Environmental Pollution 241 (2018) 451–458

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Impact of natural organic matter and increased water hardness on DGT prediction of copper bioaccumulation by yellow lampmussel (*Lampsilis cariosa*) and fathead minnow (*Pimephales promelas*)☆

Rebecca R. Philipps [a], Xiaoyu Xu [a, *], Gary L. Mills [a], Robert B. Bringolf [b]

[a] Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, USA
[b] Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, USA

ARTICLE INFO

Article history:
Received 7 November 2017
Received in revised form
4 April 2018
Accepted 17 May 2018

Keywords:
Bioavailability
Copper
DGT
Speciation
Water chemistry

ABSTRACT

We conducted an exposure experiment with Diffusive Gradients in Thin- Films (DGT), fathead minnow (*Pimephales promelas*), and yellow lampmussel (*Lampsilis cariosa*) to estimate bioavailability and bioaccumulation of Cu. We hypothesized that Cu concentrations measured by DGT can be used to predict Cu accumulation in aquatic animals and alterations of water chemistry can affect DGT's predict ability. Three water chemistries (control soft water, hard water, and addition of natural organic matter (NOM)) and three Cu concentrations (0, 30, and 60 μg/L) were selected, so nine Cu-water chemistry combinations were used. NOM addition treatments resulted in decreased concentrations of DGT-measured Cu and free Cu ion predicted by Biotic Ligand Model (BLM). Both hard water and NOM addition treatments had reduced concentrations of Cu ion and Cu-dissolved organic matter complexes compared to other treatments. DGT-measured Cu concentrations were linearly correlated to fish accumulated Cu, but not to mussel accumulated Cu. Concentrations of bioavailable Cu predicted by BLM, the species complexed with biotic ligands of aquatic organisms and, was highly correlated to DGT-measured Cu. In general, DGT-measured Cu fit Cu accumulations in fish, and this passive sampling technique is acceptable at predicting Cu concentrations in fish in waters with low NOM concentrations.

1. Introduction

Copper (Cu) is an essential element for aquatic organisms that occurs naturally in environments, but can be toxic at higher concentrations often created by anthropogenic actions. Metal toxicity is a function of the metal bioavailability, which is controlled by metal speciation (Campbell, 1995; Di Toro et al., 2001; Newman, 2010). Metal to ligand binding and competition with cations for binding sites impact Cu speciation and its bioavailability (Stumm and Morgan, 1996). Natural organic matter (NOM) is one type of complexing ligand that has been shown to have ameliorative effects on Cu bioaccumulation and toxicity to a wide range of aquatic organisms (Playle et al., 1993; Erickson et al., 1996; Di Toro et al., 2001; De Schamphelaere and Janssen, 2004; Zhong et al., 2012; Giacomin et al., 2013; Deruytter et al., 2014). Other inorganic ligands, including carbonate and hydroxide can also bind Cu, altering speciation, but the effect on bioavailability is less clear (Luider et al., 2004).

Due to the importance of metal speciation on metal bioavailability and toxicity, regulators begun considering water chemistry in making metal regulations. The US EPA first incorporated water chemistry through addition of a hardness factor in 1980 (USEPA, 1980). Additional tools, including geochemical modeling, biological, and passive sampling techniques, have been developed to increase the accuracy and efficiency of assessing metal bioavailability. Controlled toxicology studies and biomonitors have been used to assess the biological response, such as toxicity and accumulation at various water chemistries, and at considerable cost, effort, and variable accuracy (Armstead and Yeager, 2007; Meyer et al., 2007; USEPA, 2007). Models like Biotic Ligand Model (BLM) and

☆ This paper has been recommended for acceptance by B. Nowack.

* Corresponding author. Savannah River Ecology Laboratory, University of Georgia, PO Drawer E, Aiken, SC, 29802, USA.
E-mail address: xiaoyuxu@uga.edu (X. Xu).

https://doi.org/10.1016/j.envpol.2018.05.059
0269-7491/

Windermere Humic Aqueous Model were developed to bridge the gaps of water chemistry and aquatic organism toxicology by using metal complexation parameters to predict metal speciation and membrane binding (Di Toro et al., 2001; Bourgeault et al., 2013). The BLM was adopted by the US EPA for development of sit-specific Cu water quality criteria in 2003 (USEPA, 2007). At various water chemistries, the BLM has been found to over or under-predict toxicity and bioavailable metal concentrations (De Schamphelaere and Janssen, 2004; Craven et al., 2012; Erickson, 2013).

One alternative to determining metal speciation or metal bioavailability via the speciation model is the use of passive sampling devices. One such device is Diffusive Gradients in Thin-films (DGT), which is used as metal speciation devices to determine metal bioavailability (Davison and Zhang, 1994; Zhang et al., 2001; Sigg et al., 2006). The premise behind DGT is that a concentration gradient develops between the bulk solution and the device, allowing only the free and readily dissociated metals (i.e., labile complexes) to diffuse through the two diffusive membranes, and bind to the Chelex resin (Davison and Zhang, 1994, 2012). Concentrations of Chelex bound metals can be quantified and related back to the bioavailable concentration in the bulk solution during the period of deployment. Alterations of water chemistry, such as NOM addition, can affect Cu speciation in the water and thus affect concentrations of DGT-measured metal, because metals that are complexed by NOM or other strongly binding organic molecules diffuse slower through the DGT membranes. While DGT has been primarily used as a metal speciation tool, recent studies begun comparing DGT with metals accumulated by a variety of aquatic organisms (USEPA, 1975; Zhang et al., 2001; Webb and Keough, 2002; Luider et al., 2004; Tusseau-Vuillemin et al., 2004; RØyset et al., 2005; Buzier et al., 2006; Jordan et al., 2008; Schintu et al., 2008). When DGT were used to predict Cu accumulation by *Daphnia magna* that were exposed to Cu with EDTA, NTA, and humic acids, DGT was able to predict metal toxicity in natural water spiked with inert organic ligands but not in the water spiked with labile organic ligands (Tusseau-Vuillemin et al., 2004). In another study comparing DGT to *Daphnia magna,* where four types of wastewater with varying water chemistries (e.g., dissolved organic carbon (DOC), cations, and anions) were used, it was concluded that DGT may be useful as an operational tool, but that more than the bioavailable metal was measured (Buzier et al., 2006). Higher correlation ($p < 0.00001$) was found when DGT was compared to bioaccumulation of Cu by rainbow trout (*Oncorhynchus mykiss*), when exposed to different NOM solutions (Luider et al., 2004). This study also showed increasing NOM concentrations decreased Cu measured by DGT, and the Cu accumulation in rainbow trout gill. These and other studies demonstrate both the usefulness and limitations of DGT for use as a bioavailability and biomimetic device; however, more research on controlled exposure comparisons is clearly needed to better understand the potential applications of this technology.

The objectives of this study were three fold. First, we determined how NOM addition and/or elevated water hardness altered the Cu speciation in the water and thus affect DGT-measured Cu. The hard water treatment included the natural increase in pH, alkalinity, and cations as these parameters co-vary; so the focus was on all changes in water chemistries caused by elevated hardness, rather than elucidating the effect of just one water quality parameter. Second, we compared the DGT-measured Cu to the accumulation of Cu by fathead minnow (*Pimephales promelas*) and/or yellow lampmussel (*Lampsilis cariosa*) to determine if DGT mimicked the organism response. Finally, different techniques were compared. The DGT-measured Cu was plotted to BLM predicted bioavailable Cu, the species complexed with biotic ligands of aquatic organisms and defined as accumulative species in the BLM.

2. Materials and methods

2.1. Experiment setup

The exposure trial was conducted in a climate controlled animal care facility (16 h light: 8 h dark, 22.5 °C ± 0.03) at University of Georgia's Savannah River Ecology Laboratory. Nine treatments were established with 5 replicates each for a total of 45 tanks. The treatments include one factor with three levels of Cu concentrations (0, 30 and 60 μg/L), and a second experimental factor of water chemistry with three types of water: control water, addition of natural organic matter (NOM), or hard water (Table 1). Plastic (HDPE) food-grade containers (20 L) with covers were used as exposure tanks and arranged in five blocks on laboratory benches, with each randomly assigned treatment represented once per block. Synthetic water was prepared following USEPA ASTM requirement guidelines (USEPA, 1975) with Milli-Q water: soft water (48.0 mg $NaHCO_3$, 30.0 mg $CaSO_4$, 30.0 mg $MgSO_4$, 2.0 mg KCl per liter) was used for control water and NOM addition treatments, and hard water (192.0 mg $NaHCO_3$, 120.0 mg $CaSO_4$, 120.0 mg $MgSO_4$, 8.0 mg KCl per liter) was used for hard water treatment. Tanks were spiked with $CuSO_4$ to yield final Cu concentrations of 0, 30, and 60 μg/L. The Cu concentrations were chosen to be at or below the BLM-predicted LC50s for fathead minnows under each treatment condition. NOM was added to appropriate tanks using Suwanee River NOM (International Humic Substances Society) to yield the final NOM concentrations of 4 mg/L. Suwannee River NOM was isolated using a RealSoft Co. reverse osmosis system as described by Serkiz and Perdue (Serkiz and Perdue, 1990; McCurry et al., 2012), which contains 50.7% carbon. So the designed DOC concentration in NOM addition treatment was 2 mg/L. A plastic aerator was inserted into each tank to maintain dissolved oxygen levels and facilitate solution mixing. All tanks were allowed to equilibrate for 24 h prior to introduction of fish, mussels, and DGT.

2.2. Handling of aquatic animals

Clean laboratory stocks of fish and mussel were obtained from Aquatic Toxicology Lab in Warnell School of Forestry and Natural Resources, University of Georgia (Athens, GA) and North Carolina State University (Raleigh, NC),respectively. Upon arrival, fish and mussel were acclimated and held for more than 48 h in aerated synthetic soft water. On day 0, each tank was allocated 5 sub-adult fish (14.3 mg, standard error (SEM) = 1.2 mg), 2 adult mussel (1.9 g, SEM = 0.05 g) and 1 DGT. Feeding did not occur during the 6 day of exposure period. Mortalities were noted and removed from the tank daily. After day 6, fish were removed from the tank, euthanized with MS-222, rinsed with 1% nitric acid and Milli-Q water (Barnstead Nanopure Analytical Ultrapure Water System, Series 1367, >18.2 MΩ/cm), and then placed in clean, labeled, and preweighed whirl bags. Mussels were then removed, prodded with a

Table 1
Setup of exposure treatments. Treatment title is denoted by Cu concentration and water chemistry.

Treatment Title	Cu (μg/L)	Water Chemistry
0-C	0	Control
0-N	0	NOM addition
0-H	0	Hard Water
30-C	30	Control
30-N	30	NOM addition
30-H	30	Hard Water
60-C	60	Control
60-N	60	NOM addition
60-H	60	Hard Water