COPPER SPECIATION. THE EFFECTS OF DISSOLVED ORGANIC MATTER (DOM) AND SUSPENDED PARTICULATE MATTER (SPM) ON COPPER SPECIATION AND TOXICITY TO MARINE ORGANISMS

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Introduction

The speciation of waterborne copper is of fundamental importance in terms of its bioavailability and subsequent toxicity to marine and freshwater organisms. The bioavailability of copper is a reflection of how easily it can pass through the semi-permeable membranes of either the gill or the intestine of aquatic organisms were it can cause oxidative damage to internal cells. The three main copper species of importance are; 1) free cupric ion (Cu^{2+}), 2) inorganically bound copper, and 3) organically bound copper. Of these species the free ion concentration is considered the most bioavailable and toxic form followed by inorganic copper. However, copper bound to organic matter is considered to be non-bioavailable and unable to cause any toxic effect to aquatic organisms. In addition, the presence of suspended particulate matter (SPM) can also partition copper and take it out of solution, thereby reducing total dissolved copper concentrations. In many marine ecotoxicity studies copper concentrations are not measured and even when measured speciation is often ignored. This study reports on the effects of DOM and SPM loading on copper speciation using a laboratory flow-through system. Preliminary data is also reported on the mussel embryo bioassay performed within the flow-through system allowing time for copper complexation to take place prior to exposure to the mussel embryo/larvae.

Objectives

- Construction and testing of a laboratory flow-through system for copper dosing. Enable sufficient time for copper ageing before exposure to test animals.
- To investigate the effects of DOM and SPM on copper speciation.
- Determine copper 48 h EC_{50} value for embryos of the mussel, *Mytilus edulis* for both labile and total dissolved copper.
- Establish the suitability of the flow-through system to perform future chronic ecotoxicity test on a range of marine organisms, with respect to copper and DOM/SPM loading.
- Overall to demonstrate that the labile copper fraction is the bioavailable and toxic form to marine organisms.

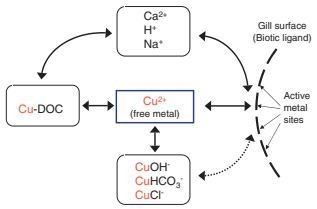

Figure 1: Biotic Ligand Model for Copper Speciation, showing the main copper species and the potential interactions that can take place.

Copper measurements

Split Copper into 2 fractions - electrochemical method.

Total Dissolved Copper
Differential Pulse Anodic Stripping Voltammetry at a hanging mercury dropping electrode (DPASV HMDE). After acidification and UV digestion.

Labile copper
(Free ion & Inorganic species) DPASV at a Thin Mercury Film on a Rotating Glassy Carbon Electrode (TMF RGCE).

Organic Copper
Difference of total dissolved Cu and labile Cu.

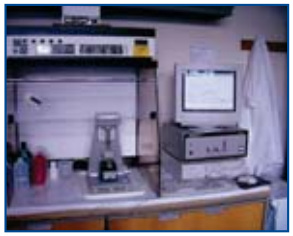

Figure 2: Photograph of the equipment used to measure copper speciation by Differential-Pulse Anodic Stripping Voltammetry (DPASV).

Flow-through system for copper dosing

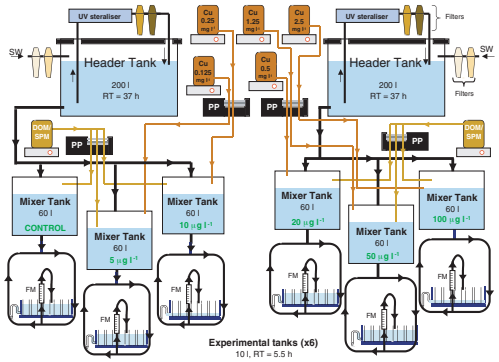

Figure 3: Schematic representation of the laboratory flow-through system for copper dosing. Natural seawater was obtained from the system installed at the CEFAS Burnham Laboratory, which obtains sand-filtered seawater from the River Crouch. The seawater was cleaned through a series of filters (glass wool & 10 µm) prior to the header tank. Header tank water was recirculated through a UV filter and passed through a 1.5 µm filter and polyfilter. A protein skimmer was also installed to reduce background DOC concentrations. Each header tank services three mixer tanks, which in turn services an experimental tank. The experimental tanks contain five holding chambers used in the mussel embryo bioassay. Dosing of copper, DOM (as humic acid) and SPM are via a peristaltic pump (PP) in to each mixer tank. A 60 l seawater volume in the mixer tanks provides a 33 h residency time (RT) for copper ageing and complexation reactions to take place before exposure to the test animals. Flow rates in to the experimental tanks are 30 ml min^{-1} set using flow meters (FM) and stop valves. Seawater temperature in the experimental tanks are maintained as 14 ± 1°C.

Figure 4: Photograph of the flow-through system set-up in a constant temperature room (14 ± 1°C) at CEFAS Burnham laboratory.

Testing of the copper dosing system

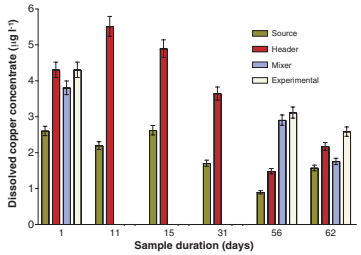

Figure 5: Background Copper Concentrations. Total dissolved copper concentrations in seawater samples taken from the tanks of the laboratory dosing system. Source = samples taken from the seawater after initial filtering but before entering the header tank. Error bars represent 5% instrument error.

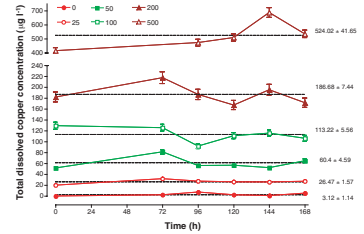

Figure 6: Maintaining Stable Copper Concentrations. Total dissolved copper concentrations of water samples taken from the experimental tanks of the flow-through system (mean ± 5% error from copper analysis). The dotted line indicates the mean for each data set. Numbers on the right side of the graph denote means and standard errors for each data set.

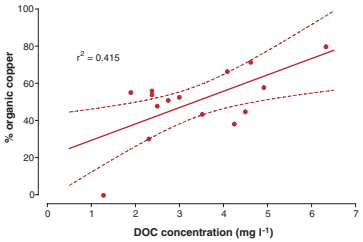

Figure 7: The effects of Dissolved organic carbon (DOC, as humic acid) on the proportion of organically bound copper (% of total dissolved copper). Copper concentrations were determined from seawater samples taken from the experimental tanks.

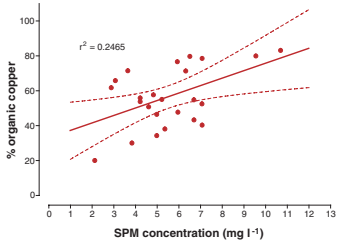

Figure 8: The effects of Suspended Particulate Matter (SPM) on the proportion of organically bound copper (% of total dissolved copper). Copper concentrations were determined from seawater samples taken from the experimental tanks.

Mussel Embryo bioassay

Procedure

- Roped mussels from Fencebay Fishery in Ayrshire, Scotland.
- Spawning induced in female by thermal shock. Fertilised egg with stripped sperm to produce developing embryo.
- Embryos allowed to develop for 48 h ± 1 h at 14 ± 1°C in the holding chambers of each experimental tank of the flow-through system.
- Frequency of normal D-stage larvae were compared to that of the control (no copper added)

Figure 9: The marine mussel, Mytilus edulis

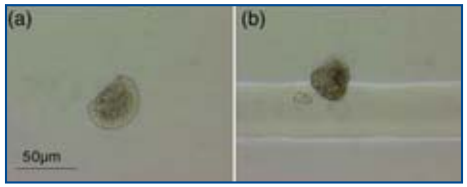

Figure 10: D-shaped larvae of the mussel, Mytilus edulis showing (a) normal and (b) abnormal development.

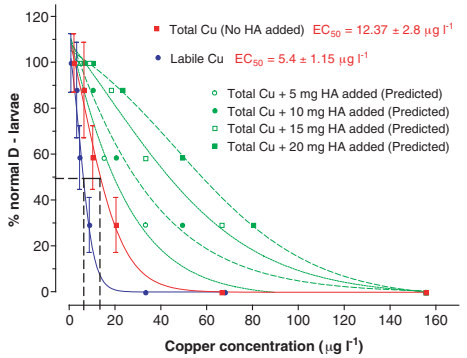

Figure 11: The effects of 48 h water borne copper exposure on the larval development of the mussel, Mytilus edulis. EC_{50} values for labile and total dissolved copper were calculated using ToxCalc Software (Tidepool Scientific). The green dotted curves denote predicted values as a result of increased DOC concentrations in the test waters.

Overall Summary

- Successful construction of a laboratory flow-through system that enable sufficient time for copper ageing before exposure to test animals.
- Background copper concentrations were between 2 and 4 µg l^{-1} after a 2 month equilibrium period.
- Total dissolved copper concentrations remained relatively stable for the 7 days of testing.
- The proportion of organically bound copper tended to increase with increased HA and SPM concentration.
- The 48 h EC_{50} value for mussel embryos was 12.37 ± 2.8 µg l^{-1} for total dissolved copper and 5.4 ± 2.8 µg l^{-1} for Labile copper.
- It is predicted in future experiments that the EC_{50} for total dissolved copper will increase with increased DOC (as HA) concentration, whilst the labile EC_{50} will remain the same. Demonstrating that the labile copper fraction is the bioavailable and toxic copper species to the mussel embryo.

Future Work

- Continue this study on copper bioavailability with respect to DOM load using the mussel embryo bioassay, in order to prove the predicted EC_{50}s shown in Figure 11.
- Copper bioavailability with respect to DOM load to the macroalgae *Fucus vesiculosus* using germling growth as the ecotoxicological endpoint.

Acknowledgements

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