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Effects of copper on early developmental stages of *Lessonia nigrescens* Bory (Phaeophyceae)

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Early developmental stages of Lessonia nigrescens are highly sensitive to copper.

Abstract

Copper effects on the early developmental gametophytic and sporophytic stages of the kelp *Lessonia nigrescens* were tested in gradients of increasing concentrations of ASV-labile copper. The results demonstrated a high sensitivity to copper of all life-history stages of the alga, where even the lowest tested concentration affected spore release as well as their subsequent settlement. More significant, concentrations higher than 7.87 $\mu g\,L^{-1}$ totally interrupted the development of the spores after they settle. This effect led to a failure in the formation of male and female gametophytes and, as a consequence, to a complete disruption of the normal life cycle of the kelp. Thus, we suggest that the absence of *L. nigrescens* from copper-enriched environments results from the high sensitivity of its early life cycle stages, which limits growth and maturation of the gametophytic microscopic phase and, as a consequence, prevents development of the macroscopic sporophytic phase.

Keywords: Brown kelps; Copper toxicity; Early developmental stages

1. Introduction

Various sources of copper, including industrial and domestic wastes, agricultural processes and discharges from copper mine activities, contribute to a progressive increase of this metal in marine coastal environments (Bryan and Langston, 1992; Correa et al., 1999; Livingstone, 2001). Although significant improvements have been introduced in modern operation of copper mines around the world (see Camaño and Silva, 1995 for a Chilean case), copper mining has been characterized by releasing large volumes of wastes on relatively reduced areas, causing long-lasting effects on the environment. Severe negative effects caused by discharges of copper mine effluents into marine habitats are reported for the coasts of England (Bryan and Langston, 1992), Canada (Marsden and DeWreede, 2000), Australia (Stauber et al., 2001) and Chile (Lancellotti and Stotz, 2004; Medina et al., 2005).

Seaweeds are fundamental components of coastal benthic ecosystems and, although copper is an essential element for them as co-factor of enzymes and key participant in several metabolic pathways (Stauber and Florence, 1987; Gaetke and Chow, 2003), at elevated concentrations it becomes toxic (Florence and Stauber, 1986; Gledhill et al., 1997). Growth, development, reproduction and photosynthesis are among the life-history traits of aquatic organisms known to be affected by copper excess (Florence and Stauber, 1986; Gledhill et al., 1997; Collén et al., 2003; Yap et al., 2004). Furthermore, negative effects of copper excess on seaweeds can directly or

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indirectly affect whole coastal benthic communities (Coelho et al., 2000; Medina et al., 2005). This seems to be the case of a coastal area in northern Chile (26° 15S′, 70° 40W′) chronically affected by wastes from a copper mine (Correa et al., 1999; Medina et al., 2005), which has resulted in persistently high levels of total dissolved copper in the seawater, from 2400 $\mu g\,L^{-1}$ at the discharge point in the past (Castilla, 1996) to 20–25 $\mu g\,L^{-1}$ in the present (Stauber et al., 2005). Recent studies have revealed that current ASV-labile copper in the area ranges from 8 to 15 $\mu g\,L^{-1}$ (Stauber et al., 2005; Andrade et al., in press). This copper enrichment is associated with a severe reduction in species richness and a complete modification of the intertidal community structure (Medina et al., 2005). The algal assemblage has been reduced to the stress-tolerant opportunistic species *Ulva compressa*, *Scytosiphon lomentaria* and *Scytosiphon tenellus,* which form scattered patches on the otherwise empty rocks. Even though a recent gradual reduction in copper levels has been reported, the kelp *Lessonia nigrescens* remains absent from the impacted sites (Medina et al., 2005).

L. nigrescens dominates the lower intertidal zone of the Chilean coast, where it facilitates algal and invertebrate recruitments and modulates biological diversity and community structure (Cancino and Santelices, 1984; Ojeda and Santelices, 1984; Santelices, 1990). In spite of the recognized ecological importance of *L. nigrescens,* studies addressing its tolerance to metals are restricted to the report on copper accumulation by adult sporophytes (Leonardi and Vásquez, 1999). However, several studies investigating the effects of copper on other seaweeds (Chung and Brinkhuis, 1986; Anderson and Hunt, 1988; Bidwell et al., 1998; Coelho et al., 2000; Nielsen et al., 2003a) suggest that the early developmental stages are more sensitive than adults to the toxic effects of the metal. *L. nigrescens* has a biphasic life history, with a macroscopic sporophytic generation alternating with a microscopic generation of separate male and female gametophytes (Avila et al., 1985).

This study addresses the hypothesis that copper enrichment at levels similar or higher to those present along the coastal area affected by copper mine tailings impairs the life cycle of *L. nigrescens* through detrimental effects on its early developmental stages. This study aims at the identification of specific factors that might have determined the disappearance of the kelp in the past, and are contributing to its current absence from the copper-enriched areas. Simultaneously, the expected results should unravel some of the factors that rule the ecology of these copper-enriched coastal areas.

2. Materials and methods

2.1. Sampling of algae and seawater

Mature fronds from 10 fertile plants of *L. nigrescens* were collected from Las Cruces, central Chile (33° 30′S, 71° 30′W). Algal material was sealed in plastic bags and transported at 4 °C to the laboratory within 3 h of collection. Seawater was simultaneously collected and stored in high-density polystyrene containers pre-treated according to the USEPA (1999). Once in the laboratory, seawater was immediately sterile-filtered through 0.2 µm pore membrane (Sartorius, Goettingen, Germany) and stored in darkness at 15 °C until used.

2.2. Copper toxicity tests

Copper toxicity included the analysis of possible effects on spore release by adult sporophytes, spore settlement, and gametophyte and sporophyte development. Five treatments consisting of filtered seawater with increasing copper concentrations and a treatment with no copper addition were included. Based on preliminary trials, copper treatments included the nominal concentrations of 10, 20, 50, 100 and 200 $\mu g\,L^{-1}$ copper as $CuCl_2$ (p.a., Merck, Germany). ASV-labile copper (Cu′) is summarized in Table 1.

2.3. Effect of copper on spore release

Individual discs of 1 cm in diameter were excised from five randomly selected sori (reproductive structures of sporophytes which release haploid spores that germinate into male and female independent gametophytes) obtained from mature fronds previously rinsed with running tap water. Each disc was blot-dried with absorbent paper and air dried on trays at room temperature for 1 h to simulate normal desiccation during low tide. Groups of five discs from different sori were placed in 50 mL glass vials pre-treated with 5% nitric acid (p.a., Merck, Germany) containing 20 mL of the test solutions. Each treatment included five replicates. Glass vials were agitated using an orbital shaker (SO1, Stuart, TX, USA) during the entire assay. The number of spores was recorded every hour, for 8 h, by counting three samples in a haemocytometer (Neubauer Improved 1/400 mm^2, Superior – Marienfeld, Germany). To avoid re-counting spores from previous time intervals, sori were removed after each count and transferred into new vials containing fresh solutions.

2.4. Effect of copper on spore settlement

To generate the initial bulk of spores, five randomly selected sori were rinsed with running tap water and placed in a 500-mL glass vial containing 250 mL of filtered seawater. After 3 h, densities of spores in suspension were determined by counting the number of cells with a haemocytometer. Aliquots of 200 µL containing average densities of 5×10^3 spores were inoculated in acid-treated plastic multi dishes (Costar 3524, Corning, NY, USA,) containing 1.5 mL of test solutions. Each treatment included five replicates. Spore settlement was monitored periodically during 48 h in an inverted microscope (Optiphot-2, NIKON, Sturbridge, MA, USA). The number of settled spores was counted in four areas of 1 mm^2 per replicate.

2.5. Effect of copper on gametophyte development

Five sori of *L. nigrescens* were treated as for the spore settlement experiment. Individual aliquots of 100 µL of spore solution (ca. 25 spores μL^{-1}) were deposited on 18 × 18 mm glass cover slips held in acid-treated plastic multi dishes containing 5 mL of the test solutions. Culture medium was changed daily and the development of gametophytic stages was recorded every 2–4 days during 42 days. Each treatment included five replicates and a total of four areas of 1 mm^2 were evaluated in each replicate. In order to classify early spore

Table 1
Nominal and AVS-labile copper concentration in all tested solutions at the beginning of the (A) spore release, spore settlement and spore development experiments and (B) sporophyte development experiment

Nominal copper ($\mu g\,L^{-1}$)	A	B
	AVS-labile copper ($\mu g\,L^{-1}$)	AVS-labile copper ($\mu g\,L^{-1}$)
Control	0.2	0.2
10	7.9	7.3
20	17.8	16.3
50	45.4	43.1
100	95.6	94.1
200	195.0	193.0