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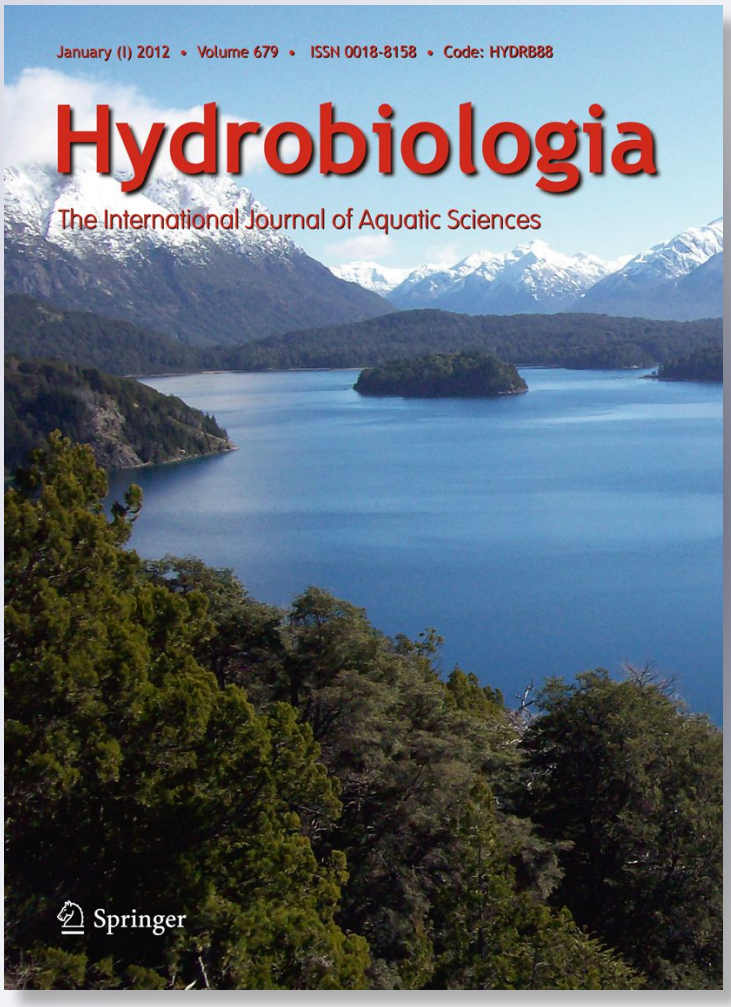

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PRIMARY RESEARCH PAPER

Cadmium and lead toxicity on tropical freshwater periphyton communities under laboratory-based mesocosm experiments

Taurai Bere · José Galizia Tundisi

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Abstract Periphyton constitutes an important community that is useful for assessment of ecological conditions in lotic systems. The objective of this study was to assess the effects of different mixtures of Cd and Pb on periphyton growth as well as Cd and Pb mixtures toxicity to diatom assemblages in laboratory mesocosm experiments. A natural periphyton community sampled from the Monjolinho River (South of Brazil) was inoculated into five experimental systems containing clean glass substrates for periphyton colonization. The communities were exposed to mixtures of dissolved Cd and Pb concentrations of 0.01 and 0.1 mg l^{-1} Cd and 0.033 and 0.1 mg l^{-1} Pb. Periphyton ash-free dry weight, growth rate, diatom cell density and diatom community composition were analyzed on samples collected after 1, 2 and 3 weeks of colonization. High Cd concentration (0.1 mg l^{-1}) has negative effects on periphyton growth while high concentration of Pb (0.1 mg l^{-1}) decreased the toxic effects of Cd on periphyton growth. Shifts in species composition (development of more resistant species like *Achnanthidium minutissimum* and reduction of sensitive ones like *Cymbopleura naviculiformis*, *Fragilaria capucina*, *Navicula cryptocephala*, *Encyonema silesiacum*, *Eunotia bilunaris*, and *Gomphonema parvulum*), decreases in species diversity of diatom communities with increasing Cd and Pb concentrations and exposure duration have been demonstrated in this study making diatom communities appropriate monitors of metal mixtures in aquatic systems.

Keywords Cd · Pb · Toxicity · Periphyton · Diatom communities

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Introduction

Periphyton, a biological community of attached autotrophic and heterotrophic organisms that are associated in complex matrix of polysaccharide exudates and detritus (Stevenson et al., 1996), are considered solar-powered biogeochemical reactors, biogenic habitats, hydraulic roughness elements, early warning systems for environmental degradation, and troves of biodiversity (Larned, 2010). Thus, they are important communities that are useful for assessment of ecological conditions in lotic systems. In particular, diatoms communities, which constitute the major part of periphyton communities, are composed by a large number of species with various ecological tolerances

and preferences, thus, constituting a well-adapted biological model for environmental monitoring (US EPA, 2001).

Periphytic diatoms are widely employed as ecological indicators of organic pollution (Descy & Coste, 1991) and eutrophication (Kelly & Whitton, 1995) in rivers. Nevertheless, validity of diatoms as indicators of metal pollution is difficult to determine in situ, e.g. even if sites show clear differences in metal concentrations, both in the water column and in the sediments, because variation in other environmental factors of natural and/or anthropogenic origin are inevitable between such sites (Gold et al., 2003a; Duong et al., 2010). To overcome the challenges associated with use of diatoms as indicators of metal pollution in situ, laboratory experiments, conducted under controlled conditions, are performed. Results of these experiments are then extrapolated to predict natural systems. However, most of these experiments are based on toxicological responses at an individual or population level with no information on complex web of symbiotic interactions (i.e. competitive exclusion, predation or parasitism during succession, mutualism or commensalism) typical of natural communities (Clements et al., 1989), making the extrapolation of this data to natural systems dubious.

More emphasis is now being given to mesocosm community experiments that better mimic field conditions compared to single species tests and enables improved accuracy in the extrapolations from laboratory bioassays to responses in natural systems. For that reason, many laboratory-based metal toxicity experiments have been realized on the effects of Cd on periphyton communities (Gold et al., 2003a; Morin et al., 2008a; Duong et al., 2010). These studies focus on Cd only, but in nature, many metals are present at a given site at the same time (Bere & Tundisi, 2011; Altenburger, 2011). For example, Pb levels in aquatic systems increased during the industrial age and have risen rapidly since Pb was added to gasoline fuel of vehicles (Nriagu, 1978; Harrison & Laxen, 1981; Waldock, 1998). At the same time, Cd is widely found in ship paints (Nriagu, 1980), so can be found in large amounts in ports where Pb (though fuels) may also occur. Thus, the objective of this study was to assess the effects of different mixtures of Cd and Pb on periphyton growth as well as Cd and Pb mixtures toxicity to diatom assemblages in laboratory mesocosm experiments.

Materials and methods

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 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 at the reference site (Bere & 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 (Biggs & Kilroy, 2000).

Two 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 rakes were carefully removed from the river and periphyton colonizing the glass substrate was brushed with a toothbrush into culture medium. The periphyton from all the glass substrates was pooled into one sample of ~2 l. This periphyton suspension was transported to the laboratory in cooler box (4°C).

Laboratory experimental design

Five closed experimental systems (hereafter referred to as experimental units; EUs) were set up to allow the exposure of natural periphytic communities to stressors under controlled conditions following Gold et al. (2003a, b; 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 Woods Hole culture medium (Nichols, 1973) modified by diluting (4×) after Gold et al. (2003a). This culture medium was kept without ethylenediaminetetraacetic acid (EDTA), which presents very high binding capacities for metals, and supplemented with silica, an essential diatom nutrient. Test medium were

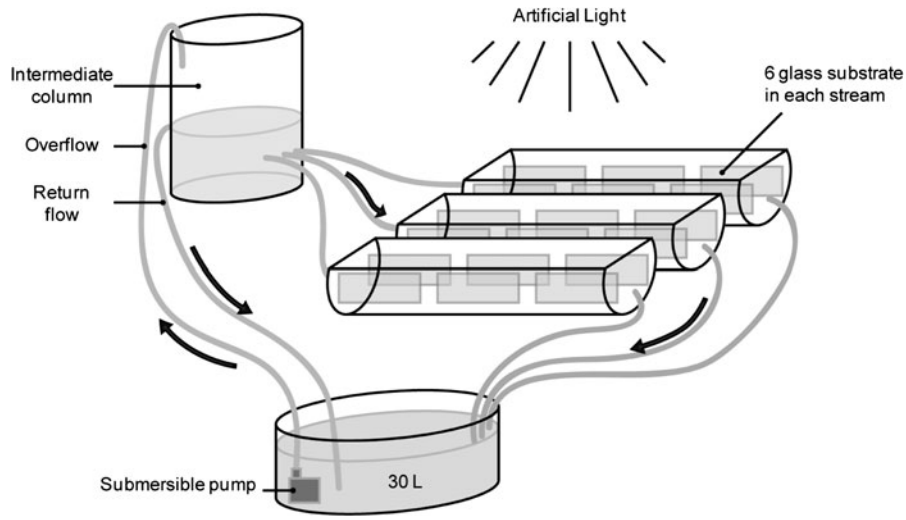

Fig. 1 Schematic representation of a closed experimental system, consisting of three artificial streams (50 cm length, 5 cm radius), each containing 6-glass substrata (6 × 15 cm). *Arrows* indicate flow direction (by: Ricardo M. Degani)

prepared from distilled water. A pump 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 six clean glass substrates (6 × 15 cm) in a slightly slanting position for periphyton colonization. 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) was maintained with a light: dark regime of 12 h/12 h.

During the course of the experiment pH, conductivity, temperature and dissolved oxygen (DO) levels for each experimental unit were recorded daily. Water samples for nutrient analysis (phosphates, silica and nitrates) were collected every 2 days from each stream. These samples were filtered through pre-combusted Whatman GF/F filters and analyzed for the nutrients following standard methods (APHA, 1988). Based on these measurements, the nutrients were adjusted accordingly.

Metal exposure

Homogenized periphyton suspension from the field was divided into five 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. EU_2 was contaminated with 0.01 mg l^{-1} (low) Cd and 0.033 mg l^{-1} (low) Pb. EU_3 was contaminated low Cd and 0.1 mg l^{-1} (high) Pb. EU_4 was contaminated with 0.1 mg l^{-1} (high) Cd and low Pb. EU_5 was contaminated with high Cd and high Pb. Cadmium chloride ($CdCl_2$, 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 and Pb concentrations were measured twice per week during the experiment by atomic absorption spectrophotometry (Varian AA 400) equipped with a model GTA graphic tube atomizer and auto- sampler. Based on these measurements, levels of Cd and Pb in contaminated systems were readjusted twice a week, to maintain relatively stable concentrations close to the required levels.

Periphyton sampling and analysis

Periphyton was collected after a colonization period of 1, 2, and 3 weeks. At each sampling time, two glass substrates were randomly removed from each stream of each EU ($n = 3$ for each EU). The periphyton from the two glasses were brushed with a toothbrush into mineral water and the resultant periphyton suspensions from the two glasses were pooled to make one sample and making the volume of the suspension to 50 ml. After each sampling time, the artificial streams were reset by new glass substrates to maintain identical flow conditions.

The periphyton suspensions were then divided into three fractions each for various analyses. The first fraction (10 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 ×400 with cell densities expressed as living algal cells (containing chlorophyll) 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 sulfuric acid and mounted in Naphrax (Northern Biological supplies Ltd. UK. RI = 1.74) following Biggs & Kilroy (2000). A total of 250–600 valves per sample were identified and counted using the phase contrast light microscope (1000×) (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), 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 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 according to 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. After final weighing, samples were ashed at 500°C for 1 h and weighed again to obtain ash-free dry mass (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 per day (Biggs, 1990). From these growth rates, the percent inhibitions of each treatment were calculated (Biggs, 1990).

Data analysis

Variations in physicochemical characteristics of the water, diatom community structure (species richness, diversity and cell densities), chlorophyll *a* and AFDM of periphyton with treatments and duration of exposure were examined by means of repeated measures analysis of variance (RM-ANOVA). Treatments were used as fixed factors among objects, and time a fixed factor within objects. Variations in periphyton growth rate and percentage growth inhibition with treatments were examined by means of one-way ANOVA. Tukey's HSD pairwise comparison test ($P < 0.05$) was used to test for significant differences in periphyton species richness, diversity, cell densities, chlorophyll *a*, AFDM and growth rate among treatments where significant ANOVA effects were detected. Taxonomic differences between the different treatments were revealed using principal component analyses (PCA) using Palaeontological Statistics (PAST) software version 1.95 (Hammer et al., 2009). RM-ANOVA and one-way ANOVA were carried out using STATISTICA software package, Release 7, Stat Soft. Inc., USA.

Results

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 1). 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 while pH increased slightly in all the systems during the cause of the experiment. On the other hand, conductivity increased significantly with increasing Cd and Pb concentration and duration of the experiment ($P < 0.05$).

Periphyton growth

Chlorophyll *a* concentration and AFDW were significantly higher ($P < 0.05$) in the control and low Cd concentration treatments compared to the high Cd concentration treatments (Fig. 2a and b, respectively). During the first week of the experiment, highest levels of chlorophyll *a* concentration and AFDW were recorded in the control, with chlorophyll *a* and AFDW being significantly higher in control and low than in high Cd treatments. However, during the 2nd and 3rd week of the experiment, an increase in chlorophyll *a* concentration and AFDW was observed in low Cd concentration treatments (being significantly higher in

Table 1 Water column physicochemical parameters (mean value and standard deviation) in all the systems measured during a 3-week experimental period

	Control	Low Cd low Pb	Low Cd high Pb	High Cd low Pb	High Cd high Pb
Temperature (°C)					
W1	23.9 ± 1.1	23.8 ± 1.3	23.7 ± 1.8	23.8 ± 1.5	23.8 ± 1.9
W2	23.8 ± 1.4	23.8 ± 1.8	23.8 ± 1.8	23.8 ± 1.6	23.9 ± 1.5
W3	23.9 ± 1.7	23.9 ± 1.5	23.9 ± 1.6	23.9 ± 1.7	23.9 ± 1.8
pH					
W1	7.31 ± 0.01	7.32 ± 0.03	7.31 ± 0.04	7.32 ± 0.01	7.32 ± 0.01
W2	8.01 ± 0.03	7.74 ± 0.02	7.83 ± 0.05	7.74 ± 0.04	7.62 ± 0.02
W3	8.02 ± 0.14	8.13 ± 0.05	8.14 ± 0.10	7.82 ± 0.17	7.84 ± 0.14
DO (mg l^{-1})					
W1	6.4 ± 0.2	6.4 ± 0.2	6.3 ± 0.3	6.3 ± 0.1	6.3 ± 0.2
W2	6.4 ± 0.2	6.4 ± 0.3	6.3 ± 0.1	6.3 ± 0.2	6.3 ± 0.2
W3	6.4 ± 0.5	6.4 ± 0.4	6.4 ± 0.1	6.3 ± 0.2	6.3 ± 0.1
Conductivity (μS cm^{-1})					
W1	210.4 ± 1.6	223.4 ± 3.1	258 ± 3.3	224 ± 5.6	241.1 ± 7.7
W2	231.3 ± 1.3	234.2 ± 5.3	243.2 ± 9.8	280.3 ± 6.4	288.2 ± 4.4
W3	303.4 ± 2.3	306.1 ± 9.1	342.4 ± 6.9	361.3 ± 6.8	328.4 ± 9.9

high Pb treatment) compared to the control. In all the systems, chlorophyll *a* concentration and AFDW increased significantly throughout the experiment with increase being higher in the control and low Cd concentrations compared to high Cd concentration ($P < 0.05$). In both low and high Cd treatments, increase in Pb concentration resulted in statistically significant ($P < 0.05$) increase in chlorophyll *a* concentration and AFDW, during the 2nd and 3rd week of the experiment.

Growth rate was also significantly higher ($P < 0.05$) in the control (0.066 ± 0.020 AFDW cm^{-2} day^{-1}) and low Cd concentration treatments (0.056 ± 0.010 and 0.064 ± 0.010 AFDW cm^{-2} day^{-1} for low Cd/low Pb and low Cd/high Pb treatments, respectively) than in high Cd concentration treatments (0.040 ± 0.020 and 0.041 ± 0.021 AFDW cm^{-2} day^{-1} for high Cd/low Pb and high Cd/high Pb treatments, respectively, Fig. 3a). In both low and high Cd treatments, increase in Pb concentration resulted in slight but statistically insignificant increase in growth rate ($P > 0.05$). Growth inhibition was significantly lower ($P < 0.05$) in low Cd concentration treatments (15.1 ± 2.0 and 2.1 ± 1.0% for low Cd/low mg l^{-1} Pb and low Cd/high Pb treatments, respectively) than in high Cd treatments (38.6 ± 5.0 and 31.8 ± 2.0% for high Cd/low Pb and high Cd/high Pb treatments, respectively, Fig. 3b). As in the case of chlorophyll *a* concentration and AFDW, an increase in Pb concentration in both low and high Cd concentration treatments resulted in corresponding significant ($P < 0.05$) decrease in growth inhibition.

Community structure and composition

Algal cell densities of control and low Cd treatments were significantly higher than in high Cd concentration treatments ($P < 0.05$). During the first week of the experiment, highest levels of algal cell densities were recorded in the control (52,246.9 ± 11,957.0 cells cm^{-2}), while lowest densities were recorded in high Cd/low Pb treatment (4,098.8 ± 3,909.4 cells cm^{-2}). However, during the 2nd and 3rd week of the experiment, a significant ($P < 0.05$) increase in algal cell density was observed in low Cd concentration treatments (low Cd/low Pb = 262,716.0 ± 10,009.1 and 377,382.7 ± 3,766.4 cells cm^{-2} for 2nd and 3rd week, respectively; low Cd/high Pb = 290,074.1 ± 5,824.6 and 404,345.8 ± 48,740.1 cells cm^{-2} for 2nd and 3rd week, respectively) compared to the control (239,555.5 ± 678.9 and 323,530.9 ± 8,022.4 cells cm^{-2} for 2nd and 3rd week, respectively).

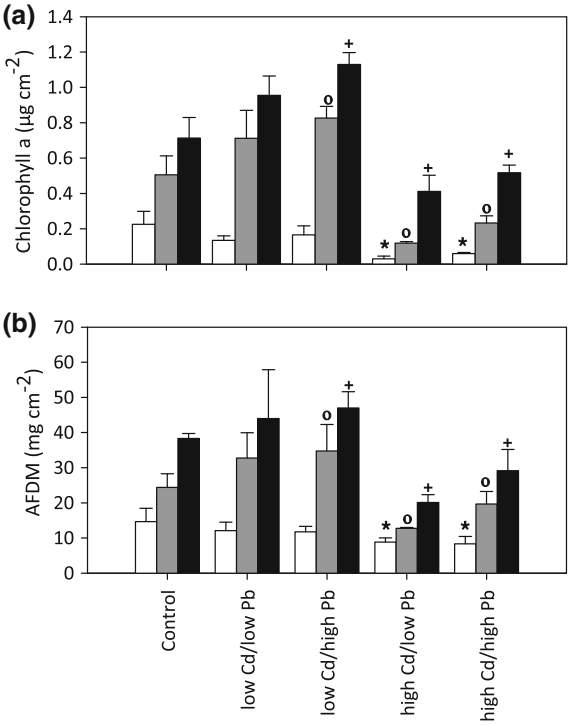

Fig. 2 The mean values and standard deviations ($n = 3$) of chlorophyll a (**a**) and AFDW (**b**) developed on glass substrates in five treatments during the first (*white*), second (*gray*), and third (*black*) week of the experiment. *Asterisk* significant difference ($P < 0.05$) with the control at first week, *open circle* significant difference ($P < 0.05$) with the control at second week and *plus* significant difference ($P < 0.05$) with the control at third week

Significantly low cell densities were recorded in high Cd/low Pb (26,814.8 ± 2,225.9 and 77,728.5 ± 9,567.4cells cm^{-2} for 2nd and 3rd week, respectively) and high Cg/high Pb (44,963.0 ± 12,317.7 and 126,197.5 ± 3027.4 cells cm^{-2} for 2nd and 3rd week, respectively) treatments compared to the control and low Cd treatments. In all the systems, cell densities increased significantly throughout the experiment with the increase being significantly higher in the control and low Cd concentrations than in high Cd concentration ($P < 0.05$). In both low and high Cd treatments, increase in Pb concentration resulted in significant increase in algal cell densities during the 2nd and 3rd week of the experiment ($P < 0.05$).

Species richness was also significantly higher in the control (82 ± 4, 77 ± 2 and 68 ± 3 for 1st, 2nd, and 3rd week, respectively) and low Cd concentrations (low Pb = 81 ± 3, 75 ± 5, and 63 ± 3; high Pb = 78 ± 8, 78 ± 3 and 97 ± 3 for the 1st, 2nd, and 3rd week respectively) compared to the higher Cd concentrations (low Pb = 80 ± 4, 77 ± 3, and 59 ± 4; high Pb = 81 ± 3, 69 ± 8 and 55 ± 4 for the 1st, 2nd, and 3rd week, respectively). Species diversity was also significantly higher in the control (3.7 ± 0.1, 3.2 ± 0.2 and 2.9 ± 0.1 for 1st, 2nd and 3rd week respectively) and low Cd concentrations (low Pb = 4.0 ± 0.1, 2.6 ± 0.1 and 2.6 ± 0.1; high Pb = 4.0 ± 0.2, 2.6, ± 0.1 and 2.5 ± 0.2 for the 1st, 2nd and 3rd week respectively) compared to the higher Cd concentrations (low Pb = 3.9 ± 0.1, 2.8 ± 0.1 and 2.1 ± 0.2; high Pb = 3.7 ± 0.1, 2.3 ± 0.1 and 1.3 ± 0.2 for the 1st, 2nd and 3rd week respectively). In all the systems, species richness and diversity decreased significantly throughout the experiment with the decrease being higher in the higher Cd concentration treatments compared to the control and low Cd concentration treatments ($P < 0.05$).

Of the 97 diatom species belonging to 40 genera that were recorded in all the EUs during the course of the study, nine 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. 4). 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.

At week 2, the dominant species were still similar with a general increase in the relative abundance of *A. minutissimum* in all the systems and a general decrease in *E. bilunaris*. The relative abundance of *A. minutissimum* increased notably with increasing Cd and Pb concentration with highest value (57.8 ± 6.5%) being recorded in high Cd/high Pb treatment. The relative abundance of *C. naviculiformis* and *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 except the control where its relative abundance decreased sharply. The relative

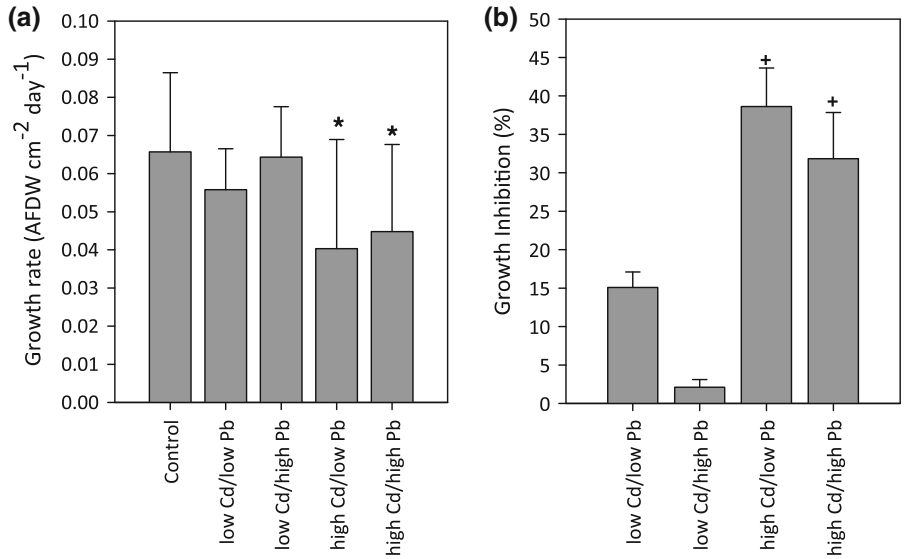

Fig. 3 Periphyton growth rate (**a**) in the control and the four treatments and percentage inhibition (**b**) in the four treatments during the experiment. *Asterisk* significant difference ($P < 0.05$) with the control and *plus* significant difference ($P < 0.05$) with low Cd concentration treatments

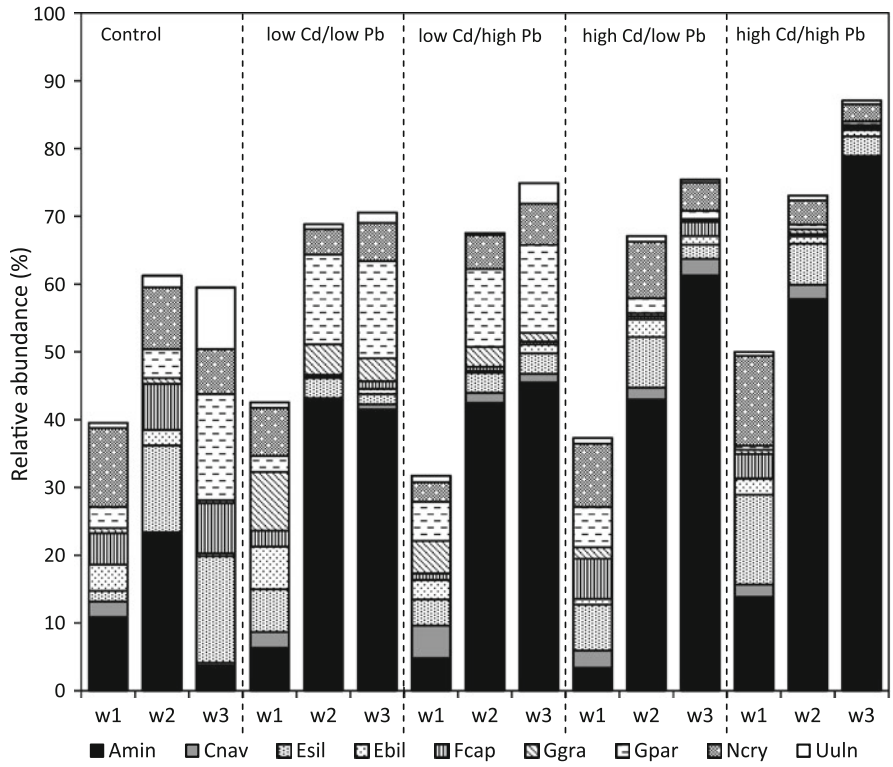

Fig. 4 The relative abundance of the nine major diatom species form diatom communities recorded in five treatments during the first, second and third week of the experiment. Amin, *Achnanthidium minutissimum* (Kützing) Czarnecki; Cnav, C*ymbopleura naviculiformis* (Auerswald) Krammer; Esil, *Encyonema silesiacum* (Bleisch) Mann; Ebil, *Eunotia bilunaris* (Ehrenberg) Mills; Fcap, *Fragilaria capucina* Desmazières; Ggra, *Gomphonema gracile* Ehrenberg; Gpar, *Gomphonema parvulum* (Kützing) Kützing; Ncry, *Navicula cryptocephala* (Grunow) Cleve; Uuln, *Ulnaria ulna* (Nitzsch) Compère

abundance of *A. minutissimum* was around 78.7% in the high Cd/high Pb treatment. The relative abundance of *Ulnaria ulna* (Nitzsch) Compère, *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 while the same tended to

decrease in other treatments with increasing exposure duration.

Taxonomic difference in diatom communities collected during the 3 weeks of the experiment in the five systems were investigated using PCA performed on the relative abundance of the 24 species with the highest cumulative abundance when all the communities were considered (Fig. 5). Changes in diatom community composition with treatment (especially change in Cd concentration) and duration of the experiment were observed during the experiment. PCA separated all communities from the 1st week of the experiment, as well as those from the control and low Cd communities of the 3rd week, from the rest of the communities along the first axis. These communities were closely related, being associated with such species as *C. naviculiformis* and *Eunotia intermedia* (Krasske & Hustedt) Nörpel & Lange-Bertalot. These communities were negatively associated with the fist axis of the PCA that accounted for 94.9% of the total variation of diatom data. Diatom communities from the 2nd and 3rd week of the experiment were also distributed along the first axis of the PCA with diatom community from the high Cd/high Pb treatment (3rd week) being notably distinct from the rest of the communities and strongly positively associated with the first PCA axis. This community was associated with *A. minutissimum*, which was also strongly positively associated with the first PCA axis. Separation along axis 2 (accounting for 2.6% of total variation) resulted mainly from other species especially *G. parvlum*.

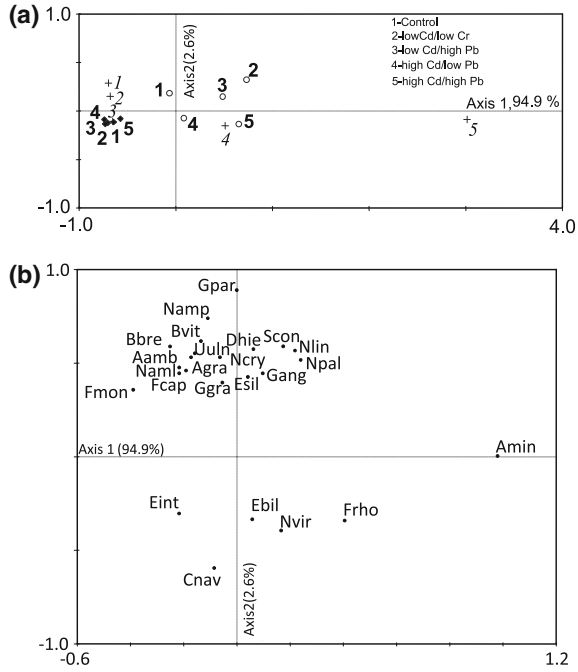

Fig. 5 Principal component analysis based on the taxonomic composition of the diatom communities recorded in five treatments (all concentrations in mg l^{-1}). **a** Projection of the communities on the first two principal component axes (1st week—diamond; 2nd week—*open circle*; 3rd week—*cross*). **b** Projection of the species with loading >0.1 for one of the two axes. Amin, *Achnanthidium minutissimum* (Kützing) Czarnecki; Aamb, *Aulacoseira ambigua* (Grunow) Simonsen; Agra, *A. granulata* (Ehrenberg) Simonsen; Bbre, *Brachysira brebissonii* Ross; Bvit, *B. vitrea* (Grunow) Ross; Cnav, *Cymbopleura naviculiformis* (Auerswald) Krammer; Dhie, *Diatoma hiemale* (Lyngbye) Heiberg; Esil, *Encyonema silesiacum* (Bleisch) Mann; Ebil, *Eunotia bilunaris* (Ehrenberg) Mills; Eint, *E. intermedia* (Hustedt) Nörpel and Lange-Bertalot; Fcap, *Fragilaria capucina* Desmazières; Fmon, *Fallacia monoculata* (Hust) Mann; Frho, *Frustulia rhomboides* (Rabenhorst) De Toni; Gang, *Gomphonema angustatum* (Kützing) Rabenhorst; Ggra, *G. gracile* Ehrenberg; Gpar, *G. parvulum* (Kützing) Kützing; Ncry, *Navicula cryptocephala* (Grunow) Cleve; Nvir, *N. viridula* (Kützing) Kützing; Naml, *Neidium ampliatum* (Ehrenberg) Krammer; Namp, *Nitzschia amphibia* Grunow; Nlin, *N. linearis* (Agardh) Smith; Npal, *N. palea* (Kützing) Smith; Scon, *Staurosira construens* Ehrenberg; Uuln, *Ulnaria ulna* (Nitzsch) Compère

Discussion

Effects of Cd and Pb on periphyton growth

Based on indoor artificial streams to study the effects of Cd and Pb mixtures on periphytic communities, we demonstrated that high Cd concentration (0.1 mg l^{-1}) has negative effects on periphyton growth. These negative effects on periphyton growth and development with increasing Cd concentration have been widely reported (Ivorra et al., 2000; Gold et al., 2003a, b; Duong et al., 2008; Morin et al., 2008a, b; Duong et al., 2010). High Cd concentrations have been shown to affect cellular processes such as global metabolism (Husaini & Rai, 1991), phosphorus metabolism and cell division (Guanzon et al., 1994) and modify cell ultrastructure (endoplasmic reticulum, mitochondria) (Wong, 1987). This explains significant reduction in periphyton growth rate with increasing Cd concentration recorded in this study.

Although Cd treatment of 0.01 mg l^{-1} exceeded Cd concentrations normally regarded protective to aquatic communities by Brazilian environmental monitoring board, Conselho Nacional do Meio

Ambiente-(CONAMA), and other international regulations such as US EPA (1995), chlorophyll a, AFDW and cell densities from this treatment were comparable with the control. On the other hand, high concentration of Pb (0.1 mg l^{-1}) also exceeding Pb concentrations regarded protective to aquatic communities by CONAMA, and US EPA (1995) did not affect chlorophyll *a* concentration and AFDW but tended to decrease the toxicity effects of Cd. This demonstrates the sufficiency of these guidelines in protecting aquatic environments.

In both low and high Cd treatments, increase in Pb concentration resulted in increase in growth rate, chlorophyll a concentration and AFDW, especially at the 2nd and 3rd week of the experiment. This is an important observation given the fact that inorganic chemical stressors usually occur as mixtures in nature (Altenburger, 2011).

Effects of Cd on diatom communities

Significant decrease in cell densities and diversity with increasing Cd concentration was recorded in this study corroborating previous studies (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 concentration treatments led to a remarkable decrease in species richness and diversity index throughout the experiment and is typical of metal polluted rivers (Morin et al., 2007).

The diatom assemblages present during the first week were similar in all the five systems. The assemblages then differentiated according to the ability of the species to grow under elevated Cd and Pb exposure with the development of more resistant species like *A. minutissimum* and reduction or exclusion of sensitive ones like *C. naviculiformis*, *F. capucina, N. cryptocephala, E. silesiacum, E. bilunaris*, and *G. parvulum* at 2nd and 3rd week of the experiment. This is supported by studies of 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 (Ivorra et al., 2000; Gold et al., 2003a, b; Duong et al., 2008, 2010; Morin et al., 2007, 2008a, b). The proliferation of *A. minutissimum* with increasing Cd concentration and duration of exposure (around 78% in the high Cd/high Pb treatments during 3th week) seems to indicate favor and tolerance of this species to Cd and Pb contamination. 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 concentration mixtures in streams in agreement with other studies (Ivorra et al., 2000; Gold et al., 2003a, b; Morin et al., 2007, 2008a; Duong et al., 2010).

Conclusions

Dissolved Cd reduces growth of periphyton communities at high Cd concentrations with addition of Pb decreasing the toxic effects of Cd on periphyton community. Shifts in species composition, decreases in species richness and diversity of periphyton communities with increasing Cd and Pb concentrations and exposure duration have been demonstrated in this study making periphyton communities appropriate monitors of metal mixtures in aquatic systems.

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