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The effects of continuous and fluctuating copper exposures on the marine alga Phaeodactylum tricornutum

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Abstract

Contaminant concentrations in aquatic systems are seldom constant. Erratic inputs such as industrial discharges, rain water flushing, and random spills may cause concentrations to increase rapidly. Environmental processes may contribute through the dispersive actions of tides and currents, adsorptive losses to or release from resuspended sediments, and contaminant losses due to photo-degradation and volatilization. Despite such variability in contaminant concentrations, environmental guidelines are derived from toxicity test data using continuous exposure, where contaminant concentrations at the beginning of the exposure are assumed to remain relatively constant over the test duration. Responses of organisms exposed to fluctuating contaminant concentrations may differ from those exposed continuously to contaminants, even for equal contaminant loads. The current knowledge gap regarding the differing responses of organisms to contaminants from continuous and pulsed exposures is impeding decision making processes of both regulatory bodies and discharging industries. We have investigated the effects of continuous and pulsed copper exposure on the growth of the copper-sensitive microalga Phaeodactylum tricornutum as an indicator of ecosystem health.

Keywords

phaeodactylum, marine, alga, tricornutum, effects, exposures, copper, fluctuating, continuous

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The Effects of Continuous and Fluctuating Copper Exposures on the Marine Alga *Phaeodactylum tricornutum*

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INTRODUCTION

Contaminant concentrations in aquatic systems are seldom constant. Erratic inputs such as industrial discharges, rain water flushing, and random spills may cause concentrations to increase rapidly. Environmental processes may contribute through the dispersive actions of tides and currents, adsorptive losses to or release from resuspended sediments, and contaminant losses due to photo-degradation and volatilization. Despite such variability in contaminant concentrations, environmental guidelines are derived from toxicity test data using continuous exposure, where contaminant concentrations at the beginning of the exposure are assumed to remain relatively constant over the test duration. Responses of organisms exposed to fluctuating contaminant concentrations may differ from those exposed continuously to contaminants, even for equal contaminant loads. The current knowledge gap regarding the differing responses of organisms to contaminants from continuous and pulsed exposures is impeding decision making processes of both regulatory bodies and discharging industries.

We have investigated the effects of continuous and pulsed copper exposure on the growth of the copper-sensitive microalga *Phaeodactylum tricornutum* as an indicator of ecosystem health.

METHODS

Algae were exposed to equal loads of copper either continuously or in different pulse exposures (varying pulse concentration and duration) and algal growth inhibition and changes in cell size/morphology were examined. Growth of *P. tricornutum* was investigated in experiments using copper spiking to create pulses. Algae were separated by centrifugation so that contaminated seawater could be replaced with clean seawater without loss of algal cells. Algal growth in controls was not affected in preliminary experiments using this technique. Rates of copper adsorption, internalization and elimination of copper were determined during and following exposure to different copper concentrations. A 30-min wash in 0.01 M EDTA (in 3.5 % NaCl) removed extracellular copper without disrupting cell integrity. An acid digest of the remaining algal pellet using concentrated HNO_3 gave the intracellular copper. Intra- and extracellular copper were measured using combinations of graphite furnace-atomic emission spectroscopy and anodic stripping voltammetry, respectively. Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and optical microscope techniques were used to investigate changes in cell size/morphology.

Bioassays used inhibition of growth (cell yield) as the test endpoint after 72 h. Initial cell densities of $4\text{-}8\times10^3$ cells/mL were chosen to reflect cell densities in natural waters in order to achieve environmentally relevant partitioning of copper in solution (Franklyn *et al.*, 2002). Copper exposures included 1, 4, and 8-h pulses and continuous exposures (over 72 h) all equivalent to a time-averaged concentration near the 72-h continuous exposure IC50 of 7 µg Cu/L (Figure 1). Clean filtered sea water with 15 mg NO_3^-/L and 1.5 mg PO_4^{3-}/L was used as

the test medium. Incubations were undertaken at 21ºC, using a 12:12 h light:dark cycle of cool white fluorescent light at 140 µmol photons/m^2/s.

MODELLING THE EFFECT OF COPPER ON ALGAL GROWTH

Algal growth is exponential when nutrients are not limiting. Normal exponential growth in control cells can be described by $N_t = N_0 e^{\mu t}$, where, N_t is the cell biomass at any time, N_0 is the initial cell biomass, μ_t is the growth rate, and t is time. As the concentration of copper increases the algal growth rate decreases resulting in a decrease in algal cell biomass at the end of the test. Continuous exposure bioassays run at the same time as pulse exposures were used to calculate model constants. A three-parameter equation was used to predict copper inhibition of growth according to $\mu_t = \mu_i - C/(1+\exp(A+B\log(C_t)))$, where μ_t is the growth rate at time t, μ_i is the initial growth rate, C_t is the bioassay dissolved concentration at time t, and A, B, and C are constants chosen to minimize the sum of the differences between the real and model inhibitions (Simpson et al., 2003).

RESULTS AND DISCUSSION

Algal growth inhibition following copper exposure to equivalent time-averaged copper concentrations was dependent on the exposure scenario (pulse concentration and duration). Intra- and extracellular copper also varied with the different exposure scenarios (pulse and continuous) of equivalent time-averaged concentrations. The 1-h pulse scenario and the equivalent continuous exposure resulted in the highest intracellular copper concentrations, and the greatest algal growth inhibition.

A model was developed that described the response of *P. tricornutum* to both continuous and pulsed copper exposures. This model used the uptake and elimination rates to account for the delay (lag) between copper exposure and the toxic response. A linear relationship was found between intra- and extracellular copper (Figure 2).

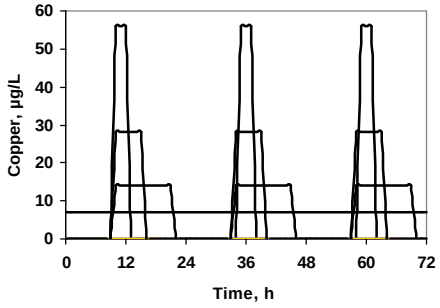

Figure 1. Exposure concentrations used to achieve the time-averaged IC50

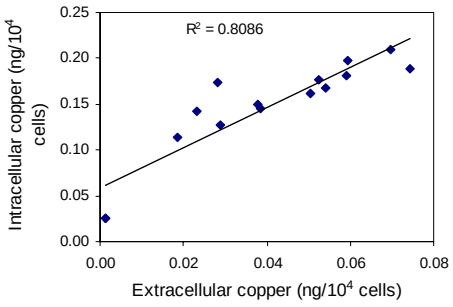

Figure 2. Intracellular vs extracellular copper

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