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Bioabsorption of cadmium, copper and lead by the red macroalga *Gelidium floridanum*: Physiological responses and ultrastructure features

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ABSTRACT

Heavy metals, such as lead, copper, cadmium, zinc, and nickel, are among the most common pollutants found in both industrial and urban effluents. High concentrations of these metals cause severe toxic effects, especially to organisms living in the aquatic ecosystem. Cadmium (Cd), lead (Pb) and copper (Cu) are the heavy metals most frequently implicated as environmental contaminants, and they have been shown to affect development, growth, photosynthesis and respiration, and morphological cell organization in seaweeds. This paper aimed to evaluate the effects of 50 μM and 100 μM of Cd, Pb and Cu on growth rates, photosynthetic pigments, biochemical parameters and ultrastructure in *Gelidium floridanum*. To accomplish this, apical segments of *G. floridanum* were individually exposed to the respective heavy metals over a period of 7 days. Plants exposed to Cd, Cu and Pb showed discoloration of thallus pigmentation, chloroplast alteration, especially degeneration of thylakoids, and decrease in photosynthetic pigments, such as chlorophyll *a* and phycobiliproteins, in samples treated with Cd and Cu. Moreover, cell wall thickness and the volume of plastoglobuli increased. X-ray microanalysis detected Cd, Cu and Pb absorption in the cell wall. The results indicate that Cd, Pb and Cu negatively affect metabolic performance and cell ultrastructure in *G. floridanum* and that Cu was more toxic than either Pb or Cd.

1. Introduction

Over the last few years, increasing human population and industrial development have led to an increase of contaminants in aquatic systems (Rocchetta et al., 2007). Accordingly, studies reporting the effects of heavy metals on aquatic organisms are currently attracting more attention, particularly those focusing on industrial and urban pollution. The contamination of coastal waters with trace metals through sewage and other anthropogenic sources has become a severe problem (Mamboya et al., 1999).

Heavy metals, such as lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), and nickel (Ni), are among the most common pollutants found in both industrial and urban effluents (Sheng et al., 2004). In low concentrations, some heavy metals (Cu, Zn, Ni, and Mn) are essential trace elements for photosynthetic organisms; however, in higher concentrations, these metals cause severe toxic effects (Hu et al., 1996).

Heavy metals affect all organisms, especially those in the aquatic ecosystem, in many important ways. Several studies have shown such effects as decreasing macroalgal growth rates (Mamboya et al., 1999), changes in photosynthetic pigments (Bouzon et al., 2012a; Rocchetta et al., 2007), and photosynthetic

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efficiency (Bouzon et al., 2012a; Mamboya et al., 1999), as well as increasing total proteins and lipid contents (Rocchetta et al., 2007). Some reports have shown changes in the ultrastructure of the red algae *Audouinella savina* (F.S. Collins) Woelkerling (Talarico, 2002), *Ceramium ciliatum* (J. Ellis) Ducluzeau (Diannelidis and Delivopoulos, 1997), *Hypnea musciformis* (Wulfen) Lamouroux (Bouzon et al., 2012a), and *Gracilaria domingensis* (Kützing) Sonder ex Dickie (Santos et al., 2012, 2013); the green algae *Dunaliella minuta* Lerche (Visvik and Rachlin, 1992) and *Enteromorpha flexuosa* (Wulfen) J.Agardh (Andrade et al., 2004); the photosynthetic euglenoid *Euglena gracilis* Klebs (Rocchetta et al., 2007); and the brown alga *Padina gymnospora* (Kützing) Sonde (Andrade et al., 2002).

Gradual increase in the discharge of heavy metals and other pollutants into the environment directly exposes marine organisms to different levels of toxicity, affecting development and decreasing both growth and biodiversity (Torres et al., 2008). Heavy metals in high concentrations are non-biodegradable pollutants (Mallick and Rai, 2001) and can be accumulated in macroalgae, thereby decreasing growth rates (Amado Filho et al., 1997).

Cadmium is one of the heavy metals most frequently implicated in environmental contamination. This metal is utilized in the manufacture of various products, such as batteries, chipsets, pigments, television receivers, and semiconductors (Hashim and Chu, 2004; Hu et al., 1996). Cadmium can bind to sulfated groups, as well as metalloproteins and metalloenzymes, thereby neutralizing their functions (Pinto et al., 2003). However, Cd has no nutritional value for algae (Visvik and Rachlin, 1992). Cadmium is not part of organic molecules, and it has been associated with decreasing photosynthesis, Cd absorption, and growth rate of seaweeds (Diannelidis and Delivopoulos, 1997; Visvik and Rachlin, 1992).

Lead is a major environmental contaminant that arises from human, agricultural and industrial activities, e.g., mining, burning of coal, effluents from storage battery manufacture, automobile exhaust, metal plating and finishing operations, fertilizers, pesticides, and additives in pigments and gasoline (Eick et al., 1999). This metal is not an essential element for biological processes, but it can be easily absorbed and accumulated in different parts of the organism (Sharma and Dubey, 2005).

On the other hand, Cu is an essential micronutrient for plant growth and development. This metal is a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress, cell wall metabolism, transcription, protein trafficking, and hormone signaling (Yruela, 2005). However, in excess, it can inhibit growth and impair important cellular processes like photosynthetic electron transport, photosynthesis, and respiration. Membrane transport systems seem to be a target of this metal, playing a central role in toxicity processes (Yruela, 2005). Several sources of Cu, including industrial and domestic waste, agricultural practices, copper marine drainage, copper-based pesticides, and antifouling paints, have led to a clear increase in Cu concentrations in aquatic environments (Callow and Callow, 2002).

Gelidium floridanum W.R. Taylor is distributed along the Brazilian coastline from Espirito Santo State (19°20′10″S; 40°20′16″W) to Rio Grande do Sul State (30°01′59″S; 51°13′48″W), Brazil. As a source of agar extraction throughout the world, this species has achieved significant importance, as agar is utilized in diverse products with gelling and bacteriological properties (Armisen et al., 1995). *Gelidium* J.V. Lamouroux species are known to produce high-quality agar with low sulfate content; as such, suppliers can command high prices (Sousa-Pinto et al., 1999).

Considering the effects of heavy metals on seaweeds, the present study aimed to evaluate and compare the biological effects of three different heavy metals (Cd, Cu, and Pb) on physiological responses and ultrastructure features in the red macroalga *G. floridanum* by studying metal bioabsorption, morphological features, growth rate, photosynthetic pigments, flavonoid contents, ultrastructure characteristics, and elementary chemical composition. This study can serve to support subsidized environmental impact reports and monitoring programs.

2. Materials and methods

2.1. Algal material

Individual *G. floridanum* samples were collected from Sambaqui Beach (27°29′18.8″S; 48°32′18.9″W), Florianopolis-SC, Brazil, in January 2012, during the summer season. The algal samples were collected from the intertidal zone during low tide and transported at ambient temperature in dark containers to LABCEV-UFSC (Plant Cell Laboratory) (Macroalgae Laboratory, Federal University of Santa Catarina, Florianopolis, Santa Catarina, Brazil). Macroepiphytes from macroalgal samples were meticulously eliminated by cleaning with a brush and filtered seawater. Apical portions were maintained in culture medium with filtered seawater plus von Stosch enrichment solution at half strength (VSES/2; Edwards, 1970) and cultivated under laboratory-controlled conditions during 14 days (experimental acclimation period) before experimental treatment with Cd, Cu, and Pb.

2.2. Culture conditions and experimental treatments

Apical thallus portions were selected ($\pm$2.0 g) from the acclimated *G. floridanum* plants and cultivated for 7 days under the experimental treatments with heavy metals in Erlenmeyer flasks containing 500 mL of natural sterilized seawater, 34 practical salinity units (p.s.u.), and enriched with VSES/2 (without EDTA, ethylene diamine tetraacetic acid). Laboratory-controlled conditions were 24 ± 2 °C, continuous aeration, 80 ± 5 μmol photons. $m^{-2} s^{-1}$ (Philips C-5 Super 84 16 W/840 fluorescent lamps; LI-COR light meter 250, USA), and 12 h photocycle (starting at 8 h). Experimental treatments were carried out with a control (without metal addition) and Cd, Cu and Pb supplied individually as $CdCl_2$, $CuCl_2$ or $PbCl_2$ at 50 and 100 μM for each metal. Eight replicates were made for each experimental group (seven treatments).

2.3. Concentration of Cd, Cu and Pb in seawater and algal samples

The concentrations of Cd, Cu and Pb in water and algal samples (initial and end of experiment) were analyzed by inductivity-coupled plasma atomic emission spectrometry (ICP-AES, ARCOS from M/s. Spectro, Germany), using the following analyte line: Cd 214.438 nm, Cu 324.754 nm and Pb 220.353 nm plasma view-axial, with a detection limit of 0.001 ppm for Cd, Cu and Pb. After 7 days of exposure to Cd, Cu and Pb, algal samples (300 mg) were washed in distilled water, dried at 65 °C and digested in nitric acid. Water samples (50 mL) were digested using concentrated nitric acid. Total metal absorption was expressed in percent, calculated through (mg of Cd/Cu and Pb in 500 mL of water by mg of Cd in 750 mg of wet weight algae). The Bioconcentration Factor (BCF) was calculated as remaining metal concentration and wet weight plant biomass (expressed in ppm) divided by initial concentration of metal added in the culture medium. All analyses were performed in quadruplicate.

2.4. Growth rates (GRs)

Growth rates were calculated using the following equation: GR ($\%.day^{-1}$)= $[(Wt/Wi)^{1/t} - 1] \times 100$, where Wi=initial wet weight, Wt=wet weight after 7 days, and t=experimental time in days (Penniman et al., 1986); where Wi=initial wet weight, Wt=wet weight after 7 days, and t=experimental time in days (Penniman et al., 1986).

2.5. Pigments analysis

The contents of photosynthetic pigments (chlorophyll *a*, phycobiliproteins and carotenoids) and flavonoids of *G. floridanum* were analyzed from frozen fresh samples (n=4) kept at −40 °C until use.

Chlorophyll a was extracted from approximately 1 g of algal material in 3 mL of dimethylsulfoxide (DMSO, Merck, Darmstadt, FRG) at 40 °C, during 30 min, using a glass tissue homogenizer (Hiscox and Israelstam, 1979; Schmidt et al., 2010a, 2010b). The homogenates were centrifuged at 2000 g for 20 min, and the supernatant containing the pigment was quantified spectrophotometrically, according to Wellburn (1994).

Phycobiliproteins were extracted from about 1 g of algal material ground to a powder with liquid nitrogen and extracted at 4 °C in darkness in 0.1 M phosphate buffer, pH 6.4. The homogenates were centrifuged at 2000 g for 20 min.