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Effects of dissolved organic matter and reduced sulphur on copper bioavailability in coastal marine environments

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ABSTRACT

Copper-induced toxicity in aqueous systems depends on its speciation and bioavailability. Natural organic matter (NOM) and reduced sulphur species can complex copper, influencing speciation and decreasing bioavailability. NOM composition in estuaries can vary, depending on inputs of terrigenous, autochthonous, or wastewater source material. At a molecular level, variability in NOM quality potentially results in different extents of copper binding. The aims of this study were to measure acute copper EC_{50} values in coastal marine and estuarine waters, and identify the relationships between total dissolved copper EC_{50} values and measured water chemistry parameters proportional to NOM and reduced sulphur composition. This has implications on the development of marine-specific toxicity prediction models. NOM was characterised using dissolved organic carbon (DOC) concentration and fluorescence measurements, combined with spectral resolution techniques, to quantify humic-, fulvic-, tryptophan-, and tyrosine-like fractions. Reduced sulphur was measured by the chromium-reducible sulphide (CRS) technique. Acute copper toxicity tests were performed on samples expressing extreme DOC, fluorescent terrigenous, autochthonous, and CRS concentrations. The results show significant differences in NOM quality, independent of DOC concentration. CRS is variable among the samples; concentrations ranging from 4 to 40 nM. The toxicity results suggest DOC as a very good predictive measure of copper EC_{50} in estuaries ($r^2=0.87$) independent of NOM quality. Furthermore, for filtered samples, CRS exists at concentrations that would be saturated with copper at measured EC_{50}, suggesting that while CRS might bind Cu and decrease bioavailability, it does not control copper speciation at toxicologically relevant concentrations and therefore is not a good predictive measure of copper toxicity in filtered samples.

1. Introduction

Metal toxicity in aquatic environments is influenced by the chemical characteristics of the water. These chemical characteristics can vary over time and by location, which may alter the bioavailability of metals. With over 53% of the United States of America (USA) population living along the coast (NOAA, 2004), the water chemistry of coastal marine and estuarine environments is impacted by increasing anthropogenic input. It is important to research the nature of these inputs and quantify the variability of water chemistry with respect to metal toxicity. Biotic ligand models (BLMs), such as developed by HydroQual (2007), are widely used for predicting deleterious effects of metals in freshwater environments (U.S. EPA, 2007). These models are organism-specific and dependent on water chemistry, which makes up the framework for calculating the chemical equilibrium within a system (Di Toro et al., 2001; Santore et al., 2001; Paquin et al., 2002). In the development of a marine-specific Cu BLM, these water chemistry measurements would be used as input parameters for a software-based approach, similar to existing freshwater Cu BLMs. The focus of the research reported here is on identifying the effects of dissolved organic matter (DOM) and chromium(II) reducible sulphide (CRS) on copper bioavailability. CRS can accurately measure reduced sulphur with approximately 95% recovery, as HS^-, metal sulphides, pyrite, polysulfides, S^0, thiosulphates, and sulphites, in nM (Bowles et al., 2003). Samples used in this study were originally characterised in DePalma et al. (in press).

DOM has been found to reduce metal bioavailability and is currently an input parameter in freshwater BLMs, measured as dissolved organic carbon (DOC). DOC is operationally defined as organic carbon that passes through a 0.45 μm membrane filter. In coastal marine and estuarine systems, copper toxicity is found to be strongly influenced by DOC, independent of any other water chemistry measurements (Arnold, 2005; Arnold et al., 2006). However, variation in DOM effects

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on bioavailability has been found in both freshwater (Schwartz et al., 2004) and marine water spiked with exogenous DOM (Nadella et al., 2009), so there is a possibility that measurements of DOM source material would improve toxicity predictions. Schwartz et al. (2004) reported specific absorption coefficients at 340 nm (SAC_{340}) of different DOM source material in freshwater as a characterisation method of DOM that could be applied to distinguish the protective effects of DOM from different sources. High SAC_{340} is indicative of terrigenous carbon originating from terrestrial run-off where low SAC_{340} is indicative of autochthonous carbon, which originates directly from the water column (McKnight et al., 2001). In toxicity studies, it has been reported that optically-dark terrigenous carbon significantly decreased bioavailability of copper, lead, and cadmium, relative to autochthonous carbon in freshwater (Schwartz et al., 2004).

Characterisation of DOM by fluorescence spectroscopy can differentiate fluorescent molecules (fluorophores) in a heterogeneous system based on different fluorescent properties. A fluorescence excitation-emission matrix (FEEM) is the result of compiling measured fluorescence data from simultaneous scanning of excitation and emission wavelengths. Based on FEEM analysis, terrigenous carbon can be detected in the Ex/Em ranges of 300–350 nm/400–450 nm and 250–390 nm/460–520 nm, suggesting the presence of terrestrially derived fulvic and humic material, respectively (Smith and Kramer, 1999; McKnight et al., 2001; Wu et al., 2003; Stedmon and Markager, 2005). Autochthonous carbon can be detected by the Ex/Em peaks within of 225–275 nm/350 nm and 225–275 nm/300 nm identifying microbially derived tryptophan-like and tyrosine-like fractions, respectively (Baker, 2001; Stedmon and Markager, 2005). Recently, Winter et al. (2007) identified humic-, fulvic-, tryptophan-, and tyrosine-like fractions in freshwater through fluorescence. In seawater, fluorescence has been a useful technique for DOM characterisation as well, through characterisation of the open ocean (Mopper and Schultz, 1993; Coble, 1996) and estuaries (Hall and Kenny, 2007). Mopper and Schultz (1993) identified a higher abundance of proteinaceous material near the surface of marine water as compared to a higher abundance of humic-like material at greater depth (300–4001 m) in samples collected from the Atlantic and Pacific Oceans, based on the fluorescence Ex/Em wavelengths of approximately 270 nm/320 nm and 310 nm/430 nm, respectively.

To quantify in relative terms, the humic-, fulvic-, tryptophan-, and tyrosine-like fractions observed by fluorescence, parallel factor analysis (PARAFAC) is used here. Through spectral deconvolution of a ‘stack’ of FEEMs, PARAFAC quantifies a minimum number of fluorescent components to describe each FEEM in a set of related samples. Stedmon and Markager (2005) resolved eight components by PARAFAC that described the fluorescent data of 1276 samples. In DePalma et al. (in press), a simple classification scheme was implemented, resolving four operationally defined fractions of humic-, fulvic-, tryptophan-, and tyrosine-like material. Here, these four components will be analysed for correlations with copper toxicity and contributions of fluorescence measurements will be addressed in terms of improving metal toxicity predictions for regulatory purposes.

The presence of reduced sulphur in marine environments has been reported at concentrations of $<0.001-162$ nM in marine and coastal waters by means of voltammetry, high performance liquid chromatography (HPLC), gas chromatography (GC), and various spectroscopic methods such as UV/Vis (Luther and Tsamakis, 1989; Al-Farawati and van den Berg, 2001; Bianchini and Bowles, 2002, and references therein). Reduced sulphur is known to bind strongly to copper in aquatic systems, primarily via (meta)stable complexation (Rozan et al., 2000). Furthermore, it may bind to copper, as Cu(I) and Cu(II), in the presence of other competing metal ions and in the presence of other ligands such as DOM (Al-Farawati and van den Berg, 2001). Very little is known of reduced sulphur in coastal marine and estuarine systems in terms of its effects on copper bioavailability. In DePalma et al. (in press), CRS (as a proxy for reduced sulphur) was measured on 72 unfiltered coastal marine and estuarine samples. Here, CRS concentrations are analysed for correlations with copper toxicity in 10 of the 72 samples measured in DePalma et al. (in press), in relation to copper bioavailability and as a potentially predictive measure of toxicity.

The objectives of this study were to (1) measure acute copper EC_{50} in coastal marine and estuarine waters, (2) identify the relationships between total dissolved copper EC_{50} values and concentrations of humic-, fulvic-, tryptophan-, and tyrosine-like fractions, SAC_{340}, and CRS, and (3) evaluate whether organic matter quality and CRS concentrations should be included as input parameters in a marine-specific BLM. The samples used in this study were a subset of the 72 samples analysed in DePalma et al. (in press). The subset was statistically selected to represent the extreme concentrations of each measured parameter for correlation analysis.

2. Experimental section

2.1. Reagent preparation

2.1.1. Synthetic seawater

Synthetic seawater was prepared by dissolving commercial-grade sea salt (Kent Marine, Atlanta, GA, USA) in MilliQ water (18.2 MΩ) and adjusting to 30‰ ± 1‰ salinity using a PINPOINT® Salinity Monitor (American Marine Inc., Ridgefield, CT). This synthetic sea salt was recommended for use in marine copper toxicity tests by Arnold et al. (2007). All marine standard solutions and dilutions were prepared using this synthetic seawater. The reference toxicity tests were performed using this synthetic seawater.

2.1.2. Copper nitrate solution

A 10^{-4} M solution of reagent grade $Cu(NO_3)_2$ ($>99.99\%$, Aldrich, USA) was prepared using synthetic seawater as described above. This stock solution was used to prepare diluted copper solutions with the ambient water samples for toxicity testing. When not in use, this $Cu(NO_3)_2$ stock was stored in a covered bottle at 4 °C.

2.2. Sample selection

A subset of samples were selected from the 72 listed in DePalma et al. (in press) to measure copper toxicity, based on the measurements of DOC, fluorescence, and CRS. A 2^3 factorial design (Box et al., 1978) was implemented to initially divide the sample set into eight categories through simultaneous comparisons of the main effects of three parameters: DOC, tryptophan, and CRS. From these categories, samples were selected that best represented extreme concentrations of these parameters. Samples representing extreme high and low concentrations of each parameter (and combinations within) would provide quantitative insight into their individual effects reducing copper bioavailability in estuarine environments. Initial categorization of the sites was based on main effects of DOC, tryptophan, and CRS using a 2^3 partial factorial design. From DePalma et al. (in press), humic and fulvic material were strongly correlated to each other and also to DOC, so only one parameter (DOC) was used to represent DOC, humic, and fulvic concentrations. Similarly, tryptophan and tyrosine were strongly correlated and, therefore, were represented as one parameter, tryptophan. Nine samples were selected for toxicity analysis, and are listed in Table 1. In fact, a *partial* factorial was actually implemented due to the nature of the water samples (i.e. not all of the conditions in a full factorial could be satisfied, which include every possible high and low combination of each parameter).