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Effects of Cr III and Pb on the bioaccumulation and toxicity of Cd in tropical periphyton communities: Implications of pulsed metal exposures

Taurai Bere [a,b,*], Mathias Ahii Chia [b], José Galizia Tundisi [a]

[a] Instituto Internacional de Ecologia, Rua Bento Carlos, 750, Centro, São Carlos, São Paulo, Brazil
[b] Universidade Federal De São Carlos, Programa de Pós-Graduação em Ecologia e Recursos Naturais, Rodovia Washington Luís, km 235 - SP-310, São Carlos - São Paulo, Brazil

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ABSTRACT

Metal exposure pattern, timing, frequency, duration, recovery period, metal type and interactions, has obscured effects on periphyton communities in lotic systems. The objective of this study was to investigate the effects of intermittent exposures of Cr III and Pb on Cd toxicity and bioaccumulation in tropical periphyton communities. Natural periphyton communities were transferred to artificial stream chambers and exposed to metal mixtures at different pulse timing, duration, frequency and recovery periods. Chlorophyll *a*, dry mass and metal accumulation kinetics were recorded. Cr and Pb decrease the toxic effects of Cd on periphyton communities. Periphyton has high Cd, Cr and Pb accumulation capacity. Cr and Pb reduced the levels of Cd sequestrated by periphyton communities. The closer the frequency and duration of the pulse is to a continuous exposure, the greater the effects of the contaminant on periphyton growth and metal bioaccumulation. Light increased toxic and accumulative effects of metals on the periphyton community.

1. Introduction

Metal contamination of freshwater environments causes significant accumulation of metals in various components of trophic chains, among which periphyton communities attract great attention from researchers. Field (Gold et al., 2003a; Nunes et al., 2003; Morin et al., 2007, 2008a; Duong et al., 2008) and laboratory studies (Gold et al., 2003b; Morin et al., 2008b; Duong et al., 2010) have shown that periphyton is effective in accumulating metals from the water column. Since periphyton communities are at the base of the food chain in lotic systems, metals accumulated by these communities strongly influence metal accumulation by organisms which feed on them.

Metal pollution of lotic system water column is erratic (in pulses) and concentrations fluctuate with time, with peak concentrations temporarily, but greatly, exceeding the background level (Zhao and Newman, 2004; Diamond et al., 2006a). This pattern of heavy metal exposure including the possible interaction between metals in complex mixtures, timing, frequency, duration and magnitude of exposure has obscured effects on periphyton communities. From our personal experience, effluents with elevated concentrations of metals are normally discharged at night and during the weekends without the knowledge of the law enforcement agents in developing countries. This timing of effluent discharge is likely to affect growth and bioaccumulation of metals in periphyton communities as they have developed complex systems, circadian clocks, to detect time and synchronize processes, actions, and behaviours to the diel cycle (Roenneberg and Merrow, 2002).

In lotic aquatic ecosystems, the bioavailability and toxicity of heavy metals to periphyton rely on their physical and chemical speciation (Lombardi et al., 2002). The knowledge of metal speciation in aquatic systems is useful for prediction and interpretation of their toxicity to algae (Sunda and Huntsman, 1996; Lombardi et al., 2002, 2007). A large number of studies have shown that metal toxicity is directly related to the free ion and labile metal concentration (Twiss et al., 2001; Lombardi et al., 2007) and the complexation reactions represent an important process regulating the availability of metal ions to the aquatic biota (Gouvea et al., 2005).

The objective of this study was to investigate interactive effects of intermittent exposures of chromium III (Cr III) and lead (Pb) on cadmium (Cd) toxicity and bioaccumulation in tropical periphyton communities. Metal mixtures were used instead of single metals

* Corresponding author.
E-mail address: taubere@yahoo.com (T. Bere).

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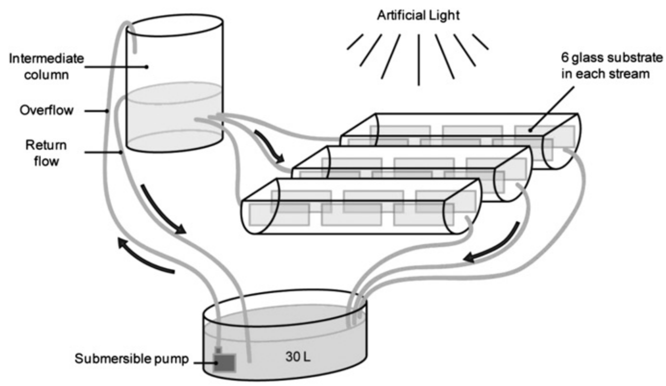

Fig. 1. Schematic representation of a closed experimental system, consisting of three artificial stream chambers (50 cm length, 5 cm radius), each containing 6-glass substrata (6 × 15 cm). Arrows indicate flow direction, (by: Ricardo M. Degani).

because in nature, many metals are present at a given site per time (Wong, 1987; Bere and Tundisi, 2011a). These metals were chosen because they were shown to be strongly correlated in the Monjolinho River and its tributaries (Bere and Tundisi, 2011a). Interactions between metals can alter the absorption, toxicokinetics, and toxicodynamics of metals (Waldock, 1998; Altenburger, 2011). The effects of frequency, duration, recovery period and timing of pulses of elevated concentrations of mixtures of Cd and Cr, and Cd and Pb on periphyton were investigated to address the following question: Does pulse timing (i.e. night-dark or day-light), exposure duration, frequency and recovery period between pulses affect growth and metal bioaccumulation of periphyton? To our knowledge, nobody has yet tried to find out the potential effects of timing of high heavy metal concentration discharge, i.e. night (dark) or day (light), on growth and metal bioaccumulation in periphyton communities in lotic systems.

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 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 20–30 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 substrates was brushed with a toothbrush into culture medium. The periphyton from all the glass substrates was pooled into one sample of approximately 2 L. This biofilm suspension was immediately transported to the laboratory in cooler box (4 °C).

2.2. Laboratory experiments

Twelve closed experimental systems (hereafter referred to as experimental units; EUs) were set up to allow the exposure of natural periphytic communities to pulses of elevated concentrations of Cd, Cr and Pb under controlled conditions following Gold et al. (2003b). Each EU consisted of three half-polyvinyl chloride (PVC) tubes 50 cm long with a radius of 5 cm as artificial stream chambers with a capacity of 2.8 L each. The three stream chambers were connected in parallel to a 30 L tank (Fig. 1). All systems were filled with Woods Hole culture medium (Nichols, 1973) modified by diluting (4×) after Gold et al. (2003b). This culture medium was kept without ethylenediaminetetraacetic acid (EDTA), which presents very high binding capacities for metals (Stauber and Florence, 1989), and supplemented with silica, an essential diatom nutrient. A pump allowed continuous circulation of the medium 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 chamber 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) was maintained with a light: dark regime of 12 h/12 h, pH of 6.5–7.0 and a temperature of 24 ± 1.0 °C. Prior to this experiment, various pilot studies were carried out to determine the optimum experimental conditions required.

2.3. Metal exposure

This research focused on five experimental factors: pulse timing (light/dark), duration, frequency, recovery period between pulses and chemical type on periphyton. Pulse frequency ranged from two to seven pulses in a given test and recovery time between pulses ranged between 12 and 60 h. Pulse durations were either 12 h or 24 h. The 12 h pulses were carried out either during the light or dark periods of the experiment for each type of metal treatment (Table 1).

Homogenised periphyton suspension from the field was divided into 8 equal volumes. Each fraction was introduced into the water column of the tank feeding 8 EUs as described below. The systems were equilibrated over night and then the desired concentrations of Cd, Cr III or Pb were obtained by addition of aliquots of the stock solutions to different systems. Cadmium chloride ($CdCl_2$, 10 mg L^{-1}, Merck, Darmstadt, Germany), lead nitrate ($Pb(NO_3)_2$ 10 mg L^{-1}, Merck, Darmstadt, Germany) and chromium (III) chloride hexahydrate ($CrCl_3{\bullet}6H_2O$, 10 mg L^{-1}, Merck, Darmstadt, Germany) were used as stock solutions.

Table 1
A Schematic depiction of the general experimental design for a given metal treatment and pulsing regimes used in experiments involving 2, 4 or 7 pulses and different recovery times between pulses. Grey, chemical exposure; white, no chemical exposure; cont, continuous; reps, replicas; L, light; D, dark.

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