importantes para a avaliação das condições ecológicas em sistemas lóticos. A aplicabilidade dos índices de avaliação da qualidade da água regionais e externas, baseados nas diatomáceas em córregos em torno de São Carlos-SP, Brasil é discutida. A relação entre as medidas da qualidade variável da água e os níveis dos índices de diatomáceas foi avaliada. Os índices, quando comparados com análises químicas, provaram ser úteis para fornecer uma indicação da qualidade das águas estudadas. Embora todos os índices aferidos sejam aplicáveis à área de estudo, pois muitas espécies de diatomáceas de ampla distribuição têm tolerâncias ambientais semelhantes aos registrados para estas espécies em outros lugares, as exigências ecológicas de algumas espécies de diatomáceas do Brasil precisam ser esclarecidas e incluídas em um protocolo

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de avaliação da qualidade da água baseado nas diatomáceas específico de determinada região**.**

Palavras-chave: diatomáceas, índices de qualidade da água, biomonitoramento

5.1 Introduction

Patterns of benthic diatom communities are responsive to the nature of the physical and chemical characteristics of lotic systems (STEVENSON et al., 1996; WEHR and SHEATH, 2003; AZIM et al., 2005). They respond rapidly to degradation of water quality, often changing in both taxonomic composition and biomass where even slight contamination occurs (ROUND, 1991; PAN et al., 1996; STEVENSON et al., 1996; BIGGS and KILROY, 2000; POTAPOVA and CHARLES, 2003; WEHR and SHEATH, 2003; AZIM et al., 2005). Therefore, the integrity of these communities provides a direct, holistic and integrated measure of the integrity of lotic systems. Thus, pollution control and monitoring programmes routinely include the examination of diatoms to investigate the ecological status of lotic systems (e.g. ROUND, 1991; PAN et al., 1996; GÓMEZ and LICURSI, 2001; LOBO et al., 2002; POTAPOVA and CHARLES, 2002, 2003). They have gained momentum in their usage as alternatives to chemical analyses because the latter techniques provide, at best, a fragmented overview of the state of lotic systems as sporadic or periodic sampling cannot reflect fluxes of effluent discharge. In contrast, diatom community structure in lotic systems gives a time-integrated indication of the water quality components (TAYLOR et al., 2007a). The unique composite picture of ecosystem conditions provided by the diatoms can only be replicated by intensive chemical monitoring studies.

Numerous studies focusing on the application of standardized methods based on diatom assemblages for water quality assessment have been carried out, especially in the northern hemisphere, in particular in European countries (e.g. DESCY and COSTE, 1991; KELLY and WHITTON, 1995; PRYGIEL et al., 1999). Several diatom-based indices have been developed most of which are based on the weighted average equation of ZELINKA and MARVAN (1961) and are general pollution indices. There are as many indices as there is the number of researchers working in the field (RIMET et al., 2005). A

discussion of the manner in which diatom indices function may be found in HARDING et al. (2005).

The wide geographic distribution and well-studied ecology of most diatom species are cited as major advantages of using diatoms as indicator organisms (McCORMICK and CAIRNS, 1994). These assumptions imply that diatom-based water-quality assessment tools should have universal applicability across geographical areas and environments. For this reason, due to lack of information on ecological preferences and tolerances of diatoms in some regions, indices developed in other regions are often borrowed. However, there is evidence that diatom metrics or indices developed in one geographical area are less successful when applied in other areas (PIPP, 2002). This is due not only to the floristic differences among regions (PAN et al., 1996; TAYLOR et al., 2007b), but also to the environmental differences that modify species responses to water quality characteristics (POTAPOVA and CHARLES, 2005). TAYLOR et al. (2007b) recommended that borrowed diatom indices can be used for gaining support and recognition for diatom-based approaches to water quality monitoring allowing for sample and data collection, which can then be used later in the formulation of a unique diatom index. Strict testing of these borrowed indices is required to ensure that diatom index scores give a realistic reflection of the specific type of environmental pollution being tested.

The use of diatoms as indicators of water quality changes has relatively fewer precedents in South America compared to North America and Europe. In Brazil, the first studies on the use of aquatic biota, particularly phytoplankton, for monitoring of the ecological status of lotic systems were carried out in the catchment areas of São Paulo City by a French researcher, Henric Charles Potel, between 1907, and 1910 based on empirical and qualitative data (ROCHA, 1992). Other studies followed up on this work based on quantitative data. All these studies confirmed diatoms as excellent indicators of environmental conditions in lotic system (e.g. LOBO and TORGAN, 1988; ROSA et al., 1988; LOBO et al., 1991, 1996, 1999; LOBO and CALLEGARO, 2000). However, in most cases, the assessment of the ecological conditions of lotic systems was determined by foreign methods (e.g. LANGE-BERTALOT, 1979; WATANABE et al., 1986; KOBAYASI and MAYAMA, 1989), because no information on diatom ecological preferences was present. This prompted the first attempts to classify diatoms in terms of tolerance of species to organic pollution in rivers in southern Brazil by LOBO et al. (1996). Subsequently, LOBO et al. (2002) determined the tolerance to organic pollution

of diatom species in different conditions of pollution. Based on this information, LOBO et al. (2002) developed the first saprobic system in the country, which uses epilithic diatoms for water quality assessment in southern Brazil. This study was completed by LOBO et al. (2004) leading to the formation of the first diatom-based water quality assessment index, the Biological Index of Water Quality (BIWQ) trophic index, that incorporate the effects of organic contamination and eutrophication.

However, this index was developed in the southern part of the country and very little was done in other lotic systems. Given the high variations in climatic conditions among different regions in Brazil due to its geographical positioning, there is need for strict testing of the BIWQ to ensure that its scores give a realistic reflection of the specific type of environmental pollution being tested in other regions of Brazil. Thus, the objective of the present study was to test the applicability of the BIWQ, together with other indices developed in other regions and calculated by the OMNIDIA version 5.3 software, to the study area. This study presents the relationship between measured water quality variables in the Monjolinho River and its tributaries and diatom index scores. Diatom index scores were calculated and correlated to concurrent physical and chemical water quality data. The results of these correlation analyses were compared to results obtained in similar studies carried out elsewhere, e.g. in Europe and South Africa.

5.2 Materials and Methods

5.2.1 Study Area

The headwaters of the Monjolinho River and the tributaries studied fall mainly within an agricultural area. From the agricultural area, the streams pass through urban area of the city of São Carlos, which covers a total area of 1,143.9 km^2. The area is characterised by a rugged topography and an average annual temperature of around 19.5 °C, with mean monthly maximum of around 21.9 °C recorded in January and February and the mean monthly minimum of around 15.9 °C recorded in July.

In 2008, the population of São Carlos was estimated at 218,080 inhabitants by Instituto Brasileiro de Geografia e Estatística. 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 so on. Streams in the study area, therefore, receive untreated or semi-treated effluent from various domestic and industrial sources as well as other diffuse sources as they pass through the city. This disorderly growth of the city results in

stream health deterioration, loss of the remaining primary vegetation, organic pollution and eutrophication amongst other problems.

Ten sites were established along the Monjolinho river and its tributaries: four sites (1, 2, 3 and 7) in the relatively less impacted agricultural and forested headwaters to act as reference sites; three sites (4, 5 and 6) in the moderately polluted urban area; and three sites (8, 9 and 10) in highly polluted downstream area after the urban area (Figure 5.1). The rational for choosing the sampling sites was to obtain a pollution gradient of all the stream systems from relatively unpolluted agricultural headwaters to highly polluted urban downstream sites. Substrate assessment, diatom, and water quality sampling were done during dry seasons (autumn and winter) when flow was stable. Four samplings were carried out, two in September and October 2008 and two in May and June 2009. Sampling was done during dry season to avoid variable effects of rainy season such as great variations in water level and velocity, floods and inundations. These factors affect diatom development, especially growth rates and relative abundance of different species (ROUND, 1991).

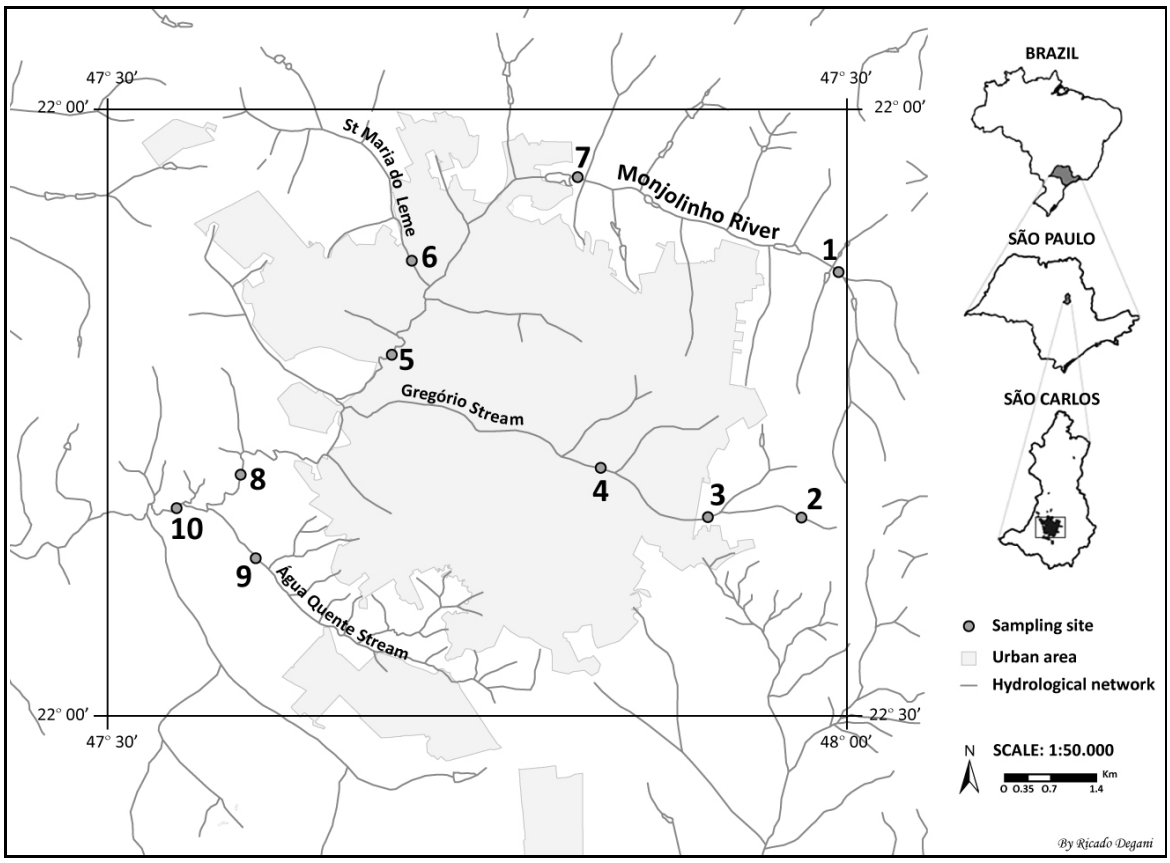

Figure 5.1: The location of the sampling sites in the study area.

5.2.2 Environmental Variables

At each site, dissolved oxygen (DO), electrical conductivity (EC), temperature, pH, and turbidity were measured using a Horiba U-23 and W-23XD Water Quality Metre (Horiba Ltd., Japan). Depth and current velocity were measured at each site with an FP 201 global flow probe (Global Water Instrumentation Inc., AK, USA). The percentage riparian vegetation cover was visually estimated at each site. Altitude was determined at each site using a GPS (Northport Systems, Inc. Toronto, Canada).

Water samples for metals, ions, total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD_5) analysis were collected at each site into acid-cleaned polyethylene containers (APHA, 1988). In the laboratory, the concentrations of TN, TP and BOD_5 in the water samples were determined following standard methods (APHA 1988). The following metals were analysed in water samples using flame atomic absorption spectrometry analytical methods (Varian Australia Pty. Ltd., Victoria, Australia): cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu) and iron (Fe). The concentrations of fluoride (F^-), chloride (Cl^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were analysed by isocratic ion analysis using suppressed conductivity detection ion chromatography method using Dionex DX-80 Ion Analyser (DX-80) (DIONEX, 2001).

5.2.3 Biological Element

At each site, epilithic diatom samples were collected as outlined in BERE and TUNDISI (2010). Dead wood was used as a substrate in the absence of boulders as suggested by KELLY et al. (1995). In the laboratory, 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 and KILROY (2000). Three replicate slides were prepared for each sample. A total of 250 – 600 valves per sample (based on counting efficiency determination method by PAPPAS and STOERMER (1996)) were identified and counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type -020-519.503 LB30T, Germany). The mean and standard deviations of counting efficiencies of diatom communities calculated according to PAPPAS and STOERMER (1996) on different substrates were as follows: macrophytes, 82.5 ±11.4 %; sand, 86.1 ±7.6 %; stones, 83.6 ±18.5 %; and silt/clay, 82.9 ±14.2 %. The diatoms were identified to

species level based on studies by LOBO et al., 2002; METZELTIN ET AL. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007).

5.2.4 Indices

The diatom species counts were entered into the diatom database and index calculation tool OMNIDIA version 5.3 (LECOINTE et al., 1993) and the following indices were calculated and tested: the Artois-Picardie Diatom Index (APDI; PRYGIEL et al., 1996); the Eutrophication/Pollution Index (EPI; DELL'UOMO, 1996); the Biological Diatom Index (BDI; LENOIR and COSTE, 1996); Schiefele and Schreiner's index (SHE; SCHIEFELE and SCHREINER, 1991); the Saprobic Index (SI; ROTT et al., 1997); the Trophic Index (TI; ROTT et al., 1999); the Watanabe index (WAT; WATANABE et al., 1986); the Specific Pollution sensitivity Index (SPI; COSTE in CEMAGREF, 1982); the Sládeček's index (SLA; SLÁDEČEK, 1986); Descy's pollution Index (DES; DESCY, 1979); Leclercq (IDSE; LECLERQ and MAQUET, 1987); the Generic Diatom Index (GDI, COSTE and AYPHASSORHO, 1991); the Commission of Economical Community Index (CEC; DESCY and COSTE, 1991); the Trophic Diatom Index (TDI; KELLY and WHITTON, 1995); the Pampean Diatom Index (PDI; GÓMEZ and LICUIRSI, 2001) and the Biological Index of Water Quality trophic index (BIWQ; LOBO et al., 2004). All these indices, except CEC, SHE, TDI and WAT, are based on the formula of ZELINKA and MARVAN (1961). The last two indices (BIWQ and PDI) were developed in South America and are here classified as regional indices while all the other indices were developed outside South America and are here classified as foreign indices. The percentage of species used in calculation of the indices, as indicated by the OMNIDIA, was also recorded.

5.2.5 Data analysis

The available environmental data consisted of 27 environmental variables (Table 5.1). Environmental variables that were not normally distributed (Shapiro-Wilk, $p \leq 0.05$) or had no equal variance (Levene's test, $p \leq 0.05$) were transformed as described in BERE and TUNDISI (2010). Two-way Analysis of Variance (Two-way ANOVA) was used to compare means of transformed environmental variables among the three sites categories and among the four sampling periods (section 5.2.1).

Pearson correlation was used to determine the relationship between the calculated index scores and measured physical and chemical water quality data. One-way ANOVA with Tukey's pairwise comparisons was used to compare values of correlations between the calculated index scores and the measured water quality variables among different indices. The same method was used to compare percentages of species used in calculation of different indices. Forward stepwise multiple regression analysis was performed on the data to determine the indices that gave the best reflection of general water quality (LENOIR and COSTE, 1996). This method can give important additional information about the factors that influence the various index scores over and above pure correlations (LENOIR and COSTE, 1996). Adjusted R^2 values were used as indicators of the level of success with which the variables were able to explain the variation in the index values; the higher the value, the more accurate the indices are as indicators of the measured water quality variables. Pearson correlation, ANOVA, Levene's test and Shapiro-Wilk were performed using PAlaeontological STatistics (PAST) software version 1.95 (HAMMER et al., 2009). Forward stepwise multiple regression was performed with STATISTICA Version 7.

5.3 Results

5.3.1 Physico-chemical variables

The values of physical and chemical variables measured in the study area during the study period are shown in Table 5.1. The pH increased slightly down the agricultural to urban gradient being slightly acidic at upstream sites and slightly alkaline/neutral at downstream sites. However, the difference in pH among the three site categories (section 5.2.2) was not statistically significant (ANOVA, $p > 0.05$). Temperature increased downstream, but as in the case of pH, the increase was not significant (ANOVA, $p > 0.05$). On the other hand, conductivity, BOD_5, TDS, turbidity, TN, TP, most of metals increased significantly downstream (ANOVA, $p < 0.05$) while DO and percentage riparian vegetation cover decreased significantly downstream (ANOVA, $p < 0.05$). The concentrations of all the ions in water increased significantly downstream (ANOVA, $p > 0.05$) (Table 5.1). No significant differences were observed in the means of environmental variables among the four sampling periods (ANOVA, $p > 0.05$). This is expected since all sampling was confined to stable base flow periods when variations in water chemistry are low compared to the rainy season.

Table 5.1: The mean (n = 4) values of physical and chemical variables measured at 10 sites during 4 sampling periods.

Site	1	2	3	7	4	5	6	8	9	10
Temperature (^{o}C)	18.3 ±1.1	20.9 ±1.6	20.6 ±1.7	20.4 ±1.5	24.0 ±2.6	21.2 ±1.7	21.2 ±1.1	24.8 ±3.0	23.0 ±1.9	21.3 ±2.2
Altitude (m)	761	837	831	761	774	794	745	724	630	627
Canopy cover (%)	80	95	60	45	20	50	4	20	50	5
BOD_5 (mg.L^{-1})	0.9 ±0.2	1.0 ±1.2	2.6 ±0.2	1.2 ±2.3	6.9 ±0.2	1.6 ±0.6	7.2 ±1.1	19.5 ±1.1	24.5 ±1.2	26.2 ±1.3
DO (mg.L^{-1})	7.3 ±2.3	8.2 ±1.0	7.6 ±0.6	7.2 ±1.5	6.8 ±2.1	6.9 ±1.0	7.6 ±1.3	1.9 ±2.8	2.1 ±1.0	0.4 ±1.2
Conductivity (μS.cm^{-1})	45.0 ±7.5	20.0 ±10.5	53.0 ±9.5	30.0 ±4.0	28.0 ±7.7	89.0 ±8.9	103.0 ±6.4	715.0 ±22.3	322.0 ±191.3	283.0 ±201.7
pH	6.6 ±0.8	6.4 ±1.0	6.3 ±1.0	6.8 ±1.0	6.7 ±0.6	6.8 ±0.9	7.2 ±0.4	7.2 ±0.5	7.2 ±0.4	7.1 ±0.4
TDS (g.L^{-1})	29.4 ±0.5	13.4 ±1.2	22.6 ±3.2	19.3 ±0.2	18.1 ±1.4	57.4 ±3.4	66.5 ±2.9	457.8 ±27.1	206.1 ±11.1	182.0 ±34.1
Turbidity (NTU)	5.1 ±1.2	4.2 ±1.1	4.7 ±1.3	7.3 ±1.6	19.5 ±4.4	11.1 ±2.9	13.2 ±3.4	45.3 ±10.7	53.2 ±12.1	60.4 ±15.4
TN (mg.L^{-1})	0.65 ±0.3	0.18 ±0.3	0.24 ±1.4	0.93 ±0.7	1.72 ±0.5	1.29 ±0.4	1.41 ±0.5	38.32 ±8.3	14.87 ±4.1	10.17 ±2.9
TP (mg.L^{-1})	0.01 ±0.0	0.01 ±0.0	0.01 ±0.1	0.02 ±0.0	0.03 ±0.0	0.16 ±0.2	0.06 ±0.1	2.97 ±1.0	1.12 ±0.2	0.75 ±0.2
Nitrite (μg.L^{-1})	<10	<10	<10	<10	423.9 ±84.8	42.4 ±50.3	24.3 ±48.6	884.9 ±105.8	2036.6 ±391.9	3164.9 ±641.7
Nitrate (μg.L^{-1})	51.9 ±22.3	195.6 ±33.1	470.0 ±38.5	98.2 ±8.8	714.8 ±232.3	819.6 ±667.2	745.0 ±595.3	1141.2 ±1025.4	176.5 ±142.5	441.1 ±273.2
Ammonium (μg.L^{-1})	11.8 ±3.6	116.1 ±23.5	11.9 ±3.7	15.0 ±3.1	858.9 ±175.5	418.8 ±401.9	141.4 ±128.2	1361.6 ±783.1	4610.8 ±2482.1	3310.5 ±703.0
Phosphate (μg.L^{-1})	15.0 ±17.8	<2	2.4 ±2.9	<2	136.2 ±49.3	26.7 ±14.3	7.7 ±9.1	21.4 ±5.0	132.5 ±51.8	190.8 ±131.2
Sulphate (mg.L^{-1})	0.1 ±0.1	1.5 ±1.0	1.1 ±0.3	0.3 ±0.1	8.3 ±1.8	4.9 ±3.1	3.1 ±1.3	3.7 ±0.9	15.1 ±3.9	9.5 ±3.9
Fluoride (μg.L^{-1})	38.3 ±12.1	45.8 ±14.7	67.5 ±40.5	43.7 ±14.8	88.8 ±28.4	95.7 ±66.5	124.7 ±88.3	287.5 ±87.3	305.4 ±93.2	262.9 ±104.3
Chloride (mg.L^{-1})	2.0 ±0.4	4.9 ±1.0	4.1 ±1.7	2.8 ±0.6	15.5 ±5.9	7.3 ±4.8	6.8 ±4.8	19.8 ±8.3	21.7 ±4.2	30.0 ±7.0
Sodium (mg.L^{-1})	2.0 ±0.8	2.3 ±0.6	2.4 ±1.2	2.2 ±0.6	7.5 ±1.5	4.1 ±2.3	4.1 ±2.6	12.4 ±6.5	15.3 ±2.9	19.7 ±4.2
Potassium (mg.L^{-1})	1.0 ±0.2	0.6 ±0.2	1.0 ±0.3	0.7 ±0.1	2.3 ±0.6	1.3 ±0.9	0.9 ±0.4	2.4 ±1.0	4.3 ±1.2	3.8 ±0.8
Magnesium (mg.L^{-1})	0.7 ±0.1	0.6 ±0.1	0.8 ±0.2	0.7 ±0.1	1.2 ±0.3	1.2 ±0.6	1.0 ±0.5	1.7 ±0.3	2.5 ±0.7	1.6 ±0.3
Calcium (mg.L^{-1})	1.4 ±0.3	1.9 ±0.4	1.3 ±0.2	2.4 ±0.5	3.9 ±1.7	5.7 ±3.5	4.5 ±2.1	7.4 ±1.5	11.9 ±2.8	8.2 ±1.6
Depth (m)	0.2 ±0.02	0.3 ±0.08	0.4 ±0.10	0.4 ±0.01	0.2 ±0.05	0.4 ±0.08	0.3 ±0.05	0.5 ±0.07	0.3 ±0.04	0.3 ±0.01
Cr (mg.L^{-1})	0.01 ±0.001	0.01 ±0.001	0.01 ±0.002	0.00 ±0.001	0.01 ±.003	0.02 ±0.001	0.01 ±0.002	0.03 ±0.001	0.03 ±0.002	0.03 ±0.002
Cu (mg.L^{-1})	0.004 ±0.001	0.004 ±0.001	0.005 ±0.001	0.004 ±0.000	0.006 ±0.000	0.004 ±0.001	0.009 ±0.003	0.0225 ±0.002	0.021 ±0.002	0.024 ±0.002
Fe (mg.L^{-1})	3.04 ±1.2	0.35 ±0.11	0.50 ±0.01	0.43 ±0.04	0.26 ±0.01	0.48 ±0.06	0.29 ±0.03	0.45 ±0.06	1.02 ±0.11	0.79 ±0.16
Cd (mg.L^{-1})	< 0.001	< 0.001	0.001 ±0.001	< 0.001	0.001 ±0.001	0.002 ±0.001	0.002 ±0.001	0.002 ±0.002	0.005 ±0.002	0.004 ±0.001
Pb (mg.L^{-1})	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02 ±0.005	0.01 ±0.002	0.02 ±0.004	0.01 ±0.005	0.02 ±0.1

5.3.2. Indices

All the diatom index scores show significant correlations ($p < 0.05$) to water quality variables (Table 5.2). With the exceptions of metals, correlations between water quality variables and all index scores ranged from R^2 ±0.62 to R^2 ±0.99 indicating strong correlations. Significantly low correlation ($p < 0.05$) was observed between metal levels,

except Cr, and all index scores compared to that between other variables (especially those related to eutrophication and organic pollution) and all index scores. Fe levels did not correlate significantly with any of the indices. Correlation coefficients between the calculated index scores and the measured water quality variables differed significantly ($p < 0.05$) among different indices. These values did not differ significantly ($p > 0.05$) between the two 'regional indices' (BIWQ and PDI) whilst they tended to differ between regional and some foreign indices like DES, IDSE, SHE, WAT, TDI, GDI, CEE, SPI and BDI. Correlation between the BIWQ scores and water quality variables was generally significantly low ($p < 0.05$) compared to that between some foreign index scores like DES, IDSE, SHE,WAT, GDI, SPI and BDI scores and water quality variables. Foreign index scores like IDSE, GDI, BDI, EPI, CEE, TI and SPI were calculated based on significantly more species ($p < 0.05$) compared to regional indices (Figure 5.2).

Table 5.2: Pearson's correlation coefficients between measured environmental variables and diatom index scores generated for sites in the Monjolinho River and its tributaries. Numerical values indicate significant correlations at p ≤ 0.05. SLA: Descy's pollution Index, DES: Leclercq, IDSE: Generic Diatom Index, SHE: Saprobic Index, WAT: Watanabe index, TDI: Trophic Diatom Index, GDI: Sládeček's index, CEC: Commission of Economical Community Index, SPI: Specific Pollution sensitivity Index, BDI: Artois-Picardie Diatom Index, APDI: Schiefele and Schreiner's index, EPI: Eutrophication/Pollution Index, SI: Biological Diatom Index, TI: Trophic Index, PDI: Pampean Diatom Index, and BIWQ: Biological Index of Water Quality trophic index.

	SLA	DES	IDSE	SHE	WAT	TDI	GDI	CEE	SPI	BDI	APDI	EPI	DI-CH	PDI	BIWQ	SI	TI
BOD_5	0.95	-0.94	-0.96	-0.97	-0.92	0.96	-0.96	-0.81	-0.97	-0.94	-0.88	0.94	0.90	0.94	0.89	0.97	0.84
DO	-0.92	0.92	0.92	0.94	0.85	-0.95	0.93	0.71	0.94	0.91	0.81	-0.91	-0.85	-0.89	-0.86	-0.94	-0.83
Conductivity	0.78	-0.76	-0.78	-0.77	-0.66	0.81	-0.77	-0.62	-0.79	-0.77	-0.67	0.75	0.69	0.71	0.73	0.79	0.73
pH	0.76	-0.86	-0.82	-0.78	-0.72	0.82	-0.78	-0.74	-0.80	-0.83	-0.73	0.78	0.86	0.82	0.85	0.82	0.82
TDS	0.78	-0.77	-0.79	-0.77	-0.66	0.82	-0.77	-0.63	-0.79	-0.78	-0.67	0.75	0.70	0.71	0.73	0.79	0.73
Turbidity	0.95	-0.96	-0.97	-0.98	-0.92	0.97	-0.97	-0.82	-0.98	-0.96	-0.90	0.95	0.92	0.95	0.91	0.98	0.87
TN	0.74	-0.70	-0.72	-0.70	-0.59	0.77	-0.72	-0.56	-0.73	-0.71	-0.58	0.69	0.62	0.64	0.67	0.72	0.65
TP	0.73	-0.70	-0.72	-0.70	-0.59	0.76	-0.71	-0.57	-0.73	-0.71	-0.59	0.69	0.62	0.64	0.67	0.72	0.65
PO_4^{3-}	0.68	-0.75	-0.74	-0.79	-0.75	0.72	-0.74	-0.66	-0.76	-0.75	-0.79	0.76	0.75	0.78	0.69	0.76	0.70
NH_4^+	0.84	-0.80	-0.80	-0.79	-0.93	0.78	-0.84	-0.63	-0.81	-0.81	-0.82	0.86	0.77	0.83	0.77	0.80	0.69
SO_4^{2-}	0.73	-0.75	-0.74	-0.70	-0.87	0.68	-0.76	-0.70	-0.73	-0.77	-0.84	0.79	0.76	0.79	0.75	0.72	0.70
Na^+	0.84	-0.89	-0.88	-0.90	-0.89	0.85	-0.89	-0.79	-0.90	-0.91	-0.92	0.90	0.88	0.90	0.85	0.89	0.84
K^+	0.80	-0.84	-0.82	-0.83	-0.93	0.78	-0.85	-0.70	-0.83	-0.85	-0.91	0.88	0.84	0.88	0.81	0.83	0.79
Mg^{2+}	0.85	-0.87	-0.86	-0.82	-0.92	0.83	-0.87	-0.70	-0.85	-0.88	-0.88	0.90	0.85	0.88	0.86	0.86	0.83
Ca^{2+}	0.85	-0.90	-0.88	-0.83	-0.91	0.85	-0.88	-0.77	-0.87	-0.90	-0.89	0.90	0.88	0.89	0.89	0.87	0.85
F^-	0.90	-0.90	-0.90	-0.86	-0.91	0.89	-0.90	-0.73	-0.89	-0.92	-0.86	0.91	0.86	0.87	0.86	0.90	0.82
Cl^-	0.76	-0.86	-0.85	-0.87	-0.84	0.80	-0.84	-0.84	-0.85	-0.88	-0.94	0.86	0.87	0.88	0.83	0.85	0.86
Cr	0.85	-0.77	-0.78	-0.79	-0.73	0.85	-0.82	-0.53	-0.82	-0.77	-0.63	0.80	0.64	0.74	0.74	0.80	0.68
Cu	0.65	-0.64	-0.66	-0.65	...	0.70	-0.64	-0.53	-0.67	-0.65	-0.53	0.62	0.55	0.58	0.63	0.66	0.65
Fe	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Cd	0.56	-0.53	-0.52	-0.55	-0.66	0.52	-0.59	...	-0.56	-0.53	-0.58	0.63	...	0.59	0.52	0.54	0.50
Pb	0.51	-0.54	-0.58	-0.61	....	0.58	-0.57	...	-0.59	-0.55	-0.58	0.60	...	0.57	0.53	0.61	0.63

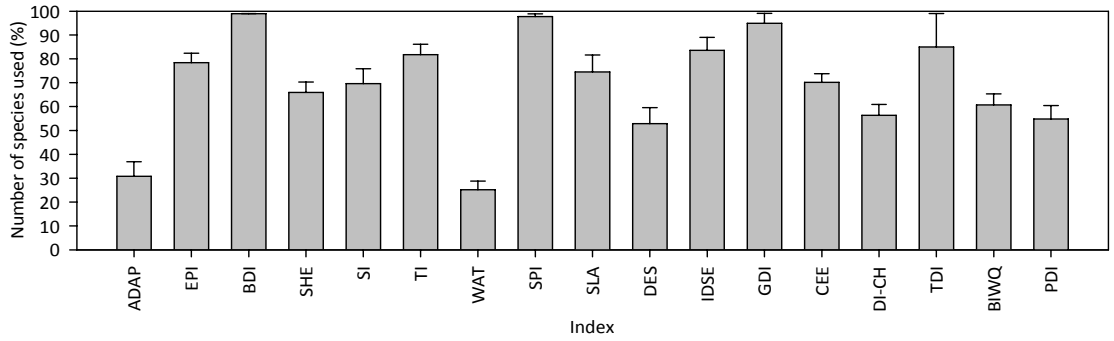

Figure 5.2: The means (n = 4) and standard deviations of the percentage of species used in calculation of different indices score in this study.

Based on forward stepwise multiple regression analysis performed to determine the indices that gave best reflection of water quality, measured water quality variables significantly ($p < 0.05$) account for the variation in all index scores. Adjusted R^2 values (which reflect the amount of variation in index scores explained by the measured water quality variables, i.e. the degree of applicability of index scores to assess water quality) ranged from 50% (CEE) to 85% (DES) (Figure 5.3). The higher the adjusted R^2 value, the more accurate the indices are as indicators of the measured water quality variables. Adjusted R^2 value for measured water quality variables and BIWQ scores (78%) was slightly low compared to those based on water quality variables and some index scores from some indices developed in Europe, USA, Argentina and other countries such as DES (85%), PDI (80%), IDSE (79%), GDI (79%) and SPI (79%). The rest of the indices had lower adjusted R^2 values compared to the BIWQ (Figure 5.3).

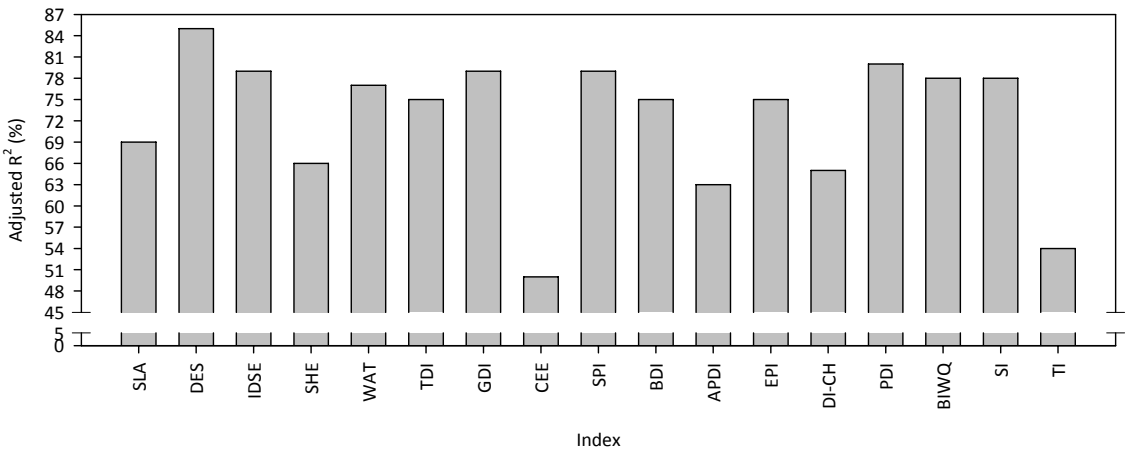

Figure 5.3: Adjusted R^2 values obtained from forward stepwise multiple regression analysis performed on index scores and measured water quality variables. Index abbreviations correspond to those in Table 5.2.

5.4 Discussion

Significant correlations between diatom-based index scores and physical and chemical characteristics of streams recorded in this study indicate the success with which diatom indices may be used to reflect general changes in water quality variables in lotic systems. Results of Pearson correlation coefficients between water quality variables and diatom indices obtained in the present study compared favourably with results between indices and water quality variables from some European and South African authors (PRYGIEL and COSTE, 1993; KWANDRANS et al., 1998; TAYLOR et al., 2007a, b) and in some cases better than the correlations demonstrated in Europe.

Although there may be concerns as to the feasibility of transferring data concerning the ecological tolerance limits of diatoms between the northern and southern hemispheres (ROUND, 1991), most of the dominant diatom species encountered in this study (detailed in BERE and TUNDISI (2010, 2011a, b)) are cosmopolitan species well-documented in international literature, especially from the Northern Hemisphere (e.g. KRAMMER and LANGE-BERTALOT, 1986-1991). This is supported by the observation that foreign index scores like IDSE, GDI, BDI, EPI, CEE, TI and SPI were calculated based on significantly more species compared to regional indices (Figure 5.2). These results are in agreement with BATE et al. (2004) who found that most dominant diatom species found in South African rivers were already recorded in international literature. For that reason, most European diatom indices may be used in the study area as they are based on the ecology of widely distributed or cosmopolitan taxa.

From the forward stepwise multiple regression analysis results, measured water quality variables significantly accounted for the variation in all index scores (Figure 5.3) indicating the success with which these indices can be used in the study region. The amount of variation in index scores accounted for by measured water quality variables compared favourably with results from some European and South African authors (PRYGIEL and COSTE, 1993; KWANDRANS et al., 1998; TAYLOR et al., 2007a, b) and was, in some cases, better than the amount of variation observed in Europe. For example, LENOIR and COSTE (1996) showed that the overall water quality analysis explained 72% of the variation in the BDI, whilst in this study measured water quality variables accounted for 75% of the variation in BDI (Figure 5.3). Thus, it can be concluded that this index is both applicable and useful for monitoring water quality in the study region. The fact that most of the variation in other index scores (in most cases above 60%, Figure 5.3) was significantly accounted for by the physical and chemical variables in the study region also indicates the applicability and usefulness of these indices.

Generally, significantly low correlation was observed between metal levels, and all index scores compared to that between other variables (especially those related to eutrophication and organic pollution) and all index scores. This is expected since most of these indices are designed to monitor organic pollution and eutrophication with no provision for assessment of metal contamination, underlining a clear vision to develop diatom-based water quality monitoring protocols that incorporate the effects of metals. It

could also be due to the overriding effects of organic pollution and eutrophication on metal pollution.

Weaker correlation of the BIWQ scores and water quality variables compared to that between water quality variables and some foreign index scores like DES, IDSE, SHE, WAT, GDI, SPI and BDI scores was observed. This can be attributed to differences in ecological characterization of many, even common, diatom species in the BIWQ classification. One striking difference is the classification of *Gomphonema parvulum* (Kützing) Kützing and *Nitzschia palea* (Kützing) Smith. Although these diatoms are reported from almost every survey of freshwater algae, the information about their distribution in relation to eutrophication and organic pollution or about their responses to eutrophication and organic pollution is often inconsistent. In their calculation of the BIWQ scores, LOBO et al. (2004) described *N. palea* as a medium and high pollution tolerant species. In this study, however, this species had high relative abundance (around 90 %) at hypereutrophic sites 8, 9 and 10 with very bad water quality (BERE and TUNDISI, 2011b), an indication that it is tolerant of high pollution. This is supported by several studies (e.g. LANGE-BERTALOT, 1979; KILHAM et al., 1986; KOBAYASI and MAYAMA, 1989; VAN DAM et al., 1994; KELLY and WHITTON, 1995). However, recent evidence from the study by TROBAJO et al., (2009) suggests that *N. palea* is taxonomically problematic. Their work illustrated that *N. palea* is almost certainly a complex of several or many separate species, which may differ subtly in morphology and may not all share the same ecology.

The work by LOBO et al. (2002) upon which the BIWQ is based considered *G. parvulum,* a relatively abundant species at downstream eutrophic sites with high organic pollution as α-mesosaprobic. There is a lot of controversy in the literature regarding the classification of this species in relation to its pollution tolerance. In streams located in the Municipal District of Mato Leitão (Brazil), LOBO et al. (1999) classified this species as belonging to both α-mesosaprobic and polysaprobic environments. In the same streams, RODRIGUES and LOBO (2000) registered the occurrence of this species in moderately polluted, β-mesosaprobic waters. In a study carried out in the same study area as the current study, SOUZA (2002) encountered this species in oligosaprobic environments. However, in studies carried out in rivers of Japan KOBAYASI and 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. KELLY and WHITTON (1995) also described this species as highly tolerant of eutrophication (indicative value = 3 and

sensitivity value = 5) in their calculation of the TDI. Diverse mophotypes of *G. parvulum*, however, exist, probably corresponding to different varieties (MORALES and JASIKI, 2002) which would explain the variety of responses attributed to this species. Diatom morphology has also been shown to vary as a result of both genetic variability and ecological variation, which may result in the formation of ecotypes (SALOMONI et al., 2006). Detailed study on the autecology of *N. palea* and *G. parvulum* are called for in order to clarify their taxonomy and ecological requirements. ROUND (2004) discovered that lumping of several similar looking taxa into one ‘‘morphospecies’’ diminishes discriminative ability of diatom metrics, while detailed taxonomic and ecological studies allow recognition of taxa with good indicator properties. With diatom taxonomy undergoing rapid changes, especially at the genus level, diatom identification guides and methods to be used in South America must be consistent and updated.

Weak correlation of the BIWQ scores and the water quality compared to other indices may also be attributed to the fact that many species that were abundant in the present study area, such as *Brachysira vitrea* (Grunow) Ross, *Eunotia bilunaris* (Ehrenberg) Mills, *Frustulia saxonica* Rabenhorst, *Lemnicola hungarica* (Grunow) Round & Basson, *Navicula radiosa* Kützing, *Nitzschia clausii* (Hantzsch) Grunow, *Pinnularia braunii* (Grunow) Cleve and *Pinnularia microstauron* (Ehrenberg) Cleve were classified as indicators of oligotrophic and oligosaprobic conditions. However, most of these species are known to be tolerant to eutrophication and organic pollution (VAN DAM et al., 1994). The ongoing discussion demonstrates the inconsistence and lack of appropriate information on ecological requirements of diatom species from South America hampering formulation of sound diatom-based water quality management protocols. In addition, the ecological characteristics of indicator species and the uniqueness of each stream are not well documented. Thus, more work is needed to elucidate the ecological requirements of diatoms in Brazil, something that is far from being understood. The species base of the BIWQ is too narrow for widespread use in Brazil and needs to be broadened. This underlines a clear vision for developing a diatom-based water quality monitoring protocol unique to different regions of Brazil.

Meanwhile, as recommended by TAYLOR et al. (2007a), BIWQ, as well as other foreign indices can be used to (a) gain support and recognition for diatom-based approaches to water quality monitoring in other regions, (b) provide an indication of water quality and allowing for the dissemination of simplified useful information to resource managers, conservationists and the general public, and (c) allow for sample and

data collection which can then be used later in the formulation of a Brazilian diatom index tailored for different regions. Combining several datasets produced by different institutions or researches might provide a good way to increase the number of samples available for particular geographical regions and types of rivers. Such work, however, would be complicated by the taxonomic inconsistencies that exist between different datasets.

5.5 Conclusions

Assessment of water quality based on diatom-based indices is deemed useful in Brazil to provide information on water quality impacts on rivers and streams for management purposes. Although all borrowed indices were applicable to the study area because many widely distributed diatom species have similar environmental tolerances to those recorded for these species elsewhere, ecological requirements of some diatom species from Brazil need to be clarified and incorporated in a diatom-based water quality assessment protocol unique to the region. These species include *B. vitrea, E. bilunaris, F. saxonica, G. parvulum*, *L. hungarica, N. radiosa, N. clausii, N. palea*, *P. braunii* and *P. microstauron.* To improve diatom-based water quality assessments in Brazil, autecology-based diatom metrics should be developed based on datasets representative of the areas or river types where the metrics will be applied and by assuring high-quality taxonomic identifications.

5.6 Reference

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CHAPTER 6

Toxicity and sorption kinetics of dissolved cadmium and the interactive effects of dissolved cadmium, chromium III and lead on tropical freshwater periphytic communities in laboratory mesocosm experiments[6]

Abstract: Understanding the cause and effect relationship between stressors and biota is crucial for the effective management, restoration and preservation of aquatic systems. The first objectives of the present study was to assess the effects of five cadmium (Cd) concentrations on tropical periphyton community growth, dissolved Cd accumulation kinetics, as well as metal toxicity to diatom communities. The second objective was to characterize periphyton growth under interactive effects of dissolved cadmium, chromium III and lead as well as Cd, Cr and Pb mixtures toxicity to diatom communities in laboratory mesocosm experiments. A natural periphyton community sampled from the Monjolinho River (South of Brazil) was inoculated into six experimental systems containing clean glass substrates for periphyton colonization. For the first objective, the communities were exposed to Cd concentrations of 0.005, 0.01, 0.03, 0.05 and 0.1 $mg.L^{-1}$. Metal accumulation (total and intracellular) in biofilms, dry weight and ash-free dry mass, growth rate, algal cell density and diatom community composition were analyzed on samples collected after 1, 2 and 4 weeks of colonization. For the second objective, the communities were exposed to mixtures of dissolved Cd and Pb (0.01 and 0.1 $mg.L^{-1}$) and Cr (0.05 and 0.2 $mg.L^{-1}$). Biofilm dry weight, ash-free dry mass, growth rate, algal cell density and diatom community composition were analyzed on samples collected after 1, 2 and 3 weeks of colonization. High Cd accumulation capacity by periphyton was demonstrated with total and intracellular Cd content in biofilms reflecting the effects of dissolved concentrations of Cd in the culture media and exposure duration. Total and intracellular Cd content generally increased in treatments in the order $0.005 < 0.01 < 0.03 < 0.05 < 0.1$ $mg.L^{-1}$ at any sampling time with increasing level of accumulated Cd with duration of exposure in all the systems. Hormesis (low Cd dose stimulation and high dose inhibition of responses like growth rate) has been demonstrated with periphyton growth and development being substantially lowered by high concentration of Cd (EC_{50} = 0.077 $mg.L^{-1}$). High Cd concentration (0.1$mg.L^{-1}$) affected periphyton growth whilst high

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concentration of Cr (0.2 $mg.L^{-1}$) and Pb (0.1$mg.L^{-1}$) had antagonistic interference on the toxic effects of Cd on periphyton growth demonstrating the importance of studying metal mixtures in field studies. Shifts in species composition (development of more resistant species like *Achnanthidium minutissimum* and reduction of sensitive ones like *Diatoma vulgare*, *Encyonema silesiacum, Eunotia bilunaris, Fragilaria capucina, Gomphonema parvulum, Navicula viridula* and *Navicula cryptocephala*), decreases in species richness and diversity and morphological alterations (deformities) of diatom cells with increasing Cd concentration and exposure duration were observed making biofilms appropriate monitors of metal pollution in aquatic systems.

Keywords: growth rate, diatom communities, morphological deformities, toxicity, bioaccumulation,

Toxicidade e cinética de sorção de cádmio dissolvido em comunidades perifíticas tropicais de água doce em experimentos mesocósmicos de laboratório

Resumo: Compreender a relação de causa e efeito entre estressores e biota é crucial para a gestão eficaz, recuperação e preservação de sistemas aquáticos. O primeiro objetivo do presente estudo foi avaliar os efeitos de diferentes concentrações de cádmio no crescimento de comunidades perifíticas tropicais, avaliando-se a cinética de acumulação de Cd dissolvido e a toxicidade do metal para comunidades de diatomáceas. O segundo objetivo foi caracterizar o crescimento de perifiton sob efeitos interativos de cádmio, cromo (III) e chumbo dissolvidos, assim como a toxicidade de misturas destes metais nas comunidades de diatomáceas em experimentos mesocósmicos de laboratório. Para o primeiro objetivo, a comunidade perifítica natural amostrada no rio Monjolinho (Sul do Brasil) foi inoculada em seis sistemas experimentais em recipientes contendo vidros como substratos para a colonização do perifíton e exposta às concentrações de 0,005, 0,01, 0,03, 0,05 e 0,1 mgL^{-1} de Cd. Acumulação de metal (total e intracelular) em biofilmes, peso seco, peso seco de cinzas, taxa de crescimento e densidade celular das algas e composição da comunidade de diatomáceas foram analisados em amostras coletadas após 1, 2 e 4 semanas de colonização. Para a segundo objetivo, as comunidades foram expostas a misturas formadas pela combinação de íons Cd(III) (0,01 e 0,1 mgL^{-1}), Cr(III) (0,05 e 0,2 mgL^{-1}) e Pb(II) (0,01 e 0,1 mgL^{-1}) em solução. Peso seco, peso seco de

cinza, taxa de crescimento e densidade celular de algas nos biofilmes e composição da comunidade de diatomáceas foram analisados em amostras coletadas após 1, 2 e 3 semanas de colonização. Observou-se elevada capacidade de acúmulo de Cd pelo perifíton, com conteúdo de Cd total e intracelular em biofilmes refletindo os efeitos das concentrações de Cd dissolvido nos meios de cultura em diferentes tempos de exposição. Conteúdos crescentes de Cd total e intracelular foram observados com o aumento de concentração na ordem 0,005 <0,01 <0,03 <0,05 <0,1 $mg.L^{-1}$, independente do tempo de amostragem e da duração da exposição para todos os sistemas. Hormese (estimulação com doses baixas Cd e inibição com doses elevadas de respostas como taxas de crescimento) tem sido demonstrada com crescimento do perifíton e o desenvolvimento do mesmo substancialmente reduzido em altas concentrações de Cd (EC_{50} = 0,077 mgL^{-1}). Alta concentração de Cd (0,1 mgL^{-1}) afetaram o crescimento de perifíton, enquanto que altas concentrações de Cr (0,2 $mg.L^{-1}$) e Pb (0,1 mgL^{-1}) interferiram de maneiras antagônicas nos efeitos tóxicos do Cd sobre o crescimento do perifíton demonstrando a importância de estudar misturas de metais em estudos de campo. Mudanças na composição (desenvolvimento de espécies mais resistentes como *Achnanthidium minutissimum* e redução de espécies mais sensíveis, como *Diatoma vulgare*, *Encyonema silesiacum, Eunotia bilunaris, Fragilaria capucina, Gomphonema parvulum, Navicula viridula* e *Navicula cryptocephala*), diminuição da riqueza e diversidade de espécies bem como alterações morfológicas (deformidades) de células de diatomáceas com o aumento da concentração de Cd e tempo de exposição sugerem que biofilmes sejam componentes adequados para o monitoramento da poluição de sistemas aquáticos por metais.

Palavras-chave: taxa de crescimento, comunidades de diatomáceas, deformidades morfológicas, toxicidade de Cd, bioacumulação

6.1 Introduction

Heavy metal contamination of freshwater environments causes significant accumulation of metals in various components of trophic chains, among which periphyton (especially diatoms) communities attract great attention from researchers because they are considered solar-powered biogeochemical reactors, biogenic habitats, hydraulic roughness elements, early warning systems for environmental degradation, and troves of biodiversity (LARNED, 2010). Periphyton sensitivity has been shown to high

metal levels in watersheds draining mining areas (GOLD et al., 2003a; NUNES et al., 2003) and to low pollution as described under experimental conditions (DUONG et al., 2008; MORIN et al., 2008a, 2008b). Different species respond differently to metal and other stressors because of differences in tolerances developed. *In situ* studies conducted at sites exhibiting high level of metals and microcosm experiments have demonstrated a decrease in productivity, diversity and changes in species composition of periphyton communities, especially diatoms, (TAKAMURA et al., 1989; DUONG et al., 2010). Thus, the composition of communities at different locations, or at different point in time, provide useful information about environmental conditions (DUONG et al., 2008; MORIN et al., 2008a, 2008b). Changes in morphology of diatom cells is also a manifestation of high concentration of metals (MORIN et al., 2007; DUONG et al., 2008; MORIN et al., 2008a, 2008b; DUONG et al., 2010).

Periphyton is a biological community of attached autotrophic and heterotrophic organisms that are associated in complex matrix of polysaccharide exudates and detritus with a complex function (STEVENSON et al., 1996). This matrix can form a protective layer thereby reducing the exposure of solid surface to the external environment and decreasing toxicity of contaminants (IVORRA et al., 2000; GOLD et al., 2003a, 2003b). In the case of metallic pollution, the protective effect of the biogenic matrix manifests itself through a less pronounced impact of metals on primary production and a less altered diatom community composition (IVORRA et al., 2000; GOLD et al., 2003a, 2003b).

Attempts to develop predictive models of metal protective role of biofilms are characterized by large amounts of unexplained variance impeding the development of protective stream metal standards. For example, IVORRA et al. (2000) and MORIN et al. (2008) showed that an early exposure of biofilms to Cd concentrations of ~10 and ~100 μg L^{-1} resulted in a 100- to 200-fold higher concentration measured in the biofilm matrix. However, the absence of experimental data between 10 and 100 μg Cd L^{-1} did not allow any precise evaluation of the Cd threshold for negative effects on biofilm biomass and diatom community structure. DUONG et al. (2010) studied this aspect using only 100 μg L^{-1} Cd and obtained the mean value of concentration factor (i.e. ratio of total accumulated to dissolved Cd) of around 30. They recommended carrying out of further experiments with different metal concentrations during the development of young biofilms to determine whether the non-exchangeable Cd fraction is proportional to Cd concentrations available in water column or depend on a limited incorporation process.

In addition, most of these studies have been carried out in temperate regions with little studies of this nature being carried out in the tropical regions. However, there is evidence that research findings developed in one geographic area are less successful when applied in other areas (PIPP, 2002). This is due not only to the floristic differences among regions (PAN et al., 1996; TAYLOR et al*.,* 2007), but also to the environmental differences that modify species responses to water-quality characteristics (POTAPOVA and CHARLES, 2005). Strict testing of research findings from temperate regions is required to ensure that they give a realistic reflection of tropical environments.

Two experimental types were carried out. The first experiment (hereafter referred to as experiment 1) was aimed at studying the effects of five Cd concentrations on tropical periphyton community growth (assessing measurements of dry weight, ash-free dry mass, chlorophyll *a* and diatom density), accumulation kinetics of dissolved Cd, as well as metal toxicity to diatom assemblages. Adsorbed and intracellular Cd levels in periphyton were hypothesised to be a function of duration of exposure and the dissolved Cd concentrations in the culture media.

The second experiment was aimed at characterizing periphyton growth (using measurements of chlorophyll *a*, dry weight, ash-free dry mass, and algal cell density) under interactive effects of dissolved Cd, Cr III and Pb as well as Cd, Cr and Pb mixtures toxicity to diatom assemblages in laboratory mesocosm experiments. These metals were chosen because they were shown to be strongly correlated in the study area (BERE and TUNDISI, 2011). Response of organisms to metal combinations can be different from what might be expected from information on the solitary action of metals. For example, two or more metals may act antagonistically where one metal ameliorate the toxicity of the other metal or the metals may act synergistically where the toxic response is greater than the sum of the individual toxicities.

6.2 Materials and Methods

6.2.1 Field periphyton collection

Periphytic communities were collected from Monjolinho River in the southern part of Brazil at a reference site after ecological park before the river pass through the city of São Carlos (21^{o}59'09.16" S; 47^{o}52'35.82" W; elevation 832m, site 7, Figure 1). Headwaters of the Monjolinho River and its tributaries fall within mainly agricultural area. Very low metal concentrations, similar to background levels in the area were measured in the water column and sediment at the reference site (BERE and TUNDISI,

2011). Sampling was done during dry season to avoid variable effects of rainy season like great variations in water level and velocity, floods and inundations. These variations affect diatom development, especially growth rate and relative abundance of different species (ROUND, 1991; BIGGS and KILROY, 2000).

For each experiment, 4 plastic racks, each fitted with 10 separate and vertical glass substrates (6 X 15 cm) were immersed at the reference site parallel to the current 20 to 30 cm below the water surface. The racks were secured accordingly and left for 4 weeks prior to sampling. On sampling, the plastic rakes were carefully removed from the river and biofilms colonising the glass substrate were brushed with a toothbrush into culture medium. The biofilms from all the glass substrates were pooled into one sample of approximately 2 L. This biofilm suspension was transported to the laboratory in cooler box (4 ^{o}C).

6.2.2 Laboratory experiments

Closed experimental systems (hereafter referred to as experimental units; EUs; 6 for experiment 1 and 10 for experiment 2) were set up to allow the exposure of natural periphytic communities to stressors under controlled conditions following GOLD et al. (2003a; Figure 6.1). Each EU consisted of three half-polyvinyl chloride (PVC) tubes 50 cm long with a radius of 5 cm as artificial streams with a capacity of 2.8 L each. The three streams were connected in parallel to a 30 L tank. All systems were filled with diluted (4x) modified Woods Hole culture medium after GOLD et al. (2003a). This culture medium was kept without ethylenediaminetetraacetic acid (EDTA), which presents very high binding capacities for metals (STAUBER and FLORENCE, 1989), and supplemented with silica, an essential diatom nutrient. Test medium were prepared from distilled water. A pump (Boyu bomba submersa SP-0100-600/h, SP-Brazil) allowed continuous circulation of the water through each system at a rate of 10 ±0.25 ml s^{-1}, corresponding to a velocity of 0.2 cm s^{-1}. Discharge was monitored daily and adjusted where necessary. Each stream was fitted with 6 clean glass substrates (6 X 15 cm) in a slightly slanting position for periphyton colonisation. Water level was kept at 0.5 cm above substrate. A light intensity of 55 ± 5 µmol s^{-1} m^{-2} at the water-air interface for photosynthetically active radiations (400-700 nm, LI-193 Spherical Quantum Sensor (LI-COR Worldwide, Brazil) was maintained with a light: dark regime of 12h/12h.

During the course of the experiment pH, temperature and dissolved oxygen (DO) level for each experimental unit were recorded daily. Water samples for nutrient analysis

(phosphates, silica and nitrates) were collected every two days from each stream. These samples were filtered through pre-combusted Whatman GF/F filters and analysed for the nutrients following standard methods (APHA, 1988). Based on these measurements, the nutrients were adjusted accordingly.

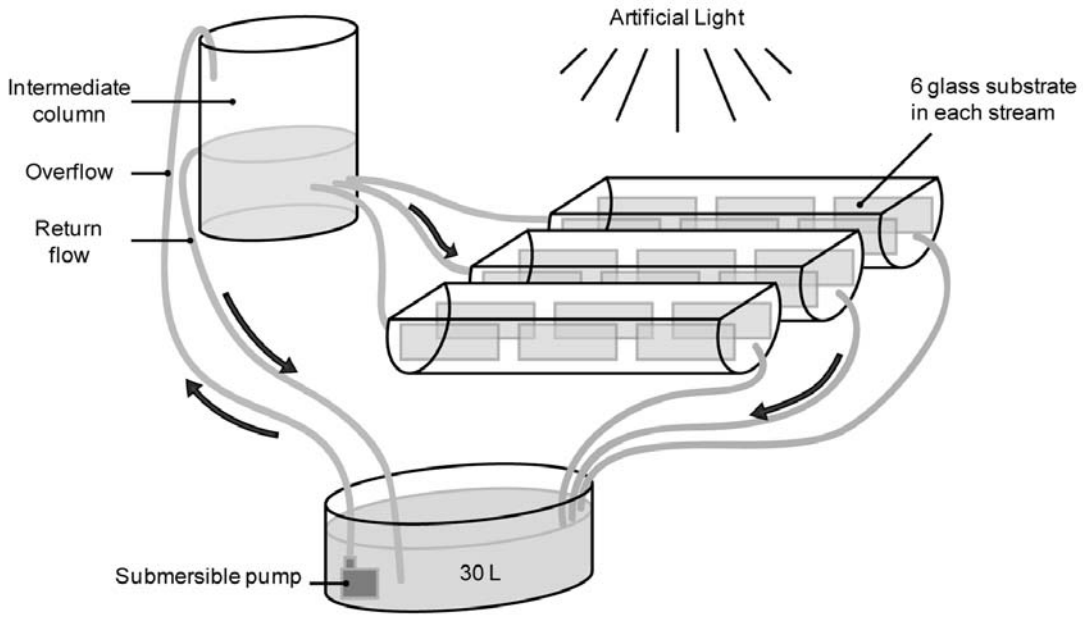

Figure 6.1: Schematic representation of a closed experimental system, consisting of three artificial streams (50 cm length, 5 cm radius), each containing 6-glass substrata (6 X 15 cm). Arrows indicate flow direction (by: Ricardo M. Degani).

6.2.3 Metal exposure

For the first experiment, homogenised periphyton suspension from the field was divided into 6 equal volumes corresponding to the number of EUs. Each fraction was introduced into the water column of the tank feeding each EU. The systems were equilibrated over night and then the desired concentrations of Cd were obtained by addition of aliquots of the stock standard solutions to different systems. EU_1 was left free of metals to act as control. EUs 2 to 6 were contaminated with Cd at concentrations of 0.005, 0.01, 0.03, 0.05 and 0.1 mg L^{-1} respectively. Cadmium chloride ($CdCl_2$, 10 mg L^{-1}, Merck, Darmstadt, Germany), used as stock solution, was added to the systems to obtain final desired concentrations for each EU. Cd concentrations were measured twice per week during the experiment by atomic absorption spectrophotometry (Varian AA 400). Based on these measurements, Cd levels in contaminated systems were readjusted, to maintain a relatively stable Cd concentration close to the required level.

For the second experiment, homogenized periphyton suspension from the field was divided into ten equal volumes corresponding to the number of EUs. As in the first

experiment, each fraction was introduced into the water column of the tank feeding each EU, the systems were equilibrated over night, and then the desired concentrations of Cd, Cr III and Pb were obtained by addition of aliquots of the stock standard solutions to different systems. EU_1 was left free of metals to act as control. EU_2 was contaminated with 0.01 $mg.L^{-1}$ Cd and 0.033 $mg.L^{-1}$ Pb. EU_3 was contaminated with 0.01 $mg.L^{-1}$ Cd and 0.1 $mg.L^{-1}$ Pb. EU_4 was contaminated with 0.1 $mg.L^{-1}$ Cd and 0.033 $mg.L^{-1}$ Pb. EU_5 was contaminated with 0.1 $mg.L^{-1}$ Cd and 0.1 $mg.L^{-1}$ Pb. EU_6 was contaminated with 0.01 $mg.L^{-1}$ Cd and 0.05 $mg.L^{-1}$ Cr III. EU_7 was contaminated 0.01 $mg.L^{-1}$ Cd and 0.2 $mg.L^{-1}$ Cr III. EU_8 was contaminated with 0.1 $mg.L^{-1}$ Cd and 0.05 $mg.L^{-1}$ Cr III. EU_9 was contaminated with 0.1 $mg.L^{-1}$ Cd and 0.2 $mg.L^{-1}$ Cr III. EU_{10} was contaminated with 0.01 $mg.L^{-1}$ Cd, 0.033 $mg.L^{-1}$ Pb and 0.05 $mg.L^{-1}$ Cr III. Cadmium chloride ($CdCl_2$, 10 $mg.L^{-1}$, Merck, Darmstadt, Germany), chromium (III) chloride hexahydrate $[(CrCl_2(H_2O)_4]Cl{\cdot}2H_2O$, 10 $mg.L^{-1}$, Merck, Darmstadt, Germany) and lead nitrate ($Pb(NO_3)_2$, 10 $mg.L^{-1}$, Merck, Darmstadt, Germany), used as stock solutions, were added to the systems to obtain final desired concentrations for each EU. Cd, Cr and Pb concentrations were measured twice per week during the experiment by atomic absorption spectrophotometry (Varian AA 400). Based on these measurements, levels of Cd, Cr and Pb in contaminated systems were readjusted, to maintain relatively stable concentrations close to the required levels.

6.2.4 Biofilm sampling and analysis

For experiment 1, biofilms were collected after a colonization period of 1, 2 and 4 weeks, while for experiment 2, biofilms were collected after a colonization period of 1, 2 and 3 weeks. At each sampling time, 2 glass substrates were randomly removed from each stream of each EU (n = 3 for each EU). The biofilm from the 2 glasses were brushed with a toothbrush into mineral water and the resultant biofilm suspensions from the 2 glasses were pooled to make one sample and making the volume of the suspension to 100 mL for experiment 1 and 60 mL for the experiment 2. After each sampling time, the artificial streams were reset by new glass substrates to maintain identical flow conditions.

For experiment 1, these biofilm suspensions were then divided into five fractions each for various analyses. The first fraction (20 ml) was fixed with 4% (final concentration) formalin for identification and cell density determination. Cells in 100 μL subsample was counted in a Nageotte counting chamber at X400 with cell densities expressed as living cells per unit area (cells cm^{-2}). For diatom identification to species

level, sub-samples of the 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 and KILROY, 2000). A total of 250 – 600 valves per sample (based on counting efficiency determination method by PAPPAS and STOERMER (1996)) were identified and counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type - 020-519.503 LB30T, Germany). The mean and standard deviation of counting efficiencies of diatom communities calculated according to PAPPAS and STOERMER (1996) on different substrates was 86.1 ±7.6 %. The diatoms were identified to species level based on studies by METZELTIN et al. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007). Changes in diatom frustules/valve morphology are important manifestations of high concentration of metals (MORIN et al., 2007; DUONG et al., 2008; MORIN et al. 2008a, 2008b; DUONG et al., 2010). Thus, individual deformities (valves with abnormal general shapes and/or diatoms with deformed valve wall ornamentation) were observed and their frequency recorded.

The second fraction (20 ml) was used for chlorophyll *a* analysis. The samples were filtered onto Whatman GF/C filters. Chlorophyll *a* from the filters was measured spectrophotometrically (at 665 nm and 750 nm) following extraction in boiling 80% ethanol (5 min) and steeping at 4°C in the dark (24 h). A phaeopigment correction was obtained by acidification NUSCH (1980).

The third fraction (20 mL) was filtered through pre-combusted GF/C filters and dried at 60 °C for 48 h to determine dry weight (DW). After final weighing, samples were ashed at 500 ^{o}C for 1 hr and weighed again to obtain ash-free dry mass (AFDM) and expressed as AFDM cm^{-2}. Growth rates inferred from AFDM measurement data were calculated for the exponential phase (BIGGS, 1990) and were expressed as micrograms of AFDM per unit area of glass substrate. From these growth rates, the percent inhibition (stimulation) of each Cd concentration was calculated (BIGGS, 1990). From these results, the EC_{50} was then determined from linear regression equation.

The fourth fraction (20 mL) was used to determine the total amount of metal accumulated in biofilm as described in section 6.2.5. To measure intracellular metal (non-exchangeable) content in biofilm, the fifth fraction (20 mL) of sample was washed with EDTA 4 mM at pH = 8, for 10 min to remove Cd adsorbed onto the surface of algal cells and most of inorganic complexes embedded in the biofilm (SOLDO et al., 2005). The resultant sample was then analysed for Cd as described in section 6.2.5. Adsorbed Cd was

calculated as the difference between the metal content before and after washing with EDTA. Concentration factors (CFs) of the biofilm for Cd were calculated according to FOSTER (1976) by dividing concentrations of Cd in biofilms (total and non-exchangeable fractions) by those in water column.

For experiment 2, biofilm suspensions were divided into three fractions each for analyses of cell densities, diatom communities, chlorophyll *a*, and biomass as in the case of experiment 1.

6.2.5 Metal analysis

Samples from fourth and fifth fractions of experiment 1 were filtered through a tarred metal free paper (0.45 µm membrane, Millipore) to obtain the dry weight after drying at 60 °C for 48h. Dried biofilm samples were first digested with nitric acid following method 3050B (Environmental Protection Agency-USA). The digestates were diluted with ultra pure water (Millipore, Simpakkr1, Simplicity 185, SP-Brazil) to 100 ml (final volume). Cd concentrations in biofilm (washed with EDTA or not) were measured by inductively coupled plasma mass spectrometry (ICP-MS), (Analytical Instrument Recycle, Inc, USA). Total and non-exchangeable Cd in biofilms was estimated for the first and second week of the experiment.

6.2.6 Data analysis

Variations in physicochemical characteristics of the water, diatom community structure (species richness, diversity, cell densities and relative abundance), chlorophyll *a*, DW, AFDM, Cd accumulation in biofilm and frequency of morphological deformities with treatments and duration of exposure were examined by means of a repeated measures analysis of variance (RM-ANOVA; STATISTICA software package, Release 7, Stat Soft. Inc., USA) after testing for homogeneity of variances (Levene`s test, $p < 0.05$) and normality of distribution (Shapiro-Wilk test, $p < 0.05$) and log transforming where necessary. Treatments were used as fixed factors among objects, and time as fixed factor within objects. Cluster analysis with unweighted pair-group average and Euclidian distance was performed based on pooled benthic diatom community data to show the main differences and similarities in community composition among the treatments. Shapiro-Wilk test, Levene`s test, and cluster analysis, were performed using PAlaeontological STatistics (PAST) software version 2.01 (HAMMER et al., 2009).

6.3 Results

6.3.1 Experiment 1

6.3.1.1 Physicochemical characteristics of the water column

Water temperature, pH and dissolved oxygen did not differ significantly ($p > 0.05$) among the systems over the 4-week experimental period (Table 6.1). The pH was generally slightly higher in the control compared to other EUs, decreasing as the concentration of Cd increased. No trend in temperature change was detected with increasing Cd concentration with the temperature ranging from 21.1 to 23.1 ^{o}C among the EUs. DO increased slightly with increasing Cd concentration to about 0.01 mg.L-1 Cd and then decreased slightly with increasing Cd concentration. The pH, temperature and DO remained relatively constant in all the EUs throughout the experiment.

In all systems, at the beginning of the experiment phosphates, nitrates and silica concentrations were around 2.9 mg.L^{-1}, 33 mg.L^{-1} and ~70mg.L^{-1} respectively. Based on measurements recoded after every two days (data not shown), nutrient concentrations were shown to decrease gradually in high Cd concentration EUs (0.1; 0.05 and 0.03 mg.L^{-1}) whereas sharp decreases were observed in the non-contaminated treatment (i.e. the control) and low Cd concentration EUs (0.005, and 0.01 mg.L^{-1}). This trend in nutrient concentrations was observed among all the EUs throughout the experiment. Adjustments of nutrient levels by additions of aliquots (depending on the rate of decrease in nutrient levels) of nutrient stock solutions to the culture medium of all the EUs restored the initial phosphates, nitrates and silica concentrations.

Table 6.1: Water column physicochemical parameters (mean value and standard deviation) in all the systems measured during a 4-week experimental period

Treatment (mg.L^{-1})	DO (mg.L^{-1})	Temperature (^{o}C)	pH
Control	6.4 ±0.50	22.6 ±0.5	8.0 ±0.2
0.005	6.5 ±0.40	22.8 ±0.5	7.9 ±0.1
0.01	6.6 ±0.02	22.5 ±0.6	7.8 ±0.2
0.03	6.4 ±0.10	22.5 ±0.6	7.8 ±0.3
0.05	6.3 ±0.04	22.4 ±0.6	7.8 ±0.1
0.1	6.4 ±0.20	22.4 ±0.5	7.8 ±0.1

6.3.1.2 Periphyton growth

Chlorophyll *a* concentrations recorded in the six EUs during the course of the experiment are shown in Figure 6.2*a*. No significant differences were observed among the control and 0.005 and 0.01 mg.L^{-1} Cd treatments but these treatments had significantly higher chlorophyll *a* concentration compared to 0.03, 0.05 and 0.1 mg.L^{-1} Cd treatments (with $p < 0.05$ for the treatment X date effect at 2nd and 4th week). A

significant increase in chlorophyll *a* concentration ($p < 0.05$ at week 4) was observed between the control (0.81 ±0.01 μg.cm^{-2}) and 0.005mg.L^{-1} Cd treatment (1.68 ±0.2 μg.cm^{-2}) at 4th week of the experiment. The control and 0.01 mg.L^{-1} Cd treatment generally exhibited similar chlorophyll *a* values during the whole course of the experiment. Highest chlorophyll *a* concentration was generally recorded in 0.005 mg.L^{-1} Cd treatment (0.081 ±0.005 at week 1 to 1.680 ±0.2 μg.cm^{-2} at week 4) whilst lowest concentrations were recorded in 0.1 mg.L^{-1} Cd treatment (0.026 ±0.001 at week 1 to 0.114 ±0.2 μg.cm^{-2} at week 4). In all the systems, chlorophyll *a* concentrations increased significantly ($p < 0.05$) throughout the experiment with the increase being significantly ($p < 0.05$) higher in the control and low Cd treatments compared to high Cd treatments.

As in the case of chlorophyll *a*, DW and AFDW were significantly high in the control and low Cd concentration treatments (0.005 and 0.01 mg.L^{-1} Cd) compared to the higher Cd concentration treatments (with $p < 0.05$ for the treatment X date effect at 2nd and 4th week; Figure 6.2*c* and *d* respectively). However, a slight but statistically insignificant increase in DW and AFDW ($p > 0.05$ at week 4) was observed between control and 0.005mg.L^{-1} Cd treatment throughout the experiment. Highest DW was generally recorded in 0.005 mg.L^{-1} Cd treatment (31.75 ±5.62 at week 1 to 85.80 ±2.83 mg.cm^{-2} at week 4) whilst lowest DW was recorded in 0.1 mg.L^{-1} Cd treatment (17.75 ±0.87 at week 1 to 24.63 ±2.41 mg.cm^{-2} at week 4). Highest AFDW was generally recorded in 0.005 mg.L^{-1} Cd treatment (19.50 ±13.14 at week 1 to 75.38 ±12.68 mg.cm^{-2} at week 4) whilst lowest AFDW was recorded in 0.1 mg.L^{-1} Cd treatment (6.63 ±2.07 at week 1 to 20.50 ±2.29 mg.cm^{-2} at week 4). In all the systems, DW and AFDW increased significantly ($p < 0.05$) throughout the experiment with the increase being significantly ($p < 0.05$) higher in the control and low Cd treatments compared to high Cd treatments.

A slight but statistically insignificant increase in periphyton growth rate ($p > 0.05$) was observed between control (0.071 ±0.024 AFDW.cm^{-2}.day^{-1}) and 0.005mg.L^{-1} Cd treatment (0.073 ±0.045 AFDW.cm^{-2}.day^{-1}; Figure 6.3*a*). Besides this slight increase, a significantly negative correlation ($R^2 = 0.94$) between growth rate and Cd concentration was observed. Highest growth rate was recorded in 0.005 mg.L^{-1} Cd treatment (0.073 ±0.045 AFDW.cm^{-2}.day^{-1}) whilst lowest growth rate was recorded in 0.1 mg.L^{-1} Cd treatment (0.031 ±0.02 AFDW.cm^{-2}.day^{-1}). Thus, growth rate was stimulated by increase in Cd concentration to around 0.005mg.L^{-1}, hence negative inhibition value (-2.24%; Figure 6.3*b*). Besides this stimulation, a strong linear positive correlation ($R^2 = 0.99$) was observed between periphyton growth inhibition and Cd concentration with 56.23%

growth inhibition being recorded in 0.1 $mg.L^{-1}$ Cd. An EC_{50} of 0.077 $mg.L^{-1}$ Cd on periphyton communities was recorded in this study.

6.3.1.3 Cadmium accumulation

Total and non-exchangeable Cd accumulated by biofilms is presented in Figure 6.4. Cd levels were below detection limit (1 $\mu g.L^{-1}$) in the control treatment. An exponential accumulation of total and non-exchangeable Cd in biofilms with increasing dissolved Cd concentration was observed. Total and non-exchangeable Cd contents were significantly different among all the treatment (with $p < 0.05$ for the treatment X date effect). Highest and lowest total and non-exchangeable Cd contents were recorded at 0.1 and 0.005 $mg.L^{-1}$ Cd treatment respectively. In all the systems, total and non-exchangeable Cd contents increased significantly throughout the experiment ($p < 0.05$).

Within any given treatment, total and non-exchangeable Cd in biofilms varied during the course of the experiment: the ratio of and non-exchangeable/total Cd was low at low Cd concentration (0.005 $mg.L^{-1}$ = 0.40 and 0.59 at 1st and 2nd week respectively and 0.01 $mg.L^{-1}$ = 0.52 and 0.64 at 1st and 2nd week respectively) compared to high Cd concentration treatments (0.03 $mg.L^{-1}$ = 0.62 and 0.65 at 1st and 2nd week respectively; 0.05 $mg.L^{-1}$ = 0.72 and 0.69 at 1st and 2nd week respectively; 0.1 $mg.L^{-1}$ = 0.76 and 0.69 at 1st and 2nd week respectively).

In addition concentration factors (CFs) of the biofilm for Cd, based on total and intracellular Cd, showed an increasing accumulation ability of the biofilms with increasing dissolved metal concentrations and duration of exposure (Figure 6.5) (with $p < 0.05$ for the treatment X date effect). Gross uptake capacity of biofilms (i.e., the ratio of total Cd accumulated to dissolved metal) increase linearly with increasing dissolved Cd concentration: from 22.6 ±6 to 38.0 ±9 and from 33.7 ±5 to 51.6 ±7 at 1st and 2nd week respectively, (R^2 = 0.92 and 0.97 at 1st and 2nd week respectively, Figure 6.5). The CFs based on non-exchangeable Cd also expressed an increased Cd sorption activity with increasing dissolved Cd concentrations in the treatments: from 9.1 ±6 to 28.0 ±4 and from 19.6 ±9 to 34.6 ±5 at 1st and 2nd week respectively, (R^2 = 0.89 and 0.93 at 1st and 2nd week respectively, Figure 6.5). Thus, the levels of accumulated Cd in the biofilms from 0.005 $mg.L^{-1}$ treatment were higher than Cd levels in the medium demonstrating biofilm metal concentrating ability.

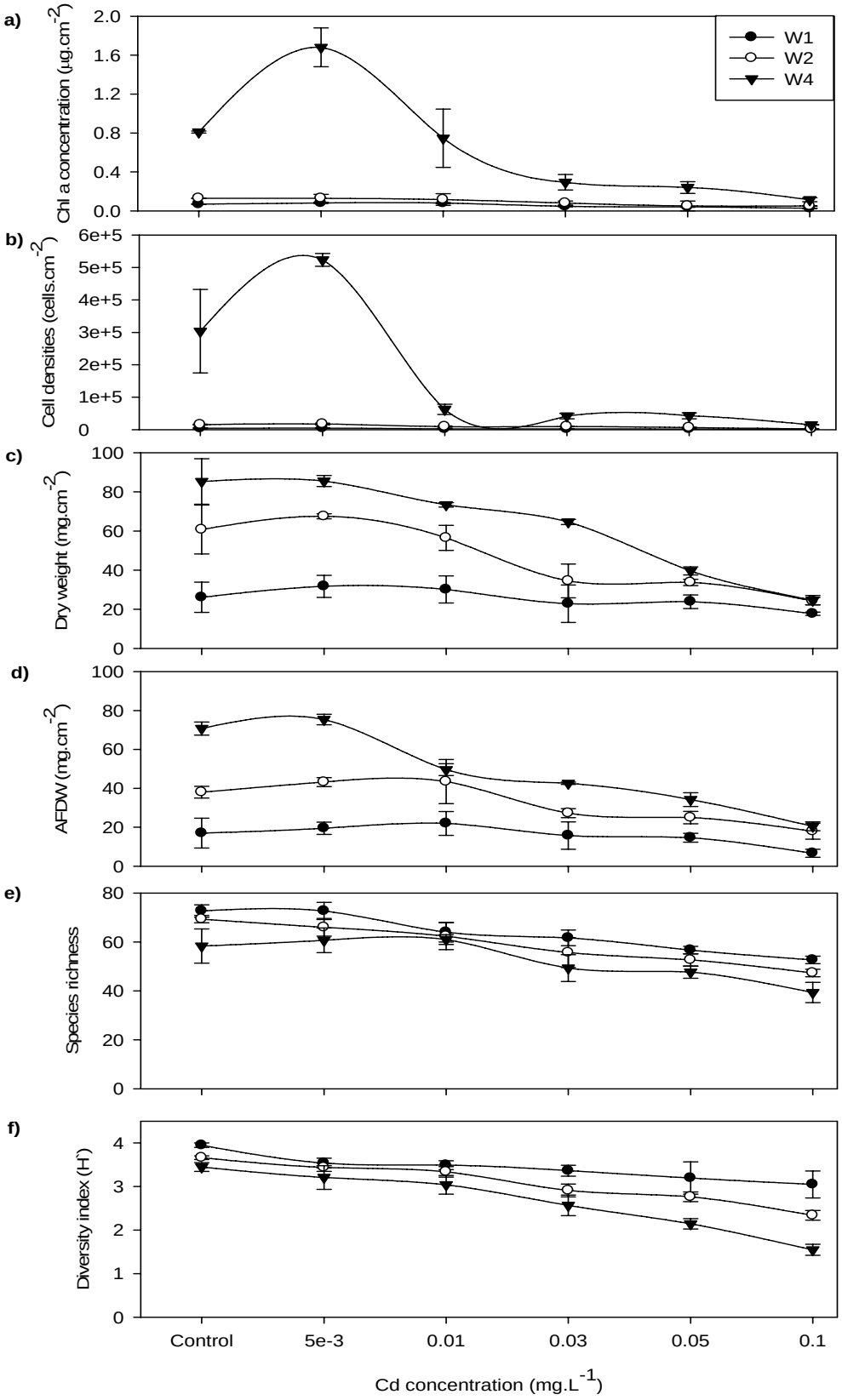

Figure 6.2: The mean values and standard deviations (n = 3) of chlorophyll *a* (a), cell densities (b), dry weight (c), AFDW (d), diatom species richness (e) and diversity (f) developed on glass substrates in six experimental units in the first, second and fourth week of the experiment.

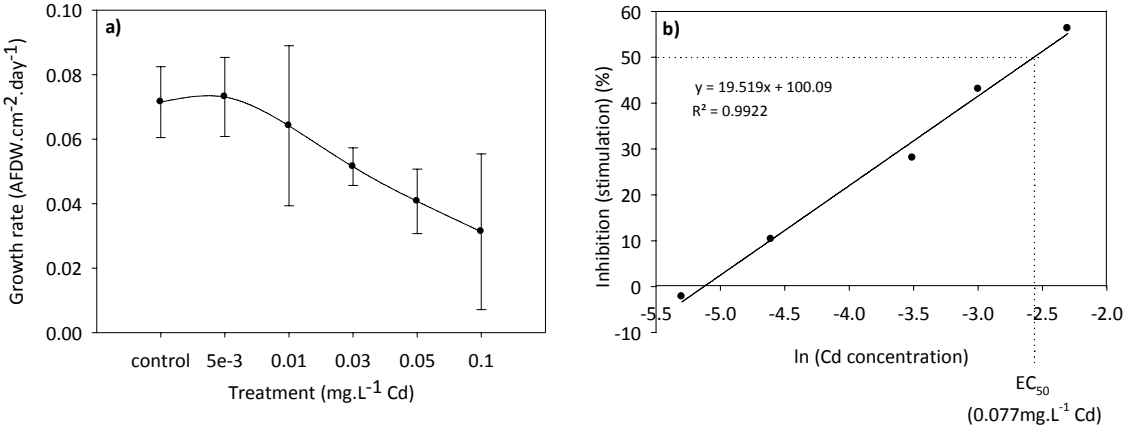

Figure 6.3: Periphyton growth rate (a) and percentage inhibition (b) in the five different Cd treatments. Error bars: standard deviation of three replicates.

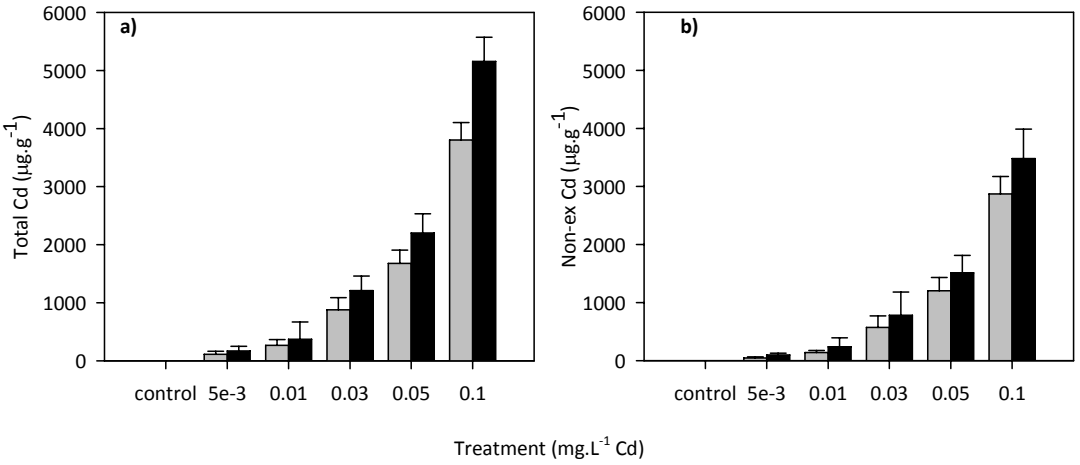

Figure 6.4 Biofilm accumulation of total (a) and non-exchangeable (b) Cd in the six experimental units: gray bars – 1st week; black bars – 2nd week. Error bars: standard deviation of three replicates.

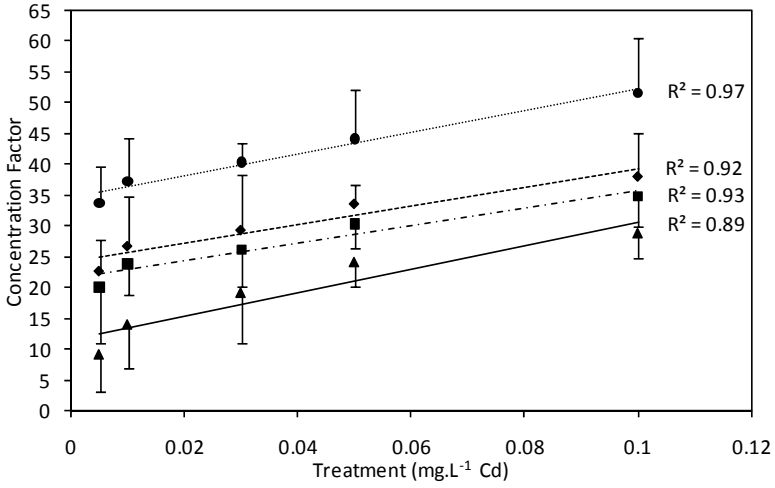

Figure 6.5: Concentration factors of total and non-exchangeable Cd with increasing dissolved Cd levels in the experimental units: diamonds and circles – based on total Cd at 1st and 2nd week respectively; triangles and squares – based on non-exchangeable Cd at 1st and 2nd week respectively. Error bars: standard deviation of three replicates.

6.3.1.4 Community composition and morphological abnormalities

As in the case of chlorophyll *a*, cell densities were significantly high in the control and low Cd concentrations (0.005 and 0.01 mg.L^{-1} Cd) compared to the higher Cd concentrations (with $p < 0.05$ for the treatment X date effect at 2nd and 4th week; Figure 6.2*b*). A significant increase in cell densities ($p < 0.05$ at week 4) was observed between control (303 704 ±128 780 cells cm^{-2}) and 0.005mg.L^{-1} Cd treatment (523 496 ±19 863 cells cm^{-2}). Highest cell densities were generally recorded in 0.005 mg.L^{-1} Cd treatment (4 800 ±1 700 at week 1 to 523 496 ±19 863 cells cm^{-2} at week 4) whilst lowest densities were recorded in 0.1 mg.L^{-1} Cd treatment (1 341 ±516 at week 1 to 15 130 ±1 013 cells cm^{-2} at week 4). In all the systems, cell densities increased significantly ($p < 0.05$) throughout the experiment with the increase being significantly ($p < 0.05$) higher in the control and low Cd treatments compared to high Cd treatments.

Species richness and diversity were significantly high in the control and low Cd concentrations (0.005 and 0.01 mg.L^{-1} Cd) compared to the higher Cd concentrations (with $p < 0.05$ for the treatment X date effect at 2nd and 4th week; Figure 6.2*e* and *f* respectively). Species richness was generally highest in the control (73 ±3 and 58 ±7 for the 1st and 4th week respectively) and lowest in 0.1 mg.L^{-1} Cd treatment (53 ±2 and 39 ±4 for the 1st and 4th week respectively) for a total of 104 species identified. Species diversity was also highest in the control (3.95 ±0.05 and 3.45 ±0.10 for the 1st and 4th week respectively) and lowest in 0.1 mg.L^{-1} Cd treatment (3.05 ±0.31 and 1.55 ± 0.13 for the 1st and 4th week respectively). In all the systems, species richness and diversity decreased significantly ($p < 0.05$) throughout the experiment with the decrease being significantly ($p < 0.05$) higher in the control and low Cd treatments compared to high Cd treatments.

Based on cluster analysis carried out to show the main differences and similarities in community composition amongst the six EUs and among the sampling dates, two major groups of communities were observed (Figure 6.6). The grouping reflected a change in community composition over the experimental period with the first group consisting of communities from all the EUs recorded at first and second week of the experiment, whilst the second group comprised of communities from all the EUs recorded at the forth week. Within these two major groups, subgroups were observed which roughly reflected changes in community composition due to changes in Cd concentration with the subgroups being more distinct in the second major group compared to the first group.

Of the 104 diatom species belonging to 38 genera that were recorded in all the EUs during the course of the study, 10 dominant diatom species with mean relative abundances >5% and present in at least two communities were described as characteristic of each diatom community developed throughout the experiment (Figure 6.7). After 1 week of colonization, diatom composition in the six systems was similar with the presence of *Navicula viridula* (Kützing) Kützing (Nvir), *Eunotia bilunaris* (Ehrenberg) Mills (Ebil), *Eunotia pectinalis* (Kützing) Rabhenhost (Epec), *Diatoma vulgare* Bory (Dvul), *Navicula cryptocephala* (Grunow) Cleve (Ncry) and *Achnanthidium minutissimum* (Kützing) Czarnecki (Amin). At week 2, the dominant species were still similar with a general increase in the relative abundance of *A. minutissimum* in all the EUs. The relative abundance of *A. minutissimum* generally tended to increase with increasing Cd concentration though it was low at 0.01 $mg.L^{-1}$ compared to other Cd concentrations. The relative abundance of *D. vulgare* increased in the control as well as low Cd concentration treatments (0.005 and 0.01$mg.L^{-1}$ Cd), whilst the same decreased in higher Cd concentration treatment between the first and the second week. The relative abundance of *N. viridula* tended to decrease in the control and low Cd concentration treatments whilst remaining relatively constant at higher Cd concentrations. After 4 weeks of colonization, the species composition in all EUs differed from that noted at week 1 and 2 with the proliferation of *A. minutissimum* and *Ulnaria ulna* (Nitzsch) Compère (Uuln) and a general decrease of *N. viridula* and *D. vulgare*. The relative abundance of *A. minutissimum* significantly increased with increasing Cd concentration (control = 9.00%; 0.005 $mg.L^{-1}$ Cd = 18.49%; 0.01 $mg.L^{-1}$ Cd =23.89%; 0.03 $mg.L^{-1}$ Cd = 28.00%; 0.05 $mg.L^{-1}$ Cd = 38.83%; 0.1 $mg.L^{-1}$ Cd = 45.00%). The relative abundance of *U. ulna* also tended to increase with increasing Cd concentration though highest relative abundance was recorded at 0.01 $mg.L^{-1}$ Cd.

In addition to changes in species composition due to Cd level and exposure duration, response of diatoms communities to metal contamination was characterized by the appearance of diatoms deformities. The frequency of teratological frustules of diatom species, which consisted of twisted valves in their apical axis or irregularity in striae arrangement, also tended to increase with increasing Cd concentration and duration of exposure hardly exceeding 1% in most affected treatments (Table 6.2). The frequency of diatom deformities in the control system remained low with around 1.9 ±0.5‰ from week 1 to week 4. Occurrence of abnormal forms significantly increased with Cd concentration and exposure duration with deformities being significantly low in the

control and low Cd concentrations (0.005 and 0.01 mg.L^{-1} Cd) compared to the higher Cd concentrations ($p < 0.05$ for the treatment X date effect at 2nd and 4th week). Highest frequencies of deformities were recorded in 0.1 mg.L^{-1} Cd treatment (3.1 ± 1.3‰ at week 1 and 23.8 ± 2.2‰ at week 4). Deformed *A. minutissimum*, *E. bilunaris*, *Fragilaria capucina* Desmazières, *Gomphonema gracile* Ehrenberg, *Nitzschia amphibia* Grunow and *U. ulna* were the most frequently observed in biofilm samples.

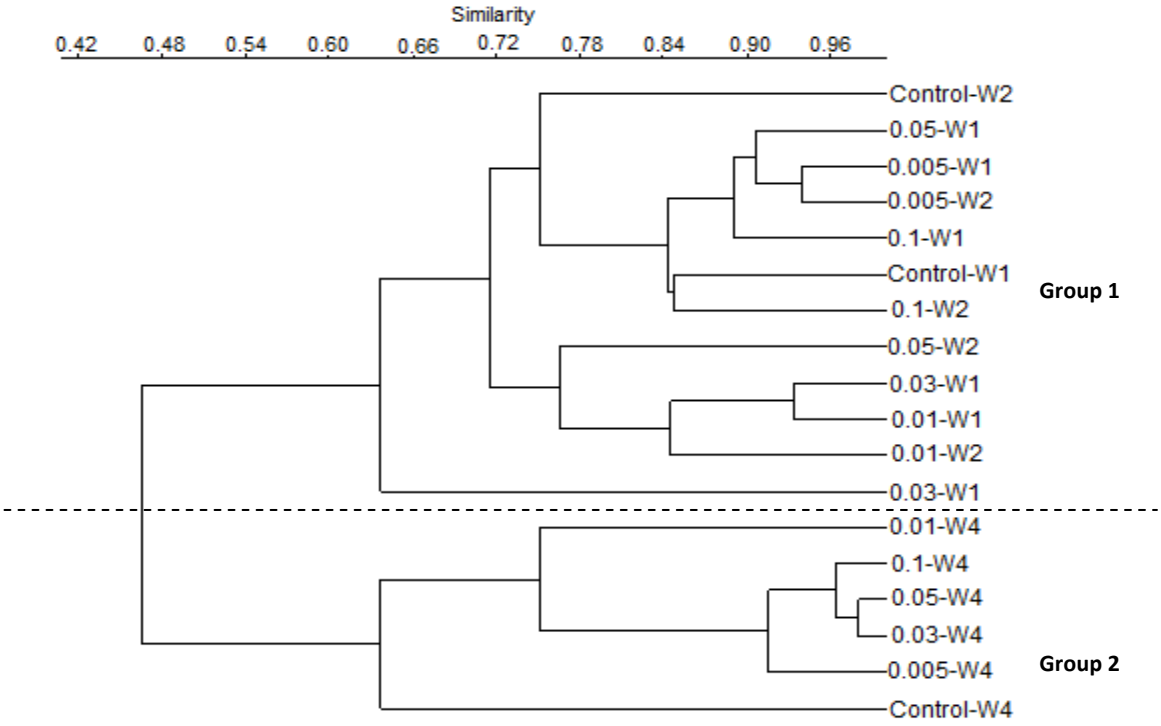

Figure 6.6: Cluster analysis to show similarities in taxonomic composition of diatom communities developed on glass substrates in six experimental units in the first, second and fourth week of the experiment.

Table 6.2: Mean and standard deviations of abnormal valve form abundances (‰) of total diatom communities from the six treatments (control and five Cd concentrations (mg.L^{-1})) during the course of the experiment.

Treatment	W1	W2	W4
Control	1.5 ± 0.9	1.9 ± 0.5	1.5 ± 0.1
0.005	1.4 ± 1.1	2.1 ± 1.2	2.6 ± 1.4
0.01	1.7 ± 0.5	2.2 ± 0.4	3.4 ± 1.2
0.03	2.5 ± 1.1	4.8 ± 2.2	9.8 ± 5.5
0.05	2.8 ± 1.8	6.7 ± 3.3	11.5 ± 5.4
0.1	3.1 ± 1.3	10.2 ± 5.4	23.8 ± 2.2

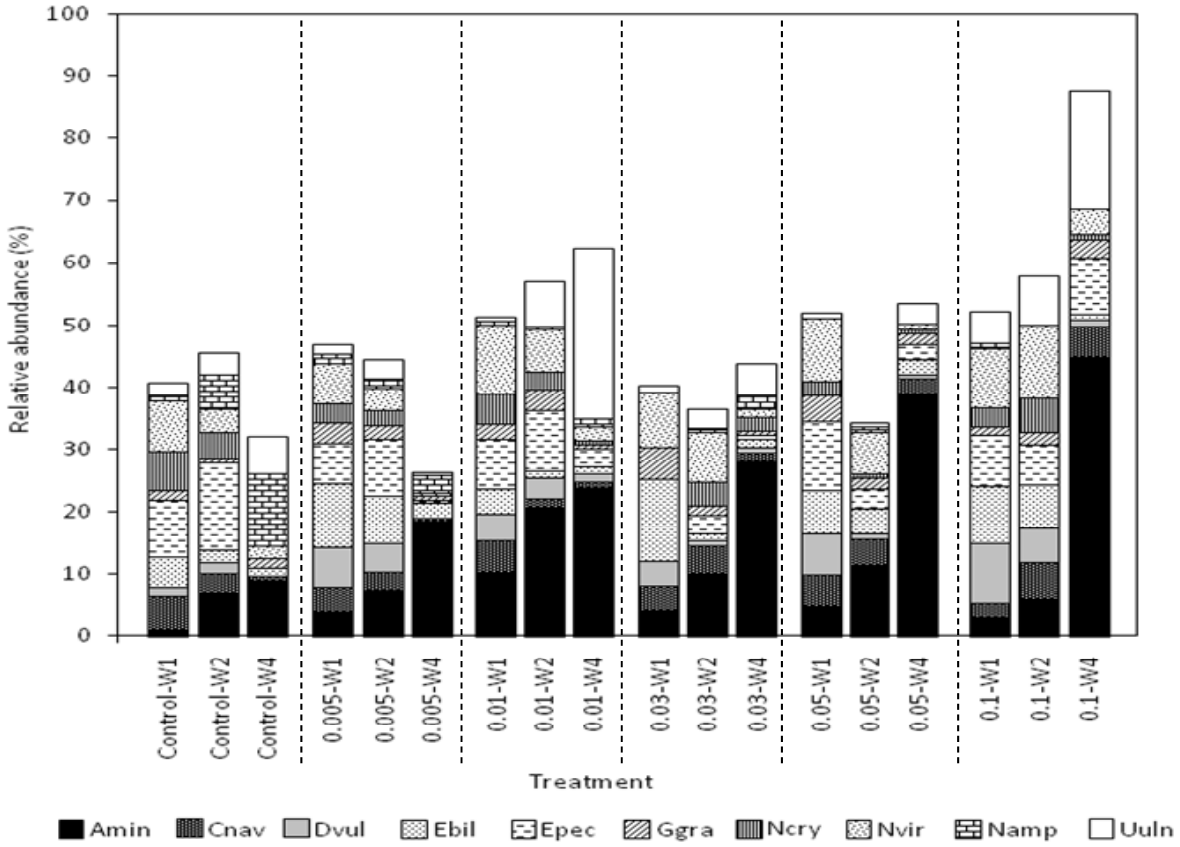

Figure 6.7: The relative abundance of the 10 major diatom species form diatom communities recorded at 5 Cd concentrations (mg.L^{-1}) during the first, second and fourth week of the experiment. Amin, *Achnanthidium minutissimum* (Kützing) Czarnecki; Cnav, C*ymbopleura naviculiformis* (Auerswald) Krammer; Dvul, *Diatoma vulgare* Bory; Ebil, *Eunotia bilunaris* (Ehrenberg) Mills; Epec, *Eunotia pectinalis* (Kützing) Rabhenhos; Ggra, *Gomphonema gracile* Ehrenberg; Ncry, *Navicula cryptocephala* (Grunow) Cleve; Nvir, *Navicula viridula* (Kützing) Kützing; Namp, *Nitzschia amphibia* Grunow; Uuln, *Ulnaria ulna* (Nitzsch) Compère.

6.3.2 Experiment 2

6.3.2.1 Physicochemical characteristics of the water column

Water temperature, pH and dissolved oxygen did not differ significantly (p > 0.05) among the systems over the 3-week experimental period (Table 6.3). However, pH and DO were generally slightly higher in the control and low Cd concentration treatments compared to high Cd concentration treatments. The temperature and DO remained relatively constant in all the EUs throughout the experiment whilst pH increased slightly in the systems during the cause of the experiment. On the other hand, conductivity increased significantly with increasing Cd, Cr and Pb concentration and duration of the experiment (p < 0.05).

Table 6.3: Water column physicochemical parameters (mean value and standard deviation) in all the systems measured during a 3-week experimental period. All concentrations are in $mg.L^{-1}$.

		EU1 [Control]	EU2 [Cd(0.01) Pb(0.033)]	EU3 [Cd(0.01) Pb(0.1)]	EU4 [Cd(0.1) Pb(0.033)]	EU5 [Cd(0.1) Pb(0.1)]	EU6 [Cd(0.01) Cr 0.05)]	EU7 [Cd(0.01) Cr(0.2)]	EU8 [Cd(0.1) Cr(0.05)]	EU9 [Cd(0.1) Cr(0.2)]	EU10 [Cd(0.01) Pb(0.033) Cr (0.05)]
Temperature (°C)	W1	23.9±1.8	23.8±1.3	23.7±1.8	23.8±1.5	23.8±1.9	23.8±1.8	23.7±1.9	23.8±1.8	23.8±1.8	23.7±1.9
	W2	23.8±1.8	23.8±1.8	23.8±1.8	23.8±1.6	23.9±1.5	23.8±1.7	23.8±1.8	23.8±1.7	23.9±1.7	23.8±1.7
	W3	23.9±1.7	23.9±1.5	23.9±1.6	23.9±1.7	23.9±1.8	23.9±1.9	23.9±1.9	23.9±1.6	23.9±1.7	23.9±1.5
pH	W1	7.3±0.01	7.3±0.03	7.3±0.04	7.3±0.01	7.3±0.01	7.3±0.03	7.3±0.04	7.4±0.01	7.5±0.01	7.3±0.04
	W2	7.7±0.03	7.7±0.02	7.8±0.05	7.7±0.04	7.6±0.02	7.6±0.01	7.7±0.03	7.5±0.04	7.6±0.02	7.6±0.04
	W3	8.0±0.14	8.1±0.05	8.1±0.10	7.8±0.17	7.8±0.14	8.3±0.26	8.0±0.15	7.8±0.16	7.8±0.11	8.0±0.01
DO ($mg.L^{-1}$)	W1	6.4±0.15	6.4±0.20	6.3±0.31	6.3±0.11	6.3±0.15	6.4±0.20	6.3±0.31	6.3±0.11	6.3±0.15	6.3±0.31
	W2	6.4±0.21	6.4±0.28	6.3±0.11	6.3±0.18	6.3±0.23	6.4±0.28	6.3±0.11	6.3±0.18	6.3±0.23	6.3±0.11
	W3	6.4±0.51	6.4±0.42	6.4±0.10	6.3±0.21	6.3±0.11	6.4±0.42	6.4±0.10	6.3±0.21	6.3±0.11	6.4±0.10
Conductivity ($\mu S.cm^{-1}$)	W1	210±1.6	223±3.1	258±3.3	224±5.6	241±7.7	223±3.1	255±4.1	254±3.5	247±2.8	241±8.1
	W2	231±1.3	234±5.3	2.43±9.8	280±6.4	288±4.4	246±5.5	2.77±3.3	280±6.4	276±5.8	2.34±7.7
	W3	303±2.3	306±9.1	342±6.9	361±6.8	328±9.9	329±6.1	375±7.0	367±3.3	328±9.9	394±6.9

6.3.2.2 Periphyton growth

DW, AFDW, chlorophyll *a* concentration and algal cell densities were high in the control and low Cd concentration compared to the high Cd concentration treatments (Figure 6.8*a*, *b* and *c* respectively). Highest DW, AFDW, chlorophyll *a* concentration and algal cell densities values were recorded in low Cd, Cr II, and Pb mixture treatment. No significant differences ($p > 0.05$) were recorded between the control and low Cd treatments but these treatments were significantly different from high Cd treatments (with $p < 0.05$ for the treatment X date effect at 2nd and 3rd week). Slight but statistically insignificant ($p > 0.05$) increase in DW, AFDW, chlorophyll *a* concentration and algal cell densities were observed in low Cd concentration treatments compared to the control, especially at 2nd and 3rd week of the experiment. In all the systems, DW, AFDW, chlorophyll *a* concentration and algal cell densities increased significantly ($p < 0.05$) throughout the experiment with the increase being significantly ($p < 0.05$) higher in the control and low Cd treatments compared to high Cd treatments. In both low and high Cd treatments, increase in Cr III and Pb concentration resulted in slight but statistically insignificant increase in DW, AFDW, chlorophyll *a* concentration and algal cell densities, especially at the 2nd and 3rd week of the experiment ($p > 0.05$). High concentration of Cr III (0.2 $mg.L^{-1}$) and Pb (0.1 $mg.L^{-1}$) did not affect DW, AFDW, chlorophyll *a* concentration and algal cell densities but tended to decrease the toxicity effects of Cd. Cr III treatments (low/high Cd) had slightly higher DW, AFDW, chlorophyll *a* concentration and algal cell densities compared to Pb treatments (low/high Cd) though the difference was not statistically significant ($p > 0.05$).

Growth rate was also high in the control and low Cd concentration treatments compared to high Cd concentration treatments (Figure 6.9a). Highest growth rate was recorded in low Cd, Cr III, and Pb mixture treatment. No significant differences ($p > 0.05$) in growth rate were recorded between the control and low Cd treatments but these treatments were significantly different from high Cd treatments (with $p < 0.05$ for the treatment X date effect at 2nd and 3rd week). In both low and high Cd treatments, increase in Cr III and Pb concentration resulted in slight but statistically insignificant increase in growth rate ($p > 0.05$). Cr III treatments (low/high Cd) had slightly higher growth rate compared to Pb treatments (low/high Cd) though the difference was not statistically significant ($p > 0.05$).

Growth inhibition was significantly low ($p < 0.05$) in low Cd concentration treatments compared to high Cd treatments (Figure 6.9b). As in the case of growth rate, an increase in Cr III and Pb concentration in both low and high Cd concentration treatments resulted in corresponding decrease in growth inhibition though this was statistically insignificant ($p > 0.05$). Cr III treatments (low/high Cd) had slightly lower growth inhibition compared to Pb treatments (low/high Cd) though the difference was not statistically significant ($p > 0.05$).

6.3.2.3 Community composition

Species richness and diversity were also significantly high in the control and low Cd concentrations compared to the higher Cd concentrations (with $p < 0.05$ for the treatment X date effect at 2nd and 3th week; Figure 6.10*a* and *b* respectively). In all the systems, species richness and diversity decreased significantly throughout the experiment with the decrease being significantly higher in the higher Cd concentration treatments compared to the control and low Cd concentration treatments ($p < 0.05$). As in the case of DW, AFDW, chlorophyll *a* concentration and algal cell densities, Cr III treatments (low/high Cd) had slightly higher species richness and diversity compared to Pb treatments (low/high Cd) though the difference was not significant ($p > 0.05$). However, unlike in the case of DW, AFDW, chlorophyll *a* concentration and algal cell densities, low Cd treatments had generally low species richness and diversity compared to the control.

Changes in diatom community composition with treatment (especially change in Cd concentration) and duration of the experiment were observed during the experiment. Communities were generally similar among the systems during the 1st week of the

experiment whilst thy differed considerably during the 2nd and 3rd week of the experiment. Of the 106 diatom species belonging to 44 genera that were recorded in all the EUs during the course of the study, 12 dominant diatom species with mean relative abundances >5% and present in at least two communities were described as characteristic of each diatom community developed throughout the experiment (Figure 6.11). After 1 week of colonization, diatom composition in the five systems was relatively similar with the presence of *Cymbopleura naviculiformis (*Auerswald) Krammer, *Fragilaria capucina* Desmazières, *Navicula cryptocephala* (Grunow) Cleve, *Encyonema silesiacum* (Bleisch) Mann, *Eunotia bilunaris* (Ehrenberg) Mills and *Achnanthidium minutissimum* (Kützing) Czarnecki. The relative abundance of the latter generally tended to increase with increasing metal concentrations.

At week 2, the dominant species were still similar with a general increase in the relative abundance of *A. minutissimum* and *Nitzschia palea* (Kützing) Smith in all the systems and a general decrease in *E. bilunaris* and *C. naviculiformis*. The relative abundance of *A. minutissimum* and *N. palea* increased notably with increasing metals concentration with highest values being recorded in high Cd/high Pb treatment. The relative abundance of *N. cryptocephala* decreased in high Cd concentration treatments and that of *Gomphonema parvulum* (Kützing) Kützing increased in low Cd concentration treatments. After 3 weeks of colonization, the species composition in all treatments differed from that noted at week 1 and 2 with the proliferation of *A. minutissimum* in all the systems. The relative abundance of *A. minutissimum* was around 74.7 % in the high Cd/high Pb treatment. The relative abundance of *E. silesiacum* and *G. parvulum* increased in the control and low Cd concentration treatments and decreased in high Cd concentration treatments. The relative abundance of *F. capucina* remained relatively constant in the control throughout the experiment whilst the same tended to decrease in other treatments with increasing exposure duration.

Taxonomic difference in diatom communities collected during the three weeks of the experiment in the 10 systems were investigated using PCA performed on the relative abundance of the 29 species with the highest cumulative abundance when all the communities were considered (Figure 6.12). Changes in diatom community composition with treatment (especially change in Cd concentration) and duration of the experiment were observed during the experiment. PCA clearly separated all communities from the 1st week of the experiment from the rest of the communities. Communities from the first week of the experiment were closely related being associated with such species as

Brachysira brebissonii Ross, *Brachysira vitrea* (Grunow) Ross, *C. naviculiformis, Diatoma hiemale* (Lyngbye) Heiberg, *Encyonema mesianum* (Cholnoky) Mann, *Encyonema neomesianum* Krammer, *E. bilunaris, F. capucina, Fragilaria intermedia* Grunow, *Gomphonema accuminatum* Ehrenberg, *Gomphonema pseudoaugur* Lange-Bertalot, *Gomphonema subtile* Ehrenberg, *Navicula cryptotenella* Lange-Bertalot, *Navicula viridula* (Kützing) Kützing; *Nitzschia amphibia* Grunow, *Nitzschia linearis* (Agardh) Smith and *Staurosira construens* Ehrenberg. These communities were negatively associated with the fist axis of the PCA that accounted for 39.7 % of the total variation. In contrast, diatom communities from the 2nd and 3rd week of the experiment overlapped each other along the first axis of the PCA with diatom community from the high metal treatments (2nd and 3rd week) being notably distinct from the rest of the communities and strongly positively associated with the first PCA axis. These communities were highly associated with *A. minutissimum* and *N. palea*, which were also strongly positively associated with the first PCA axis. Separation along axis 1 (accounting for 14.6% of total variation) resulted mainly from other species especially *G. parvlum, E. bilunaris C. naviculiformis* and *Ulnaria ulna* (Nitzsch) Compère.

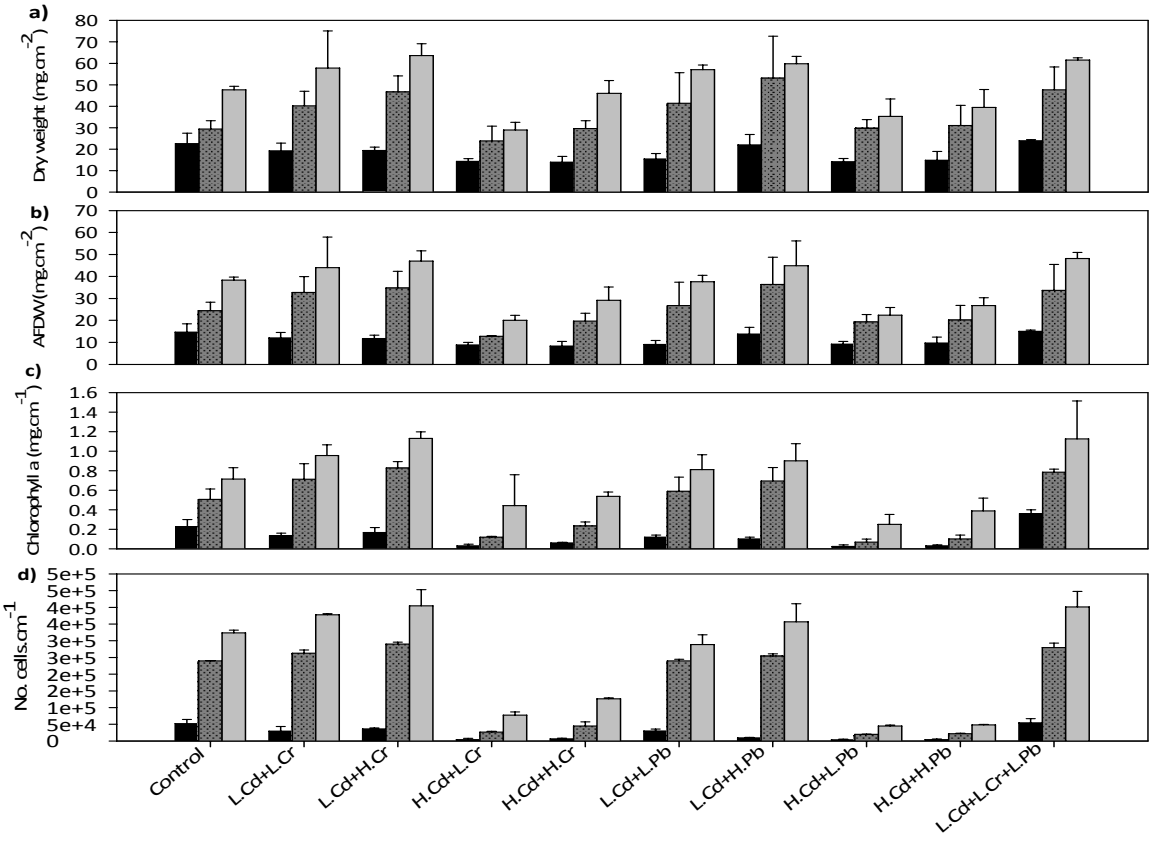

Figure 6.8: The mean values and standard deviations (n = 3) of dry weight (DW) (a), ash-free dry weight (AFDW) (b), chlorophyll *a* (c) and algal cell densities (d) developed on glass substrates in 10 treatments during the first (black), second (dark gray-dotted) and third (light gray) week of the experiment (L = low, H = high).

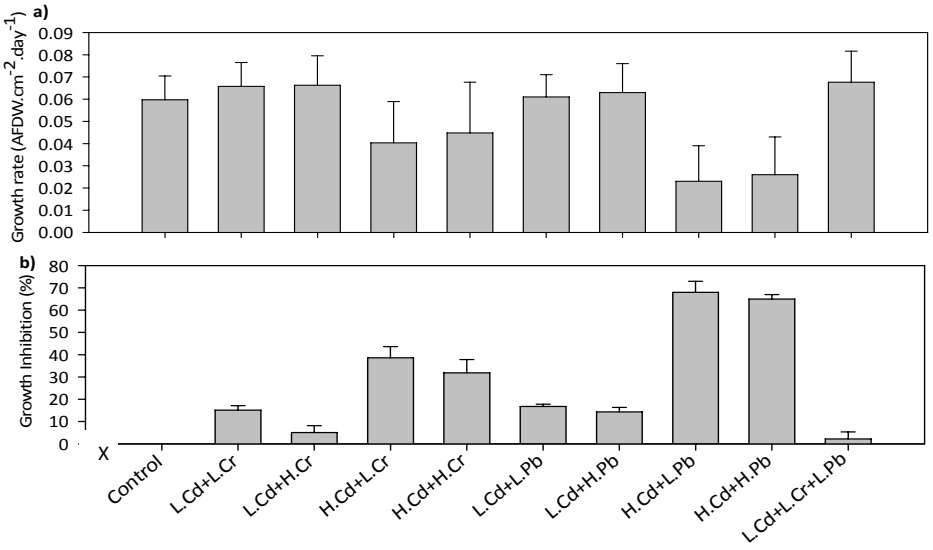

Figure 6.9: Periphyton growth rate (a) in the control and the nine treatments and percentage inhibition (b) in the nine treatments during the experiment (L = low, H = high X = not determined due to the formula used).

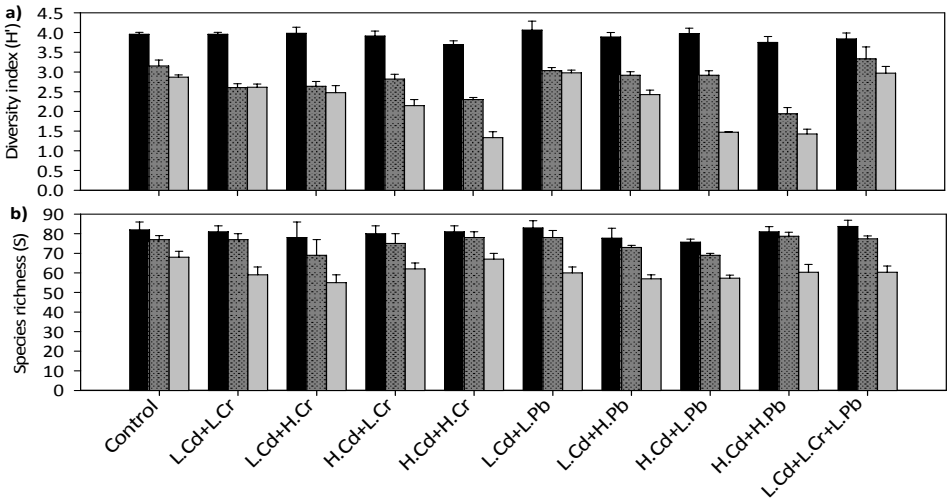

Figure 6.10: The mean values and standard deviations (n = 3) of species diversity (a) and richness (b) developed on glass substrates in 10 treatments during the first (black), second (dark gray-dotted) and third (light gray) week of the experiment (L = low, H = high).

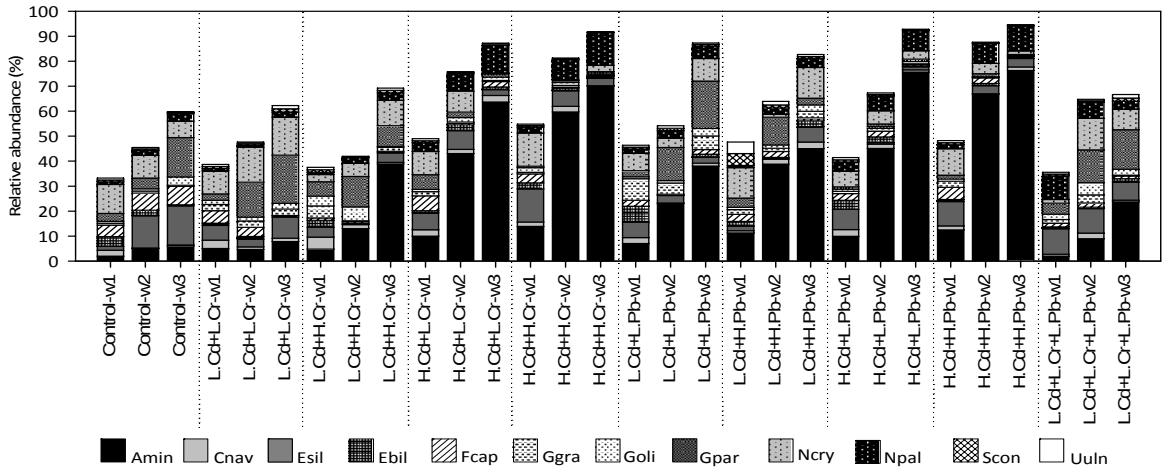

Figure 6.11: The relative abundance (%) of the 12 major diatom species form diatom communities recorded in 10 treatments during 1st, 2nd and 3rd week of the experiment. L = low, H = high, w = week. Taxa codes correspond to those in Figure 7.6.

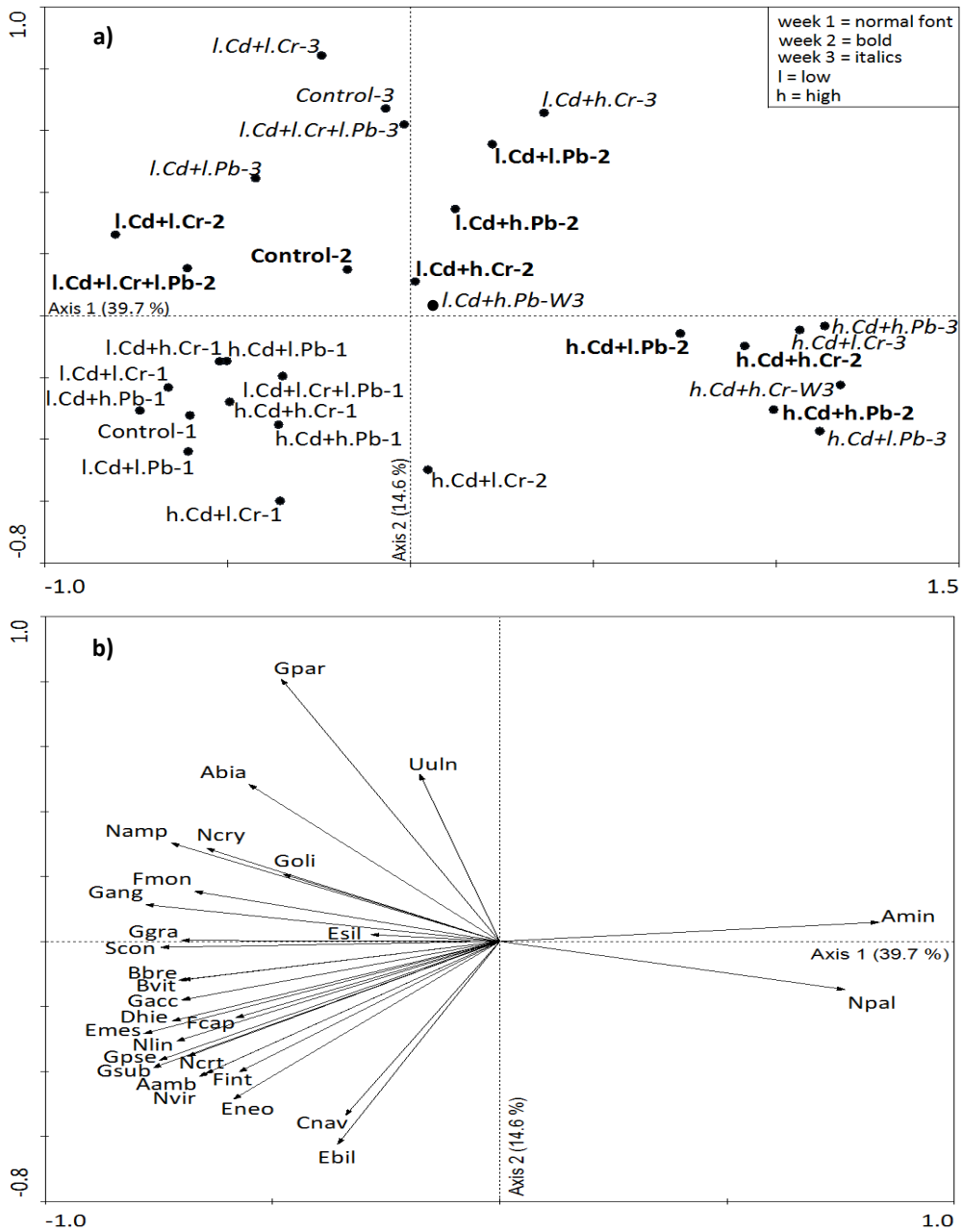

Figure 6.12: Principal component analysis based on the taxonomic composition of the diatom communities recorded in 17 treatments. (a) Projection of the communities on the first two principal component axes. (b) Projection of the species with loading > 0.2 for one of the two axes. Abia, *Achnanthidium biasolettianum*; Amin, *Achnanthidium minutissimum*, Aamb, *Aulacoseira ambigua*; Bbre, *Brachysira brebissonii*; Bvit, *Brachysira vitrea*; Cnav, *Cymbopleura naviculiformis*; Dhie, *Diatoma hiemale*; Emes, *Encyonema mesianum*, Eneo, *Encyonema neomesianum*; Esil, *Encyonema silesiacum*; Ebil, *Eunotia bilunaris*; Fmon, *Fallacia monoculata*; Fcap, *Fragilaria capucina*; Fint, *Fragilaria intermedia*; Gacc, *Gomphonema accuminatum*; Gang, *Gomphonema angustatum*; Ggra, *Gomphonema gracile*; Goli, *Gomphonema olivaceum*; Gpar, *Gomphonema parvulum*; Gpse, *Gomphonema pseudoaugur*; Gsub, *Gomphonema subtile*, Ncry, *Navicula cryptocephala*; Ncrt, *Navicula cryptotenella*; Nvir, *Navicula viridula*; Namp, *Nitzschia amphibian*; Nlin, *Nitzschia linearis*; Npal, *Nitzschia palea*; Scon, *Staurosira construens*, Uuln, *Ulnaria ulna*.

6.4 Discussion

6.4.1 Effects of Cd, Cr and Pb on periphyton growth

6.4.1.1 Effects of Cd only (experiment 1)

The present study demonstrates hormesis, a dose response phenomenon characterized by a low dose stimulation and high dose inhibition of responses like growth rate (GENTER, 1996) also demonstrated by DEVI PRASAD and DEVI PRASAD, (1982) on three green algae subjected to low concentrations of Cd, Pb, and Ni. A slight increase in Cd concentration (from control to around 0.005mgL.$^{-1}$) resulted in a significant increase in chlorophyll *a* concentration and cell densities, stimulation of growth (-2.24% inhibition) and slight increase in growth rate, DW and AFDW. This could be attributed to two factors. Firstly, although Cd is generally viewed an environmental problem and toxic element that has no known biological functions, studies on the marine diatom *Thalassiosira weissflogii* have discovered a metalloenzyme (carbonic anhydrase) that specifically uses Cd to achieve its biological function, especially in the absence of zinc (LEE and ROBERTS, 1995; LANE and MOREL, 2000; LANE et al., 2005), demonstrating the need to revise the general opinion on Cd. Although the presence of the equivalent mechanism in other diatoms, especially from the freshwater environments, has not yet been studied, the results of the present study strongly suggest a possible role of Cd in biological functions of freshwater periphytic algae at low concentrations. Due to the nature of the current study, it is difficult to determine the exact mechanism and to pinpoint the specific species that employ this mechanism; necessitating more work to elucidate the possible role of low concentrations of Cd on freshwater diatom biological mechanisms.

Secondly, NALEWAJKO (1995) showed that moderate additions of Cd (up to 0.005 mg.L^{-1}) would not be toxic to phytoplankton photosynthesis probably because of the formation of non-toxic Cd chlorides as well as nutrient (especially phosphorus) bioavailability. Nutrient bioavailability has been shown to interact with the toxicant and slightly favour biofilm development (LOZANO and PRATT, 1994), nutrient concentrations being higher in 0.005 and 0.01mg.L^{-1} Cd treatment during the course of this experiment compared to the control. IVORRA et al. (2002) noted that increased dissolved phosphorus concentrations might mitigate metal toxicity to biofilms or exert a protective role, by making coordination complexes of trace metals with phosphorus. BARRANGUET et al. (2002) also described nutrient-stimulated biomass accumulation in photosynthetic biofilms affected by metals. This complicates the assessment of toxic

effects of low Cd concentrations on periphyton biofilms necessitating more work to shade more light on this aspect.

Although Cd treatment of 0.01 $mg.L^{-1}$ (EU_3) exceeded Cd concentrations normally regarded protective to aquatic communities by Brazilian environmental monitoring board, Conselho Nacional do Meio Ambiente-CONAMA (2005), and other international regulations such as U.S. EPA (1995), chlorophyll *a*, DW, AFDW and cell densities from this treatment were comparable with the control. This demonstrates the sufficiency of these guide lines in protecting aquatic environments.

However, increasing the concentration of Cd beyond 0.01 $mg.L^{-1}$ negatively affected periphyton growth and development: chlorophyll *a* concentration, cell densities, DW, AFDW and growth rate were significantly high in low (control, 0.005, 0.01 $mg.L^{-1}$) compared to high Cd concentration treatments (0.03, 0.05 and 0.01 $mg.L^{-1}$). Growth inhibition was low at low compared to high Cd concentrations and an EC_{50} of 0.077 $mg.L^{-1}$ Cd on periphyton communities was recorded in this study. These negative effects on periphyton growth and development with increasing Cd concentration have been widely reported (e.g. WONG, 1987; TAKAMURA et al., 1989; HUSAINI and RAI, 1991; GUANZON et al., 1994; IVORRA et al., 2000; GOLD et al., 2003a, 2003b; NUNES et al., 2003; DUONG et al., 2008; MORIN et al., 2008a, 2008b; DUONG et al., 2010). Inorganic chemical stress affects algae at biochemical, cellular, population and community level of biological organization with cellular level effects influencing growth rate, development, and abundance of algal populations observed in this study (GENTER, 1996). High Cd concentrations have been shown to affect cellular processes such as global metabolism (HUSAINI and RAI, 1991), phosphorus metabolism and cell division (GUANZON et al., 1994) and modify cell ultrastructure (endoplasmic reticulum, mitochondria) (WONG, 1987). High metal concentration have a pivotal effect on enzyme systems that control biochemical and physiological functions like photosynthesis, respiration and the synthesis of biological molecules (RAI et al., 1981). This explains significant reduction in growth and development with increasing Cd concentration recorded in this study.

No much information is available in the literature on EC_{50} of Cd at periphyton community level to compare with our EC_{50} estimate of 0.077 $mg.L^{-1}$. As far as we know, our estimate of EC_{50} is the first available in the literature and suggests that periphyton growth is a sensitive measure of Cd toxicity. Similar studies assessing the effects of 0.01 and 0.1 $mg.L^{-1}$ Cd indeed demonstrated a significant reduction in growth rate at 0.1 $mg.L^{-}$

[1] Cd (IVORRA et al., 2000; MORIN et al., 2008a; DUONG et al., 2010). However, the absence of experimental data between 0.01 and 0.1 $mg.L^{-1}$ did not allow any precise evaluation of the EC_{50} as was possible in this study.

Toxicity studies based on single species (such as HILL et al., 2000; IRVING et al., 2009) offers relatively different values from the one reported in this study. This, in part, could be attributed to the acute nature of these studies compared to the present study. However, single species tests have been blamed for lacking environmental realism because they do not take into account the multiplicity of biotic factors (inter-specific competition, grazing, etc.) which are potentially involved (SCHMITT-JANSEN and ALTENBURGER, 2005) that were reflected to some extent in this study. Thus, the present study better mimics field conditions compared to single species tests and enables improved accuracy in the extrapolations from laboratory bioassays to responses in natural systems. Care should be taken when extrapolating the results of this study to natural systems as the current design did not take into consideration important factors like periphyton biofilm maturity that have been shown to be important determinants of the response of biofilms to Cd (GOLD et al., 2003a; MORIN et al., 2008a, b; DUONG et al., 2010).

6.4.1.2 Interactive effects of Cd, Cr and Pb (experiment 2)

High Cd concentration (0.1 $mg.L^{-1}$) affects periphyton growth whilst high concentration of Cr (0.2$mg.L^{-1}$) and Pb (0.1 $mg.L^{-1}$) did decreases the toxicity effects of Cd. Depending on factors like pH and water hardness, Cd is generally more toxic than Pb, which is in turn more toxic than Cr III (GENTER, 1996) as supported by the results of the present study. Thus, Cd play a more important role in regulating periphyton growth and development compared to Pb and Cr III, with periphyton growth rate being high at low compared to high Cd concentration treatments due to reasons discussed in section 6.4.1.1.

In both low and high Cd treatments, increase in Cr and Pb concentration resulted in slight increase in growth rate, chlorophyll *a* concentration, DW and AFDW, especially at the 2nd and 3rd week of the experiment. This suggests antagonistic interference of Cr and Pb on the toxic effects of Cd on periphyton community level that, as far as we know, has not been demonstrated in the literature. This is an important observation given the fact that inorganic chemical stressors almost always occur as mixtures in nature. This antagonism has been demonstrated by DEVI PRASAD and DEVI PRASAD (1982)

between Ni-Cd and Pb-Cd mixtures for three freshwater green algae, but not on algal communities. BRÆK et al. (1980) and RAI et al. (1981) found that the same algae may behave synergistically to one metal combination and antagonistically to another metal combination, and that a particular metal combination may act antagonistically on one metabolic process and synergistically on another metabolic process for the same algae. Hence, the uniqueness of various physiological and biochemical processes is important in determining response to metal mixtures. Due to the nature of this study, it is difficult to determine which species or processes behave antagonistically to Cd and Cr/Pb mixtures shown in this study necessitating more work to shade more light on this aspect. The fact that antagonism between Cd and Cr/Pb became more pronounced at 2nd and 3rd week of the experiment suggests that the interaction may depend on the duration of exposure as support by (PREVOT and SOYER-GOBILLARD, 1986).

Slight increase in chlorophyll *a* concentration, DW, AFDW and algal cell densities was observed in low Cd concentration treatments compared to the control, especially at 2nd and 3rd week of the experiment. This could be due to antagonism of Cr and Pb on the toxicity effects of Cd described above. This also seems to demonstrate hormesis and the possible role of Cd in algal biological functions as discussed in section 6.4.1.1.

6.4.2 Sorption kinetics of cadmium in the biofilms (experiment 1)

As previously hypothesised, total and non-exchangeable Cd content in biofilms reflected the effects of dissolved concentrations of Cd in the culture media of each experimental unit and exposure duration. Hence, biofilm Cd levels (total and non-exchangeable) generally increased in treatments in the order $0.005 < 0.01 < 0.03 < 0.05 < 0.1$ mg.L^{-1} at any sampling time with increasing Cd concentration with duration of exposure in all the systems (Figure 6.4). Cd concentration was below detection limit in the control while the concentration was detected in all the other treatments, demonstrating biofilm Cd accumulation capacity. Good biofilm Cd accumulation capacity in support of the present research findings has been demonstrated by studies such as GUANZON et al. (1994), HILL et al. (2000), GOLD et al. (2003a), MORIN et al. (2008a, b), and DUONG et al. (2010). This makes biofilms appropriate monitors of river metal pollution the same way as measurements of metals in the sediment and suspended solids are used (FUCHS et al., 1996).

GADD (1988) observed that algae have the capacity to concentrate inorganic ions to amounts several thousand folds greater than in external dilute solutions by a variety of biological, chemical and physical mechanisms involving adsorption, precipitation and metabolism–dependent processes that operate simultaneously or in sequence. This is supported by the results of this study where total Cd levels in the biofilms from different treatments was 22.6 to 51.7 folds higher than in the water column, with a positive linear relationship between water column Cd concentrations and CFs (Figure 6.5). For this reason, chemical analysis of water by itself may not be sufficient for assessing environmental stress because periphyton can decrease dissolved metal concentrations to background levels, so measuring metal levels in periphyton is necessary for environmental assessment. Algae are sometimes as effective as commercial resins for removal of metals from wastewater (GENTER, 1996).

Microbial uptake often follows two phases: first is a rapid metabolism-independent phase with binding or adsorption to cell walls and external surfaces; second is a slower metabolism-dependent phase with transport across the cell membrane. Most metals accumulated by the first method are easily removed by washing algae with distilled water alone or with a chelator (EDTA). Biofilms have been demonstrated to have a large number of metal binding sites located in either organic matrix (produced by algae, bacteria and fungi) at the surface of cells or in the organic particles trapped by the biofilm (RAI et al., 1981; WONG, 1987; HUSAINI and RAI, 1991; GUANZON et al., 1994; GENTER, 1996). These substances can play an important role in the sorption of metals from water column. As observed in this experiment, large amounts of metals assayed in the biofilms were not actually taken up by the cells; ~40-76% (section 6.3.3) of the metal was rather absorbed on the cell surface and hence eliminated by the EDTA wash. Increasing Cd sorption with increasing biofilm biomass underline a significant contribution of subsurface cells to metal sorption, these cells providing additional sorption sites (HILL et al., 2000). It can also be attributed to the slow metabolism-dependent uptake process.

6.4.3 Effects of Cd, Cr and Pb on diatom communities

Unlike in the case of chlorophyll *a*, growth rate and cell densities where hormesis was demonstrated, slight increase in Cd concentration resulted in reduction in species richness and diversity. Similar community-level toxicity tests using periphyton have also shown that inorganic stressors at concentrations near the Water Quality Criteria of the

U.S.E.P.A. alter species composition (GENTER et al., 1988). Significant decrease in cell densities, species richness and diversity with increasing Cd concentration was recorded in this study in agreement with previous studies (e.g. IVORRA et al., 2000; GOLD et al., 2003a; MORIN et al., 2008a; DUONG et al., 2010). A general slow development of diatom cells at 0.1 $mg.L^{-1}$Cd was reported by DUONG et al. (2010) explaining the low cell densities, species richness and diversity recorded at this treatment in this study. A strong effect of metal contamination on the densities of diatom communities was also reported by GOLD et al. (2003b), possibly corresponding to a reduction in the rate of cell division of diatom species as demonstrated by RIVKIN (1979). This inhibition of cell division coupled with the development of a few species at high Cd concentrations (0.03; 0.05 and 0.1 $mg.L^{-1}$ Cd) led to a remarkable decrease in species richness and diversity index throughout the experiment and is typical of metal polluted rivers (GENTER, 1996; MORIN et al., 2007).

In all the experiments, the diatom assemblages present during the first week were similar in all the systems. The assemblages then differentiated according to the ability of the species to grow under elevated Cd exposure with the development of more resistant species like *A. minutissimum* and reduction or exclusion of sensitive ones like *D. vulgare*, *F. capucina, E. silesiacum, E. bilunaris* and *G. parvulum N. viridula* and *N. cryptocephala* at 2^{nd} and 4^{th} week of the experiment. This is supported by studies by RAI et al. (1981) and GENTER et al. (1988) which demonstrated that exposure to inorganic chemical stress often places a selection pressure on the community that either decreases abundance of pollution-sensitive species and increases or does not change abundance of pollution-tolerant species. Algae may tolerate inorganic chemical stress at the cellular level by a decreased number of binding sites at the cell surface, inhibition of metabolism-dependent uptake stage, physiological development of exclusion mechanisms, genetic adaptation, morphological changes, and internal detoxifying mechanisms or safe storage sites (RAI et al., 1981). Differential sensitivity among species leads to different growth rates and is expected to alter species composition in communities (GENTER, 1996).

A. minutissimum has already been reported in metal-contaminated environments (e.g. WONG, 1987; TAKAMURA et al., 1989; HUSAINI and RAI, 1991; GUANZON et al., 1994; IVORRA et al., 2000, 2002; GOLD et al., 2003a, 2003b; NUNES et al., 2003; DUONG et al., 2008; MORIN et al., 2007, 2008a, 2008b; DUONG et al., 2010). The proliferation of *A. minutissimum* with increasing Cd concentration and duration of exposure (45.00% at 0.1 $mg.L^{-1}$ Cd during 4^{th} week) seems to indicate favour and

tolerance of this species to Cd contamination. Indeed TAKAMURA et al. (1989) reported 50% inhibition of photosynthesis at Cd concentration > 12.7 $mg.L^{-1}$ on populations of *A. minutissimum* extracted from high Cu environments, concentration much higher than the highest concentration used in this study. Changes in diatom species composition and abundance with increasing Cd concentration observed in this study demonstrates the usefulness of diatom communities in identifying high or low metal concentrations in streams in agreement with other studies (e.g. IVORRA et al., 2000; GOLD et al., 2003a, 2003b; MORIN et al., 2007, 2008a; DUONG et al., 2010).

The influence of Cd on diatom assemblages (experiment 1) also manifests itself through morphological deformities of some diatom cells in the communities. In experiment 1, an increase in abnormal valve frequency with increasing Cd concentration and duration of exposure was observed in agreement with previous studies (e.g. IVORRA et al., 2000; GOLD et al., 2003a, 2003b; MORIN et al., 2007, 2008a; DUONG et al., 2010). This demonstrates the possible role or importance of diatom morphological deformities as an indicator of aquatic ecosystem health. The occurrence of some deformed valves in the control is not related to metal exposure, but could be a result of nutrient limitation (THOMAS et al., 1980) in the culture medium or mechanical effects like crowding (DRUM, 1964). Frustule morphological deformities, commonly found in cultures (BATES, 1998) and the size reduction at each cell asexual division may also lead to alteration of the morphological characteristic of the frustules in the control, but do not occur as often as in contaminated conditions (DUONG et al., 2010). As the percentage of abnormal cells increased with increasing Cd concentration and duration of exposure, it seems that Cd is the causative agent for deformed diatoms in this experimental study. The frequency of deformities recorded in this study compares favourably with those reported by GOLD et al. (2003a) and DUONG et al. (2010).

Most of the diatom-based global indices routinely used in routine biological monitoring of lotic systems (e.g. ROUND, 1991; KELLY and WHITTON, 1995; PAN et al., 1996; BIGGS and KILROY, 2000) are not accurate enough to diagnose toxic pollutions (MORIN et al., 2008a). Most of these methods are used to monitor eutrophication and organic pollution. Interactions between these types of pollution and toxicants, like metals, that are common in nature, are not evidenced through current indices (MORIN et al., 2008b). Thus, there is a growing need to take into account priority substances such as metals for the improvement of diatom-based biological monitoring of these pollutants. Evidence of heavy-metal toxicity on freshwater diatom communities

from previous studies suggests that morphological traits may be informative for investigating the relationship between metal pollution and organisms' response (DUONG et al., 2010; MORIN et al., 2008b). Evidence on the influence of Cd contamination on morphological traits has been gathered in this study with the aim of stimulating the interest of researchers in this potential metal stress indicator. Since morphological abnormalities on benthic diatoms are not ascribed exclusively to metal pollution, further investigations are needed to standardize and, as possible, to automatize the use of those biological traits in order to take them into account for routine biological monitoring. It is still necessary to investigate the effects of single and multiple contaminants and their additional and synergic effects on diatom communities and monospecific strains in order to consider morphological traits a reliable tool in the assessment of ecological conditions of lotic system.

6.5 Conclusions

Hormesis (low Cd dose stimulation and high Cd dose inhibition of responses like growth rate) has been demonstrated with periphyton growth and development being substantially lowered by high concentration of Cd (EC_{50} = 0.077 $mg.L^{-1}$). Dissolved Cd reduces growth of periphyton communities at high Cd concentrations with addition of Cr and Pb having antagonistic interference on the toxic effects of Cd on periphyton community. Shifts in species composition, decreases in species richness and diversity and morphological alterations (deformities) of diatom cells with increasing Cd concentration and exposure duration have been demonstrated in this study making biofilms appropriate monitors of metal pollution in aquatic systems. Field validation of the observed effects remains an interesting subject for further investigations.

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CHAPTER 7

Effects of pulsed Cd, Cr III and Pb exposures on periphyton communities: integrating pulse frequency, duration, recovery period, timing and chemical type in aquatic life protection.[7]

Abstract: The objective of this study was to investigate ecotoxicological effects of pulsed heavy metal exposures on periphyton communities with the overarching aim of discussing ecotoxicity in the context of increased realism in the exposure scenarios. A natural periphyton community was used in a complex experimental setup devoted to study combined effects of frequency, duration, recovery period, chemical type and timing of pulses with elevated Cd, Cr III and Pb concentrations on periphyton communities by measurements of chlorophyll *a*, dry mass, ash-free dry mass, cell densities and diatom taxonomic composition. The closer the frequency and duration of the pulse is to a continuous exposure (continuous exposures resulting in greater effects of the pollutant on test organisms than pulsed exposures), the greater the effects of the contaminant on aquatic life. The higher the frequency of 'short' duration pulses the more likely they are to produce effects similar to that of long duration exposures. Light might have a potential role in modulating the effects of metal toxicants on aquatic life. Thus, factors other than the traditionally used magnitude and duration of contaminants must be considered in order to accurately predict response of aquatic life to pulsed exposures. To this effect, better information is needed on effluent variability, and specifically, frequency, duration, timing and type of pulses as well as the magnitude of the pulse. Changes in diatom species composition and relative abundance with different exposure scenarios observed in this study demonstrates the usefulness of diatom communities in detecting high metal concentration mixtures in streams under different exposure scenarios that can only be detected by sophisticated chemical analyses. Field validation of the observed effects remains an interesting subject for further investigations.

Keywords: exposure scenarios, heavy metals, diatoms, water quality guidelines

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Efeitos das exposições pulsantes de Cd, Cr III e Pb nas comunidades perifíticas: integração da freqüência de pulso, duração, período de recuperação, tempo e tipo de produto químico na proteção da vida aquática

Resumo: O objetivo deste estudo foi investigar os efeitos ecotoxicológicos de exposições pulsante de metais pesados nas comunidades perifíticas, com o objetivo primordial de discutir ecotoxicidade no contexto de maior realismo nos cenários de exposição. A comunidade perifítica natural foi utilizada em uma configuração complexa experimental dedicada ao estudo dos efeitos combinados de freqüência, duração, período de recuperação, tipo de produto químico e tempo de pulsos com elevada concentrações de Cd, Cr III e Pb nas comunidades perifíticas, através de medições de clorofila, peso seco, peso seco de cinza, densidade celular e composição taxonômica de diatomáceas. Quanto mais próximo a freqüência e duração do pulso se aproximam, uma exposição contínua (exposição permanente, resultando em maiores efeitos dos poluentes sobre os organismos de ensaio de exposição pulsada), maiores serão os efeitos dos contaminantes sobre a vida aquática. Quanto maior a freqüência de pulsos de "curta" duração é mais provável a produção de efeitos semelhantes aos das exposições de longa duração. A luminosidade mostrou ter um papel importante na modulação dos efeitos de toxicidade de metais sobre a vida aquática. Assim, outros fatores, além da magnitude e duração de contaminantes tradicionalmente utilizadas, devem ser consideradas a fim de prever com precisão a resposta da vida aquática para exposições pulsantes. Para este efeito, é necessária uma melhor informação sobre a variabilidade do efluente e, especificamente, freqüência, duração, periodicidade e tipo de pulsos, assim como a magnitude do pulso. Mudanças na composição de espécies e abundância relativa das diatomáceas, com diferentes cenários de exposição, observados no estudo presente, demonstra a utilidade das comunidades de diatomáceas na detecção de misturas de alta concentração de metais em córregos sob diferentes cenários de exposição, que só pode ser detectada através de análises químicas sofisticadas. Validação dos efeitos observados no campo é um tema interessante para futuras investigações.

Palavras-chave: cenários de exposição, os metais pesados, diatomáceas, diretrizes de qualidade da água.

7.1 Introduction

Sustainable management of the aquatic ecosystems requires a reliable river heavy metal toxicity assessment. Through standardized laboratory ecotoxicological procedures, test results for a given heavy metal are converted to water quality criteria values that are used by environmental agents to set legally enforceable water quality guidelines. These standard ecotoxicological tests are usually designed to evaluate the effect of a heavy metal that is present at a constant concentration over a pragmatically determined time-frame. Unfortunately, such a design does not reflect environmental pollution well, since pollution is erratic and concentrations fluctuate with time (ZHAO and NEWMAN, 2004; DIAMONT et al., 2006a). Inputs of heavy metals to aquatic environments often occur in pulses when peak concentrations temporarily, but greatly, exceeds the background level (HANDY, 1994; LIESS et al., 1999). As pulses with elevated concentrations are brief, to calculate permit-limits for effluents from averages of concentrations over time may not be protective enough (DIAMOND et al., 2006a).

The pattern of heavy metal exposure including the possible interaction between metals in complex mixtures, timing, frequency, duration and magnitude of exposure cannot be discarded. The results of the interplay of these factors can be difficult to predict. Previous studies examining pulsed chemical exposures offers contrasting results with higher (e.g., VAN DERHOEVEN and GERRITSEN, 1997; BRENT, and HERICKS, 1998) or lesser (e.g., HOSMER et al. 1998) effects on test organisms than would be predicted based on results using a constant exposure at similar concentrations, depending on the chemical, test species, and test design used.

From our personal experience, effluents with elevated concentrations of metals are normally discharged at night and during the weekends without the knowledge of the law enforcement agents in developing countries. This timing of effluent discharge is likely to affect the resultant periphyton communities of lotic systems as they have developed complex systems, circadian clocks, to detect time and synchronize processes, actions, and behaviours to the diel cycle (ROENNEBERG and MERROW, 2002). In addition, microbial uptake of metals often follows two phases: first, is a rapid metabolism-independent phase with binding or adsorption to cell walls and external surfaces; second is a slower metabolism-dependent phase in which metal ions are transported across the cell membrane. The latter phase is inhibited by absence of energy sources (light), among other factors (GADD, 1988; GARNHAM et al., 1992). To our knowledge, nobody has yet tried to find out the potential effect of timing of high heavy

metal concentration discharge, i.e. night (dark) or day (light), on periphyton communities in receiving lotic systems.

Most of the studies on the pulse chemical effects and ecotoxicology studies upon which current water quality guidelines are base are focussed on single species assays and population effects (e.g. ABEL, 1980; ZHAO and NEWMAN, 2004; ASHAUER et al., 2006; CONNELL and YU, 2008). These studies lack environmental realism because they do not take into account the multiplicity of biotic factors (inter-specific competition, grazing, etc.) which are potentially involved (SCHMITT-JANSEN and ALTENBURGER, 2005). This necessitates carrying out toxicity tests on higher levels of ecological organisation, at least on the level of biological communities, which better mimic field conditions enabling improved accuracy in extrapolation from laboratory bioassays to natural systems. Studies on periphyton communities are particularly recommended because they are considered solar-powered biogeochemical reactors, biogenic habitats, hydraulic roughness elements, early warning systems for environmental degradation, and troves of biodiversity of lotic systems (LARNED, 2010).

Studying the effects of intermittent exposures on microalgal communities, GOLDSBOROUGH and ROBINSON (1986) found that ecosystem functions recover after disturbance due to functional redundancy but community structure was profoundly affected for several weeks. PUSEY et al. (1994) exposed artificial streams to the insecticide chlorpyrifos and noted both direct effects of reduced populations of sensitive species and indirect effects of increased populations of species who are early colonisers. Pollution induced community tolerance (PICT) is a concept that explains how communities are shaped during pollution events (BLANCK et al., 1988; BLANCK, 2002). The selection pressure exerted by the polluting compound removes individuals (i.e. phenotypes) and species that are sensitive to a pollutant. The community goes through a toxicant induced succession (TIS) (BLANCK, 2002).

The objective of this study was to investigate ecotoxicological effects of intermittent heavy metal exposures on periphyton communities with the overarching aim of discussing ecotoxicity in the context of increased realism in the exposure scenarios. The effects of frequency, duration, recovery period and timing of pulses of elevated concentrations of mixtures of cadmium (Cd) and chromium III (Cr) and Cd and lead (Pb) were investigated on periphyton communities to address the following questions: 1) Does exposure duration affect metal toxicity and does the toxicity go away once the exposure is gone depending on recovery period? 2) Does timing of exposure (i.e. night (dark) or

day (light)) affect the toxicity of heavy metals on periphyton communities? 3) Do the metal combinations act antagonistically or synergistically on periphyton communities?

7.2 Materials and Methods

7.2.1 Field periphyton collection

Periphytic communities were collected from Monjolinho River in the southern part of Brazil at a reference site after ecological park before the river pass through the city of São Carlos (21°59'09.16" S; 47°52'35.82" W; elevation 832m, site 7, Figure 1). Headwaters of the Monjolinho River and its tributaries fall within mainly agricultural area. Very low metal concentrations, similar to background levels in the area were measured in the water column and sediment at the reference site (BERE and TUNDISI, 2010). Sampling was done during dry season to avoid variable effects of rainy season like great variations in water level and velocity, floods and inundations. These variations affect diatom development, especially growth rate and relative abundance of different species (BIGGS and KILROY, 2000).

Four plastic racks, each fitted with 10 separate and vertical glass substrates (6 X 15 cm) were immersed at the reference site parallel to the current 20 to 30 cm below the water surface. The racks were secured accordingly and left for 4 weeks prior to sampling. On sampling, the plastic rakes were carefully removed from the river and biofilms colonizing the glass substrates were brushed with a toothbrush into culture medium. The biofilms from all the glass substrates were pooled into one sample of approximately 2 L. This biofilm suspension was immediately transported to the laboratory in cooler box (4 °C).

7.2.2 Laboratory experiments

Thirteen closed experimental systems (hereafter referred to as experimental units; EUs) were set up to allow the exposure of natural periphytic communities to pulses of elevated concentrations of Cd, Cr and Pb under controlled conditions following GOLD et al. (2003). Each EU consisted of three half-polyvinyl chloride (PVC) tubes 50 cm long with a radius of 5 cm as artificial streams with a capacity of 2.8 L each. The three streams were connected in parallel to a 30 L tank (Figure 7.1). All systems were filled with diluted (4x) modified Woods Hole culture medium by GOLD et al. (2003, Table 1). This culture medium was kept without ethylenediaminetetraacetic acid (EDTA), which presents very high binding capacities for metals (STAUBER and FLORENCE, 1989),

and supplemented with silica, an essential diatom nutrient. Nutrient levels were between the high and optimal values found in culture media, and corresponded to the lower values of natural river waters. Test medium were prepared from distilled water. A pump (Boyu bomba submersa SP-0100-600/h, SP-Brazil) allowed continuous circulation of the water through each system at a rate of 10 ± 0.25 ml s^{-1}, corresponding to a velocity of 0.2 cm s^{-1}. Discharge was monitored daily and adjusted where necessary. Each stream was fitted with 6 clean glass substrates (6 X 15 cm) in a slightly slanting position for periphyton colonisation. Water level was kept at 0.5 cm above substrate. A light intensity of 55 ± 5 µmol s^{-1} m^{-2} at the water-air interface for photosynthetically active radiations (400-700 nm, LI-193 Spherical Quantum Sensor (LI-COR Worldwide, Brazil) was maintained with a light: dark regime of 12h/12h. Prior to this experiment, various pilot studies were carried out to determine the optimum experimental conditions required.

7.2.3 Metal exposure

This research focused on six experimental factors: pulse timing (light/dark), duration, frequency, recovery period between pulses, chemical type, and 'maturity' of periphyton communities. These were found to be particularly important for predicting effects of pulsed exposures (DIAMOND et al., 2002; 2006a, b; DUONG et al., 2010). Pulse frequency ranged from two to seven pulses in a given test and recovery time between pulses ranged between 12 and 60h (Table 7.1). Pulse durations were either 12h or 24h. The 12h pulses were carried out either during the light or dark periods of the experiment for each type of metal treatment.

Homogenised periphyton suspension from the field was divided into 9 equal volumes. Each fraction was introduced into the water column of the tank feeding 9 EUs as described below. The systems were equilibrated over night and then the desired concentrations of Cd, Cr III or Pb were obtained by addition of aliquots of the stock solutions to different systems. Cadmium chloride ($CdCl_2$, 10 $mg.L^{-1}$, Merck, Darmstadt, Germany), lead nitrate ($Pb(NO_3)_2$ 10 $mg.L^{-1}$, Merck, Darmstadt, Germany) and chromium (III) chloride hexahydrate $[(CrCl_2(H_2O)_4]Cl{\cdot}2H_2O$, 10 $mg.L^{-1}$, Merck, Darmstadt, Germany) were used as stock solutions.

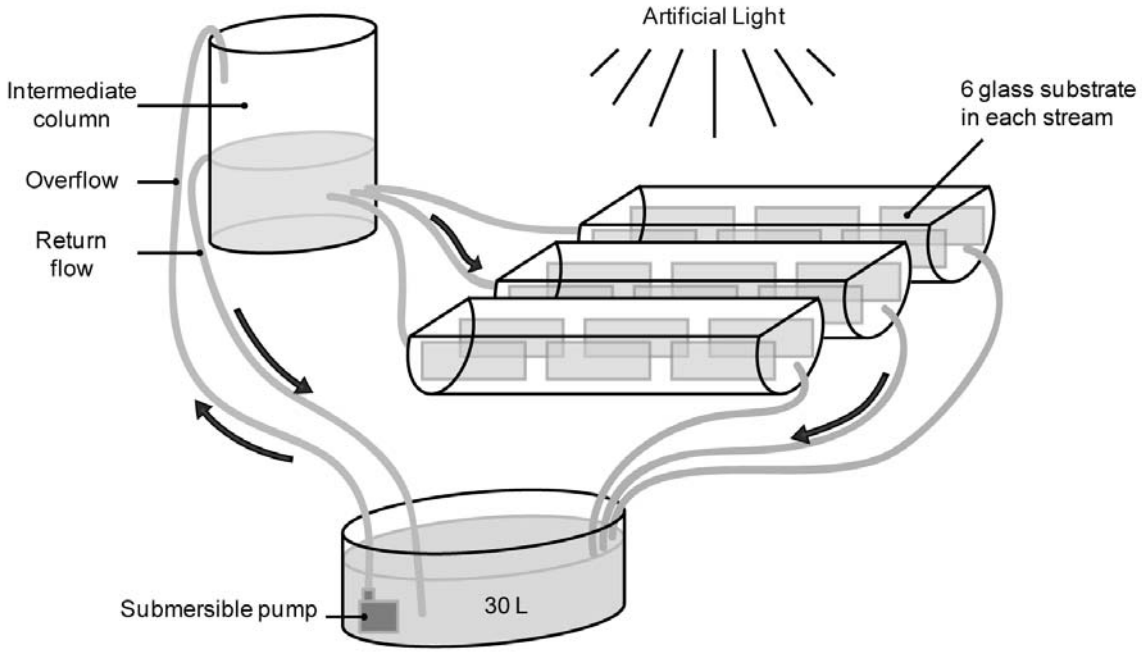

Figure 7.1: Schematic representation of a closed experimental system, consisting of three artificial streams (50 cm length, 5 cm radius), each containing 6-glass substrata (6 X 15 cm). Arrows indicate flow direction, (by: Ricardo M. Degani).

EU1 was left uncontaminated throughout the experiment to act as a control. EUs 2, 3, 4 and 5 had two replicates each i.e. one contaminated and one uncontaminated EU. The latter was not inoculated with periphyton and had no glass slides fitted in it. Sets of two glass slides from each stream from the contaminated EUs were subjected to metal pulse exposures based on scheme in Table 7.1. Chemical pulses were achieved by transferring glass slides from contaminated EUs to uncontaminated EUs and vice verse for a prescribed duration to achieve the desired exposure duration, timing, recovery period and frequency (Table 7.1). Pulse concentration was constant for a given treatment when multiple pulses were applied during a test. Contaminated systems for EU2 and 3 were contaminated with a mixture of 0.1 $mg.L^{-1}$ Cd + 0.1 $mg.L^{-1}$ Pb, whilst those for EU4 and 5 were contaminated with a mixture of 0.1 $mg.L^{-1}$ Cd + 0.2 $mg.L^{-1}$ Cr III. EU6 was left uncontaminated for three days (72hr) to allow the development of a 'mature' biofilm and then continuously exposure to a mixture of 0.1 $mg.L^{-1}$ Cd + 0.1 $mg.L^{-1}$ Pb for the rest of the experiment. After overnight equilibration, EU7, 8 and 9 were continuously contaminated with 0.1 $mg.L^{-1}$ Cd; 0.1 $mg.L^{-1}$ Cd + 0.1 $mg.L^{-1}$ Pb; and 0.1 $mg.L^{-1}$ Cd + 0.2 $mg.L^{-1}$ Cr III respectively (Table 7.1). The concentrations of Cd, Cr and Pb used in this study were chosen to reflect pulses discharge regime typical of field observations where peak concentrations temporarily, but greatly, exceeds the background level

(HANDY, 1994; LIESS et al., 1999). Metal mixtures were used instead of single metals because in nature, many metals are present at a given site at the same time (WONG et al., 1987; BERE and TUNDISI, 2010).

Table 7.1: A Schematic depiction of the general experimental design for a given metal treatment and pulsing regimes used in experiments involving 2, 4 or 7 pulses and different recovery times between pulses. Gray, chemical exposure; white, no chemical exposure; cont, continuous; reps, replicas; L, light; D, dark.

Pulse concentration ($mg.L^{-2}$)	Eu	Pulse duration and timing	1st Stream slides (3reps/Eu)	# of pulses	Recovery period between pulses (h)	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
						L	D	L	D	L	D	L	D	L	D	L	D	L	D
Control	1		2	none	none														
Cd (0.1) + Pb(0.1)	2	12hr (L)	2	7	12														
			2	4	36														
		24hr	2	4	24														
	3	12hr (D)	2	7	12														
			2	4	36														
		24hr	2	2	60														
Cd (0.1) + Cr(0.2)	4	12hr (L)	2	7	12														
			2	4	36														
		24hr	2	4	24														
	5	12hr (D)	2	7	12														
			2	4	36														
		24hr	2	2	60														
Cd (0.1) + Pb(0.1)	6	72hr	2	Cont	none														
Cd (0.1)	7	Cont	2	Cont	none														
Cd (0.1) + Pb(0.1)	8	Cont	2	Cont	none														
Cd (0.1) + Cr(0.2)	9	Cont	2	Cont	none														

7.2.4 Biofilm sampling and analysis

Biofilms were collected after a colonization period of 1 week. During sampling, two glass substrates (section 7.2.3) were separately removed from each stream of each EU (n = 3 for each EU). The biofilm from the two glasses were brushed with a toothbrush into mineral water and the resultant biofilm suspensions from the two glasses were pooled to make one sample and making the volume of the suspension to 60 ml. The biofilm suspensions were then divided into three fractions each for the following analyses. The first fraction (20 ml) was fixed with 4% (final concentration) formalin for identification and cell density determination. Cells in 100 μL subsample were counted in a Nageotte counting chamber at X400 with cell densities expressed as living algal cells per unit area ($cells.cm^{-2}$). For diatom identification to species level, sub-samples of the 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 and KILROY, 2000). A total of 250 – 600 valves per sample were identified and

counted using the phase contrast light microscope (1000 X) (Leica Microsystems, Wetzlar GmbH, Type - 020-519.503 LB30T, Germany). The diatoms were identified to species level based on studies by METZELTIN et al. (2005), BICUDO and MENEZES (2006) and METZELTIN and LANGE-BERTALOT (1998, 2007).

The second fraction (20 ml) was used for chlorophyll *a* analysis. The samples were filtered onto Whatman GF/C filters. Chlorophyll *a* from the filters was measured spectrophotometrically (at 665 nm and 750 nm) following extraction in boiling 80% ethanol (5 min) and steeping at 4°C in the dark (24 h). A phaeopigment correction was obtained by acidification NUSCH (1980). The third fraction (20 mL) was filtered through pre-combusted GF/C filters and dried at 60 °C for 48 h to determine DW. After final weighing, samples were ashed at 500 ^{o}C for 1 hr and weighed again to obtain AFDM.

7.2.5 Data analysis

Statistical significance of main effects (i.e., pulse frequency, duration, recovery period, timing and chemical type) that were varied within a given experiment were analyzed using analysis of variance (ANOVA) after testing for homogeneity of variances (Levene`s test, $p < 0.05$) and normality of distribution (Shapiro-Wilk test, $p < 0.05$) and log transforming where necessary. Tukey's pairwise comparison test ($p < 0.05$) was used to test for significant differences in periphyton response among treatments in a given experiment if ANOVA indicated significant effects. Separate ANOVAs and a Tukey's pairwise comparison tests were conducted for each type of response (DW, AFDW, chlorophyll *a*, cell densities, species richness and diversity) for each treatment. Taxonomic differences among the different treatments were revealed using principal component analyses (PCA). ANOVA, Tukey's pairwise comparison test, Shapiro-Wilk test, Levene`s test, and PCA, were performed using PAlaeontological STatistics (PAST) software version 2.01 (HAMMER et al., 2009).

7.3 Results and Discussion

All the response variables (DW, AFDW, chlorophyll *a*, cell densities, species richness and diversity) elicited significant differences among the 17 treatments assigned to periphyton communities (ANOVA, $p < 0.05$). This emphasises the importance of metal pulse frequency, duration, recovery period, timing and chemical type on aquatic life as has been previously reported (e.g. DIAMOND and BUTCHER, 2002; DIAMOND et al., 2006a, 2006b). However, discrepancies in Tukey`s pairwise comparisons of the 17

treatments based on the five response variables were observed (Table 7.2). The comparisons were relatively similar for DW, AFDW, chlorophyll *a* and cell densities compared to species richness and diversity. Several studies have cautioned against the use of diversity indices in biological monitoring of aquatic systems (e.g. ROUND, 1991; BIGGS and KILROY, 2000; DE LA REY et al., 2008). In addition, DW and AFDW were affected by organic material brought together with the periphyton suspension from the field whilst for cell densities, living and dead cells were hard to separate in some cases. The only response that gave reliable results with no perceived interferences was chlorophyll *a*. Thus, our conclusions and recommendations are based more on chlorophyll *a* than other responses.

7.3.1 Frequency and recovery time

Periphyton response was partly a function of pulse frequency and recovery time. Increasing pulse frequency from 4 to 7 pulses (decreasing recovery period between pulses from 36 to 12hr) for 12 hr pulse duration experiment resulted in significant decrease in DW, AFDW, chlorophyll *a* and cell densities both for Cd/Pb and Cd/Cr mixtures regardless of the timing of exposure (ANOVA, $p < 0.05$, Figure 7.2-4). On the other hand, increasing pulse frequency from 2 to 4 pulses (decreasing recovery period between pulses from 60 to 24 hr) for 24 hr pulse duration experiment also resulted in decrease in the above response variables but the decrease was only significant for Cd/Cr mixture (ANOVA, $p < 0.05$). This emphasizes the importance of pulse frequency, recovery period, exposure duration and chemical type and is consistent with previous research observations that the closer the frequency and duration of the pulse is to a continuous exposure, the greater the effects of the contaminant on aquatic life (e.g. BRENT and HERRICKS, 1998; REINERT et al., 2002; ZHAO and NEWMAN, 2004; DIAMOND et al., 2006a, b). The current study suggests resistance of periphyton communities to pulsed metal exposures depending on pulse frequency and the interval between pulses as has been reported in natural populations (NIEMI and YOUNT, 1990).

Table 7.2: Results of analysis of variance (ANOVA) with Tukey`s pairwise comparisons for the 17 treatments used in this study. + = significant differences and ... = no significant difference ($p < 0.05$), L = light, D = dark, cont = continuous, P = pulse, R = recovery.

		Control	L-Cd+Pb(12P-12R)	L-Cd+Pb(12P-36R)	D-Cd+Pb(12P-12R)	D-Cd+Pb(12P-36R)	Cd+Pb(24P-24R)	Cd+Pb(24P-60R)	L-Cd+Cr(12P-12R)	L-Cd+Cr(12P-36R)	D-Cd+Cr(12P-12R)	D-Cd+Cr(12P-36R)	Cd+Cr(24P-24R)	Cd+Cr(24P-60R)	Cd+Pb(72Mat-72P)	CdCont	Cd+PbCont
DW/AFDW	Control																
	L-Cd+Pb(12P-12R)	+															
	L-Cd+Pb(12P-36R)	+	+														
	D-Cd+Pb(12P-12R)	+	...	+													
	D-Cd+Pb(12P-36R)	+	+	...	+												
	Cd+Pb(24P-24R)	+	+	+	+	+											
	Cd+Pb(24P-60R)	+	+	+	+	+	...										
	L-Cd+Cr(12P-12R)	+	...	+	+	+	...	...									
	L-Cd+Cr(12P-36R)	+	+	...	...	...	+	+	...								
	D-Cd+Cr(12P-12R)	+	...	+	...	+	+	...	...	+							
	D-Cd+Cr(12P-36R)	+	+	...	...	...	+	...	+	...	+						
	Cd+Cr(24P-24R)	+	...	+	+	+	...	+	+	+	+	+					
	Cd+Cr(24P-60R)	+	...	...	...	...	+	...	+	+	+	...	+				
	Cd+Pb(72Mat-72P)	...	+	+	+	+	+	+	+	+	+	+	+	+			
	Cd Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	Cd+Pb Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+	...	
	Cd+Cr Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+	...	...
Chlorophyll a	Control																
	L-Cd+Pb(12P-12R)	+															
	L-Cd+Pb(12P-36R)	+	+														
	D-Cd+Pb(12P-12R)	+	...	+													
	D-Cd+Pb(12P-36R)	+	+	...	+												
	Cd+Pb(24P-24R)	+	...	+	...	+											
	Cd+Pb(24P-60R)	+	...	+	...	+	...										
	L-Cd+Cr(12P-12R)	+	...	+	...	+	...	...									
	L-Cd+Cr(12P-36R)	+	+	...	...	...	+	+	+								
	D-Cd+Cr(12P-12R)	+	....	+	...	+	....	...	...	+							
	D-Cd+Cr(12P-36R)	+	+	...	...	...	...	...	+	...	+						
	Cd+Cr(24P-24R)	+	....	...	...	...	...	...	...	+	...	+					
	Cd+Cr(24P-60R)	+	....	+	...	+	...	...	...	+	+	+	+				
	Cd+Pb(72Mat-72P)	+	+	+	+	+	+	+	+	+	+	...	+	+			
	Cd Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	Cd+Pb Cont	+	+	+	+	+	+	+	+	+	+	+	+	...	+	...	
	Cd+Cr Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+	...	...
Cell densities	Control																
	L-Cd+Pb(12P-12R)	+															
	L-Cd+Pb(12P-36R)	+	+														
	D-Cd+Pb(12P-12R)	+	...	+													
	D-Cd+Pb(12P-36R)	+	+	...	+												
	Cd+Pb(24P-24R)	+	...	+	...	+											
	Cd+Pb(24P-60R)	+	...	+	...	+	...										
	L-Cd+Cr(12P-12R)	+	...	+	+	+	...	...									
	L-Cd+Cr(12P-36R)	+	+	...	...	+	+	+	+								
	D-Cd+Cr(12P-12R)	+	...	+	...	+	...	...	...	+							
	D-Cd+Cr(12P-36R)	+	...	...	...	+	+	...	+	...	+						
	Cd+Cr(24P-24R)	+	...	+	...	+	...	...	...	+	...	+					
	Cd+Cr(24P-60R)	+	...	+	...	+	...	...	+	+	+	+	+				
	Cd+Pb(72Mat-72P)	+	+	+	+	+	+	+	+	...	+	+	+	+			
	Cd Cont	+	+	+	+	+	+	+	...	+	...	+	...	+	+		
	Cd+Pb Cont	+	...	+	+	+	...	+	...	+	...	+	...	+	+	...	
	Cd+Cr Cont	+	+	+	+	+	...	+	...	+	...	+	...	+	+	...	...
Species diversity	Control																
	L-Cd+Pb(12P-12R)	+															
	L-Cd+Pb(12P-36R)	+	...														
	D-Cd+Pb(12P-12R)	+	...	...													
	D-Cd+Pb(12P-36R)	+	...	...	...												
	Cd+Pb(24P-24R)	+	...	...	...	...											
	Cd+Pb(24P-60R)	+	...	...	...	...	...										
	L-Cd+Cr(12P-12R)	+	...	...	...	...	...	...									
	L-Cd+Cr(12P-36R)	+	+	...	...	...	...	...	...								
	D-Cd+Cr(12P-12R)	+	...	...	...	...	...	...	...	...							
	D-Cd+Cr(12P-36R)	+	+	...	+	...	+	...	...	...	...						
	Cd+Cr(24P-24R)	+	...	+	...	...	...	...	...	...	...	+					
	Cd+Cr(24P-60R)	+	+	...	+	+	...	+	+	+	+	...	+				
	Cd+Pb(72Mat-72P)	...	+	+	+	+	+	+	+	+	+	+	+	+			
	Cd Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	Cd+Pb Cont	+	...	...	...	...	...	...	...	...	...	...	+	...	+	+	
	Cd+Cr Cont	+	...	...	...	...	...	...	...	...	...	...	+	...	+	+	...
Species richness	Control																
	L-Cd+Pb(12P-12R)	+															
	L-Cd+Pb(12P-36R)	+	+														
	D-Cd+Pb(12P-12R)	+	...	...													
	D-Cd+Pb(12P-36R)	+	+	...	...												
	Cd+Pb(24P-24R)	+	...	+	...	+											
	Cd+Pb(24P-60R)	+	...	...	...	...	+										
	L-Cd+Cr(12P-12R)	+	+	...	...	...	+	...									
	L-Cd+Cr(12P-36R)	+	+	...	...	...	...	...	...								
	D-Cd+Cr(12P-12R)	+	...	...	...	...	+	...	...	...							
	D-Cd+Cr(12P-36R)	+	+	...	+	...	+	...	...	...	+						
	Cd+Cr(24P-24R)	+	...	...	...	...	...	...	...	...	...	...					
	Cd+Cr(24P-60R)	+	+	...	...	...	+	...	...	...	+	...	...				
	Cd+Pb(72Mat-72P)	+	+	...	+	...	+	...	...	...	...	...	...	...			
	Cd Cont	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	Cd+Pb Cont	+	...	+	...	...	...	...	...	...	...	...	...	...	...	+	
	Cd+Cr Cont	+	...	+	...	...	...	...	...	...	...	...	...	...	...	+	...

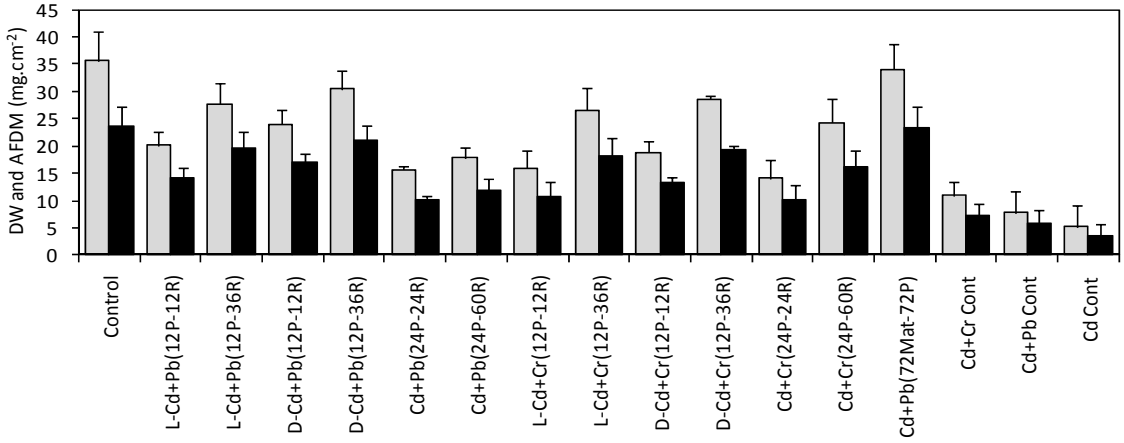

Figure 7.2: The mean values and standard deviations (n = 3) of dry weight (gray bars) and AFDW (black bars) developed on glass substrates in 17 treatments during the course of the experiment. L = light, D = dark, cont = continuous, P = pulse, R = recovery.

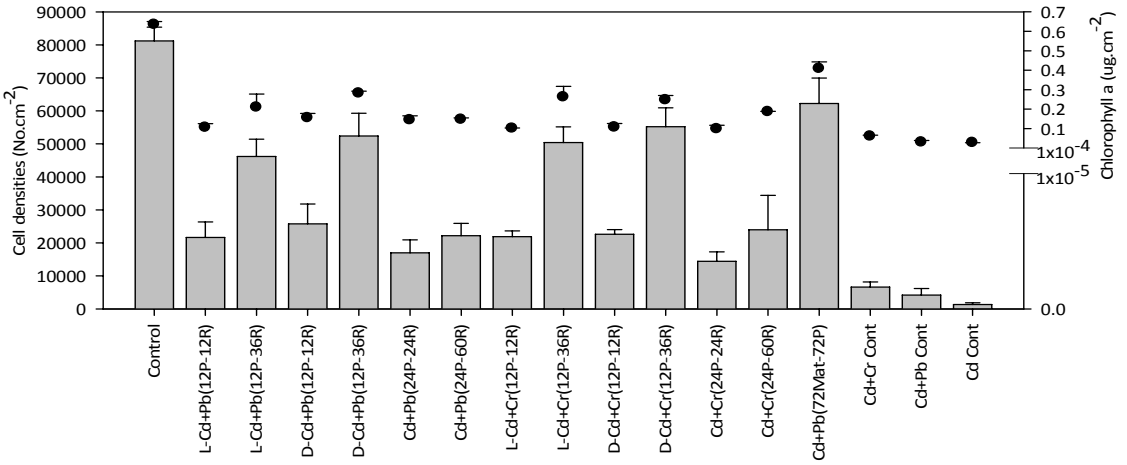

Figure 7.3: The mean values and standard deviations (n = 3) of algal cell densities (gray bars) and chlorophyll *a* concentrations (back circles) developed on glass substrates in 17 treatments during the course of the experiment. L = light, D = dark, cont = continuous, P = pulse, R = recovery.

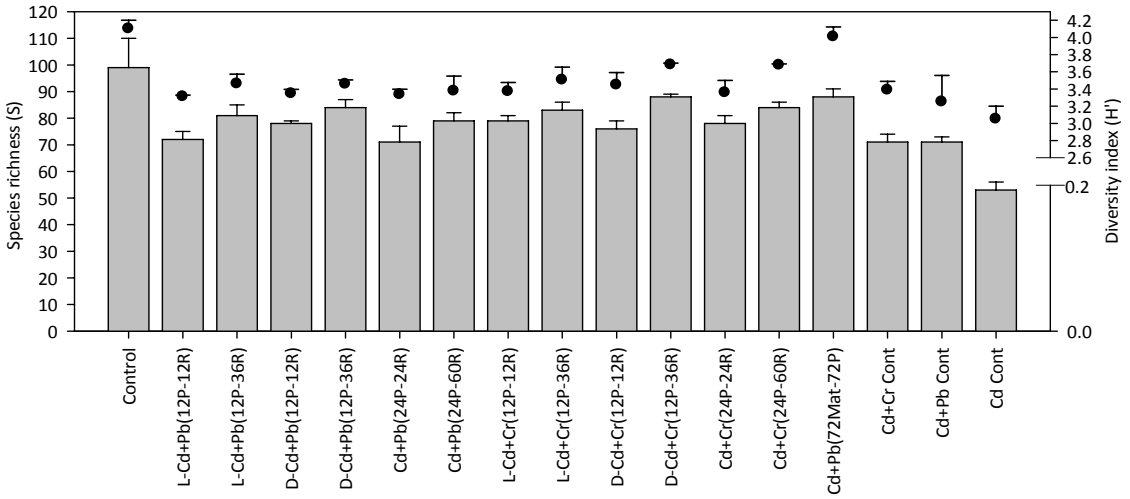

Figure 7.4: The mean values and standard deviations (n = 3) of species richness (S) (gray bars) and diversity (H`) (black circles) developed on glass substrates in 17 treatments during the course of the experiment. L = light, D = dark, cont = continuous, P = pulse, R = recovery.

7.3.2 Duration

Response of periphyton communities to increased pollutant pulse durations was also demonstrated. Increasing metal pulse duration from 12 to 24 hr resulted in significant decrease in DW and AFDW for all the contaminants regardless of pulse timing and recovery period between pulses (ANOVA, $p < 0.05$). Previous researchers (e.g. DIAMOND et al., 2006a, b) have demonstrated the importance of pollutant pulse duration in aquatic systems in agreement with the results of this study. Increasing metal pulse duration from 12 to 24 hr also resulted in decrease in chlorophyll *a* and cell densities for all the contaminants regardless of pulse timing. However, chlorophyll *a* and cell densities were only significantly high in 12h pulse/36h recovery period experiments (light and dark) compared to the all the 24 hr pulse experiments (ANOVA, $p < 0.05$). On the other hand, there were no significant differences in chlorophyll *a* and cell densities between 12h pulse/12h recovery period experiments (light and dark) and all the 24 hr pulse experiments (ANOVA, $p > 0.05$). Thus, the higher the frequency of 'short' duration pulses the more likely they are to produce effects similar to that of long duration pulses suggesting an interaction of pollutant pulse frequency and duration in shaping aquatic life, which is poorly reflected in regional and international water quality guidelines.

For both 12 and 24hr pulse treatments (regardless of pulse frequency, recovery period timing, and chemical type), DW, AFDW, chlorophyll *a* and cell densities were significantly higher than in all continuous exposure treatments (ANOVA, $p < 0.05$). Other researcher (e.g. ERICKSON et al., 1986; DIAMOND et al., 2006a) observed that continuous exposures results in greater effects of the pollutant on test organisms than pulsed exposures of the same concentrations as supported by the present research finding. The levels of metals used in this study were very high hence; all the treatments were different from the control. However, in the light of the significant differences in the measured variables between pulsed and continuous exposures (the difference increasing with decreasing pulse duration and increasing recovery period (section 7.3.2)) recorded in this study, it would make intuitive sense to suggest that infrequent and widely separated metal pulses of low concentration are likely to have little effects on aquatic life. Indeed, from our field studies, we observed that Cd, Pb and Cr III levels slightly exceeding regional and international guidelines on water quality at some points in streams around São Carlos city were not closely associated with the observed changes in diatom communities (BERE and TUNDISI, 2010). These results suggest that factors other than

the traditionally used magnitude and duration of contaminants must be considered in order to accurately predict response of aquatic life to contaminants.

7.3.3 Timing

Chlorophyll *a*, cell densities, DW and AFDW were generally higher in dark pulse exposures compared to light pulse exposures of the same duration and frequency for all the contaminants. This suggests a potential role of light in modulating the effect of toxicants as supported by researches such as GUASCH and SABATER, (1998), GUASCH et al., (1997, 1998, 2003), RAGNI and D`ALCALA (2007) and LAVIALE et al., (2010). This is quite understandable considering that most aquatic organisms have developed complex systems, circadian clocks, to detect time and synchronize processes, actions, and behaviours to the diel cycle (ROENNEBERG and MERROW, 2002). In addition, as previously noted, the metabolism-dependent phase of uptake of some metal such as Cd by some algal species is inhibited by absence of light (GADD, 1988; GARNHAM et al., 1992). Pulse timing is currently not explicitly considered in aquatic life water quality criteria paradigm.

However, differences in chlorophyll *a*, cell densities, DW and AFDW between dark pulse and light pulse exposures were not statistically significant (ANOVA, $p < 0.05$). This could be because other factors such as pulse magnitude, duration, frequency and recovery time became more important than pulse timing. According to (DIAMOND et al., 2006a), the scenarios of magnitude and duration of metal pulses used in this experiment were extraordinary, having been adopted due to almost inexistence of data on actual scenarios of these factors in lotic systems in Brazil. Low magnitude, short duration metal scenarios experiments should be carried out to confirm the present results.

7.3.4 Chemical type

As previously noted (section 7.3.1) increasing pulse frequency from 2 to 4 pulses (decreasing recovery period from 60 to 24 hr) for 24 hr exposure experiments only resulted in significant decrease in DW, AFDW, chlorophyll *a* and cell densities for Cd/Cr III mixture and not for Cd/Pb mixture experiment. This demonstrates the need to consider pollutant type in formulating water quality guidelines for protection of aquatic life as has been widely recognized. In addition, for continuous exposures experiments, DW, AFDW, chlorophyll *a* and cell densities were higher in Cd/Cr III followed by Cd/Pb and then Cd experiment though the differences were not statistically significant (ANOVA, $p < 0.05$).

Depending on factors like pH and water hardness, Cd is generally more toxic than Pb, which is in turn more toxic than Cr III (GENTER, 1996). Thus, Cd play a more important role in regulating periphyton growth and development compared to Pb and Cr III. The negative effects of high Cd levels on periphyton growth and development have been widely reported (e.g. IVORRA et al., 2000; GOLD et al., 2003; DUONG et al., 2008; MORIN et al., 2008a, 2008b; DUONG et al., 2010).

Continuous exposure of Cd alone proved to be more toxic than the continuous exposures of Cd/Pb and Cd/Cr III mixtures suggesting an ameliorative effect of Pb and Cr III on the toxicity of Cd i.e. antagonistic interference of Pb and Cr III on the toxic effects of Cd. This, as far as we know, has not been demonstrated in the literature and is an important observation given the fact that metals usually occur as mixtures in nature (BERE and TUNDISI, 2010). DEVI PRASAD and DEVI PRASAD (1982) have demonstrated this antagonism between Ni/Cd and Pb/Cd mixtures for three freshwater green algae, but not at community level.

7.3.5 Maturity

In the experiment where the periphyton communities were left to grow ('mature') for 72 hr (3 days) and then subjected to continuous Cd + Pb exposure for the rest of the experiment, chlorophyll *a* and cell densities were significantly higher than in all the other exposure scenarios except the control (ANOVA, $p < 0.05$). In the same experiment, DW and AFDW were also significantly higher than in all the exposure scenarios (ANOVA, $p < 0.05$) with no significant differences from the control (ANOVA, $p > 0.05$). Periphyton is a biological community of attached autotrophic and heterotrophic organisms that are associated in complex matrix of polysaccharide exudates and detritus with a complex function. This matrix can form a protective layer thereby reducing the exposure of solid surface to the external environment and decreasing toxicity of contaminants (IVORRA et al., 2000; GOLD et al., 2003). In the case of metallic pollution, the protective effect of the biogenic matrix manifests itself through a less pronounced impact of metals on primary production and a less altered diatom community composition (IVORRA et al., 2000; GOLD et al., 2003). The protective role of this matrix increases with maturity of periphyton communities (IVORRA et al., 2000; MORIN et al., 2008; DUONG et al., 2010) explaining why 'mature' periphyton communities showed more resilience to metal contamination compared to 'young' communities in the present study.

7.3.6 Diatom community composition in relation to exposure scenarios

Differences in metal exposure scenarios also manifested themselves through corresponding changes in diatom community composition. Relatively similar patterns to those of DW, AFDW, chlorophyll *a*, and cell densities were observed for diatom species richness and diversity with increasing pulse frequency and duration (decreasing recovery period), and with different pulse timing, metal mixtures and periphyton 'maturity' (Figure 7.4). This is due to reasons discussed in the previous sections (sections 7.3.1 to 7.3.5) emphasising the importance of metal pulse frequency, duration, recovery period, timing and chemical type on aquatic life. However, contrary to the case of DW, AFDW, chlorophyll *a*, and cell densities, differences in diatom species richness and diversity were not statistically significant among some exposure scenarios (ANOVA, $p > 0.05$; Table 7.2) reinforcing the unreliability of diversity indices in biological monitoring of aquatic systems (DE LA REY et al., 2008).

Of the 118 diatom species belonging to 45 genera that were recorded in all the treatments during the course of the study, 12 dominant diatom species, with mean relative abundances >5% and present in at least two communities, were described as characteristic of each diatom community developed throughout the experiment (Figure 7.5). Increasing pulse frequency and duration (decreasing recovery period) resulted in corresponding increase in *Achnanthidium minutissimum* (Kützing) Czarnecki and *Nitzschia palea* (Kützing) Smith and decrease in *Encyonema silesiacum* (Bleisch) Mann, *Eunotia bilunaris* (Ehrenberg), *Gomphonema angustatum* (Kützing) Rabenhorst, *G. gracile* Ehrenberg, *G. parvulum* (Kützing) Kützing, *Navicula cryptocephala* (Grunow) Cleve, *Nitzschia amphibia* Grunow, and *N. linearis* (Agardh) Smith (Figure 7.5). The relative abundances of *A. minutissimum* and *N. palea* were highest in continuous exposure scenarios, especially Cd treatment. *A. minutissimum* and *N. palea* were generally higher in dark pulse exposures compared to light pulse exposures of the same duration and frequency for all the contaminants.

Changes in relative abundance of dominant diatom taxa with increasing pulse frequency and duration (decreasing recovery period) (Figure 7.5) were also confirmed by the PCA conducted to investigate taxonomic differences among the different treatments (Figure 7.6). The PCA clearly separated communities from 12 hr pulse/12 hr recovery, 24 hr pulse/24 hr recovery period and continuous exposures from the rest of the communities regardless of pulse timing and metal mixture (Figure 7.6a). These communities were positively associated with the fist axis of the PCA that accounted for 78.6 % of the total

variation, with communities from continuous exposure treatments being more positively associated to this axis compared to other communities. The first PCA axis was also strongly positively associated with *A. minutissimum* followed by *N. palea* emphasising the strong association of these species with increased frequency and duration of metal pulses (Figure 7.6b). The remaining communities (all negatively associated to the first PCA axis) were in turn separated in to two groups i.e. the control, Cd/Cr III 12 hr pulse/36 hr recovery (light and dark) and Cd/Cr III 24 hr pulse/60 hr recovery were separated from the rest of the Cd/Pb treatment communities. This separation was attributed to differences in metal mixtures resulting in low relative abundance of *Fragilaria capucina* Desmazières, in the control, Cd/Cr III 12 hr pulse/36 hr recovery (light and dark) and Cd/Cr III 24 hr pulse/60 hr recovery compared to the rest of the Cd/Pb treatments. The former communities were associated with such species as *Diatoma vulgare* Bory, *E. silesiacum*, *E. bilunaris*, *G. angustatum*, *G. gracile* and *N. amphibia*, whilst the latter were associated with such species as *G. parvulum*, *N. cryptocephala* and *N. linearis*. This shows the importance of types of metal mixtures on aquatic life. *F. capucina* was strongly positively associated with the second PCA axis that accounted for 8.6 % of the total variance.

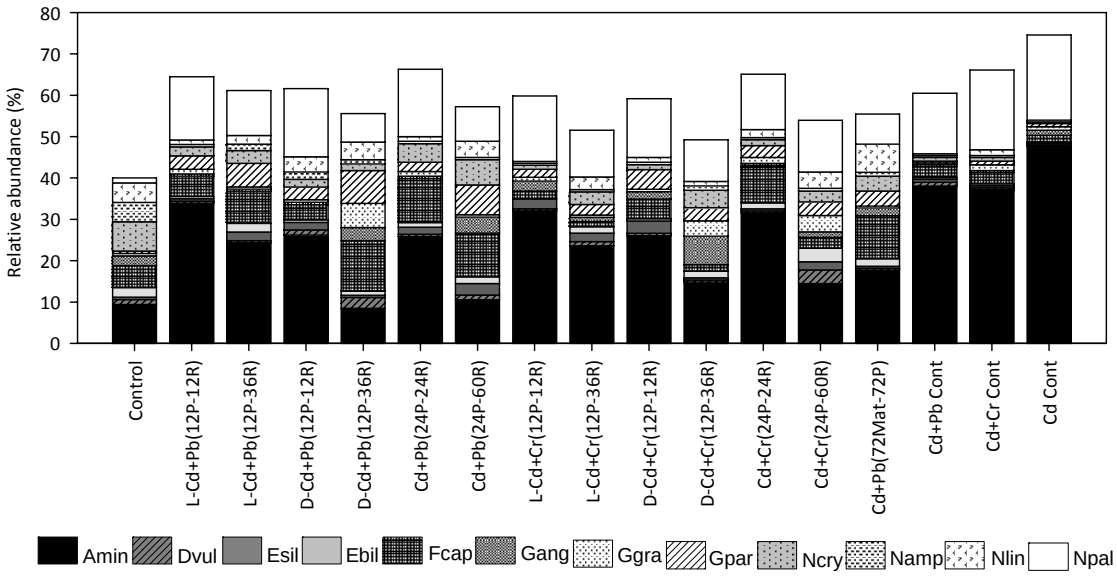

Figure 7.5: The relative abundance of the 12 major diatom species form diatom communities recorded in 17 treatments during the experiment. L = light, D = dark, cont = continuous, P = pulse, R = recovery. Amin, *Achnanthidium minutissimum* (Kützing) Czarnecki; Dvul, *Diatoma vulgare* Bory; Esil, *Encyonema silesiacum* (Bleisch) Mann; Ebil, *Eunotia bilunaris* (Ehrenberg) Mills; Fcap, *Fragilaria capucina* Desmazières; Gang, *Gomphonema angustatum* (Kützing) Rabenhorst; Ggra, *G. gracile* Ehrenberg; Gpar, *G. parvulum* (Kützing) Kützing; Ncry, *Navicula cryptocephala* (Grunow) Cleve; Namp, *Nitzschia amphibia* Grunow; Nlin, *N. linearis* (Agardh) Smith; Npal, *N. palea* (Kützing) Smith.

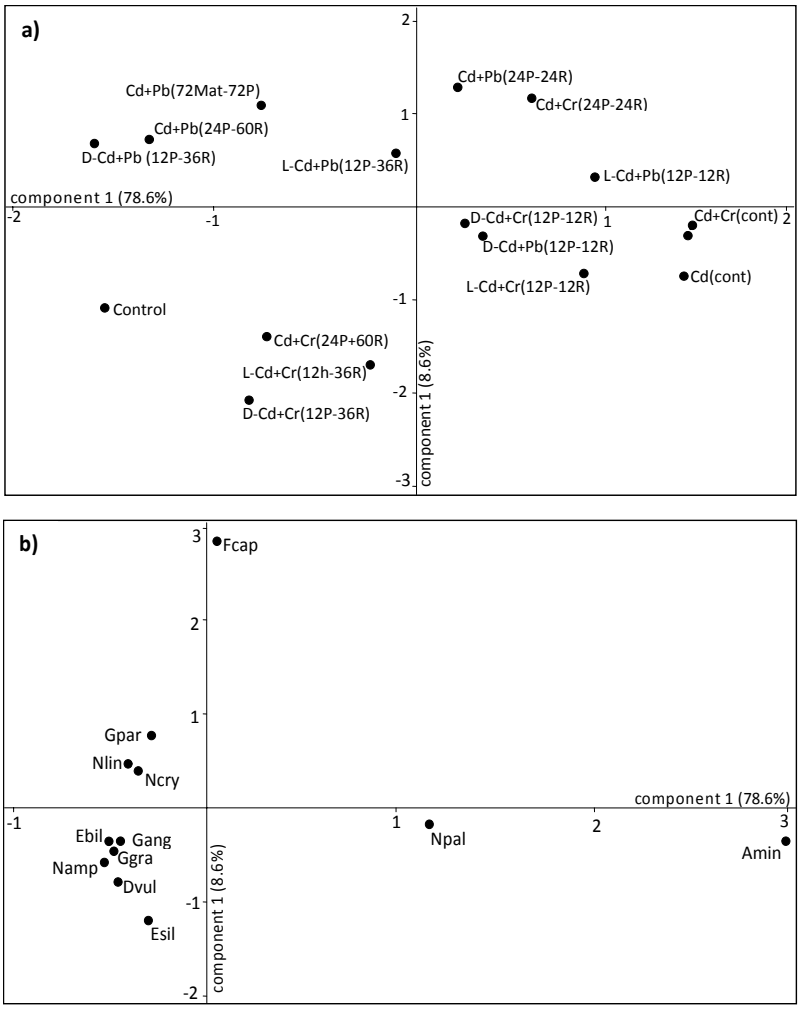

Figure 7.6: Principal component analysis based on the taxonomic composition of the diatom communities recorded in 17 treatments. (a) Projection of the communities on the first two principal component axes. (b) Projection of the species with loading >0.2 for one of the two axes. L = light, D = dark, cont = continuous, P = pulse, R = recovery. Taxa codes correspond to those in Figure 7.5.

Thus, the communities differentiated according to the ability of the species to grow under increased metal pulse frequency duration and metal mixture type in support of the pollution-induced community tolerance (PICT) concept (BLANCK et al., 1988; BLANCK, 2002). With increasing metal pulse frequency and duration, the communities goes through a toxicant induced succession (TIS) (BLANCK, 2002) leading to development of more resistant species such as *A. minutissimum* and *N. palea* and reduction or exclusion of sensitive ones such as *E. silesiacum, E. bilunaris, G. angustatum, G. gracile, G. parvulum, N. cryptocephala, N. amphibia,* and *N. linearis*. This is supported by studies by RAI et al., (1981) and GENTER et al., (1988) which demonstrated that exposure to inorganic chemical stress often places a selection pressure on the community that either decrease abundance of pollution-sensitive species and increase or does not change abundance of pollution-tolerant species. Algae may tolerate

inorganic chemical stress at the cellular level by a decreased number of binding sites at the cell surface, inhibition of metabolism-dependent uptake stage, physiological development of exclusion mechanisms, genetic adaptation, morphological changes, and internal detoxifying mechanisms or safe storage sites (RAI et al., 1981). Differential sensitivity among species leads to different growth rates and is expected to alter species composition in communities (GENTER, 1996).

A. minutissimum and *N. palea* have already been reported in metal-contaminated environments (e.g. IVORRA et al., 2000; GOLD et al., 2003; DUONG et al., 2008; MORIN et al., 2008a, 2008b; DUONG et al., 2010). The proliferation of *A. minutissimum and N. palea* with increasing pulse frequency and duration (around 47.65 % and 20.60 % respectively in continuous Cd exposure treatment) seems to indicate favour and tolerance of these species to frequent and continuous metal exposures. Changes in diatom species composition and abundance with different exposure scenarios observed in this study demonstrates the usefulness of diatom communities in detecting high metal concentration mixtures in streams under different exposure scenarios in agreement with other studies (e.g. IVORRA et al., 2000; GOLD et al., 2003; MORIN et al., 2008a; DUONG et al., 2010).

7.4 Conclusion

Factors other than the traditionally used magnitude and duration of contaminants must be considered in order to accurately predict response of aquatic life to pulsed exposures. To this effect, better information is needed on effluent variability, and specifically, frequency duration, timing and type of pulses as well as the magnitude of the pulse. Thus, environmentally relevant scenario experiments should be carried out to confirm the results of the present study. Field validation of the observed effects remains an interesting subject for further investigations.

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GENERAL CONCLUSIONS

- Benthic diatoms are excellent organisms for biological monitoring with the potential of reflecting changes in water quality due to changes in catchment land-use patterns.
- Diatom-based water quality monitoring indices developed in other regions are useful in providing an indication of the quality of the investigated waters. However, ecological requirements of some diatom species from Brazil need to be clarified and incorporated in a diatom-based water quality assessment protocol unique to different the regions.
- The results of diatom-based multivariate water quality assessment based on different substrates may be interchangeable. Only one substrate has to be collected at each site for water quality assessment surveys, thus, avoiding unnecessary expensive and time-consuming over sampling. Given the limitations of artificial substrates, sampling of natural substrates is highly recommended.
- Periphyton communities have good metal accumulation capacity.
- Hormesis (low Cd dose stimulation and high dose inhibition of responses like growth rate) has been demonstrated with periphyton growth and development being substantially lowered by high concentration of Cd (EC_{50} = 0.077 $mg.L^{-1}$).
- Pb and Cr III decrease the toxicity effects of Cd on periphyton communities suggesting antagonism.
- The closer the frequency and duration of pollutant pulse is to a continuous exposure, the greater the effects of the contaminant on aquatic life.
- The higher the frequency of 'short' duration pulses the more likely they are to produce effects similar to that of long duration exposures.
- Light might have a potential role in modulating the effects of metal toxicants on periphyton.
- Factors other than the traditionally used magnitude and duration of contaminants must be considered in order to accurately predict response of aquatic life to pulsed exposures. To this effect, better information is needed on effluent variability, and specifically, frequency, duration, timing and type of pulses as well as the magnitude of the pulse.

- Shifts in species composition with increasing metal concentrations and exposure duration and different exposure scenarios demonstrated in this study makes periphyton communities appropriate monitors of metal in aquatic systems.

OUTLOOK FOR FUTURE RESEARCH

1. There is need for clarification of ecological requirements of some diatom species from Brazil to produce a diatom-based water quality assessment protocol unique to different regions. Thus, resources should be channelled towards tackling challenges associated with diatom-based biological monitoring, principally taxonomic studies, training of skilled manpower and acquiring and maintaining the necessary infrastructure. Other bioindicators should also be taken into consideration, i.e. fish, macrophytes, macroinvertebrates and other groups of algae, and supplemented by physical and chemical methods.

2. There is need to adopt new paradigms in environmental monitoring such as a shift from an anthropocentric to an ecosystem-cantered holistic approach, with man viewed as an element of the ecosystem and the principle consumer of its services. Environmental monitoring boards, responsible for setting targets for required ecological health or integrity of lotic systems, should rely on biological monitoring to assess progress towards achieving these goals.

3. There is need for establishment of networks of competence at national, regional and global levels to improve biological monitoring through research and innovative practices that are ecologically oriented. This should be followed by fostering two-way interactions between scientists, on the one hand and the general public and decision makers, on the other.

4. Due to the interaction of the effects of variables (ionic strength, eutrophication, organic and metal pollution and so on) in the environment, laboratory experiments performed under controlled conditions must be performed to confirm the observed effects of these variables in this study. Complex experiments (incorporating the interactive effects of various factors such as ionic strength, eutrophication, organic and metal pollution, nutrient availability, light intensity and photoperiodicity and

multiplicity of biotic factors such as inter-specific competition, grazing e.t.c) that better mimic mimics field conditions must be carried out to enable improved accuracy in the extrapolations from laboratory bioassays to responses in natural systems.

5. For sufficient characterisation and monitoring of pollutants, future research should include the development of better guidance for sampling and characterising effluent variability, so as to have better information in regarding the frequency, magnitude, timing and duration of pulses (if they occur). Such information would help inform environmental monitoring and law enforcement agents on appropriate monitoring requirements and water quality guidelines. In addition, better characterisation of effluent variability, and exceedances if and when they occur, would help determine whether a given effluent is likely to pose risk to aquatic life. Field studies, examining actual effects of pulsed exposures, would help interpret the findings of laboratory experiments and better inform future implementation of chemical-specific criteria.

ANNEX: ADITIONAL PUBLICATIONS

Applicability of the Pampean Diatom Index (PDI) to streams around São Carlos-SP, Brazil[8]

Abstract: The objective of the current study was to assess the applicability of the Pampean Diatom Index (the PDI) to natural communities other than epipelic diatom communities as well as those growing on artificial substrates in Monjolinho River and its tributaries, São Carlos-SP, Brazil. Benthic diatoms and water quality sampling was done at 10 sites during summer base flow period (2008 and 2009). The PDI scores were calculated based on epilithic, epiphytic, epipsammic and epipelic diatom communities as well as those growing on bricks and glass substrates. Pearson correlation was used to determine the relationship between the PDI scores from different substrates sampled and measured physical and chemical water quality data. Two-way ANOVA was used to compare these correlation values among substrates. The PDI scores based on all the substrates showed significant correlations with physical and chemical variables. Insignificant differences in the PDI index scores based on different natural substrates were recorded, with all substrates classifying the sites into to roughly the same categories. In the light of these results, the PDI can be applied to communities other than epipelic, and is applicable to the study area. The choice of substrate sampled may not affect accuracy of the PDI-based water quality assessment.

Epipsammic diatoms in streams influenced by urban pollution, São Carlos-SP, Brazil[9]

Abstract: Epipsammic diatoms have important implications for ecosystem processes in lotic environments. Most of the studies on benthic diatoms concentrate on epilithic diatoms and very little is known about epipsammic diatoms. The objective of this study was to assess epipsammic diatom communities in streams in relation to environmental conditions. Epipsammic diatoms and water quality sampling was done at 7 sites during summer base flow period (2008). Forward stepwise multiple regression and canonical correspondence analysis (CCA) were used to determine environmental gradients along which species vary with physical and chemical variables. A total of 112 diatom species distributed among 44 genera were recorded. The process of eutrophication played a significant role in structuring diatom communities in the study region.

Weighted average regression and calibration of conductivity and pH of benthic diatom assemblages in streams influenced by urban pollution – São Carlos-SP, Brazil [10]

Abstract: The importance of conductivity and pH in structuring benthic diatom communities was assessed. Changes in diatom communities along an agricultural-to-urban conductivity and pH gradient were assessed during summer base flow period (September to October 2008). Habitat assessment, diatom and water quality sampling was done at 10 sites. Weighted averaging regression and calibration were used to quantify relationships between individual diatom taxon's relative abundance and conductivity and pH. The predictive abilities of models where assessed in terms of correlation between observed and inferred values of conductivity and pH. Frequently occurring diatom were distributed continuously along gradient of conductivity and pH with the upstream, relatively low conductivity and slightly alkaline sites being characterised by such species as *Aulacoseira ambigua, Aulacoseira granulata* and *Cymbopleura naviculiformis*, while downstream, high conductivity and slightly acidic, sites were characterized by *Gomphonema parvulum, Nitzschia palea, Nupela praecipua, Rhoicosphenia abbreviata and Sellaphora pupula.* Conductivity and pH optima for these diatoms ranged from 25.96 to 324.76 μS cm^{-1} and 6.61 to 7.54 respectively. The autecological information gained through this study augments previous works on diatom species-environmental relationships in streams in other regions and is a stepping stone towards further understanding of diatom ecology and the development of diatom biological monitoring protocol that is suitable for the study area.

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