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Effects of cadmium stress and sorption kinetics on tropical freshwater periphytic communities in indoor mesocosm experiments

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ABSTRACT

Understanding the cause and effect relationship between stressors and biota is crucial for the effective management, restoration and preservation of aquatic systems. The objective of the present study was to assess the effects of five Cd concentrations on tropical periphyton community growth, Cd accumulation kinetics, as well as the effects of Cd on diatom community structure and composition. Natural periphyton communities were transferred to artificial stream chambers and 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 analysed on samples collected after 1, 2 and 4 weeks of colonization. Periphyton growth and development were significantly lowered by Cd concentrations > 0.03 mg.L^{-1}. High Cd accumulation capacity by periphyton was demonstrated with total and intracellular Cd content in biofilms reflecting the effects of 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. Shifts in species composition (development of more resistant species like *Achnanthidium minutissimum* and reduction of sensitive ones like *Diatoma vulgare*, *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. The results give valuable information on Cd impact of freshwater biofilms.

1. Introduction

Heavy metal contamination of freshwater environments causes significant alterations of various components of trophic chain in lotic systems, among which periphyton (especially diatoms) communities attract great attention from researchers. This is 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 and urban areas (Gold et al., 2003a; Nunes et al., 2003; Fechner et al., 2012) and to low pollution as described under experimental conditions (Duong et al., 2008; Morin et al., 2008a, 2008b). Different species respond differently to metals and other stressors because of differences in tolerances. 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 points in time, provides useful information about environmental conditions (Duong et al., 2008; Morin et al., 2008a, 2008b). Changes in morphology of diatom cells are also a manifestation of high concentration of metals (Morin et al., 2007, 2008a, 2008b; Duong et al., 2008, 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 inner layers to the external environment and decreasing toxicity of contaminants (Ivorra et al., 2000; Gold et al., 2003a, 2003b). In 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 the effects of metals in biofilms are characterized by large amounts of unexplained variance. For example, Ivorra et al. (2000) and Morin et al. (2008a) showed that exposure of non-mature biofilms to Cd concentrations of ~10

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and ~100 μg L^{-1} resulted in negative effects on biofilm growth structure and composition. 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 total 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.

The objective of the present study was to assess the effects of five Cd concentrations on tropical periphyton community growth, accumulation kinetics of Cd, as well as the effects of Cd diatom community structure and composition as a precursor to field experiments. Adsorbed and intracellular Cd levels in periphyton were hypothesised to be a function of duration of exposure and the total Cd concentrations in the culture media.

2. Materials and methods

2.1. Field periphyton collection

Periphytic communities were collected from Monjolinho River in the southern part of Brazil at a reference site after the Ecological park before the river passes through the city of São Carlos (21°59′ 09.16″ S; 47°52′35.82″ W; elevation 832 m). 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 (<0.001 mg.L^{-1} and <1 mg kg^{-1} sed. respectively) at the reference site (Bere and Tundisi, 2011a). Sampling was done during dry season to avoid variable effects associated with the rainy season in the form of great water level and velocity variations, 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 (6×15 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 racks were carefully removed from the river and biofilms colonizing the glass substrate were brushed with a toothbr into Woods Hole culture medium (WC) modified according to Gold et al. (2003a). The biofilms from all the glass substrates were pooled into one sample of approximately 2 L. This biofilm suspension was transported in a cool environment to the laboratory where it was inoculated in the systems described below within 30 min of sampling.

2.2. Laboratory experiments

Six closed experimental systems (hereafter referred to as experimental units; EUs); were set up to allow the exposure of natural periphytic communities to Cd under controlled conditions following Gold et al. (2003a) and modified by Bere and Tundisi (2011b, 2011c; Fig. 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 water from the reference site that was supplemented with silica (~3.5 mg.L^{-1} final concentration), an essential nutrient for diatoms. The medium was replaced with fresh water from the reference site twice every week during the course of the experiment. 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}. Flow velocity was monitored daily and adjusted where necessary.

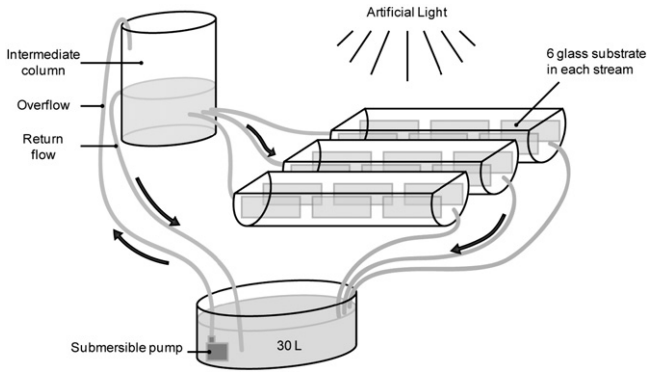

Fig. 1. Schematic representation of a closed experimental system, consisting of three artificial streams (50 cm length, 5 cm radius), each containing 6-glass substrata (6×15 cm). Arrows indicate flow direction (by: Ricardo M. Degani).

Each stream was fitted with 6 clean glass substrates (6×15 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 12 h/12 h. During the course of the experiment pH, temperature and dissolved oxygen (DO) level for each experimental unit were recorded daily using appropriate probes. Nitrates, orthophosphates and silica concentrations were measured daily by isocratic ion analysis using suspended conductivity detection chromatography (Dionex, DX-80, Ion Analyser).

2.3. Cd exposure

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 periphyton was acclimatised 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. This was done at each change of medium to maintain a relatively stable Cd concentration close to the required level. All solutions were undersaturated with respect to any solid oxide, hydroxide or carbonate phase of Cd (Table 1) as verified by calculation using MINEQL version 4.62.3 speciation software (Westall et al., 1976).

Table 1
Concentrations of Cd species calculated using MINEQL. The concentrations of all the labial forms of Cd were very low and thus undersaturated in all the treatments, hence did not interfere with the toxicity of Cd.

Species	Concentration (mg.L^{-1})
Free ion	5.96×10^{-2}
Hydroxides	6.97×10^{-9}
Nitrates	2.29×10^{-5}
Carbonates	1.02×10^{-5}
Chlorides	9.57×10^{-5}
Sulphates	3.46×10^{-4}

2.4. Biofilm sampling and analysis

Biofilms were collected after a colonization period of 1, 2 and 4 weeks. At each sampling time, 2 glass substrates were randomly removed from each stream chamber of each EU (n = 3 for each EU). The biofilm from the 2 glasses was brushed with a toothbrush into mineral water (drinking 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. 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 were 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 treatments was 86.1 ± 7.6%. 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, 2008a, 2008b; Duong et al., 2008, 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 °C for 1 h and weighed again to obtain ash-free dry weight (AFDW) and expressed as AFDW cm^{-2}. Growth rates inferred from AFDW measurement data were calculated for the exponential phase (Biggs, 1990) and were expressed as micrograms of AFDW 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 by representing the % growth as a function of log ([Cd]).

The fourth fraction (20 mL) was used to determine the total amount of metal accumulated in biofilm as described in Section 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 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.

2.5. Cd analysis

Samples from the fourth and fifth fractions were filtered through a tarred metal free paper (0.45 μm membrane, Millipore) to obtain the dry weight after drying at 60 °C for 48 h. The filters were rinsed with 100 mL of 5% nitric acid to remove any metals attached before use. 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), (Wuxi Jiebo Electrical Technology Co., Ltd., China). Total and non-exchangeable Cd in biofilms was estimated for the first and second weeks of the experiment because of financial constrains.

2.6. Data analysis

Each EU received a different treatment, but stream chambers, and glass slides in each chamber within an EU were not completely independent of each other, and they were consequently considered as subsamples. Because the treatments were not 'truly' replicated, the experimental design required the use of mixed-effects modelling techniques, and the results need to be taken with caution.

Variations in physicochemical characteristics of the water, diatom community structure (species richness, diversity, cell densities and relative abundance), chlorophyll *a*, DW, AFDW, 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).

3. Results

3.1. 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 2). The pH was generally slightly higher in the control compared to other EUs, decreasing as the concentration of Cd increased. This can be attributed to the acidic nature of the Cd stock solution added to the culture medium. However, the shift in pH was minor and of little consequence on Cd bioavailability. No trend in temperature change was detected with increasing Cd concentration with the temperature ranging from 21.1 to 23.1 °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 1.2 $mg.L^{-1}$, 24.4 $mg.L^{-1}$ and 3.5 $mg.L^{-1}$ respectively. Based on daily measurements (Table 2), 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 is because of high growth rate recorded at low compared to high Cd (>0.03 $mg.L^{-1}$) concentrations (Section 3.2). This trend in nutrient concentrations was observed among all the EUs throughout the experiment, with change of culture medium twice a week restoring nutrients to initial levels in all systems.

Table 2
The mean (n = 28) and standard deviation of physicochemical parameters in all the systems measured daily during a 4-week experimental period.

Treatment ($mg.L^{-1}$)	DO ($mg.L^{-1}$)	Temperature (°C)	pH	Cd ($mg.L^{-1}$)	N-NO_3 ($mg.L^{-1}$)	P-PO_4 ($mg.L^{-1}$)	SO_2 ($mg.L^{-1}$)
Control	6.4 ± 0.50	22.6 ± 0.5	8.0 ± 0.2	<0.001	21.8 ± 2.3	0.8 ± 0.4	2.2 ± 1.3
0.005	6.5 ± 0.40	22.8 ± 0.5	7.9 ± 0.1	0.005 ± 0.001	21.4 ± 2.1	0.7 ± 0.4	2.1 ± 1.1
0.01	6.6 ± 0.02	22.5 ± 0.6	7.8 ± 0.2	0.010 ± 0.001	21.7 ± 2.2	0.7 ± 0.5	2.3 ± 1.5
0.03	6.4 ± 0.10	22.5 ± 0.6	7.8 ± 0.3	0.030 ± 0.001	22.6 ± 2.0	0.9 ± 0.2	2.8 ± 0.7
0.05	6.3 ± 0.04	22.4 ± 0.6	7.8 ± 0.1	0.050 ± 0.002	23.4 ± 1.7	0.9 ± 0.3	2.9 ± 0.5
0.1	6.4 ± 0.20	22.4 ± 0.5	7.8 ± 0.1	0.100 ± 0.092	23.9 ± 1.4	0.9 ± 0.3	3.1 ± 0.4

3.2. Periphyton growth

Chlorophyll *a* concentrations recorded in the six EUs during the course of the experiment are shown in Fig. 2a. 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 × date effect at the 2nd and 4th weeks). A significant increase in chlorophyll *a* concentration ($p<0.05$ at week 4) was observed between the control (0.81 ± 0.01 $\mu g.cm^{-2}$) and 0.005 $mg.L^{-1}$ Cd treatment (1.68 ± 0.2 $\mu g.cm^{-2}$) at the 4th week of the experiment.

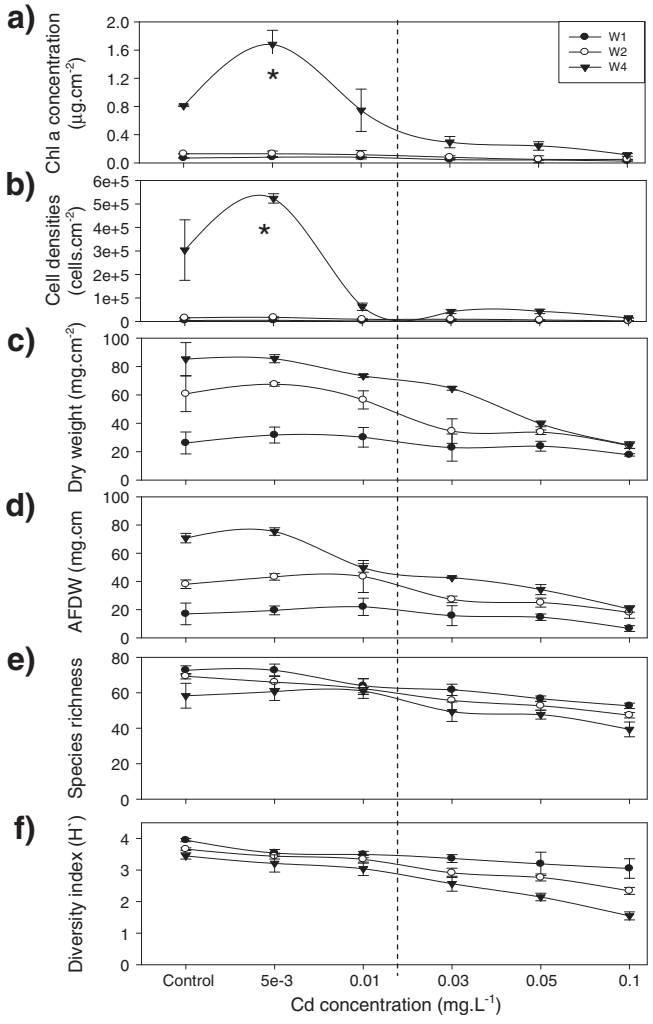

Fig. 2. The mean values and standard deviations (n = 3) of chlorophyll *a* (a), cell densities (b), dry weight (c), AFDW (d), diatom species richness (e) and diversity (f) developed on glass substrates in six experimental units in the first, second and fourth weeks of the experiment. The dashed line separates treatments that are significantly different from the control, whilst the star indicates treatments that are significantly different from the control at some stage 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 $\mu g.cm^{-2}$ 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 $\mu g.cm^{-2}$ 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 higher 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 × date effect at the 2nd and 4th weeks; Fig. 2c 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 0.005 $mg.L^{-1}$ 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 0.005 $mg.L^{-1}$ Cd treatment (0.073 ± 0.045 $AFDW.cm^{-2}.day^{-1}$; Fig. 3a). 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 0.005 $mg.L^{-1}$, hence negative inhibition value (−2.24%; Fig. 3b). 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.

3.3. Cadmium accumulation

Total and non-exchangeable Cd accumulated by biofilms is presented in Fig. 4. Cd levels were below the detection limit (1 $\mu g.L^{-1}$) in the control treatment. An exponential accumulation of total and non-exchangeable Cd in biofilms with increasing total Cd concentration was observed. Total and non-exchangeable Cd contents were significantly different among all the treatment (with $p<0.05$ for the treatment × 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

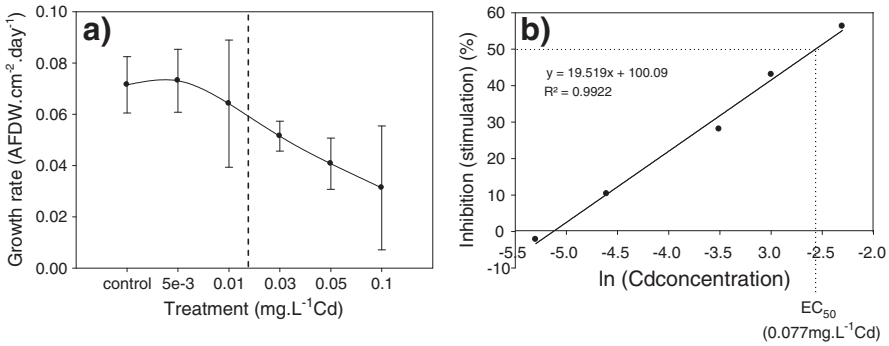

Fig. 3. Periphyton growth rate (a) and percentage inhibition (b) in the five different Cd treatments. Error bars: standard deviation of three replicates. The dashed line in (a) separates treatments that are significantly different from the control.

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 non-exchangeable/total Cd ratio was low at low Cd concentration (0.005 mg.L^{-1} = 0.40 and 0.59 at the 1st and 2nd weeks respectively and 0.01 mg.L^{-1} = 0.52 and 0.64 at the 1st and 2nd weeks respectively) compared to high Cd concentration treatments (0.03 mg.L^{-1} = 0.62 and 0.65 at the 1st and 2nd weeks respectively; 0.05 mg.L^{-1} = 0.72 and 0.69 at the 1st and 2nd weeks respectively; 0.1 mg.L^{-1} = 0.76 and 0.69 at the 1st and 2nd weeks 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 metal concentrations and duration of exposure (Fig. 5) (with $p<0.05$ for the treatment × date effect). Gross uptake capacity of biofilms (i.e., the ratio of total Cd accumulated to total metal) increases linearly with increasing Cd concentration: from 22.6 ± 6 to 38.0 ± 9 and from 33.7 ± 5 to 51.6 ± 7 at the 1st and 2nd weeks respectively, ($R^2 = 0.92$ and 0.97 at the 1st and 2nd weeks respectively, Fig. 5). The CFs based on non-exchangeable Cd also expressed an increased Cd sorption activity with increasing Cd concentrations in the treatments: from 9.1 ± 6 to 28.0 ± 4 and from 19.6 ± 9 to 34.6 ± 5 at the 1st and 2nd weeks respectively, ($R^2 = 0.89$ and 0.93 at the 1st and 2nd weeks respectively, Fig. 5).

3.4. 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 × date effect at the 2nd and 4th weeks; Fig. 2b). A significant increase in cell densities ($p<0.05$ at week 4) was observed between control (303 704 ± 128 780 cells.cm^{-2}) and 0.005 mg.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 (4800 ± 1700 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 (1341 ± 516 at week 1 to 15 130 ± 1013 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 × date effect at the 2nd and 4th weeks; Fig. 2e and f respectively). Species richness was generally highest in the control (73 ± 3 and 58 ± 7 for the 1st and 4th weeks respectively) and lowest in 0.1 mg.L^{-1} Cd treatment (53 ± 2 and 39 ± 4 for the 1st and 4th weeks 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 weeks 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 weeks 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 (Fig. 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 the first and second weeks of the experiment, whilst the second group comprised of communities from all the EUs recorded at the fourth week. Within these two major groups, subgroups were observed which roughly reflected changes in community composition due to changes in Cd

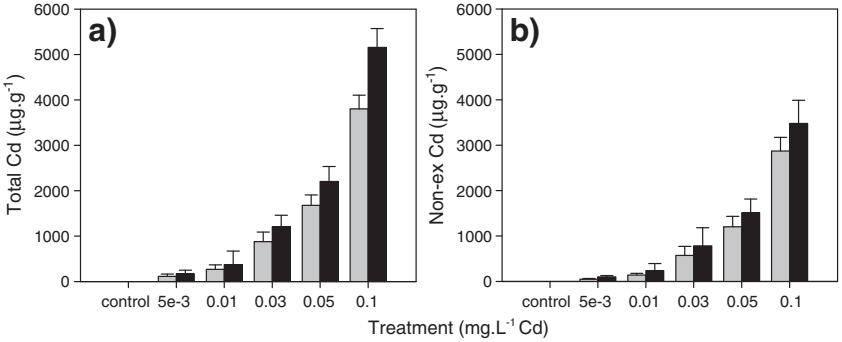

Fig. 4. Biofilm accumulation of total (a) and non-exchangeable (b) Cd in the six experimental units: grey bars — 1st week; black bars — 2nd week. Error bars: standard deviation of three replicates.

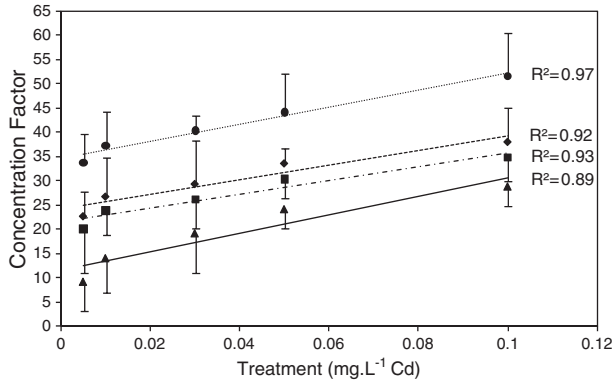

Fig. 5. Concentration factors of total and non-exchangeable Cd with increasing total Cd levels in the experimental units: diamonds and circles — based on total Cd at the 1st and 2nd weeks respectively; triangles and squares — based on non-exchangeable Cd at the 1st and 2nd weeks respectively. Error bars: standard deviation of three replicates.

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 (Fig. 7). After 1 week of colonization, diatom composition in the six systems was similar with the presence of *Navicula viridula* (Kützing) Kützing, *Eunotia bilunaris* (Ehrenberg) Mills, *Eunotia pectinalis* (Kützing) Rabhenhost, *Diatoma vulgare* Bory, *Navicula cryptocephala* (Grunow) Cleve and *Achnanthidium minutissimum* (Kützing) Czarnecki. 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 weeks 1 and 2 with the proliferation of *A. minutissimum* and *Ulnaria ulna* (Nitzsch) Compère 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 diatom communities to metal contamination was characterized by the appearance of diatom deformities. The frequency of diatom frustules 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 (Table 3). The percentage of abnormal frustules reached 1% at W2 for 0.1 mg.L^{-1} Cd and W4 for 0.03, 0.05 and 0.1 mg.L^{-1} Cd. 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 × date effect at the 2nd and 4th weeks). 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.

4. Discussion

4.1. Effects of Cd on periphyton growth

The present study showed that slight increase in Cd concentration (from control to around 0.005 mg.L^{-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 is a scarcely reported phenomenon that is interesting and notable given that Cd is usually considered a non-essential metal although a marine algal species has been shown to use it as an essential metal (Lee and Roberts, 1995; Lane and Morel, 2000; Lane et al., 2005). Other authors have reported a small and often

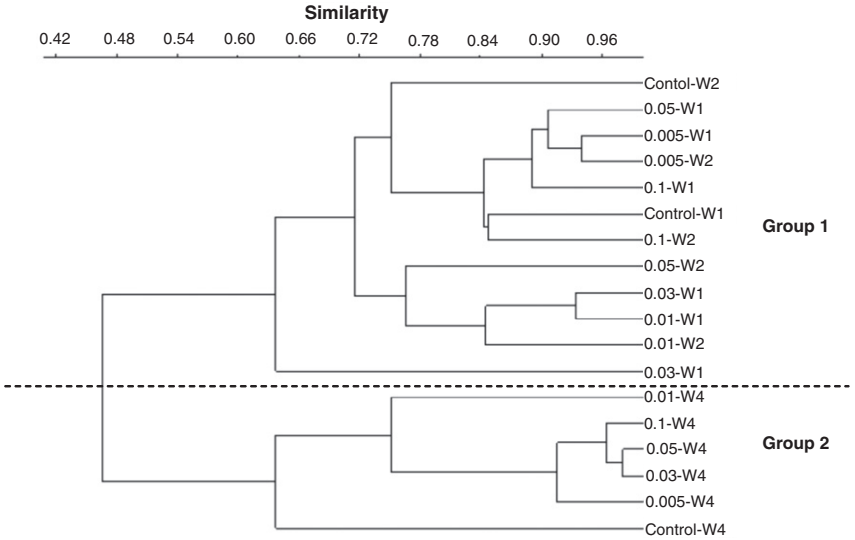

Fig. 6. Cluster analysis showing similarities in taxonomic composition of diatom communities developed on glass substrates in six experimental units in the first, second and fourth weeks of the experiment.

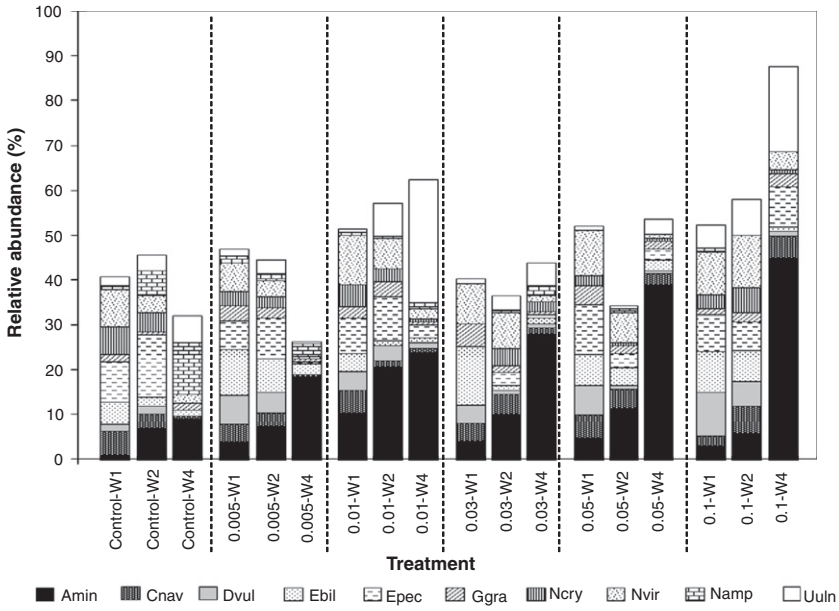

Fig. 7. The relative abundance of the 10 major diatom species from diatom communities recorded at 5 Cd concentrations ($mg.L^{-1}$) during the first, second and fourth weeks of the experiment. Amin, *Achnanthidium minutissimum* (Kützing) Czarnecki; Cnav, *Cymbopleura naviculiformis* (Auerswald) Krammer; 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.

statistically insignificant increase in biofilm growth at low metallic exposure levels as in this study (e.g. Morin et al., 2008a) though the mechanism is not clear. The results need to be replicated in studies that utilized more exposures in the low dose range to carefully explore the underlying mechanisms. According to Gadd (1988), this could be due to the fact that low Cd concentrations could reduce the abundance of some of the most sensitive taxa, providing a competitive advantage to relatively more tolerant taxa, such as *A. minutissimum* (the relative abundance of this species increased between weeks 2 and 4 in the 0.005 $mg.L^{-1}$ treatment). For that reason, care should be taken when using periphyton communities to assess low levels of Cd as total biomass estimates, like chlorophyll *a*, DW and AFDW, by themselves may not respond to stress at the community level if the abundance of sensitive populations is replaced by tolerant species (Gadd, 1988). More work is needed to elucidate the mechanism behind the simulation of periphyton growth at low Cd concentrations.

However, increasing the concentration of Cd beyond 0.01 $mg.L^{-1}$ reduced periphyton growth and development. 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; Morin et al., 2008a, 2008b; Duong et al., 2008, 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 (>0.01 $mg.L^{-1}$ used in this study) 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 Cd concentration (>0.01 $mg.L^{-1}$) has 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 beyond this concentration.

Little 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. 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. However, the complexity of periphyton communities used in this study makes interpretation of causative factors more difficult. In addition, 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).

Table 3
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. The dotted line separate treatments that are significantly different from the control.

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

4.2. Sorption kinetics of cadmium in the biofilms

As previously hypothesised, total and non-exchangeable Cd content in biofilms reflected the effects of 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 and duration of

exposure in all the systems (Fig. 4). Cd concentration was below 1 μg.L^{-1} in the control mesocosm but higher and therefore quantifiable in other mesocosms. 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 potential monitors of river metal pollution the same way as measurements of metals in the sediment and suspended solids are used (Fuchs et al., 1996). However, using biofilms to monitor metal pollution is complicated, for instance, due to the possible production of metal-binding EPS by microorganisms (algae and bacteria) as a possible response to metal pollution. This factor is likely to influence biofilm metal accumulation and is dependent on biofilm species composition. Thus, the results suggest that biofilms may be used for metal monitoring but further studies are needed before they are routinely used for that purpose.

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 were 22.6 to 51.7 folds higher than in the water column, with a positive linear relationship between water column Cd concentrations and CFs (Fig. 5). For this reason, chemical analysis of water by itself may not be sufficient for assessing environmental stress because periphyton can significantly decrease dissolved metal concentrations, 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).

Algal cell 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 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 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 can be attributed to algal growth, with new cells providing additional sorption sites (Hill et al., 2000). It can also be attributed to the slow metabolism-dependent uptake process.

4.3. Effects of Cd on diatom communities

Slight increase in Cd concentration resulted in reduction in species richness and diversity. Similar community-level Cd toxicity tests using periphyton have also shown that inorganic stressors like Cd, Pb, Ni and so on 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 throughout the experiment and is typical of metal polluted rivers (Genter, 1996; Morin et al., 2007).

The diatom assemblages present during the first week were similar in all the six systems. At week 4, cluster analysis separates the control from the Cd-exposed biofilms and the low exposure level (0.005) from the other metal concentrations but no such result can be obtained at W1 and W2. This is supported by results for chlorophyll *a*, cell densities, DW and AFDW (Fig. 2). This seems to suggest that the results obtained at W1 and W2 do not help in assessing the impacts of Cd exposure, whereas clear effects can be seen at W4. This could be due to the presence of too little material or too much variability on the parameters during the first two weeks of the experiment.

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*, *N. viridula* and *N. cryptocephala* at the 2nd and 4th weeks 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 exposed 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; Morin et al., 2007, 2008a, 2008b; Duong et al., 2008, 2010). The proliferation of *A. minutissimum* with increasing Cd concentration and duration of exposure (45.00% at 0.1 mg.L^{-1} Cd during the 4th week) seems to indicate favour and tolerance of this species to Cd contamination. Indeed Takamura et al. (1989) showed that *A. minutissimum* extracted from high Cu environments could still grow at Cd concentration > 12.7 mg.L^{-1} in acute experiments, 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 demonstrate 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 also manifests itself through morphological deformities of some diatom cells in the communities. In this study, an increase in abnormal valve frequency with increasing Cd concentration and duration of exposure was observed corroborating 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 biological monitoring of lotic systems (e.g. Round, 1991; Kelly and Whitton, 1995; Pan et al., 1996; Biggs and Kilroy, 2000) are non-specific in diagnosing toxic pollutions. 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 incorporate these biological traits in 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.

5. Conclusions

Periphyton growth and development are substantially lowered by high concentration of Cd ($EC_{50} = 0.077$ mg.L^{-1}). Levels of adsorbed and intracellular Cd levels in periphyton are a function of duration of exposure and the total Cd concentrations in the culture media. 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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References

Bates SS. Ecophysiology and metabolism of ASP toxin production. In: Anderson DM, Cembella AD, Hallegraeff GM, editors. Physiological ecology of harmful algal blooms. Springer-Verlag: Heidelberg; 1998. p. 405–26.

Bere T, Tundisi JG. The effects of substrate type on diatom-based multivariate water quality assessment in a tropical river (Monjolinho), São Carlos, SP, Brazil. Water Air Soil Pollut 2011a;216:391–409.

Bere T, Tundisi JG. Toxicity and sorption kinetics of dissolved cadmium and chromium III on tropical freshwater periphyton communities in laboratory mesocosm experiments. Sci Total Environ 2011b;409:4772–80.

Bere T, Tundisi JG. Cadmium and lead toxicity on tropical freshwater periphyton communities under laboratory-based mesocosm experiments. Hydrobiologia 2011c;680:187–97. http://dx.doi.org/10.1007/s10750-011-0917-8.

Bicudo CEM, Menezes M. Gêneros de água de águas continentais do Brasil: chave para identificação e descrições. São Carlos-SP, Brazil: Rima Editora; 2006.

Biggs BJF. Use of relative specific growth rates of periphytic diatoms to assess enrichment of a stream. N Z J Mar Freshw Res 1990;24:9-18.

Biggs BJF, Kilroy C. Stream periphyton monitoring manual. Christchurch, New Zealand: NIWA; 2000.

Drum RW. Notes on Iowa diatoms. VI. Frustular aberrations in *Surirella ovalis*. J Iowa Acad Sci 1964;71:51–5.

Duong TT, Morin S, Herlory O, Feurtet-Mazel A, Coste M, Boudou A. Seasonal effects of cadmium accumulation in periphytic diatom communities of freshwater biofilms. Aquat Toxicol 2008;90:19–28.

Duong TT, Morin S, Coste M, Herlory O, Feurtet-Mazel A, Boudou A. Experimental toxicity and bioaccumulation of cadmium in freshwater periphytic diatoms in relation with biofilm maturity. Sci Total Environ 2010;408:552–62.

Fechner LC, Gourlay-Francé C, Bourgeault A, Tusseau-Vuillemin MH. Diffuse urban pollution increases metal tolerance of natural heterotrophic biofilms. Environ Pollut 2012;162:311–8.

Foster P. Concentrations and concentration factors of heavy metals in brown algae. Environ Pollut 1976;10:45–53.

Fuchs S, Haritopoulou T, Wilhelmi M. Biofilms in freshwater ecosystems and their use as a pollutant monitor. Water Sci Technol 1996;34:137–40.

Gadd GM. Accumulation of metals by microorganisms and algae. In: Rehm HJ, editor. Biotechnology, 60. VCH Weinheim; 1988. p. 401–34.

Genter RB. Ecotoxicology of inorganic chemical stress to algae. In: Stevenson RJ, Bothwell ML, Low RL, editors. Algal ecology: freshwater benthic systems. New York: Academic press; 1996. p. 404–69.

Genter RB, Cherry DS, Smith EP, Cairns Jr J. Attached-algal abundance altered by individual and combined treatments of zinc and pH. Environ Toxicol Chem 1988;7:723–33.

Gold C, Feurtet-Mazel A, Coste M, Boudou A. Effects of cadmium stress on periphytic diatom communities in indoor artificial streams. Freshw Biol 2003a;48:316–28.

Gold C, Feurtet-Mazel A, Coste M, Boudou A. Impacts of Cd and Zn on the development of periphytic diatom communities in artificial streams located along a river pollution gradient. Arch Environ Contam Toxicol 2003b;44:189–97.

Guanzon NG, Nakahara H, Yoshida Y. Inhibitory effects of heavy-metals on growth and photosynthesis of three freshwater microalgae. Fish Sci 1994;60:379–84.

Hammer O, Harper DAT, Ryan PD. PAST — PAlaeontological STatistics. version 2.5. http://folk.uio.no/ohammer/past.

Hill WR, Bednarek AT, Larsen IL. Cadmium sorption and toxicity in autotrophic biofilms. Can J Fish Aquat Sci 2000;57:530–7.

Husaini Y, Rai LC. Studies on nitrogen and phosphorusmetabolism and the photosynthetic electron-transport system of *Nostoc linckia* under cadmium stress. J Plant Physiol 1991;138:429–35.

Ivorra N, Bremer S, Guasch H, Kraak MHS, Admiraal W. Differences in the sensitivity of benthic microalgae to Zn and cadmium regarding biofilm development and exposure history. Environ Toxicol Chem 2000;19:1332–9.

Ivorra N, Hettelaar J, Kraak MHS, Sabater S, Admiraal W. Responses of biofilms to combined nutrient and metal exposure. Environ Toxicol Chem 2002;21:626–32.

Kelly MG, Whitton BA. The trophic diatom index: a new index for monitoring eutrophication in rivers. J Appl Phycol 1995;7:433–44.

Lane T, Morel FFM. A biological function for cadmium in marine diatoms. Biochem 2000;97:4627–31.

Lane T, Saito MA, George GN, Pickering IJ, Prince RC, Morel FFM. Isolation and preliminary characterization of a cadmium carbonic anhydrase from a marine diatom. Nature 2005;435:42-142.

Larned ST. A prospectus for periphyton: recent and future ecological research. J N Am Benthol Soc 2010;29:182–206.

Lee JG, Roberts SB. Cadmium: a nutrient for the marine diatom *Thalassiosira weissflogii*. Limnol Oceanogr 1995;40:1056–63.

Metzeltin D, Lange-Bertalot H. Tropical diatoms of South America I. Iconogr Diatomol 1998;5:1-695.

Metzeltin D, Lange-Bertalot H. Tropical diatoms of South America II. Iconogr Diatomol 2007;18:1-877.

Metzeltin D, Lange-Bertalot H, García-Rodríguez F. Diatoms of Uruguay. Iconogr Diatomol 2005:1-736.

Morin S, Vivas-Nogues M, Duong TT, Boudou A, Coste M, Delmas F. Dynamics of benthic diatom colonization in cadmium/zinc-polluted river (Riou-Mort, France). Fundam Appl Limnol 2007;168:179–87.

Morin S, Duong TT, Dabrin A, Coynel A, Herlory O, Baudrimont M, et al. Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. Environ Pollut 2008a;151:532–42.

Morin S, Duong TT, Olivier H, Feurtet-Mazel A, Coste M. Cadmium toxicity and bioaccumulation in freshwater biofilms. Arch Environ Contam Toxicol 2008b;54:173–86.

Nunes ML, Ferreura da Silda E, de Almeida SFP. Assessment of water quality in the Caima and Mau river Basins (Portugal) using geochemical and biological indices. Water Air Soil Pollut 2003;149:227–50.

Nusch EA. Comparison of different methods for chlorophyll and phaeopigment determination. Arch Hydrobiol Beih Ergeb Limnol 1980;14:14–36.

Pan Y, Stevenson RJ, Hill BH, Herlihy AT, Collins GB. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. J N Am Bethol Soc 1996;15:481–95.

Pappas JL, Stoermer EFF. Quantitative method for determining a representative algal sample count. J Phycol 1996;32:693–6.

Rai LC, Gaur JP, Kumar HD. Phycology and heavy-metal pollution. Biol Rev Camb Philos Soc 1981;56:99-151.

Rivkin RB. Effects of lead on growth of the marine diatom *Skeletonema costatum*. Mar Biol 1979;50:239–47.

Round FE. Diatoms in river water-monitoring studies. J Appl Phycol 1991;3:129–45.
Soldo D, Hari R, Sigg L, Behra R. Tolerance of the *Oocystis nephrocytioides* to copper: intracellular distribution and extracellular complexation of copper. Aquat Toxicol 2005;71:307–17.
Stevenson RJ, Bothwell ML, Lowe RL. Algal ecology—freshwater benthic ecosystems. San Diego: Academic Press; 1996. p. 750.
Takamura N, Kasai F, Watanabe MM. Effects of Cu, Cd and Zn on photosynthesis of freshwater benthic algae. J Appl Phycol 1989;1:39–52.
Thomas WH, Hollibaugh JT, Seibert DLR. Effects of heavy metals on the morphology of some marine phytoplankton. Phycologia 1980;19:202–9.
Westall JC, Zachary JL, Morel FMM. A computer program for the calculation of chemical equilibrium composition aqueous systems. Tech. Note No. 18. R.M.Parsons Lab for Water Resources and Environmental Engineering, MIT, Cambridge, Department of Civil Engineering; 1976.
Wong PTS. Toxicity of cadmium to freshwater microorganisms, phytoplankton, and invertebrates. In: Nriagu JO, Sprague JB, editors. Cadmium in the aquatic environment. New York: Wiley; 1987. p. 117–38.