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Dissolved organic carbon reduces the toxicity of copper to germlings of the macroalgae, *Fucus vesiculosus* ☆

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Abstract

This study investigates the effects of waterborne copper exposure on germling growth in chemically defined seawater. Germlings of the macroalgae, *Fucus vesiculosus* were exposed to a range of copper and dissolved organic carbon (DOC as humic acid) concentrations over 14 days. Germling growth was found to be a sensitive indicator of copper exposure with total copper (TCu) and labile copper (LCu) EC_{50} values of approximately 40 and 20 μg/L, respectively, in the absence of added DOC. The addition of DOC into the exposure media provided germlings with protection against copper toxicity, with an increased TCu EC_{50} value of 117.3 μg/L at a corrected DOC (cDOC from humic acid only) concentration of 2.03 mg/L. The LCu EC_{50} was not affected by a cDOC concentration of 1.65 mg/L or less, suggesting that the LCu concentration not the TCu concentration was responsible for inhibiting germling growth. However, at a cDOC concentration of approximately 2 mg/L an increase in the LCu EC_{50} suggests that the LCu concentration may play a role in the overall toxicity to the germlings. This is contrary to current understanding of aquatic copper toxicity and possible explanations for this are discussed.

Keywords: Copper; Humic acid; DOC; Macroalgae; Fucus vesiculosus; Ecotoxicology; Effect concentration

1. Introduction

The toxicity of copper in aquatic systems is believed to be dependent on its chemical form, with the free copper ion considered as the most bioavailable and thus most toxic (Di Toro et al., 2001a, b; Santore et al., 2001). The proportion of copper present in the free ion state is highly dependent on the physicochemical properties of the seawater, mainly due to the strong tendency of these free copper ions to form complexes with both inorganic and organic ligands. Although inorganically bound copper has been reported to be partly bioavailable (MacRae et al., 1999), copper bound to organic ligands is considered non-bioavailable and therefore non-toxic (Arnold, 2005). The proportion of these three copper species and the factors that can alter their frequency are therefore extremely important in predicting the toxicity of copper in aquatic systems.

The Biotic Ligand Model (BLM) has been developed for copper in freshwater systems, which has enabled copper toxicity to be predicted on the basis of certain water parameters (Di Toro et al., 2001a, b). Although the BLM to predict copper toxicity in seawater systems is yet to be fully validated, the influence of physicochemical parameters such as dissolved organic carbon (DOC), pH and several of the main ion concentrations (e.g. Na^+, Ca^{2+}, Cl^-) have been acknowledged to have significant effects on copper speciation.

Macroalgae are important primary producers of coastal and estuarine waters; thus, negative impacts on macroalgae populations through copper exposure can have detrimental

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effects on whole coastal benthic communities (Coelho et al., 2000). Due to their dominant position within the marine food web and their widespread distribution, macroalgae have been suggested as a useful group for the development of standardised toxicity testing, to provide regulators with information on the effect concentration (EC) values of different compounds to be used in risk assessments (Eklund and Kautsky, 2003). These authors have also suggested the use of *Fucus vesiculosus*, above all other macroalgae, for regular use in standardised toxicity tests.

The present study investigates the effects of DOC, as humic acid (HA), on copper speciation and its bioavailability and subsequent toxicity to the germlings of the macroalgae, *F. vesiculosus*, following a 14 day exposure. The exposure media was measured at intervals during the 14 day test for total copper (TCu) and labile copper (LCu) concentrations by differential pulse anodic stripping voltammetry (DPASV). Other parameters including DOC, suspended particulate matter (SPM), the main cation and anion concentrations, and the physicochemical properties of the test media were measured to increase the understanding of copper speciation and its toxicity to marine macroalgae.

2. Method

2.1. Test conditions—flow-through system

Fucus germlings were exposed to a range of copper concentrations within a flow-through system. The flow-through system enabled copper aging to occur within the seawater matrix for approximately 32 h before exposing the germlings. This was considered to be sufficient time for copper complexation reaction with the seawater to stabilise (Ma et al., 1999; Campos and van den Berg, 1994). The flow-through system also ensured that the germlings were provided with a constant supply of fresh nutrients and would not be responsible for limiting germling growth.

The test seawater was passed through a series of filters (glass wool, 10 μL filter) to remove any SPM. The seawater was also treated with UV-filtration and protein skimming to reduce background DOC to its lowest limits. Protein skimming (or foam fractionation) enables hydrophobic DOC to be removed from the seawater by absorbing them onto the surface of fine bubbles that are rising in a closed contact column against a counter-current flow. The bubbles rise to the top of the protein skimmer column, burst and form stable foam, which rolls over into a collection cup.

A peristaltic pump (Watson Marlow) and chemically resistant tubing was used to add different stock solutions of copper ($CuCl_2.2H_2O$, Sigma in distilled water), to each mixer tank (excluding the control tank) to obtain nominal concentrations of 20, 40, 80, 160 and 320 μg/L copper. Humic acid (HA as Na^+ salt, Acros) was used as the source of DOC. To aid solubility, stock solutions of HA were made by dissolving in 1 mM sodium hydroxide solution. Where appropriate, HA stock solutions were dosed into each mixer tank (excluding the control) with the aid of a peristaltic pump to achieve concentrations of 2, 4, 8 and 12 mg/L HA. In tests where HA was added, a second control tank was used.

2.2. Fucus germling growth test

2.2.1. Collection of algae and test preparation

Approximately 20 adult plants with swollen receptacles were collected during low tide from Holliwell Point, Burnham-on-Crouch, Essex, UK. Plants were transported back to the Cefas Burnham laboratory in 5 L plastic buckets. Once within the laboratory, plants were rinsed in filtered seawater and covered with a damp paper towel to undergo mild desiccation overnight at 20±2 °C.

Receptacles were then removed from the plant and placed in beakers containing 200 mL of filtered seawater. Approximately 60–100 receptacles, taken from several plants, were placed in each beaker of filtered seawater to ensure the presence of both male and female receptacles. Receptacles were left for a minimum of 2 h (average 6 h) at 20±2 °C to allow for the release of reproductive bodies. The resulting zygote suspension was filtered through a 90 μm sieve to remove excessive debris and collected on a 25 μm sieve. The zygotes were washed from the sieve into a separate beaker with filtered seawater. The quality and density of the zygotes were assessed by microscopic examination (×20 magnification). A density of 500–1000 zygotes per mL was used for transplantation on to microscope slides.

2.2.2. Attachment to microscope slides

The entire surface area of a plastic tray (50 × 30 × 3 cm) was covered with 40–50 microscope slides, and covered to a depth of at least 2 cm with filtered seawater. Using a 5 mL pipette, equal amounts of the zygote suspension (1–4 mL) was placed carefully on to each individual slide. The tray of submerged slides containing zygotes was covered with a clear plastic sheet to prevent evaporation and left for 48 h to enable the zygotes to attach and develop into germlings.

2.3. Initiation of the toxicity test

A minimum of 20 germlings were photographed and measured from each slide, using an inverted microscope with camera (QICam, Qimaging) connected to a PC with Image-Pro plus v.5.1. imaging software (Media Cybernetics Inc). A minimum of five slides were placed in separate microscope slide racks. These racks were immediately transferred to the experimental tanks (one rack per tank) of the flow-through system.

Germling growth was determined by measuring the total length from rhizoid to apical hair in randomly selected germlings on days 0, 4, 7, 10 and 14. Lights were positioned above the experimental tanks to obtain optimum conditions for germling growth. A 12 h light/12 h dark photo-cycle was maintained throughout the duration of the tests. Seawater temperature was maintained at 21±1 °C throughout the duration of the tests.

2.4. Calculations of growth and EC

Relative growth rate (RGR) was calculated using the formula:

$$\mathrm{RGR/day} = \frac{\ln(a_2) - \ln(a_1)}{t_2 - t_1},$$

where a_n was the measured length at time t_n.

The total germling growth on day 14 for each test was analysed using a special case of a segmented regression model (Lerman, 1980), sometimes referred to as a 'Hockey Stick' model because of its characteristic shape (Barrowman and Myers, 2000; Pires et al., 2002). The model assumes that ln(Fucus length) remains at a constant "No effect" level (S_0) until the concentration exceeds the no effect concentration (NEC)—at which stage the response decreases linearly with ln concentration. Formally, we can define the model as

$$\ln(\text{Fucus length}) = S_0 + \text{error} \quad \text{Cu} \leqslant \text{NEC},$$

$$\ln(\text{Fucus length}) = S_0 + \beta \ln(\text{Cu}/\text{NEC}) + \text{error} \quad \text{Cu} \geqslant \text{NEC},$$

where $\beta \leqslant 0$, S_0 is a parameter representing the natural logarithm of shoot length at concentration zero and the error is assumed to be normally distributed with mean zero and constant variance.

To estimate the EC_{50}, we need to find the value of concentration at which the model predicts shoot length will be half that at concentration zero—that is $\exp(S_0)/2$ in the above model. Thus, we need to solve for EC_{50} in

$$\exp(S_0)/2 = \exp(S_0 + \beta \log(\mathrm{EC}_{50}/\mathrm{NEC})).$$