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Impacts of Effluent and Stormwater Runoff Sources on Metal Lability and Bioavailability in Developed Streams

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Impacts of Effluent and Stormwater Runoff Sources on Metal Lability and Bioavailability in Developed Streams

Hongwei Luan, PhD

University of Connecticut, 2016

Abstract: Urban streams receive Cu from both treated wastewater and stormwater runoff sources. Both sources have significant quantities of organic matter (OM), which is known to control Cu speciation and bioavailability. Individual and mixed water samples from wastewater treatment plant effluents, stormwater runoff, and streams from developed areas were characterized with respect to OM concentration and spectral properties and metal concentration and size distribution. In addition, asymmetric flow-field flow fractionation coupled to inductively coupled plasma mass spectrometry was used to measure concentration, size distribution and association of metals in the colloidal size range. Results reveal that Fe, Cu, Zn and Pb in the colloidal size range were mainly associated with the less than 5 nm, or less than 10 kDa size range. Cu was most strongly associated with OM, while Zn and Pb were mixed between Fe and OM. Effluent showed higher binding capacity for metals, while stormwater, even with higher OM concentrations showed more exchangeable metals. Upon mixing of source waters, colloidal metal concentrations and size distributions were conserved.

Dissolved OM was either left intact or separated into small (0-650 Da), medium (650 Da-50 kDa), or large (50 kDa-0.45 μ m) size fractions using tangential flow filtration (TFF). Conditional stability constants of the OM were determined using competitive ligand exchange-solid phase extraction. Metal ligand concentrations were determined from Cu titrations. Conditional stability constants of the whole effluent OM were 0.5 to 1 log unit higher than those of stormwater OM for both hydrophilic and hydrophobic OM (Hydrophilic: $\text{Log } K_{\text{Cu-eff-OMphi}} = 8.2 \pm 0.2$, $\text{Log } K_{\text{Cu-storm-OMphi}} = 7.5 \pm 0.3$; hydrophobic OM: $\text{Log } K_{\text{Cu-eff-OMpho}} = 8.9 \pm 0.4$, $\text{Log } K_{\text{Cu-storm-OMpho}} = 7.9 \pm 0.1$). The stability constants of the source OM were

all higher than those of algae ($\text{Log } K_{\text{M-total}} = 6.3$ and $\text{Log } K_{\text{M-intra}} = 6.8$). Based on short-term uptake experiments, the results show there was 46% less total Cu uptake in the presence of effluent OM than stormwater OM, while intracellular Cu was about 25% less with effluent. This difference corresponded to the higher conditional stability constants measured for effluent OM than stormwater OM. We also found Cu mainly bound hydrophilic OM (80%) in effluent while it mainly bound hydrophobic OM (77%) in stormwater. Incorporating the conditional stability constants and metal ligand concentrations into an algal uptake model, the Cu uptake could be predicted. As for attachment to algal surfaces, stormwater OM promoted more accumulation of Cu on the algal surface, but this accounted for a small percentage of the total algal Cu content. Furthermore, there was a difference in Cu bio-uptake and stability constants between effluent and storm size fractions. For effluent OM, the Cu uptake and stability constants across size fractions were similar to the whole effluent. For stormwater OM the Cu stability constants were the lowest for the small size stormwater OM, resulting in more Cu uptake as stormwater OM size decreased. Differentiation between OM sources could lead to better predictions of metal uptake to algae or higher trophic level organisms.

Impacts of Effluent and Stormwater Runoff Sources on Metal Lability and Bioavailability in Developed Streams

Hongwei Luan

B.A., Huazhong University of Science and Technology, 2011

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APPROVAL PAGE

Doctor of Philosophy Dissertation

Impacts of Effluent and Stormwater Runoff Sources on Metal Lability and Bioavailability in Developed Streams

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Chapter 1 Introduction

The assessment of risk from metals in streams is coupled to an understanding of availability of a metal to an organism, or bioavailability. Stream systems receive water from various sources that contain metals in different size, stability and lability classes which can affect their mobility, bioavailability, and toxicity (van Leeuwen et al. 2005, Brooks et al. 2007, Bradac et al. 2010). Two major sources that typically contribute elevated metals from developed landscapes compared to the background waters include treated wastewater effluent and stormwater runoff. In addition to metals, these flows carry organic matter (OM) which is known to strongly bind metals in urban streams (Warren et al. 1994, Breault et al. 1996). These sources may drive increased uptake of metals through the food web. Ultimately, the concern of excess metals is increased biotic uptake leading to decreased organism diversity and abundance (Rauch et al. 1999, Beasley et al. 2002, Duarte et al. 2008), particularly of macroinvertebrate populations. However, a clear understanding of the differences in effluent and stormwater runoff effects on metal bioavailability in urban streams is still lacking.

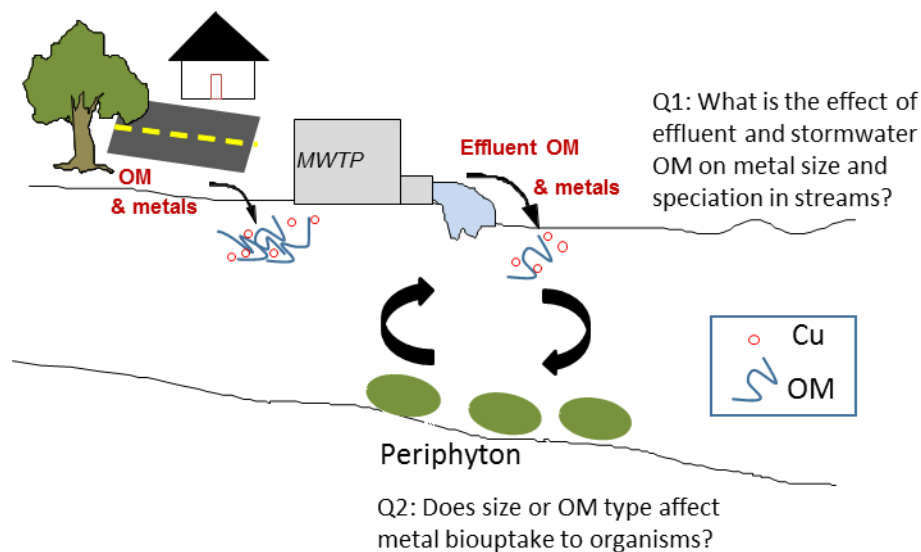


Figure 1 conceptual model of metal source impacts on bioavailability in streams leading to impairment. OM=organic matter

The purpose of this research is (1) to characterize the OM and size distribution of trace metals in different stream waters and source waters in developed landscapes; (2) to investigate the accumulation of Cu in periphyton under the exposure to either effluent or stormwater sources of metals and OM; (3) to assess the use of DGT as a bioavailability indicator for periphyton in streams; and (4) to model the uptake and attachment of Cu and OM from different sources to one component of periphyton, the freshwater algae *Selenastrum capricornutum*. A more comprehensive understanding of metal inputs and their role in bio-uptake at the base of the food chain as a function of both OM characteristics and exposure will help clarify the observed biotic impairments (EPA, 2014) and contribute to more comprehensive ecosystem modeling efforts involving metals.

1.1. Background

1.1.1. Size and lability characteristics of OM and associated metal inputs to streams

There are previous measurements of OM and inorganic colloid size distributions and metal-OM binding constants in natural streams, but fewer observations have been made in distinct anthropogenic inputs to these streams, such as wastewater effluent or stormwater runoff. Effluent discharges from municipal wastewater treatment plants contribute a significant source of OM to streams, around 5 mg C L⁻¹ (Quaranta et al. 2012). This OM is mostly in the small size range, less than 10 kDa, with some components in higher size ranges >50 kDa (Worms et al. 2010, Quaranta et al. 2012). The OM characteristics of effluent are more similar to microbial sources typical of the bacterial-based treatment processes. Advanced size fractionation techniques such as flow field-flow fractionation (F4) coupled to highly sensitive metal detectors like inductively coupled plasma-mass spectrometry (ICP-MS) have shown variations in the molar mass, size distribution, and metal and OM associations in effluent. Metals such as Cu, Zn, and Cd are typically associated with the low molecular mass fraction, while Al, Fe, and Pb were distributed between both size fractions (Worms et al., 2010). On the other hand, storm runoff brings variable concentrations of OM to streams. Storm OM originates from terrestrial surface soil OM. A higher molecular mass distribution was observed in streams impacted by large rain events in a mostly pristine river (Li et al. 2003), but a closer

look at the size distribution, metal ligands, and metal stability constants of the dissolved OM fractions entering with stormwater runoff has not been conducted for streams in developed areas.

Studies have reported differences in metal-OM binding strength and capacity in streams. From effluent, small but strong complexing ligands such as nitrilotriacetic acid (NTA) can have a significant influence on metal binding capacity in streams (Nowack et al. 2005). This corresponds to OM in the <1 kDa fraction from treated effluent which has a greater capacity to chelate metals (Jarvie et al. 2012). A recent study comparing road runoff and effluent OM suggest similar conditional stability constants with Cu, log K' of 7.2 and 7.4, respectively, but that effluent data fit to a two-ligand model with a second log K' of 6.4 with an overall 2-3 fold higher concentration of binding ligands in effluent compared to road runoff (Chaminda, Nakajima et al., 2010). Our studies suggest that one watershed had similar binding capacities for Zn in stormwater and effluent, despite the 3-fold difference in OM concentration, while stormwater runoff had about a 5-fold higher binding capacity for Cu than effluent (Luan and Vadas, 2015). In addition, metal binding capacity is also known to vary with OM size. The ultrafilterable (<3 kDa) organic ligands (log $K \sim 13.5$) in freshwater rivers with moderate organic carbon content and low inorganic colloid content have 0.5 log unit higher binding constants than the 0.45 μm filterable fraction (Hoffmann et al. 2007). A contradicting result was observed in other freshwater samples, with high molecular weight (>1 kDa) DOM generally having higher metal binding constants with Cu than bulk (unfractionated) and low molecular weight (<1 kDa) DOM, though the difference was less than 1 log unit (Chen et al. 2013). Though neither observation saw a big difference with size in natural stream samples, size fractions of effluent are likely to show a more significant difference attributed to the small synthetic chelator compounds like EDTA that are poorly removed in the treatment process (Weyrauch et al. 2010). What potentially plays a more important role is the abundance of ligand sites and therefore capacity for binding Cu which in our observation was much higher in stormwater than in effluent sources.

1.1.2. Assessment of Bioavailability to periphyton

Understanding metal speciation in solution is important for modeling and understanding which metal form is responsible for biouptake (Luoma et al. 2005). However, in field observations, indicators of bioavailability have often been used to aggregate the influence of metal exposure. Periphyton has been used as a biological indicator of water quality due to its abundance in freshwater systems and high sensitivity to changes in the surrounding aquatic environment (Wetzel 1983). With respect to metal uptake and trophic transfer, the importance of periphyton metal content in the overall food web has often been overlooked. For example, existing toxicity assays do not address metal uptake from algal food sources. One area lacking in understanding is the role of OM in controlling total and intracellular periphyton metal content. Effluent may have stronger metal binding ligands which may reduce the metal availability to organism uptake processes. Stormwater may have more hydrophobic metal binding OM that would contribute more surface adsorbed metals to the organism. Most studies thus far are field based and have focused on intracellular metal content in periphyton biofilms because they were only concerned with metals available to that organism (Brooks et al. 2004; Meylan et al. 2003). Field studies have generally observed that Cu, Zn and Cd uptake in periphyton are variable, but often correlate to exchangeable metal concentrations defined as the fraction of Cu in a free or weakly complexed form that exchanges with the coppercatechol complexes (Meylan et al. 2003). Within the context of a system with both stormwater and effluent sources, the goal is not only to understand periphyton metal uptake, but also variations over time as exposure to these sources change (e.g. stormwater), and metals attached on the surface of the periphyton since it contributes to trophic transfer.

1.1.3. Diffusive gradient in the thin film (DGT) devices as bioavailability indicators

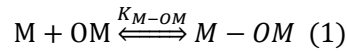
As a potential alternative tool for assessing bioavailable metals in streams, diffusive gradient in the thin film (DGT) devices are potentially useful. These have been recognized as a reliable indicator of metal uptake by plant roots (Zhang et al. 2001), but their use has not been fully investigated in stream systems. The standard DGT device consists of three layers. A resin layer contains Chelex-100 which is a strong

sorbent for most transition metals. On top of that, a diffusive polyacrylamide gel and a nitrocellulose filter are placed to fix the exposure of the gel to particles less than 0.45 µm and limit the transport processes to diffusion (Zhang and Davison, 2000). Only one study has compared DGT to periphyton uptake and found total and intracellular Cd periphyton contents were well correlated with DGT measured Cd in recirculating exposure channels (Bradac, Navarro et al., 2009). However, in the field, results were less consistent (Bradac et al. 2009). DGT will be evaluated as an indicator of Cu bioavailability to periphyton from both stormwater and effluent sources.

1.1.4. Influence of OM characteristics on metal binding and uptake by algae

The interaction of a metals with an aquatic organism normally involves the following processes:

- 1) Trace metals, and their complexes, must first diffuse from the bulk solution to the biological surface (mass transport).
- 2) Metal speciation varies as OM, pH, or hardness cation concentrations change, resulting in association or dissociation of metal-OM complexes.



$$[M - OM] = K_{M-OM} \cdot [M] \cdot [OM] \quad (2)$$

where M is the free metal in solution, OM is the organic ligand, K_{M-OM} is the stability constant of metal M binding with ligand OM.

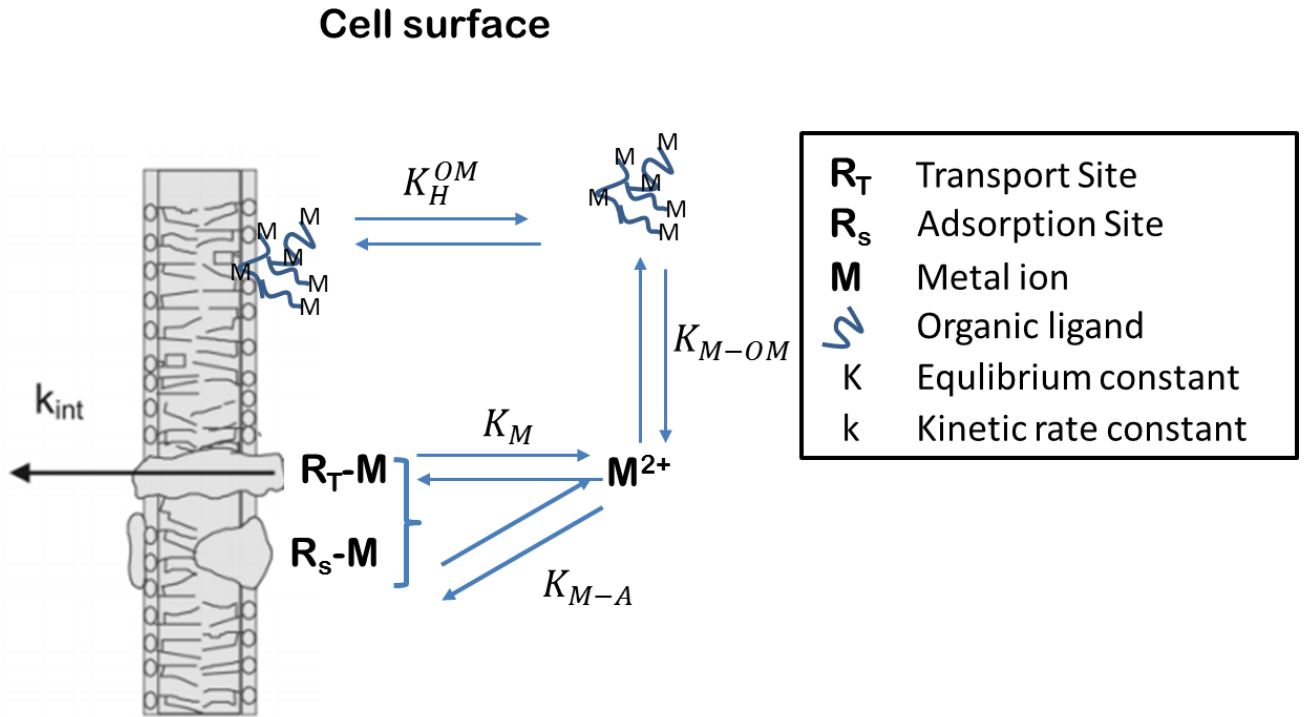
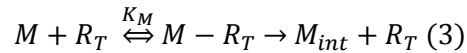


Figure 2 Conceptual model of metal-alga interactions. Size and source water are two possible factors affecting metal uptake (Campbell et al. 2002).

3) . To have an effect, the metal must adsorb to or complex with a site on the biological membrane



$$[M-R_T] = K_M \cdot [R_T] \cdot [M] \quad (4)$$

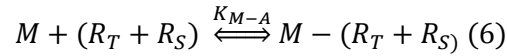
where R_T is the concentration of a physiologically active metal binding site on the surface of the organism (e.g. a transporter protein) (Slaveykova et al. 2005).

The uptake model assumes that only free metal, e.g. Cu^{2+} , interacts with the transport sites. The intracellular metal concentration, C_{int} , is described by the Michaelis-Menten saturation equation (Sunda et al. 1998):

$$C_{int} = \frac{C_{max,int}[M]K_M}{[M]K_M + 1} \quad (5)$$

Where [M] is the concentration of either the free metal ion. K_M is an affinity constant for the binding of metal M to the transport site (which can compete with aqueous organic ligands for M), C_{max} is the maximum metal concentraion achieved at metal saturation of the transport sites.

Similarly, for a fixed exposure time, the total cellular metal concentration (internalized plus adsorbed, C_{cell}) can be fitted by the same hyperbolic equation, assuming that metal adsorbs simultaneously to two types of binding sites, but only one is followed by internalization (Lamenas et al. 2008):



where R_s is the concentration of adsorption metal binding site on the surface of the organism.

$$C_{cell} = \frac{C_{max,cell}[M]K_{M-A}}{[M]K_{M-A} + 1} \quad (7)$$

Where $V_{max, cell}$ is the max cellular metal concentration and K_{M-A} is the effective average binding constant of metal to algae, representing metal affinity for both adsorption and internalization.

4) In addition, it has been demonstrated that OM will adsorb to algal surfaces (Campbell et al. 1997).

Therefore, the total metal content in a system containing algae, metal, and OM needs to consider both the metal uptake by algae and metal bound to OM and adsorbed to algae.

$$C_{cell,tot} = C_{cell} + C_{OM, adsorbed} \quad (8)$$

$$C_{cell,tot} = \frac{C_{max,cell}[M]K_{M-A}}{[M]K_{M-A} + 1} + K_H^{OM}[OM][M]K_{M-OM} \quad (9)$$

Where K_H^{OM} is the linear regime adsorption coefficient.

Metal association with periphyton is affected by metal speciation and adsorption processes. The majority of previous work on algal uptake focuses on the free ion activity model (FIAM), where free Cu^{2+} is assumed to be the only available metal species. In several cases this model was not valid, e.g. Pb uptake was higher than predicted in the presence of Suwannee River fulvic acid (SRFA) (Slaveykova et al. 2003)

and colloidal effluent OM (Worms et al. 2010). This was attributed to the formation of a ternary complex between Pb, algae, and dissolved OM that promoted transport into the cells due to the proximity to the transporter active site (Lamelas and Slaveykova, 2007) (Campbell et al. 1997, Knauer et al. 2001, Lamelas et al. 2005). The only observed difference between Pb and other metals in effluent sources, was that Pb was primarily bound to the higher molecular weight fraction while other metals were not (Worms et al. 2010). Worms et al. (2010) also found that Cd uptake closely followed the exchangeable fraction of Cd, not just the free Cd^{2+} . While Pb may be a special case, it seems more likely that for most metals, the readily exchangeable, i.e. the free metal and the weakly OM associated metals are available for transport by the organism.

In addition to actual metal transport into the algal cell, the interaction of metals and OM with cell surfaces is important due to its role in trophic transfer. OM interaction with cell surfaces can involve cation bridging, electrostatic, hydrogen-bonding or van der Waals attraction between the cell surface and different domains of the OM (Campbell et al. 1997). In experiments with humic acid associated metals, a high hydrophobic character or high aromaticity typical of humic substances were suggested to promote the (Lamelas et al. 2005, Lamenas et al. 2008). In studies mentioned above with Cd and Pb, Worms et al. (2010) suggested that effluent OM adsorption to cell surfaces was not significant due to the relatively low SUVA values (i.e. low aromaticity) determined for the colloidal fractions and the relatively high pH. Since stormwater runoff generally has higher aromaticity (Luan et al. 2015), it is expected to sorb to a greater extent to algal surfaces. In order to form a more complete model of algal cell metal association, both metal transport and surface adsorption will be investigated with respect to effluent and stormwater OM sources.

The metal-OM species association with cell surfaces is likely also affected by its size. In contrast to smaller OM, larger OM could generate higher electrostatic fields and will have greater probability of forming multidentate metal binding sites than small molecules. Larger molecules are also likely to have more aromatic C, leading to greater association of hydrophobic compounds (Cabaniss et al. 2000). In

addition to assessing optical and metal binding differences in OM characteristics with size, the adsorption of different size fractions of source OM onto algae cell surface will be assessed.

1.2. Research questions and hypotheses

Two key points arise from past research. First, that there are differences in organic-metal binding capacity and lability depending on the source water and the metal (Chaminda, Nakajima et al. 2010). This is likely to play a role in controlling transport into simple organisms such as algae. Second, that metal lability is not the only controlling factor, even in simple organisms. Attachment of the organic bound metal to the cell surface may increase total metal content and could promote uptake (Lamelas and Slaveykova 2007). Both factors are important to assess as metals associated with colloidal OM or single-celled organisms like algae are ingested by higher trophic level organisms.

This dissertation will focus primarily on the influence of OM sources in urban streams on metal uptake in algae. The first chapter, already published (Luan and Vadas 2015), assesses chemical and size differences between stormwater and effluent sources of metals and OM. The second chapter focuses on how total and intracellular metal content of periphyton change over time when exposed to either effluent and/or stormwater metal sources and how they compare to DGT devices as chemical surrogates for bioavailability. In stream exposures, I hypothesized that effluent would increase the Cu accumulation within periphyton, while stormwater would result in a spike in total metal accumulation followed by release. Although we found several distinct differences in the OM and metal size and chemical characteristics of stormwater and effluent, the field assessment of metal accumulation in periphyton was ambiguous with regards to what was driving accumulation in or on periphyton. Was it just free metal concentrations, was it related to differences in stability constants of the OM ligands, was it attachment of particulate metals, etc. While we could control for which source water periphyton was exposed to in streams and compare periphyton metal uptake to DGT measured metals (a measure of readily exchangeable metals), we could not well control the chemical concentrations and speciation, which varied significantly for storm events. The final chapter therefore focuses on more controlled laboratory studies with isolated size fractions of OM and Cu from different

sources exposed to a culture of freshwater algae. I hypothesize that OM with a more hydrophobic character and larger size will promote more metal adsorption on algal cells, while transport will be controlled by metal-OM stability constants. I will build on an existing model framework to incorporate variations in size fractionation (dissolved/colloidal/particulate) and source waters on accumulation of Cu within or on the surface of algae. If significant differences are observed based on size, stability constants, or OM characteristics, then future field work and modeling can focus on the most relevant OM fractions and metal species.

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Chapter 2. Size characterization of dissolved metals and organic matter in source waters to streams in developed landscapes

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2.1. Introduction

Stream systems receive water from various sources that contain metals in different size, stability and lability classes which can affect their mobility, bioavailability, and toxicity (Buffle et al. 1995, Imai et al. 2002, Bradac et al. 2010). Two major sources that typically contribute elevated metals compared to the background waters include treated wastewater effluent and stormwater runoff. From these sources, typical sampling only distinguishes between particulate and dissolved fractions by filtration through a 0.45 μm filter, but the dissolved fraction contains a continuum of sizes of both organic and inorganic colloids that can bind metals to varying degrees (Campbell et al. 1997, Leenheer et al. 2003) as well as free metals. The environmental fate of metals in the dissolved fraction may vary depending on dissociation, aggregation, sedimentation, sorption, or biouptake processes.

Several studies have measured size distribution of dissolved phase metals in streams and large rivers and found that iron oxide and organic matter colloids are important for binding and transporting metals (Stolpe et al., 2013, Vega and Weng, 2013 and Dahlgvist et al., 2007). Measured metal concentrations as a function of filtration and ultrafiltration of waters have shown that in river and lake systems that are not highly polluted, the greatest percentage of metals are in the colloidal phase (Town and Filella, 2002). The fraction in the higher end of the colloid size range typically coincide with increased Fe concentrations, while the lower size range typically associates with the low molecular mass organic matter fraction. Organic matter (OM) and iron-rich colloids have exhibited notably different affinities for trace elements (Neubauer et al., 2013 and Stolpe et al., 2005). While many studies have found that Cu is typically associated with

dissolved organic carbon (DOC) of less than 5 nm (Neubauer et al., 2013 and Vega and Weng, 2013), Pb and Zn are more typically associated with larger inorganic particles.

While previous measurements of stream waters and natural inputs suggest typical OM and inorganic colloid size distributions and metal associations, fewer observations have been made from anthropogenic inputs to these streams, such as wastewater effluent or stormwater runoff, specifically in urban streams. Effluent discharges from municipal wastewater treatment plants contribute a significant source of organic matter to streams, around 5 mg C L^{-1} (Quaranta et al., 2012). This organic matter is mostly in the small size range, less than 10 kDa, with some components in higher size ranges $>50 \text{ kDa}$ (Quaranta et al., 2012 and Worms et al., 2010). The organic matter characteristics are more similar to microbial sources typical of the bacterial-based treatment processes. Advanced size fractionation techniques such as flow field-flow fractionation (F4) coupled to highly sensitive metal detectors like inductively coupled plasma-mass spectrometry (ICP-MS) have shown variations in the molar mass and size distribution of metal complexes in effluent. Metals such as Cu, Zn, and Cd are typically associated with the low molecular mass fraction, while Al, Fe, and Pb were distributed between both size fractions (Worms et al., 2010). Less is known about stormwater runoff inputs. A higher molecular mass distribution was observed in streams impacted by large rain events in a mostly pristine river (Li et al., 2003), but a closer look at the dissolved fractions entering with stormwater runoff has not been conducted for streams in developed areas.

Organic matter and trace metals in impacted streams are expected to exhibit different size distributions dependent on source waters and when subjected to changing hydrochemical conditions, i.e. varying ionic strength or hardness cation concentrations (Bolea et al., 2006 and Ledin et al., 1993). While the source waters may enter with one set of characteristics, upon mixing of effluent or stormwater runoff with stream water, redistribution of metals to different binding phases or size fractions may occur. The importance of size in stream systems relates to feeding habits of macroinvertebrates and higher organisms. The high molecular mass (HMM) organic fraction can play a major role in increasing the uptake of metals by some filter feeders (Roditi et al., 2000) or be subject to aggregation and sedimentation with mineral colloids when

the ionic strength is elevated (Stolpe and Hasselov, 2007). In contrast, smaller size colloidal fractions of organic matter in effluent may concentrate metals which can disproportionately influence bioavailability in primary producers, such as the benthic periphyton, and increase trophic transfer to macroinvertebrates. Also, small organic matter is expected to be more mobile in aquatic ecosystems (Stolpe and Hasselov, 2007) and will likely promote longer distance transport of metals.

The purpose of this study was to characterize the organic matter and size distribution of trace metals in different stream waters and source waters in developed landscapes. We sampled from three development impacted streams and treatment plants, as well as stormwater runoff over the course of a storm event and compared the particulate, dissolved and colloidal size fractions of metals using both standard and advanced tools such as asymmetric flow-field-flow fractionation (AF4) coupled to ICP-MS. Our objectives were to characterize differences in metal association as a function of colloid type and whether significant changes would occur upon mixing of the source waters. The influence of size distributions and OM properties with respect to transport and bioavailability are discussed.

2.2. Methods

2.2.1. Site description and sampling

Three wadeable streams in developed areas of Connecticut including, including the Hockanum River (HR), Quinebaug River (QR) and Willimantic River (WR) were selected for this research. The ten-year average annual discharge of these three rivers was 4, 40 and 6.8 m³ s⁻¹, respectively, at the United States Geological Survey (USGS) gauging stations. The wastewater treatment plants (WWTP) on each of these rivers are at minimum secondary treatment, with the QR and WR WWTPs also implementing advanced nitrogen removal. None of the WWTP received combined sewer and stormwater loads. The HR WWTP contributes about 10-40% of the flow of the HR at the point of input, while the others contribute less than 10%.

2.2.2. Sample collection and preparation

Upstream water (H_{up} , W_{up} , Q_{up}) and WWTP effluent (H_{eff} , W_{eff} , Q_{eff}) were collected from each of these watersheds during May and June. Upstream samples were obtained prior to the discharge point of the WWTP effluent. Stormwater runoff samples (H_{st}) were collected from a stormdrain outfall downstream of the WWTP. Samples were collected in 20 L HDPE carboys that had previously been acid washed with 0.1 M HNO_3 and rinsed with deionized water ($>18.2\text{ M}\Omega$) to limit background metal contamination, and preserved in the dark at 4 °C before analysis. The pH and conductivity of the samples were measured in the field. The samples were divided into total and dissolved fractions by filtering through a 0.45 μm pore size nitrocellulose membrane. To assess any changes that may occur in the dissolved phase following mixing, the effluent and first time point stormwater runoff samples from the Hockanum River were mixed with upstream samples from the same catchment at a ratio of 3:7 or 1:1, respectively, common mixing ratios for receiving streams. In addition, samples were spiked with a 10-fold increase in Cu, Zn and Pb, and in some cases 2- or 10-fold increase in Ca concentrations to assess metal binding capacity of the different size fractions or the influence of an exchangeable cation on metal distribution. The mixed samples were shaken for 24 h prior to analysis.

2.2.3. Metal analysis of bulk samples

The total and dissolved metal samples were acidified to 2% HNO_3 (trace metal grade). Total metal samples were analyzed according to EPA Method 200.8 (1994). Inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700x with He collision cell, Agilent, Delaware, USA) was used for determination of the elements in this work. For batch sample analysis, Bi, Ho, In, and Sc were used as internal standards. Standards and QC checks were prepared from independent high purity standards (Spex-Certiprep, Metuchen, NJ, USA and VHG Labs, Inc., Manchester, NH, USA). Quality control samples, internal standards, and spike recoveries were within 10% or better of expected values. Particulate metal concentrations were calculated from the difference between total and dissolved metals.

2.2.4. Characterization of dissolved organic carbon

The DOC concentration was determined using a total organic carbon analyzer (Apollo 9000, Tekmar-Dohrmann, Mason, OH, USA). Potassium hydrogen phthalate was used for standards (Ricca Chemical, Arlington, TX, USA), and laboratory prepared standards from the same chemical were used for quality control samples, all of which were within 20% or better of expected values.

The absorbance spectra of samples was scanned between 200 to 550 nm by a Cary 50 spectrophotometer (Australia). Specific UV absorbance (SUVA in $\text{L mg}^{-1} \text{m}^{-1}$) was calculated by normalizing the UV-absorbance at 254 nm (in cm^{-1}) by the DOC-concentration (mg L^{-1}) and then multiplying by 100 (Weishacar et al 2003). The pH of samples for fluorescence analysis was adjusted to 2 by the addition of HCl. All fluorescence data was collected on a Cary Eclipse fluorescence spectrometer (Australia) with a xenon flash lamp, 5 nm slit widths, and a scan rate of 1200 scans per second. Excitation wavelengths from 200 to 450 nm in 10nm increments, as well as emission wavelengths from 250 to 550 nm in 2nm increments were scanned. Excitation-emission matrices (EEMS) were corrected and plotted following established protocols (Quaranta et al 2012). The fluorescence index (FI) was calculated as the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm for an excitation wavelength of 370 nm (Schwede-Thomas et al. 2005).

2.2.5. Size fractionation using asymmetric field flow field fractionation (AF4) coupled to ICP-MS

Concentration and size distribution of colloidal metal complexes was characterized by asymmetric flow field flow fractionation (AF4, Postnova Analytics, Landsberg, Germany) coupled on-line to UV detection at 254 nm for aromatic DOC determination and ICP-MS for trace metal determination. The AF4 2000 Control software (Postnova Analytics) was used for data collection and analysis of UV signals and size calculations, while Agilent Chemstation with MassHunter analysis software was used for time resolved analysis of metals. Prior to injection into the ICP-MS introduction system, a 6% nitric acid solution containing 500 ppb Sc was mixed in as an internal standard and to acidify the neutral samples. The AF4

system was metal free and equipped with a 275 mm long trapezoidal channel cartridge and different size spacers depending on the method. The mobile phase was 10 mM NaNO₃ with pH matched to the source water. Samples were analyzed in two different modes to capture more resolution in the large or small size colloidal fractions. Capture of the large size fraction was achieved using a 10 kDa cut-off polyethersulfone (PES) membrane and a 350 µm spacer. Samples were injected to the channel using a 1 mL sample loop at an injection flow of 0.2 mL min⁻¹ for 6 min. After a 1 min transition time, samples were separated using a channel flow of 1 mL min⁻¹ and a constant cross flow of 1 mL min⁻¹ over 40 min. To capture the small size fraction, a 300 Da cut-off PES membrane was used with a 500 µm spacer. We justified the use of a 300 Da cut-off PES membrane for the smaller size fractions as opposed to 1 kDa PES or regenerated cellulose (RC) membranes typically used in the literature (Stolpe et al., 2010 and Worms et al., 2010) based on recovery of metals during analysis with or without cross-flow. Use of the 300 Da membrane resulted in the highest recovery for both the stormwater and effluent source waters we examined (Table 1), suggesting there was less sample interaction with the 300 Da membrane as opposed to the 1 kDa PES or RC membranes. A channel flow of 0.5 mL min⁻¹ and a constant crossflow of 3 mL min⁻¹ over 20 min was used to separate the small size fraction. Only iron oxide and organic matter colloids were detectable using AF4-ICP-MS, while Al or Si based colloids were not.

Table 1 Metal recovery from a 1 kDa regenerated cellulose (RC), 1 kDa polyethersulfone (PES) and 300 Da PES membrane used in the AF4 system. The system was run without crossflow to assess the dissolved phase metal attachment to the membrane.

| Membrane | Recovery | Fe | Cu | Zn | Cd | Pb |
|------------|-------------|------|------|------|------|------|
| RC 1 kDa | QC 20ppb | 109% | 122% | 103% | 105% | 106% |
| | Storm water | 82% | 58% | 63% | ND | ND |
| | Effluent | 69% | 100% | 106% | ND | ND |
| PES 1 kDa | QC 20ppb | 98% | 101% | 98% | 101% | 94% |
| | Storm water | 92% | 71% | 69% | ND | ND |
| | Effluent | 72% | 107% | 110% | ND | ND |
| PES 300 Da | QC 20ppb | 109% | 117% | 103% | 102% | 104% |
| | Storm water | 104% | 88% | 83% | ND | ND |
| | Effluent | 77% | 97% | 93% | ND | ND |

2.2.6. Size calibration of organic matter

A series of polystyrene sulfonate (PSS) reference standards in sodium salt form (Polysciences; Eppelheim, Germany) of different molecular weights (1K, 1.8K, 4.6K, 18K, 35K Daltons) were used for size calibration. The PSS standards were prepared individually in mobile phase for analysis at 100 mg/L. The UV detector was set to analyze the PSS standards at 224nm. An aliquot of 100 μ L of the PSS dispersion was injected to the AF4 at the same flow conditions as above. Using the calibration curve, the x-axis of the chromatograms was converted from time to log molecular weight. In addition, the AF4 analysis software (Postnova Analytics, Landsberg, Germany) was used to calculate hydrodynamic diameter based on flow conditions.

2.2.7. Quantification of metal concentrations

The metal concentration measured by AF4-ICP-MS was quantified using an external calibration curve. Matrix-matched standards containing 0-100 ppb of Fe, Cu, Zn, Cd, and Pb were prepared from single element ICP standards (VHG Labs, Inc., New Hampshire, USA). Standards were spiked with EDTA to a final concentration of 0.5mM and pH adjusted to the pH of the mobile phase to minimize metal attachment to the membrane. The standards, quality control standards, and samples were injected in the same manner as the samples for size distribution, but without any cross flow. A standard curve was generated based on peak area of each metal and excellent fits were obtained ($r^2 > 0.99$). The measurements of total colloidal phase metal concentrations were calculated based on total integrated peak areas. The method detection limits for Fe, Cu, Zn, and Pb were 1.6, 0.2, 1.0, and 0.1 ppb as determined by standard methods (APHA, 2005). Truly dissolved metal concentrations (< 300 Da) were calculated from the difference between dissolved and colloidal phase metal concentrations.

2.3. Results and discussion

2.3.1. Organic matter concentrations and spectral characteristics

The DOC concentrations were similar in upstream and effluent samples, with the exception of QR effluent having nearly triple the DOC concentration (Table 2). The stormwater samples had much higher DOC concentrations, ranging from 14 to 34 mg L⁻¹, with no obvious trend over time. The SUVA values indicated that effluent organic matter was similar to microbially derived material (Park et al., 2010 and Imai et al., 2002), ranging from 1.5 to 1.76 L mg⁻¹ m⁻¹, and the stream organic matter had higher SUVA values, above 3 L mg⁻¹ m⁻¹, indicating similarity to other standard river and low order stream organic matters (Stutter et al., 2013 and Weishaar et al., 2003). The stormwater SUVA values were between 1.9 and 2.7 L mg⁻¹ m⁻¹, with a general increase over time during the runoff event. This may be indicative of a lower rate

Table 2. Summary of dissolved organic carbon (DOC), specific ultraviolet absorbance (SUVA) and fluorescence index (FI) for source water samples.

| Sample | DOC (mg L ⁻¹) | SUVA (L mg ⁻¹ m ⁻¹) | FI |
|--------------|---------------------------|--|------|
| H_up | 4.89 | 3.27 | 1.32 |
| W_up | 5.22 | 3.71 | 1.22 |
| Q_up | 5.07 | 4.09 | 1.33 |
| H_eff | 5.28 | 1.5 | 1.9 |
| W_eff | 3.91 | 1.64 | 1.97 |
| Q_eff | 14.7 | 1.76 | 1.69 |
| H_st 15 min | 34.33 | 1.91 | 1.41 |
| H_st 30 min | 18.54 | 1.92 | 1.42 |
| H_st 45 min | 14.59 | 2.19 | 1.22 |
| H_st 60 min | 18.86 | 2.42 | 1.26 |
| H_st 105 min | 27.29 | 2.74 | 1.44 |
| H_st 120 min | 25.07 | 2.22 | 1.28 |

of solubility or leachability of more aromatic DOC sources. The fluorescence index (FI) values for effluent samples were the highest, 1.69 and above, similar to what others have observed for effluent organic matter isolates and microbial sources (McKnight et al., 2001 and Worms et al., 2010), while stream and stormwater samples had similar values between 1.22 and 1.44, indicating terrestrial origins (McKnight et al., 2001). While the effluent and stream source waters had typical DOC concentrations and spectroscopic

characteristics relative to the expected sources of organic matter, the much higher concentration of DOC in stormwater and the variable spectroscopic properties could indicate differences in metal reactivity during a storm (Baken et al., 2011).

The EEMs matrices showed humic-like fluorophores characteristic of natural organic matter (peaks A and C) in all samples, whereas only effluent from H and Q had strong tryptophan-like organic matter (peaks B and T1). Even between the two effluent sources, the A, B and T peaks vary greatly in range with Q_eff having a broad and intense peak spanning from $\lambda_{Ex/Em} = 230 \text{ nm}/300\text{--}450 \text{ nm}$, and H_eff having a higher percentage of the intensity in the A peak region. This broad and narrow peak in the effluent samples looks more similar to the hydrophobic fraction than the transphilic fraction of organic matter from Quaranta et al. (2012). The third W_eff had the lowest DOC concentration, and showed the lowest intensity, but had clear peaks in A and the fulvic-like region of C at $\lambda_{Ex/Em} = 275 \text{ nm}/400 \text{ nm}$, a peak unique to that sample, and the humic-like fraction of C at $\lambda_{Ex/Em} = 350 \text{ nm}/400 \text{ nm}$. The humic-like peak in C is also discernible in most other samples. Even though W_eff does not show a strong T peak, it still has about 21% of the area normalized fluorescence intensity associated with that region, compared to about 28% for Q_eff and H_eff. The bulk of the fluorescence intensity for all samples is represented in the A peak, accounting for about 40–50% of the intensity in the effluent and stream samples, and greater than 60% in the stormwater samples. The shape of the A peak differs between samples as well, with the stream and stormwater samples exhibiting much broader peaks across the excitation wavelengths compared to effluent samples. Between the stormwater and stream samples, the center of the A peak shifts left in stormwater samples compared to stream samples. Another shift occurs in the effluent samples, with the peak near $\lambda_{Ex/Em} = 320 \text{ nm}/430 \text{ nm}$ in streams shifted to the left in effluent samples. A noticeable difference in this fulvic-like region was also observed in effluent colloidal isolates compared to suwanee river fulvic acids in Worms et al. (2010).

Depending on the lability of these organic matter fractions, effluent and/or stormflow could alter bioavailability in the stream. The T peaks are thought to be the most labile fraction of organic matter and associated with the growth of microorganisms (Hudson et al., 2007). This is likely the fraction that promotes

the enhanced microbial activity observed downstream of wastewater treatment plants (Wakelin et al., 2008). Since the stormwater samples don't show distinct T peaks, perhaps the overall lability of this source is reduced; however, stormflow in natural ecosystems has been shown to promote a 5-fold higher DOC utilization rate (Buffam et al., 2001). Both the rate of DOC utilization as well as the differences in binding affinities may alter metal bioavailability in streams.

2.3.2. Metal distribution between particulate, colloidal and truly dissolved fractions

The bulk metal distributions in stream, effluent and stormwater samples showed large variations. Upstream and stormwater samples usually had more total Fe and higher colloidal Fe fractions than the effluent, with the exception of H_{up} (Fig 2a). More than 30% of Fe was present in the particulate form especially for the stormwater (above 60%) which correlates with particulate Pb concentrations in solution. On the other hand, the effluent and stormwater samples had much higher Cu and Zn concentrations than the upstream samples. There is some ambiguity in distinguishing the truly dissolved from the colloidal size fractions due to the reduced recovery of metals when using ultrafiltration membranes (Table 1). The recovery is lowest for Cu and Zn from stormwater sources and Fe from effluent sources, suggesting we may be underestimating those sources due to sample interaction with the membrane. Recovery for Cu and Zn in effluent and Fe in stormwater is within the generally accepted range of 10% for ICP-MS analysis of metals. The observed metal concentrations vary in their environmental concern. Regulations based on dissolved concentrations set acute and chronic toxicity limits for Zn at 65 $\mu\text{g L}^{-1}$ for both, and for Cu at 14.3 and 4.8 $\mu\text{g L}^{-1}$, respectively (CT DEEP, 2013). While some of the reported concentrations of effluent or stormwater are at or above these levels, they would only be a concern if the dilution ratios were low in effluent receiving stream reaches, or if concentrations remained elevated for days in stormwater receiving stream reaches. With respect to size distribution, Cu and Zn were mostly found in the truly dissolved fractions (usually >50%), 20–50% in the colloidal phase, and a typically smaller fraction in the particulate phase which is similar to previous observations of effluent sources (Worms et al., 2010). The colloidal size

fraction, which can contain up to 50% of the dissolved metals, covers a large size distribution, and samples were further analyzed to assess the metal association between organic matter and iron colloids.

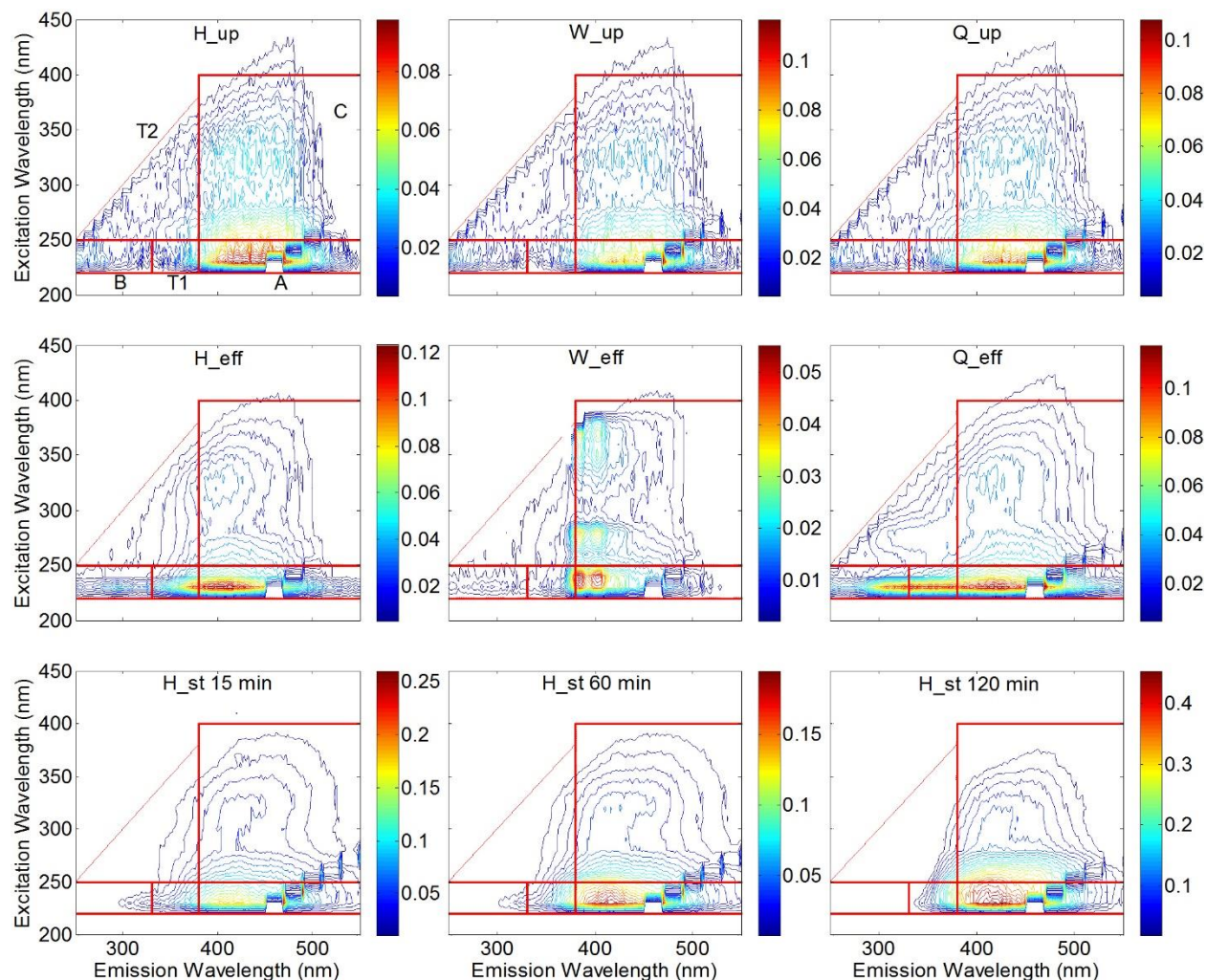


Figure 1. Excitation-emission matrices of dissolved samples in upstream, effluent or stormwater samples. The outlines across the EEMs spectra and associated letter labels represent humic-like (A and C) or protein-like (B, T1 and T2) fluorescence regions as identified by others (Hudson et al., 2007).

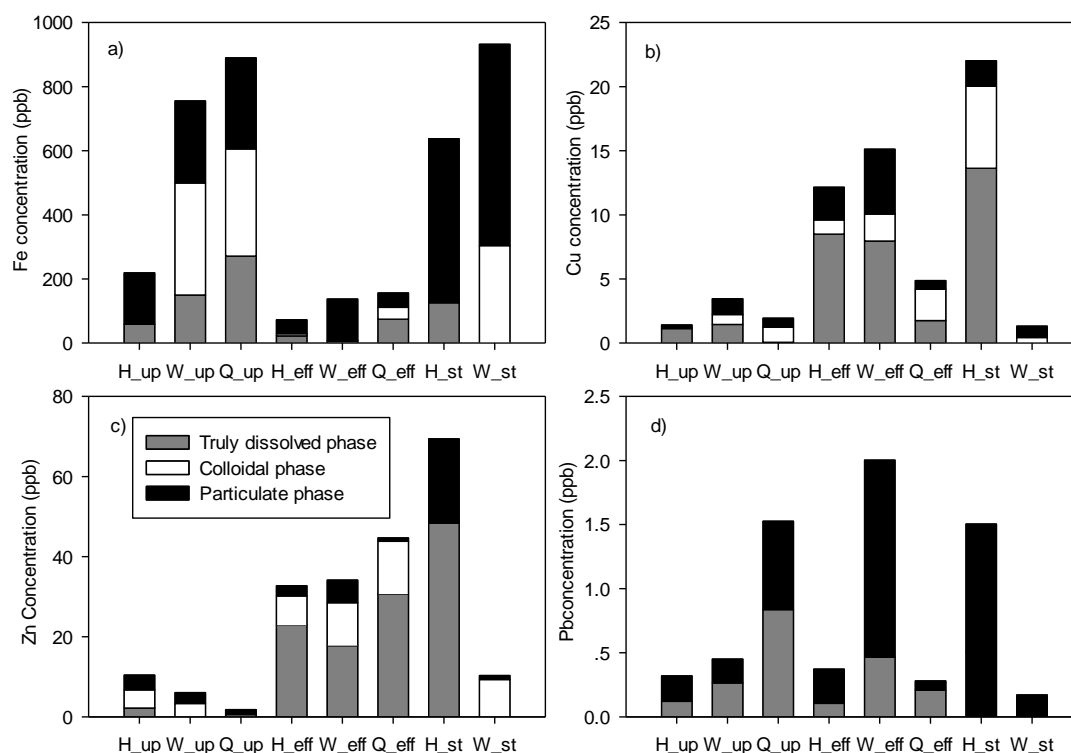


Figure 2 Metal distribution between particulate, colloidal, and truly dissolved phases in upstream, effluent and storm runoff samples.

2.3.3. Colloidal size distribution of metals

The colloidal fractions of Fe in Q_{up} and W_{up} showed both small (0.5–3 nm; 10–20% of the mass) and much more dominant large (3–80 nm; 70–80% of the mass) size distributions, whereas Fe in effluent samples was only observed in the small size range. The large size fraction of Fe never co-eluted with UV measurable organic matter or other metals measured in this study, and was likely primarily composed of iron oxides (Perret et al., 2000 and Stolpe et al., 2013). The only observable difference in the large size range Fe was a broader peak in the Q_{up} compared to the W_{up} which could have been due to differences in suspended iron oxides as a function of stream velocity or water chemistry. Further analysis focused on a higher resolution analysis of the smaller size range colloids with both metals and organic matter present.

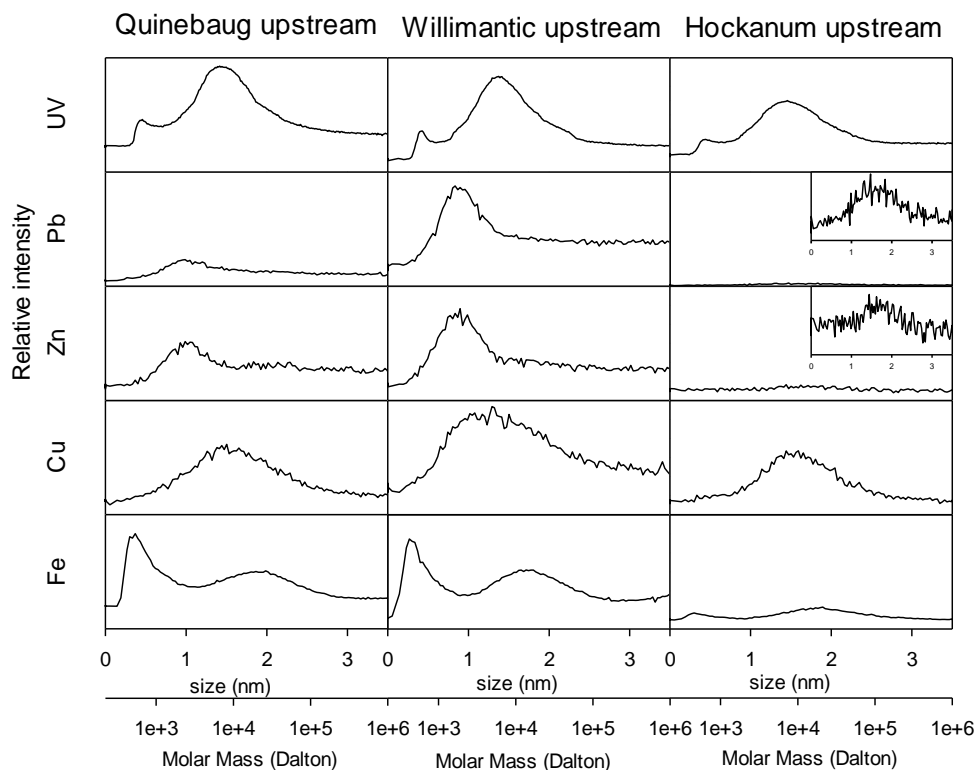


Figure 3 Size distribution of organic matter and metals in upstream water samples. The scale within the same element is identical, but signals are scaled to show the relative size and shape differences between different elements.

For all samples measured via AF4-ICP-MS, the majority of colloid associated metals, besides Fe, were found in the less than 3 nm size range. However, the distribution and association with OM or Fe was different depending on the metal. The size distributions of UV-absorbance, as a surrogate for OM, showed two peaks in both effluent and upstream samples, one centered around 0.5 nm and one typically larger peak centered around 2 nm (Fig. 3, Fig. 4). The Fe signal exhibited a similar pattern for upstream samples, but the magnitude of the 0.5 nm peak was larger for Fe. The smaller size range of OM corresponded to molecular weights of less than 1 kDa, while the larger size range fell between 1 kDa and 30 kDa. The smaller size fraction is likely more fulvic-rich, while the larger size is likely more humic-rich acids (Beckett et al., 1987). Only a few differences existed between effluent and upstream samples. In effluent samples, while the size distribution of UV absorbance still showed two peaks, the magnitude of the signals decreased and the maximum of the second peak shifted to a lower kDa size range in W_eff and H_eff samples. Even though DOC concentrations were of the same order of magnitude for upstream and effluent samples,

organic matter from effluent is typically skewed to the smaller size range (Quaranta et al., 2012). The fulvic acid signal observed in the EEMs spectra for effluent samples was not as strong as in upstream samples, thus this smaller size contribution likely came from the much larger T1 peak signals observed in effluent (Fig 1). The size distribution of the Q_eff organic matter remained similar to the upstream waters. The first peak of Fe was mostly absent or non-detectable in the effluent samples and the maximum of the second peak was more closely associated with organic carbon. While the relative Fe mass in the two size ranges differed between samples, they each overlapped, suggesting the presence of either an organic matter bound Fe ion, or an iron oxide and organic matter aggregate.

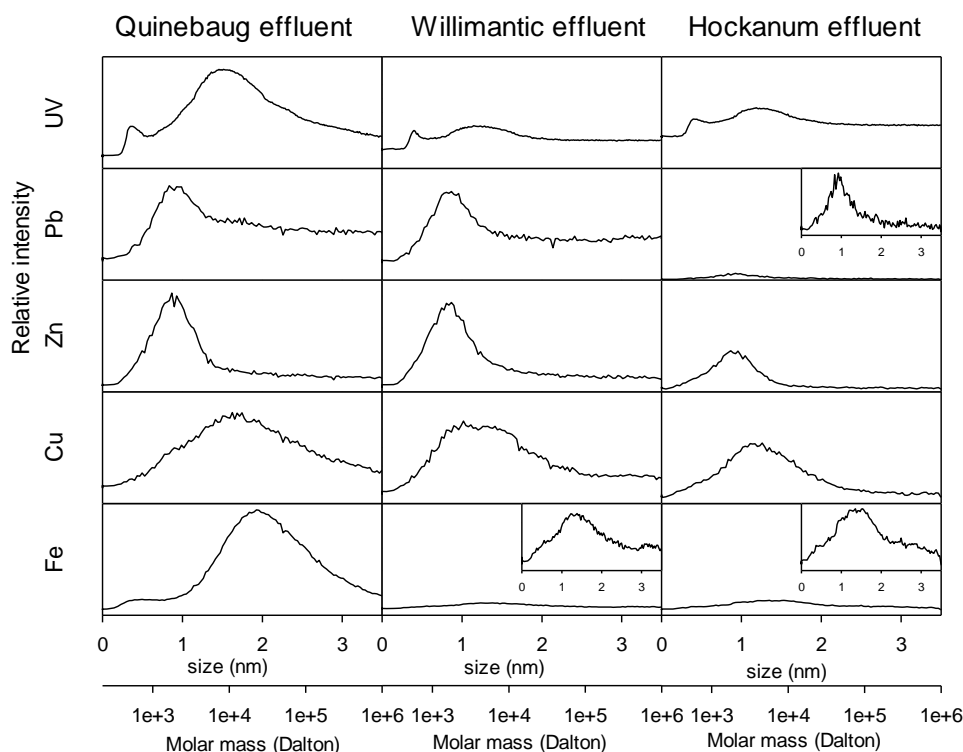


Figure 4 Size distribution of organic matter and metals in wastewater treatment plant effluent samples. The scale within the same element is identical, but signals are scaled to show the relative size and shape differences between different elements.

Most of the single peaks of Pb and Zn in effluent and upstream samples fell between the peaks of Fe and OM, suggesting some binding to both size ranges of colloids. The Zn and Pb signals in particular had long shoulders in the larger size range, similar to the UV signal. Only in the H_up samples were the Pb and Zn peaks aligned with the UV signal, but these were much lower concentrations and in that case there was

very little colloidal Fe present. The single peak of Cu lined up directly with the UV signal, but also overlaps a portion of the Fe signal. The strong association with organic matter was expected for Cu (Tipping, 1998), while Pb typically associates with iron oxides, and Zn association falling somewhere in between (Stolpe et al., 2010). However, since all signals overlap, there are potentially a variety of associations present, including mixed aggregates of iron oxides, organic matter, and metals bound to organic ligands or iron oxide surfaces.

During the storm event, all colloidal metal and UV peaks showed variation in magnitude, and Fe and Pb in particular showed shifts in size distributions over time. The colloidal size distributions of Fe in stormwater samples collected during the first hour were predominated by a single peak centered at about 2 nm, extending over a slightly larger range of sizes compared to the upstream or effluent samples. More than an hour after the storm started, Fe in even larger size ranges were observed, with a peak around 5 nm with a long tail extending into larger size distributions increasing over time. Meanwhile, the size distribution of UV-absorbance only exhibited one single peak at all times centered around 1 kDa, similar to the upstream and effluent samples, but the magnitude increased by a factor of 3 from the earlier to later times of stormwater runoff. This is consistent with the generally higher SUVA values observed later in the storm (Table 2). The colloidal size distributions of Cu and Zn are similar throughout the storm event, only varying in magnitude with the highest concentrations observed later in the storm and the lowest observed in the 45 min sample. During the storm, both Cu and Zn are more closely associated with organic matter, though Zn peaks at a slightly lower size. The size distribution of Pb is a lot less uniform and varies significantly over time. In the first 15 min, the size distribution was very broad, spanning a size range of about 1–5 nm, and exhibited a double peak (Fig 5). The two peaks correspond to the peaks in UV and Fe, suggested a role of both organic matter and iron oxides in binding Pb during a storm. Over time, the peaks change and shift in relative magnitude and are shifted to the lower size range later in the storm. The colloidal metal concentrations decreased for the first 45 min then increased, following the same trend as the dissolved metal concentrations in storm runoff. This shift in metal concentrations was likely due to differences in sources

and transport times in the storm drainage system. The appearance of larger size colloidal Fe and the increased Fe and DOC concentrations could have been due to release from the upper organic-rich soil horizons because the size of the second peak was similar to the typical colloids found in the soil pore-water (Pokrovsky et al., 2005 and Stolpe et al., 2013). It is also possible that iron oxide formation or aggregation processes during the travel time resulted in the about 3-fold increase in colloidal Fe concentration over the course of the storm.

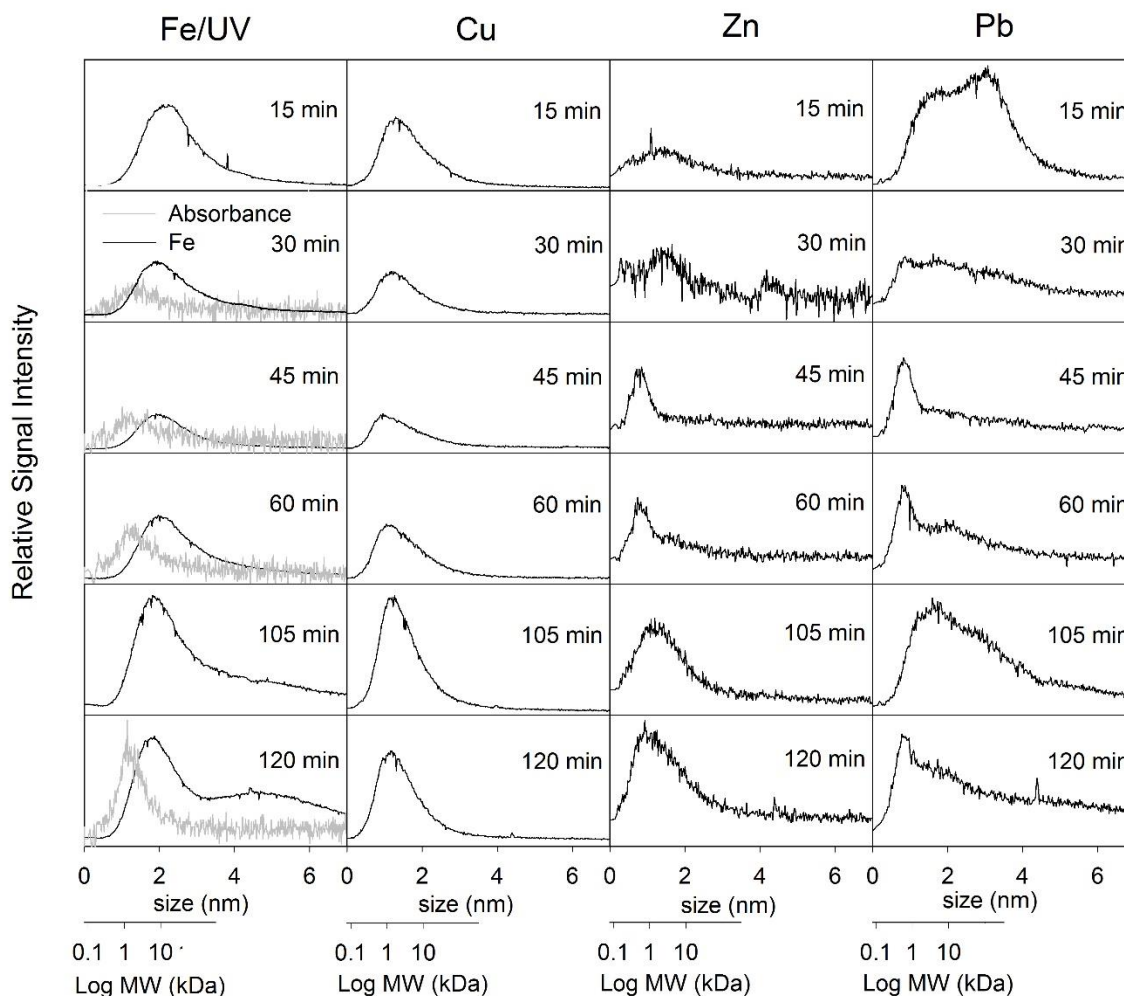


Figure 5 Size distribution of organic matter and metals in stormwater runoff into the Hockanum River over time. The scale within the same element is identical, but signals are scaled to show the relative size and shape differences between different elements.

2.3.4. Alterations in colloidal distributions upon mixing and spiking

Since these source waters all mix in the receiving stream, we assessed changes in colloidal metal distribution and size upon mixing with stream water or upon addition of metals or competing cations. The size distributions of mixed samples ($H_{up}:H_{eff}$ of 7:3 or $H_{up}:H_{st}$ of 1:1) were similar to the calculated distribution based on the previously measured individual sample distributions and while accounting for the mixing ratios. This suggests that the colloids originally present in the source water were maintained upon entering stream waters. Spiking samples with Cu, Zn and Pb at 10-fold background concentrations revealed differences depending on the metal. Total colloidal Cu increased by 8-fold in effluent and only 6-fold in stormwater samples, but the size distribution and association with organic matter changed little with the exception of a longer tail on the peak. The lower increase in colloidal Cu in stormwater suggests that even though the dissolved concentration of organic matter was about 4-fold lower in the effluent mixture, it still had excess binding capacity compared to the stormwater. The percent colloidal Zn and Pb were already low in the samples, and spiking increased their concentration to a lesser extent than Cu, but again did not change its size range. However, in these cases, stormwater showed a much larger increase for Pb and Zn than effluent. A 3–5 fold increase was observed in stormwater compared to only a 2-fold increase in effluent, suggesting more binding capacity for Zn and Pb in stormwater runoff. The addition of excess Ca did not alter the colloidal size distribution or concentrations in effluent mixtures, but did significantly change the stormwater mixtures. At a doubling of the Ca concentration, the total colloidal metal concentration slightly increased, possibly due to enhanced bridging of smaller truly dissolved organic matter. However, at a 10-fold increase in Ca concentration, the total colloidal metal concentration was reduced, suggesting weaker binding sites in the stormwater DOC than the effluent DOC.

2.4. Environmental significance

The form or size fraction of metals in urban streams has major implications for both transport and bioavailability. Colloidal phase metals are transported downstream similarly to truly dissolved metals, but are less likely to be impeded by other processes such as sorption or precipitation reactions. Alternatively,

they may be subject to removal via flocculation during major changes in water chemistry, such as ionic strength or pH that may occur as different source waters mix. Metals may also repartition between phases as component concentrations change and we saw some evidence for this when spiking mixtures with either metals or other cations. The issue of bioavailability is particularly important with respect to colloidal organic matter bound metals. While some researchers suggest colloidal phase metals are less bioavailable (Ross and Sherrell, 1999), it really depends on the lability of the metal and the fate of the organic colloid surrounding the metal. In addition, the exposure patterns of organisms to these different size fractions varies in time scales, i.e. short durations over storm events, or constant from effluent during baseflow. The differences observed in stormwater and effluent sources of organic matter and associated metals have implications for bioavailability since different organisms feed on different sized organic matter particles. In addition, different organic matter sources are unique with regard to spectral properties, and therefore functional groups, and have differences in metal lability as observed during the spiking reactions. The differences in hydrophilic or hydrophobic organic fractions will also alter the sorption to surfaces such as periphyton, which could alter the direct uptake by those organisms and trophic transfer to higher organisms. In addition, the differences observed in binding capacity and changes in metal binding due to competing elements imply differences in ambient exposure to organisms, as opposed to ingestion, due to exchangeability of metals to the organism surface. Understanding the dynamics of both altered transport processes as well as biological exposure in streams coupled to the lability of metals in these different source and size fractions would be valuable to targeting metal source management in impaired water bodies.

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Chapter 3. Lab and field comparison of DGT and periphyton response during baseflow and storm events in developed streams

3.1. Introduction

Streams in urban areas are impaired due to multiple stressors acting on them, one of which is metal contamination (Paul et al. 2001). Common sources of metals include stormwater runoff, wastewater effluents, legacy streambed contamination, or groundwater discharges from local landfills (Wuana et al. 2011, Hibbs et al. 2012). The management of metal contamination in impaired waters is often based on point source load reductions and implementation of stormwater best management practices (BMPs). However, this does not discriminate based on the importance of the contamination source to metal bioavailability in terms of either metal speciation or hydrologic loading. Metal bioavailability is controlled by metal concentrations and speciation in surface waters, and organic matter from wastewater treatment plants, stream production or land surface runoff often plays a critical role. WWTP effluent often has consistently higher metal and OM concentrations compared to the stream waters while storm runoff is variable and less well characterized. Ultimately, understanding bioavailability of metals from different sources to the organisms in the stream can more precisely guide regulations to reduce stream impairment from these sources.

Periphyton are the primary producers in streams and are a component of dietary metal exposure for many higher organisms. Periphyton are known to accumulate metals (Laxen et al. 1981) and are subject to short-term fluctuations in metal source or speciation. Most studies focus on the intracellular metal content in periphyton biofilms because it is assumed that internalization of metals must occur for toxicity effects to appear in algae (Campbell et al. 2002). Since our ultimate goal is to investigate the metal bioavailability to macroinvertebrates or higher trophic level organisms in aquatic systems, metals attached to the outside of the periphyton are also important. Brooks et al have observed that periphyton and macroinvertebrate responses were affected by high Cd levels (143 $\mu\text{g/L}$) but not by low Cd levels (15 $\mu\text{g/L}$) both in lab and field in an effluent-dominated stream (Brooks et al. 2004), but they focused on the toxicity tolerance instead

of metal accumulation in periphyton and examination of source OM effects were explored. Meylan et al (2003) investigated the accumulation of Cu and Zn in periphyton in a freshwater stream during storm events and observed a uptake corresponding to dynamic variations in stream metal speciation, which makes periphyton promising to assess changes in bioavailability during storm events. However, the differences in accumulation of metals in periphyton under different source waters is still unknown.

A simple chemical tool that can assess potentially bioavailable metals is highly desirable. Diffusive gradient in the thin film (DGT) techniques have been used effectively to assess bioavailable metals in soils for plants (Gheorghiu et al. 2010, Manceau et al. 2010) but fewer studies have considered DGT in relation to bioavailability in aquatic systems than in soils (Zhang et al. 2015). There is less consistency in aquatic systems than soils, e.g. Schintu (2010) only found a significant correlation between algal Pb and DGT Pb among several metals. Total and intracellular Cd periphyton contents were well correlated with DGT measured Cd in circulating channels (Bradac et al. 2009). While DGT measurements appear to be a promising predictor of uptake for periphyton in some studies, the evaluation of DGT in relation to bioavailability in exposures to effluent and stormwater sources in streams need further examination.

The purpose of this study was to investigate the change of Cu lability and subsequent accumulation in periphyton under exposures to effluent and stormwater sources in a stream in a developed landscape. The possibility of using DGT as a bioavailability indicator was also assessed. Periphyton and DGT devices were exposed to effluent and stream or stormwater and stream mixtures in the lab under natural or spike metal conditions. Periphyton and DGT devices were also deployed in a local river both upstream and along a distance gradient downstream from a WWTP effluent discharge and several stormwater outfalls during both baseflow and storm events. Total and dissolved metal concentrations, DOC concentrations and periphyton content were measured and compared with DGT devices in from the laboratory and field studies.

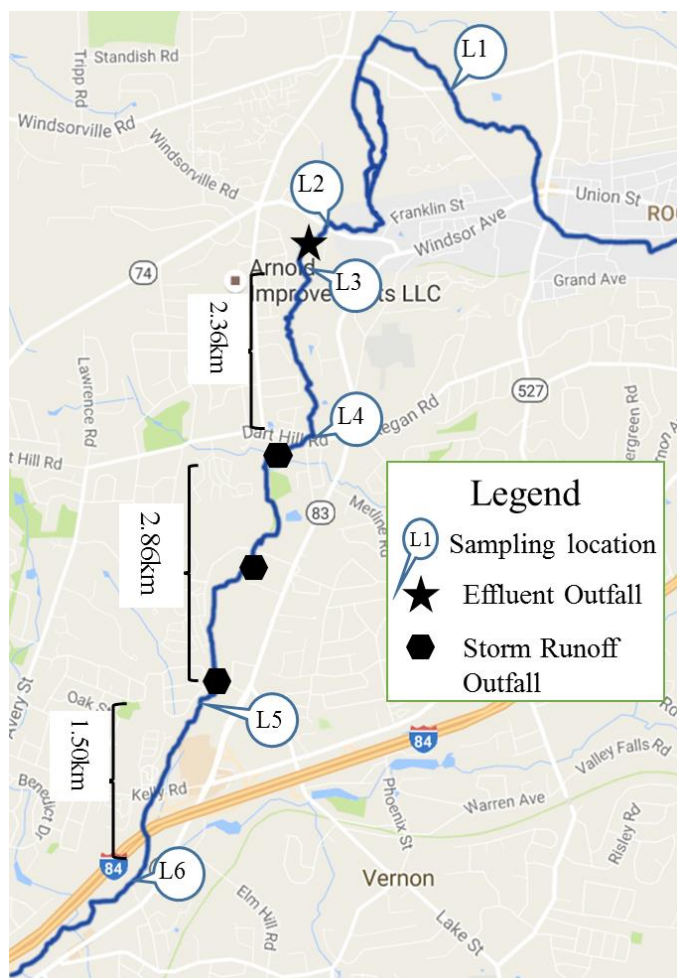


Figure 2. Study site map, sampling locations and distances.

3.2. Material and Methods

3.2.1. Site description

The Hockanum River originates at the outlet of Shenipsit Lake and flows 36.4 km through Vernon, Ellington, Manchester, and East Hartford, Connecticut, USA before it flows into Connecticut River. The research site was approximately an 8.19 km reach that flowed through a primarily suburban area of Vernon, Connecticut, USA. The Hockanum River has an average annual flow of $0.57 \text{ m}^3/\text{s}$ over the last three years. Within this reach, a WWTP discharges treated effluent into the river, with average daily effluent discharges of $0.11 \text{ m}^3/\text{s}$, which depending on the time of year contributes between 10-50% of the downstream flow. In addition, separate storm sewer outfalls exist and discharge into the river at three distinct locations along

this reach. Six sampling locations were selected along this reach and represent two upstream locations (L1, L2), one downstream of the WWTP input (L3) after complete mixing with the stream based on boron data (Weyrauch et al. 2010), and three locations downstream of storm sewer outfalls (L4, L5, L6; Figure 1). The distance between locations is marked on the map. Travel times during baseflow sampling were determined using a fluorescent tracer (rhodamine WT) following USGS methods (Wetzel 1983) and travel times are noted in Table 1. Stormwater runoff used in laboratory experiments was collected from the discharge pipe at L5 which drains both residential and commercial areas. Wastewater effluent was collected directly following the dechlorination tank at the WWTP. Streamwater was collected upstream from the treatment plant at L2. Samples were collected in 5 gallon HDPE buckets (approximately 19 L) and stored at 4 C prior to use. For field experiments, baseflow observations were made during the late summer (July and August), while storm events were observed during the fall (September-November).

3.2.2. Chemical supplies and preparation

All plastic containers and bottles, DGT devices, and tools used for DGT preparation were acid washed prior to use. Deionized water ($>18\text{ M}\Omega$) was used for rinsing and preparing solutions. DGT devices were purchased from DGT Research (Lancaster, UK). AcrylAide crosslinker for DGT gel preparation was purchased from Lonza (Walkersville, MD, USA).

3.2.3. Periphyton colonization

Colonization of periphyton on microscope slides was adapted from Meylan et al (2003). Microscope slides were installed into fabricated acrylic racks that were floated in the Fenton River, CT, USA, a pristine stream reach with low background Cu ($<1\text{ }\mu\text{g/L}$) and no major wastewater or stormwater outfall sources. The glass slides were submerged about 10 cm below the water surface and oriented vertically on edge and in-line with flow to minimize the accumulation of suspended particles. Periphyton were colonized for 3 weeks prior to translocation to the lab or the Hockanum River for experiments.

3.2.4. DGT gel preparation and device assembly

The procedure for preparing DGT gels (Zhang and Davison 1995) was completed on a clean bench (AirCleanSystems) to prevent trace metal contamination. Briefly, a polyacrylamide gel solution consisting of 15% acrylamide (Acros Organics) and 0.3% AcrylAide cross-linker by volume were prepared. The recipe for the diffusive gel was combining 10 mL of gel solution with 70 μ L of freshly prepared 10% ammonium persulfate solution (APS) followed by the addition of 30 μ L of Tetramethylethylenediamine (TEMED; Acros Organics, 99%) catalyst. The resin gel solution was prepared by adding 60 μ L of freshly prepared APS followed by 20 μ L of TEMED catalyst to a mixture of 10 mL of gel solution and 2 g of Chelex-100 resin (Na form, 200-400 wet mesh, Bio-Rad). The prepared diffusive and resin gel solutions were immediately cast between two glass plates separated by plastic spacers and incubated at 40 ± 5 °C for 45 min to cure. A spacer of 0.2 mm and 0.5 mm thick were used for diffusive and resin gel respectively. All gels were hydrated in deionized water for at least 24 h then cut into 2.54 cm diameter circles for use in DGT devices. After hydration in 0.1 M NaNO₃, the diffusive gels were 0.8 mm thick and the resin gels 0.4 mm thick. Gels were stored in a solution of 0.1 M NaNO₃ prior to use. Gel holders based on a tight-fitting piston design with a 2-cm diameter sampling window were used. To prepare the DGT devices for use, the resin gel was placed on the DGT holder with resin side facing out. A layer of diffusive gel was placed on top of the resin gel, followed by a 0.45 μ m pore size cellulose nitrate membrane (Millipore, USA). The front cap was then pressed down tightly to seal and complete assembly (Zhang et al. 2000).

3.2.5. Laboratory comparison of DGT and periphyton Cu exposures

Exposure of DGT devices and periphyton slides were compared in the laboratory to assess the ability of DGT to predict periphyton Cu uptake. The exposure solutions consisted of mixtures of stream and stormwater or effluent at different ratios. Exposures were first conducted with Cu concentrations as collected, and second with Cu concentrations spiked to 15 μ g/L, which represents the highest observed Cu concentration in collected effluent. Mixtures were equilibrated for 24 hours, followed by a 24-hour

exposure to DGT devices or periphyton slides. Exposures were conducted in 1 L teflon bottles with devices suspended in the middle with nylon string and continuously stirred at 21 °C and ambient light.

3.2.6. Field exposure experiments

Water samples at sampling locations L1, L4, L5 were taken automatically at 1 h intervals by a Teledyne ISCO 6700 portable autosampler (Lincoln, Nebraska) during storm events while manual grab samples were taken at L1, L4, L5 and L6 during baseflow conditions. Samples were split for total and dissolved metals, and pH measurements.

DGT devices and periphyton slides were placed at locations L1-L6 in the Hockanum River. DGT devices were tied to a 10 cm x 20 cm plexiglass plate, while periphyton slides were first inserted into a plexiglass slide holder, and then each were tied to a brick (Figure 2). The bricks were deployed in the bottom of the river and retrieved after 24 h exposure for both baseflow and storm events. During baseflow conditions, 4 periphyton slides were deployed with 6 DGT devices and retrieved after a 24 h exposure. During storm events, 48 periphyton slides were translocated to the Hockanum River at least one day prior to the storm event to equilibrate with baseflow, and 4 slides were sampled daily or twice a day during a storm event along with 4 DGT devices that were deployed daily for 24 hr exposures.



Figure 2. Field deployable units of DGT devices and periphyton slides

At the time of sampling, 4 microscope slides were thoroughly rinsed with filtered river water (0.45 μm). The natural periphyton biofilm was then scraped from the slide and suspended in filtered river water. The suspension was afterward split evenly into two fractions. One fraction (20 mL) was treated for 10min with 4.0mM EDTA to remove the metals adsorbed to the cell wall following (Meylan et al 2003). This process allowed for the measurement of the intracellular metal content of periphyton. The other fraction was used to measure the total metal accumulated in periphyton. Three aliquots of each fraction were filtered (cellulose nitrate 0.45 μm) to obtain the dry weight (dw) of each sample after drying at 50 °C. The filters were digested following standard methods (EPA Method 3050B). Briefly, the filters were heated at 95°C in 2 mL of concentrated nitric acid (70%) in a 15 mL digestion tube until brown fumes subsided. Subsequently, hydrogen peroxide (30%) was added stepwise until the effervescence is minimal or until the general sample appearance was unchanged.

3.2.7. Sample handling and analysis

Total metal concentrations were determined by digesting 15 mL of acidified water samples (2% HNO_3) at 90 °C for 1 h (Luoma et al. 2005). Samples for dissolved metals and DOC were first filtered through a 0.45 μm cellulose nitrate filter cartridge (Whatman). The alkalinity was determined by titration with sulfuric acid to pH=4.5. The pH was measured with an electrode (Fisher Scientific). All samples were stored at 4 °C in the dark prior to analysis.

After retrieved from field, the resin gel from DGT samplers was removed and transferred to a 2 mL polypropylene microcentrifuge tube containing 1mL of 1M HNO_3 (trace metal grade) and soaked overnight. The elution solution was diluted 5 times with 1% HNO_3 matrix before measurement. The metal concentrations were then measured by inductively coupled plasma mass spectrometry (ICP-MS). Calculations for labile metal concentrations followed Zhang and Davis (2006).

The total and dissolved metal samples were acidified to 2% HNO_3 (trace metal grade). Total metal sample were analyzed according to EPA Method 200.8 (Creed et al. 1994). Inductively coupled plasma

mass spectrometry (ICP-MS, Agilent 7700x with He collision cell, Agilent, Delaware, USA) was used for determination of the elements in this work. For batch sample analysis, Bi, Ho, In, and Sc were used as internal standards. Standards and QC checks were prepared from independent high purity standards (Spex-Certiprep, Metuchen, NJ, USA and VHG Labs, Inc., Manchester, NH, USA). Quality control samples, internal standards, and spike recoveries were within 10% or better of expected values.

The DOC concentration was determined using a total organic carbon analyzer (Apollo 9000, Tekmar-Dohrmann, Mason, OH, USA). Potassium hydrogen phthalate was used for standards (Ricca Chemical, Arlington, TX, USA), and laboratory prepared standards from the same chemical were used for quality control samples, all of which were within 20% or better of expected values.

3.3. Results and Discussion

3.3.1 Comparison of DGT and periphyton exposure in laboratory

In comparisons of periphyton slides and DGT device exposures to different mixtures of filtered effluent or stormwater with streamwater, generally both periphyton Cu content and DGT-labile Cu concentrations increased with an increase in percent effluent, but decreased with an increase in percent stormwater (Figure 3). While the effluent ratio increased from 0 to 100 %, both total dissolved organic matter and Cu concentrations increased from about 5 to 10 mg/L DOC and 2 to 14 $\mu\text{g/L}$ Cu, respectively. Due to that difference, total periphyton Cu contents increased from about 8 to 50 $\mu\text{g/g}$ while intracellular periphyton Cu contents increased from 6 to 15 $\mu\text{g/g}$, suggesting the net internalization rate decreased as both effluent OM and Cu concentrations increased. Considering the large increase of dissolved Cu concentrations, yet intracellular Cu contents did not increase dramatically, suggests a likely Cu regulation by periphyton. It is unclear whether the high total periphyton content suggests the role of Cu exchange with the periphyton external surface, or also some adsorption of effluent OM bound Cu, though the higher hydrophilicity of the effluent OM suggests lower adsorption (Worms et al. 2010). We would expect free Cu concentration in solution regulated by OM to control periphyton Cu content and DGT-labile Cu, all things being equal. However, there are known differences in hydrophobicity and metal binding between the

two sources in terms of organic matter characteristics (Quaranta et al. 2012, Luan et al. 2015). To assess the impact of changes in organic matter concentrations and characteristics, additional experiments compared the same mixtures but with a fixed Cu concentration. In similar experiments of increasing effluent percentage with a fixed spiked Cu concentration of 15 µg/L, both total and intracellular periphyton content decreased (Figure 4). This suggests a protective role of effluent OM in limiting Cu uptake by periphyton. In this experiment however, DGT-labile Cu increased from about 4.5 to 6 µg/L as the effluent fraction changed from 0 to 30%, and stayed at about 6 µg/L with increasing effluent. We would have expected a similar decreasing trend as in periphyton since as more effluent OM is present, more Cu is bound to the higher quantity of stronger ligand binding sites. The increase of DGT labile Cu in the presence of effluent could be explained by the smaller organic matter molecules in effluent. A significant amount of labile organic metal complexes was accumulated in the DGT resin in the effluent even when using restricted gels which had smaller pore sizes than our devices (Buzier et al. 2006). DGT was also less effective at estimating bioavailable Cu in the case of algae exudate solutions and might overestimate the bioavailable Cu concentrations in downstream reaches where the DOM is less humic (Tusseau - Vuillemin et al. 2004) due to degradation. Considering the more autochthonous origin and less humic composition in effluent DOM, more labile effluent Cu complexes could pass through the diffusive gel and adsorb on the resin gel, leading to an increase of DGT labile Cu in the presence of effluent. These labile effluent complexes might also have higher Cu binding constants than the organism binding sites, thus the labile effluent DOM Cu complex is not available to periphyton and it reduced the Cu uptake to periphyton in this study.

In contrast to effluent, with an increase in stormwater proportion, the Cu concentration did not increase dramatically (only from 2.5 to 3.6 µg/L), nor did DOC (from 5 to 3 mg/L), which resulted in the periphyton Cu concentrations and DGT-labile concentrations slightly decreasing, though not significantly ($p=0.191$ and 0.201 respectively; Figure 3). In the similar spiked experiments, with Cu fixed at 15 µg/L, total and intracellular periphyton Cu content decreased with increasing stormwater proportion. In this case, DGT-labile Cu also decreased, suggesting the role of stormwater OM in limiting Cu uptake by periphyton.

In assessment of correlations of the combined effluent or stormwater results, the DGT-labile Cu is not significantly correlated with either total or intracellular periphyton Cu content for effluent, but is for stormwater ($R=0.97$, $p<0.05$). Only in the case of correlating total periphyton with DGT-labile Cu in the unspiked effluent exposure was there a significant correlation for effluent. The high correlation coefficient suggests the intracellular periphyton Cu content could be predicted given a DGT-labile Cu value. However, the opposite trends of labile Cu and periphyton Cu contents in effluent revealed the performance of DGT devices were affected by source OM characteristic and thus the use of DGT as bioavailability may be source dependent.

The reduction of bioavailable Cu in the presence of effluent and storm runoff demonstrated effluent and storm OM both had protective effects on Cu uptake compared to stream water and could compete with periphyton uptake sites. It appeared that effluent OM had stronger binding constants than the stormwater OM because periphyton Cu contents decreased more in the presence of effluent, which was consistent with our previous study (Luan and Vadas, 2015). In the comparison of direct mixtures and spiked experiments, a bigger difference between total and intracellular periphyton contents in the absence of effluent or stormwater was observed when spiked with high levels of Cu, indicating internalization is a slower process than adsorption (Sarathy et al. 2005). The internalization capacity is much lower than the adsorption capacity at high Cu concentrations. At low concentrations, most of the adsorbed Cu was internalized within 24 hours. However, only 30% adsorbed Cu was internalized at total Cu concentrations of 15 ppb. Therefore, the measured bioavailable Cu in our batch study may also be affected by uptake kinetics depending on Cu concentrations and the uptake rates should be considered in dynamic systems in a future study.

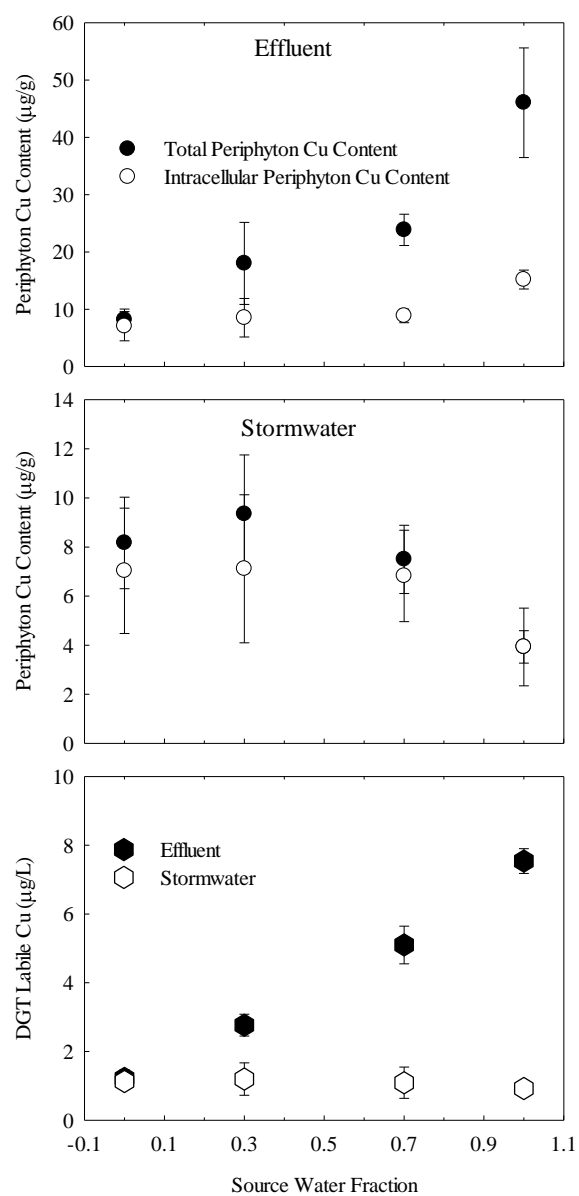


Figure 3. Laboratory exposures of periphyton and DGT to unspiked varying ratio mixtures of sourcewater fractions with stream water. The error bar represents one standard deviation (n=3).

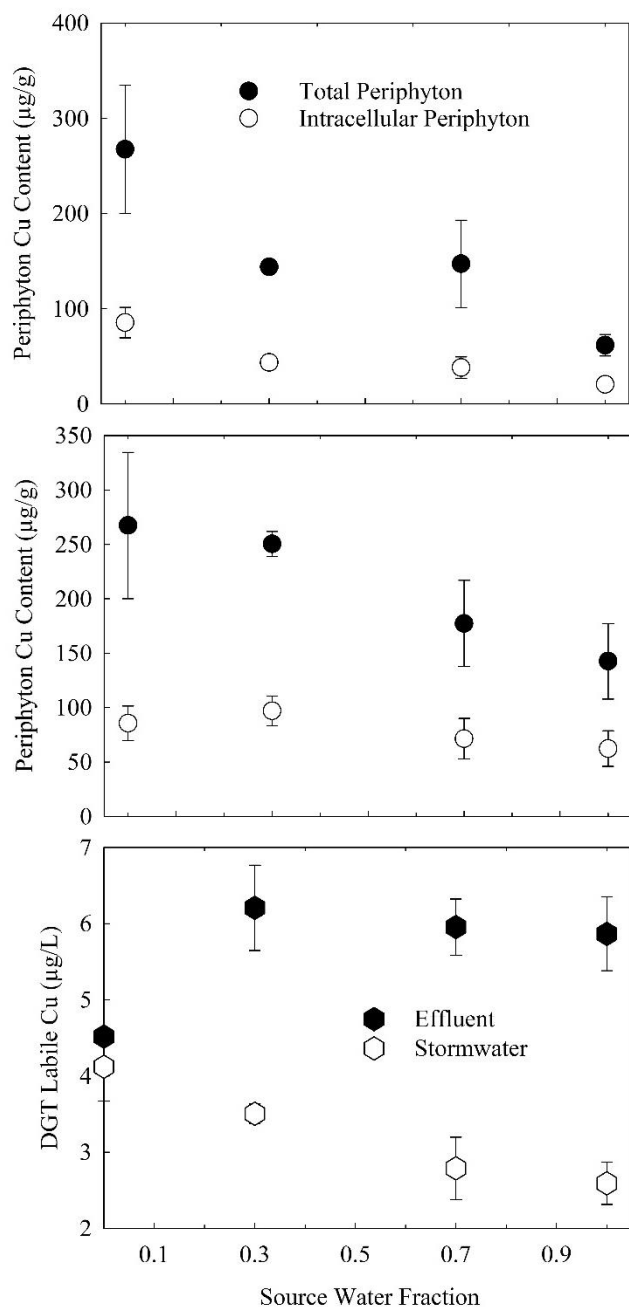


Figure 4. Laboratory exposures of periphyton and DGT to varying ratio mixtures of sourcewater fractions with stream water. Solutions were spike to a constant 20 ppb Cu. Three replicates were used for each data point. The error bar represents one standard deviation.

Table 1. Travel time during baseflow to each downstream sampling location determined based on rhodamine WT tracer study

| | WWTP | L3 | L4 | L5 | L6 |
|------------------|------|------|------|-------|-------|
| Travel Time (hr) | 0 | 4.88 | 8.75 | 10.83 | 12.42 |

3.3.2. Field exposure of DGT and periphyton during baseflow conditions

Baseflow exposure experiments were meant to isolate the influence of effluent on metal uptake. Device placement and sampling times were selected based on known travel times (Table 1) in order to ensure that exposure was similar at each site (i.e. the same volume of water released from the WWTP was exposed to each device). Both DGT-labile Cu and periphyton Cu content increased downstream of effluent discharge during baseflow condition but there was no significant difference between sampling locations downgradient from the effluent outfall. The total and dissolved Cu concentrations in streams increased from 1.5 ± 0.4 to 4.5 ± 0.9 ppb after the effluent discharge, then slightly decreased further downstream (L6) by about 1 ppb for total and 0.6 ppb for dissolved, respectively. The DGT-labile Cu concentrations, which doubled after effluent discharge (Fig 5), then remained elevated further downstream of effluent. A strong correlation between dissolved Cu concentrations in streams and DGT-labile Cu concentrations ($R=0.98$, $p<0.05$) during the baseflow conditions explained the corresponding pattern of labile Cu and dissolved Cu concentrations from upstream to downstream. On the contrary, the labile Cu ratio (i.e. DGT-labile Cu normalized to dissolved Cu) was approximate 50% for both upstream and downstream locations, indicating that at this proportion, effluent didn't alter the labile Cu fractions in streams, just the total concentration of each. As for the bioavailable Cu, both total and intracellular periphyton Cu contents was significantly elevated after effluent discharge but there was no clear trend between downstream locations. The uptake of Cu in periphyton in downstream locations was still higher than that in upstream locations when normalized to the dissolved Cu concentrations. The extracellular periphyton Cu contents (difference between total and intracellular periphyton contents) for downstream locations were greater than those at upstream locations where the extracellular periphyton contents were at a minimum. This indicates the elevated Cu cannot all be internalized within 24-hour exposure period and either the internalization may be a rate-limiting step for Cu uptake or the organisms are self-regulating uptake. There were no big changes in pH, major cation

concentrations, TOC concentrations or organic spectroscopic properties observed within the distance gradient downstream, which expounded a similar pattern of change in labile Cu speciation and periphyton Cu contents along downstreams locations.

Although DGT devices were not strongly correlated to periphyton in laboratory effluent samples, there was a significant correlation between DGT measured Cu and intracellular periphyton Cu contents ($p < 0.05$) in field samples. However, the Pearson correlation coefficient R value was 0.5, lower than the lab results, thus the prediction of intracellular periphyton Cu content based on DGT-labile Cu may differ with the effluent input based on the poor correlation in the presence of effluent from the lab results and the possible issues with effluent organic matter bound Cu and DGT measurements (Tusseau - Vuillemin et al. 2004, Buzier et al. 2006). Although a significant correlation between dissolved Cu and intracellular Cu contents ($p < 0.05$) was found, the low R square (0.657) suggests that Cu uptake regulation by the organism could have added to the variability. The organism metal uptake regulation is different from the chemical assessment tool, DGT which has an infinite sink, especially under the influence of effluents or anthropogenic inputs, Therefore, the use of DGT as a bioavailability indicator needs more constraints.

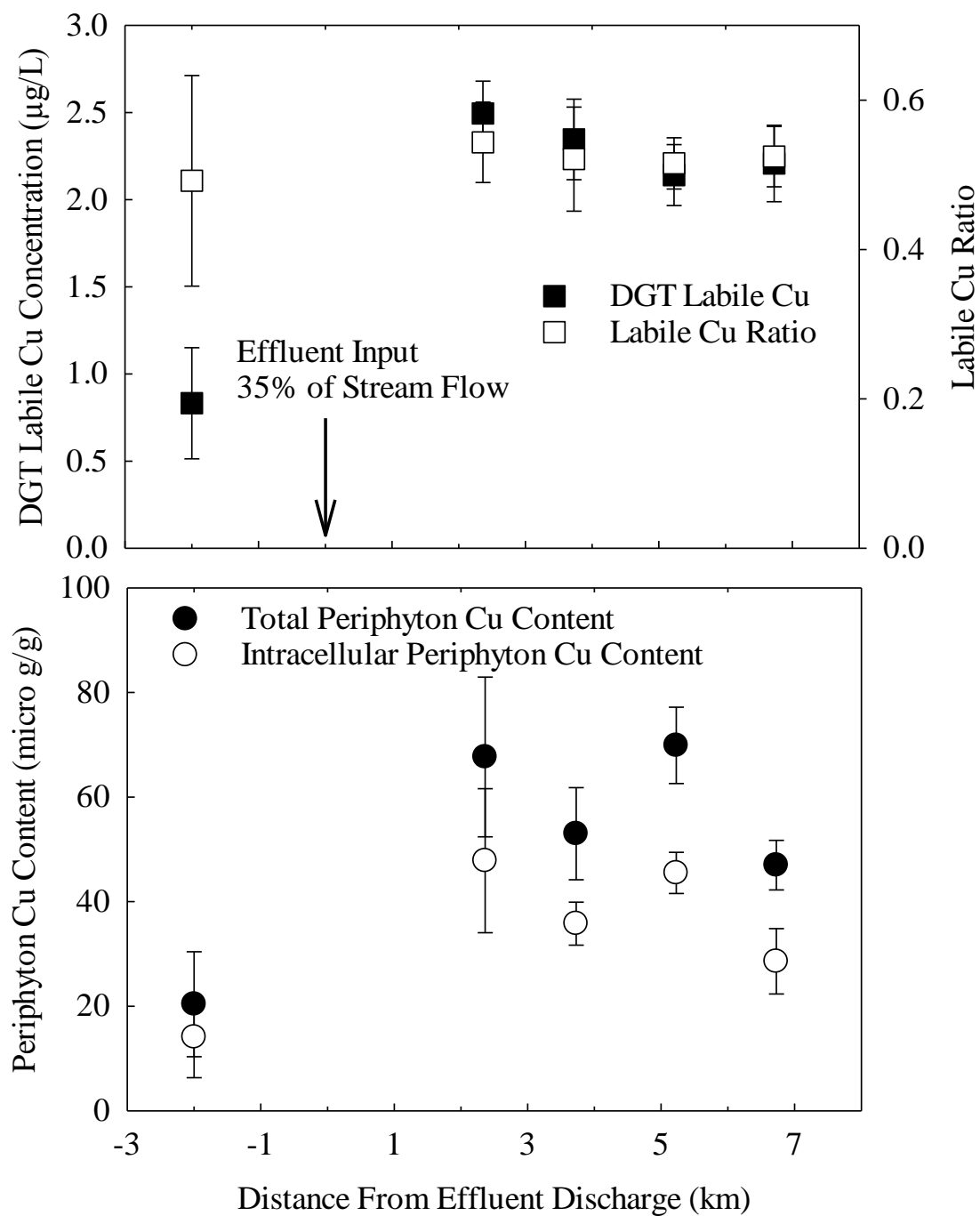


Figure 5: DGT and periphyton Cu measurements during baseflow conditions. The error bar represents one standard deviation (n=3).

3.3.3. Storm events

Total and dissolved Cu concentrations in streams were not diluted or increased by storm events except the storm event of September 2012 in which the Cu concentrations in streams during the storm event were about two times higher than that during baseflow conditions. In the storm events of September 2013 (Figure 6), total and dissolved Cu increased slightly after the storm started at the upstream location, but periphyton Cu content slightly decreased. Total and intracellular periphyton Cu content was around 20-30 $\mu\text{g/g}$ and the difference between total and intracellular periphyton Cu content was less than 10 $\mu\text{g/g}$. The storm elevated particulate Cu concentrations for a couple of days while dissolved Cu fell back to the pre-storm level after a day. This likely explained the bigger difference in total and intracellular periphyton Cu content immediately after the storm started.

As for downstream locations, both total and dissolved Cu concentrations were higher than in upstream locations and slightly decreased by 1 ppb for total and 0.6 ppb for dissolved, respectively with the distance downstream gradient. Total and intracellular periphyton Cu content increased to 60-70 $\mu\text{g/g}$ and the difference between total and intracellular Cu concentrations became larger compared to upstream locations. This was the same case as reported in the baseflow results. The elevated Cu concentrations in water column downstream may result in the bigger difference between total and intracellular periphyton Cu content because internalization was not as efficient as low Cu concentrations, similar to the laboratory results of unspiked effluent. Daily average total and dissolved Cu concentrations were not altered by storm at downstream but the daily cycle pattern of Cu concentrations before storm was interrupted. Periphyton Cu content was not varied after the storm at 1.5 km downstream but continuously increasing at 2.5 km downstream. A sudden increase of periphyton Cu content at the end of sampling period was noticed and could be attributed to the lower tolerance of periphyton after exposing to higher Cu concentrations for longer term.

Periphyton Cu contents were relatively stable during storm events unless Cu concentrations in streams showed a big change while the labile Cu concentrations decreased in the following days after storm events.

Periphyton Cu contents remained the same or slightly increased a few days delayed following a storm event for most storm events. The only exception was the 2012 September one as well. Both total and intracellular periphyton Cu contents were about 4 times higher right after a storm event than those during baseflow conditions, indicating a fast response and an amplifying effect of the periphyton to the change of Cu concentrations in streams. The delayed slight increase of periphyton contents after a storm event, however, suggested the equilibrium between periphyton and stream Cu was slow to reach. In addition, the difference between total Cu and intracellular Cu in periphyton samples was very small at the beginning of the storm but it became larger along the storm event. This meant more particulate Cu or non-bioavailable Cu was attached on the periphyton samples rather than digested by the periphyton during the storm. On the other hand, labile Cu concentrations in streams dropped after the storm started for all locations then back to the initial values. This decrease was likely due to the reduced exposure area of DGT device caused by the thin coverage of the suspended solids which are elevated during storm events (Jacob et al. 2011). The decrease of labile Cu concentrations in the presence of storm runoff was consistent with the lab results. In addition, periphyton Cu and Pb contents showed similar trends while Fe and Zn behaved as a pair. (ref to explain)

Total and dissolved Fe was usually increasing after a storm started but the periphyton Fe content decreased, suggesting storm introduced more Fe which were not available for periphyton. The difference between total and intracellular Fe content was relatively small. This could be explained by fast internalization of Fe (ref). Also, the difference between total and intracellular Fe content at upstream was larger than that at downstream locations. The higher particulate Fe concentrations at upstream explained this bigger difference, supporting the theory of more particulate metal resulting in larger difference between total and intracellular periphyton metal content mentioned above for Cu.

Both Zn concentrations in water column and periphyton Zn content was not varied much by stormwater input (Figure 9-11). Zn concentrations was always about 2 µg/L upstream and 10 µg/L downstream. The difference between total and dissolved Zn concentrations was very small, suggesting most Zn was present in dissolved fraction. However, a big difference between total and intracellular periphyton Zn content still

existed and usually downstream locations had bigger difference. The periphyton may start to regulate Zn in their body because intracellular periphyton Zn content stayed the same (about 200 µg/L) while total periphyton Zn content was only doubled from upstream to downstream even dissolved Zn concentration increased 5 times. The increase of total periphyton Zn content suggested adsorption of metal on the organism surface was still possible when organisms started regulating metals. Therefore, when metal concentration is elevated, the metal transfer in trophic chain was likely promoted due to the adsorption of metals on the cell surface although the intracellular content was stable.

Similar trends were observed for the storm events of October and November but there were some differences worth to address. Periphyton Cu content at 1 km upstream and 1.5 km downstream was higher than that during the storm event of September. This is likely due to the elevated Cu concentrations in water column especially at 1.5 km downstream (increased from 4.5 to 6 µg/L and 2.5 to 4.5 µg/L for total and dissolved Cu respectively) or the seasonal change of periphyton structure. Also, the difference between total and intracellular periphyton Cu content at 1.5 km downstream during the storm of October was about 30-40 µg/g, much higher than other observed storms. A larger difference between total and dissolved Cu was observed as well, indicating more particulate Cu may lead to a bigger difference between total and intracellular periphyton Cu content.

The stormwater did have two impacts on Cu concentrations in streams. First, it blurred the daily pattern of Cu concentrations in downstream locations during baseflow conditions (Figure 6-8) due to the daily change in discharge of effluent. Second, it increased the Cu loads to streams considering the increased flow rate during storm events, although the Cu concentrations remained the same. The elevated Cu loads may result in a delayed increase of periphyton Cu content if some remains in the sediment in the system.

Correlation between inter-elements and periphyton content of different metals revealed metal distribution in streams and uptake mechanism to periphyton. A significant correlation ($R=0.412$, $p<0.05$) between dissolved Cu and intracellular periphyton Cu content was observed. Although this indicated dissolved Cu regulates intracellular periphyton Cu content to some extent, the low R value suggested

dissolved Cu alone is not the only predictor of periphyton Cu content. A better predictor may be free Cu , but measurements of that in this dynamic system are not available. Significant pairwise correlations ($p < 0.05$) were found among Cu, Zn, and Pb for both total and dissolved concentrations and they were significantly correlated with DOC as well ($p < 0.05$), indicating these three metals may bind with organic matter in streams considering the strong Cu, Pb binding capacity of NOM or source OM. On the contrary, Fe was independent from the other three metals and possibly formed iron oxide which has little interaction with organic matter. Periphyton Fe, Cu, Zn, and Pb content was significantly pairwise correlated, suggesting these metals may have similar uptake mechanisms in periphyton and share some transporters. Therefore, competing ion effects on metal speciation and uptake need to incorporate into bioavailability prediction model in the future.

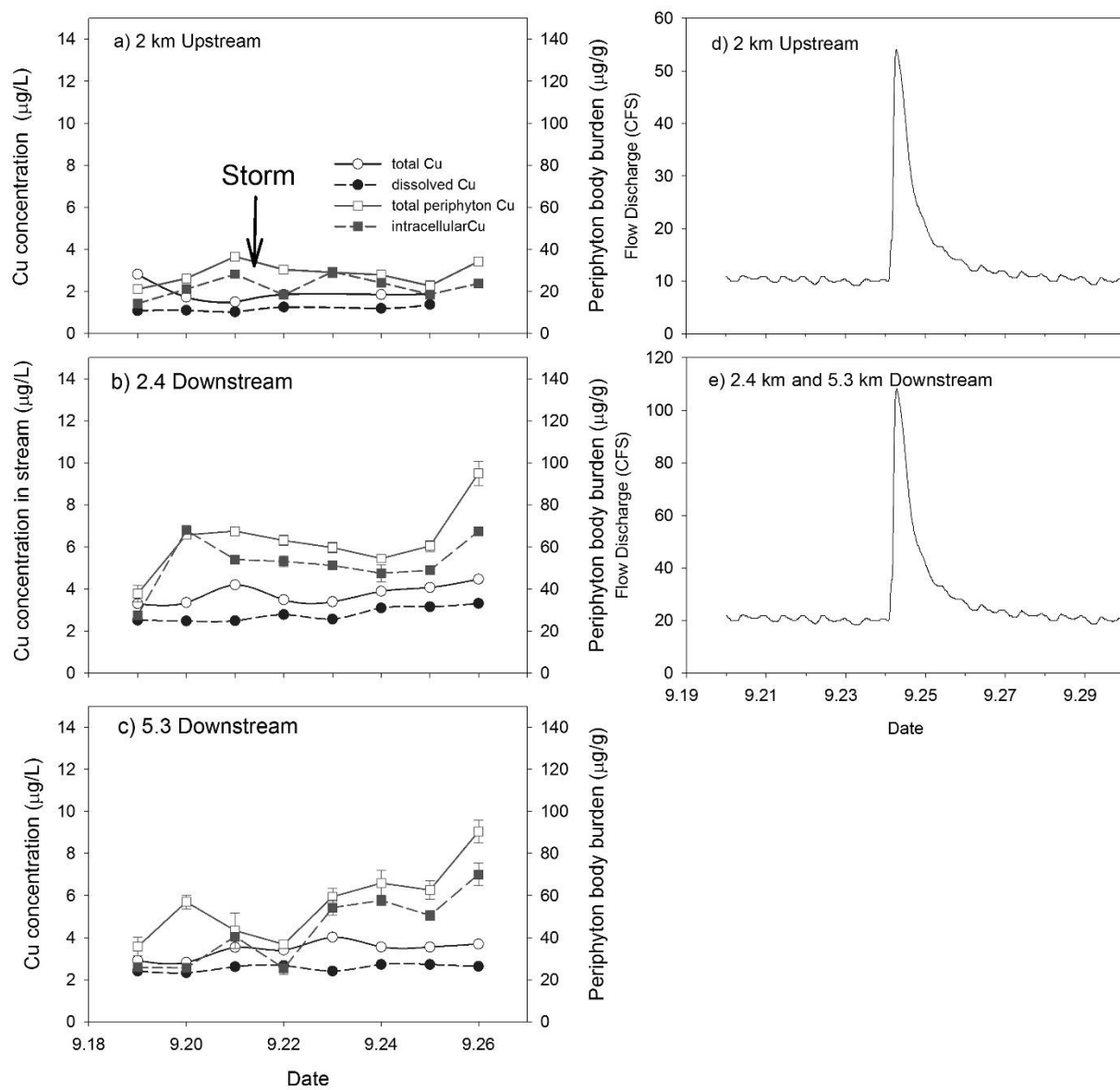


Figure 6. Variations of Cu concentrations and periphyton Cu content during storm event of September 2013 at a) 2 km upstream; b) 2.4 km downstream; c) 5.3 km downstream.

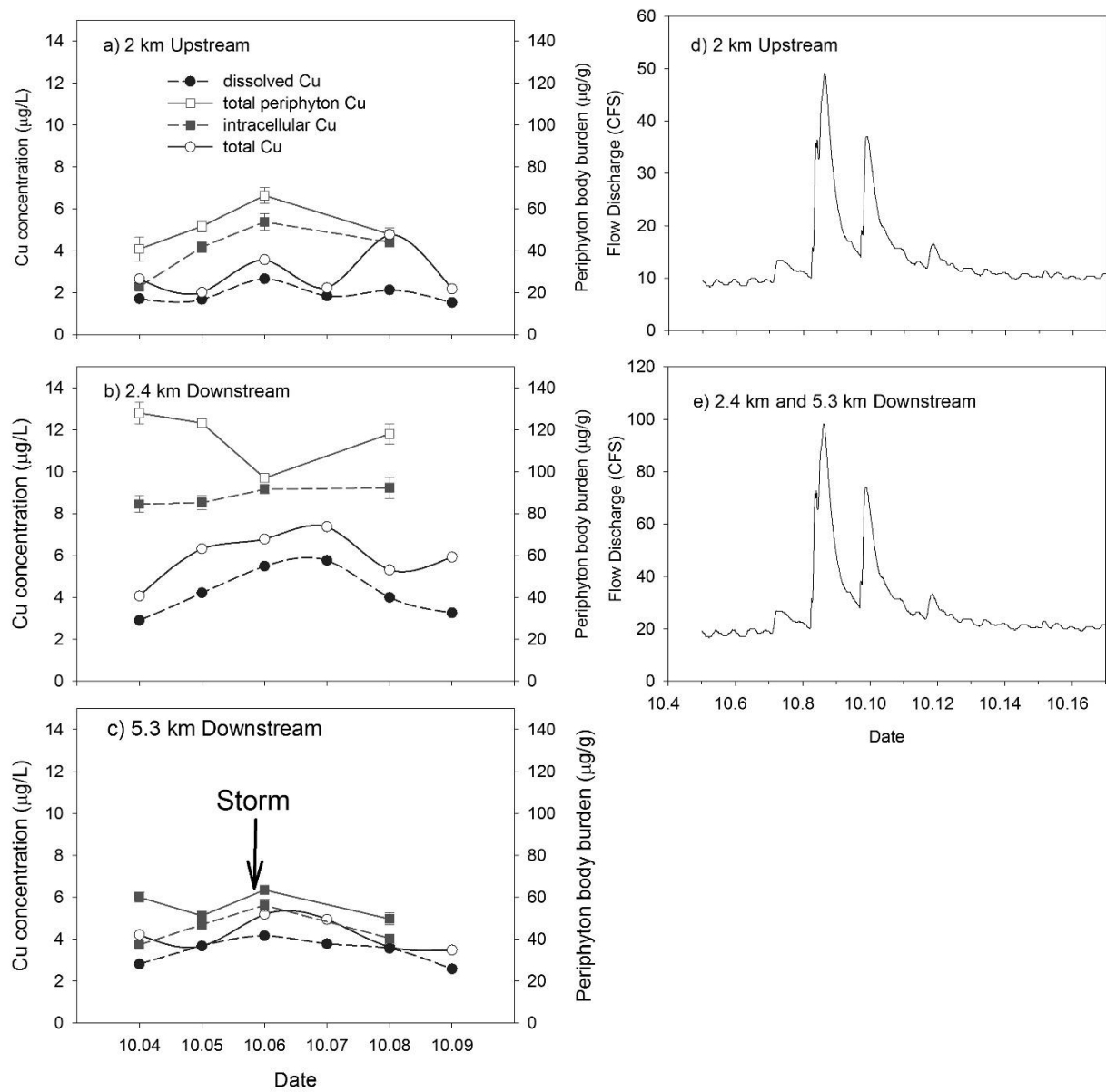


Figure 7. Variations of Cu concentrations and periphyton Cu content during storm event of October 2013 at a) 2 km upstream; b) 2.4 km downstream; c) 5.3 km downstream.

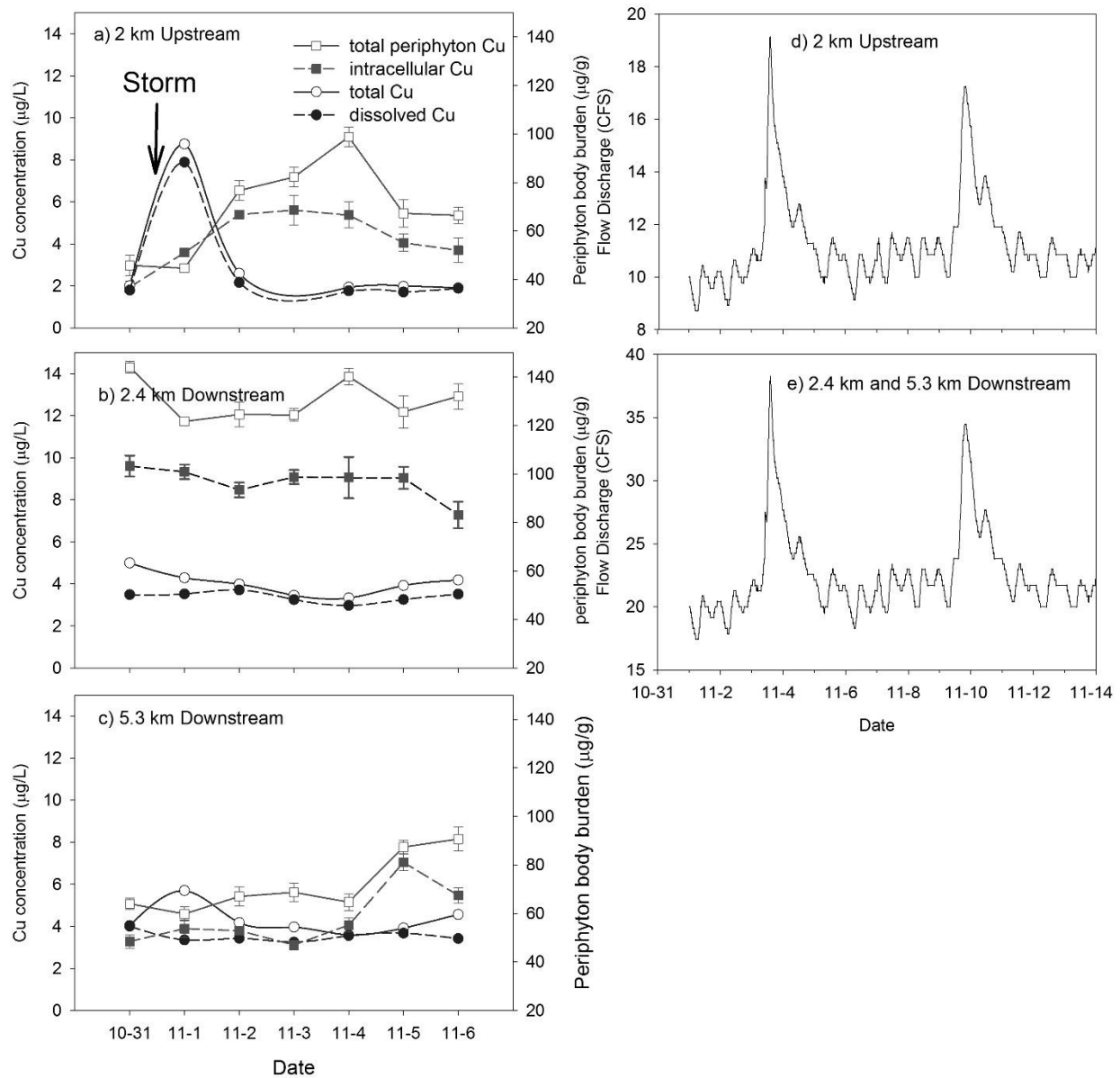


Figure 8. Variations of Cu concentrations and periphyton Cu content during storm event of November 2013 at a) 2 km upstream; b) 2.4 km downstream; c) 5.3 km downstream.

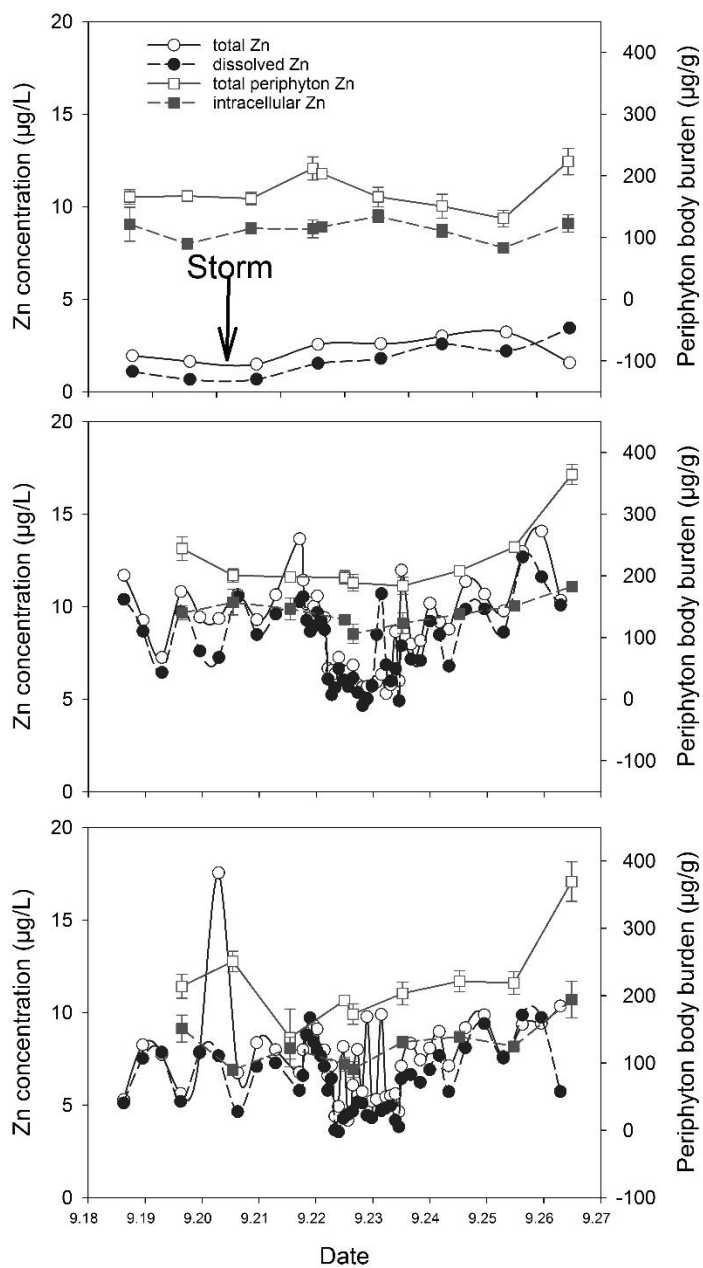


Figure 9. Variations of Zn concentrations and periphyton Zn content during storm event of September 2013 at a) 2 km upstream; b) 2.4 km downstream; c) 5.3 km downstream.

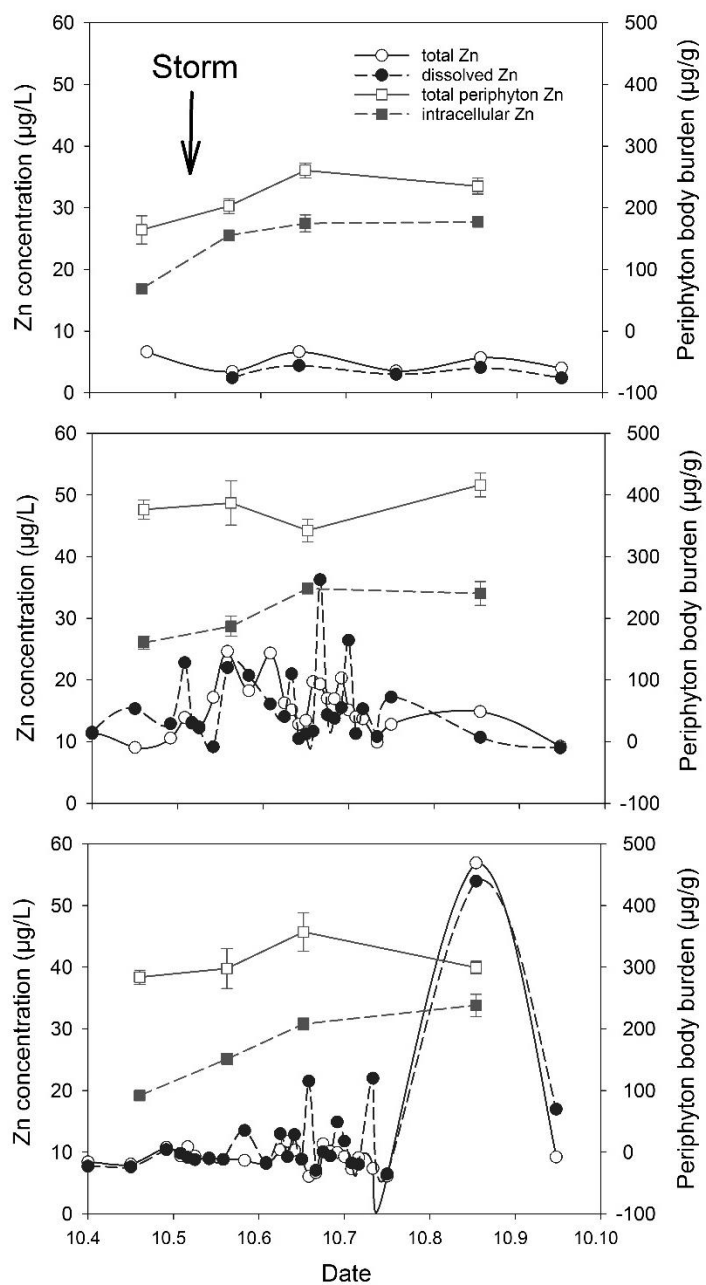


Figure 10. Variations of Zn concentrations and periphyton Zn content during storm event of October 2013 at a) 2 km upstream; b) 2.4 km downstream; c) 5.3 km downstream.

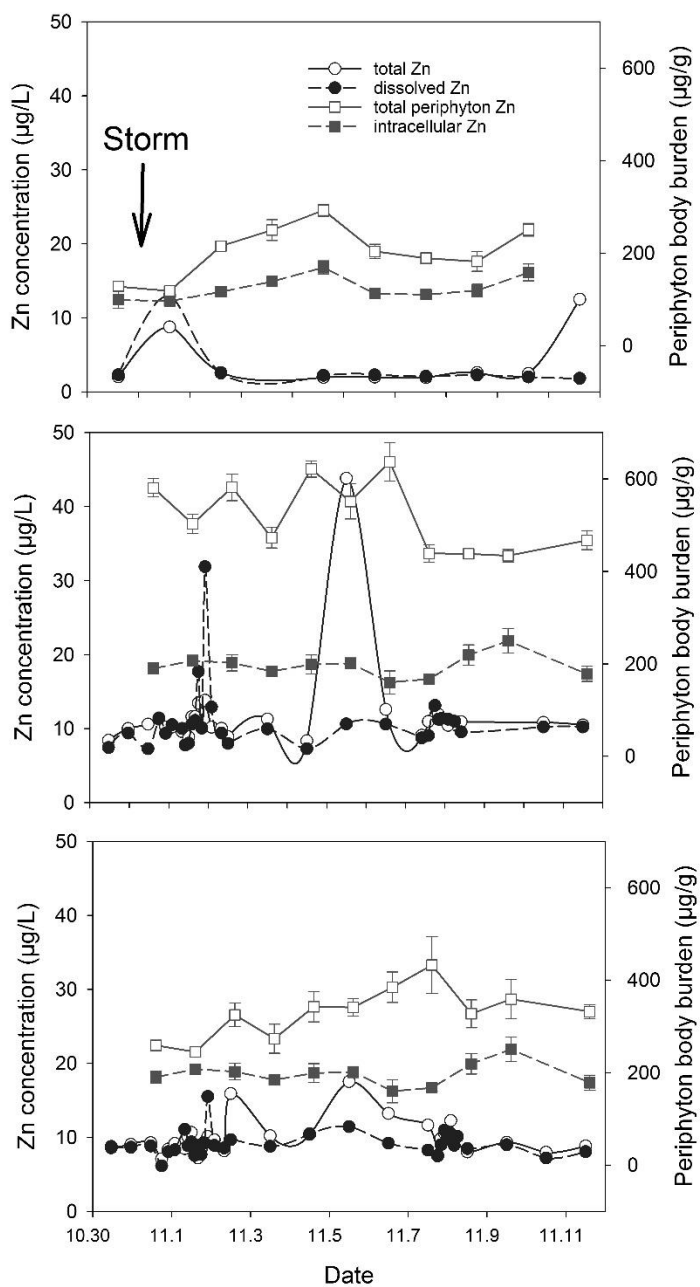


Figure 11. Variations of Zn concentrations and periphyton Zn content during storm event of November 2013 at a) 2 km upstream; b) 2.4 km downstream; c) 5.3 km downstream.

3.4. Environmental significance

Organic matter source and characteristics influence metal lability and bioavailability. Effluent OM increased periphyton Cu contents but reduced DGT-labile Cu possibly due to its higher hydrophilicity and smaller size. This contradiction confined the use of DGT as a bioavailability predictor especially for the presence of anthropogenic hydrophilic OM input. The assumption that the DGT-labile Cu increased with increasing effluent percentage and the reason to explain this contradicted assumption need to be explored in the future. Stormwater OM behaves more like natural OM but is less protective than effluent OM for periphyton uptake. The results of this study implied different source OM has influence on metal lability and bioavailability to different extent, which is opposite to the use of general natural OM like humic or fulvic acid in current bioavailability prediction model or regulation. However, the metal binding capacity was not quantified in this study. The metal binding constants and OM ligand concentration in different source need to be compared and correlated to bioavailable Cu in the future.

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Chapter 4. Comparison of Cu biouptake by freshwater algae in the presence of organic matter from wastewater effluent and stormwater runoff

4.1. Introduction

Organic matter (OM) in streams plays an important role in controlling metal speciation, transport, and uptake into organisms. Wastewater treatment plant (WWTP) effluent and stormwater runoff are two major sources of OM to streams. These two sources of OM are different in size, optical and chemical characteristics like hydrophobicity, specific ultraviolet absorbance (SUVA) or metal binding (Luan and Vadas, Chaminda, Saratha and Allen; Sedlak et al 1997) and thus have different effects on metal speciation and bioavailability in streams. However, the combination of OM properties and metal binding characteristics which control free Cu concentrations and uptake to algae from these various sources to streams are not often assessed. Since both effluent and stormwater runoff are also major sources of metals to streams, a closer look at how the source OM characteristics control metal uptake and association with algae is important for understanding source impacts on ecosystem health.

It is generally accepted that metal bioavailability to algae is controlled by the free metal ion concentration (Campbel 1995, Campbell et al. 1997, Ginneken et al. 1999). The prediction of metal speciation by the free ion activity model (FIAM; (Morel 1983, Campbel 1995) requires knowledge of metal-ligand stability constants. In natural waters, calculating metal speciation in the presence of OM is often predicted using OM models such as Windermere Humic Aqueous Model (WHAM; (Tipping 1994) or the NICA-DONNAN model (Kinniburgh et al. 1996). The models have been developed based on humic and fulvic acids from natural OM, and are not specific to OM observed in human influenced streams. Effluent OM has lower specific ultraviolet absorbance (SUVA) values, is generally considered more hydrophilic, and has more proteinaceous structures (Hudson et al. 2007, Luan et al. 2015). Lower average molecular weight and in particular higher N and S functional groups, suggest that DOM from treated effluent might have stronger metal binding potential (Sarathy et al. 2005, Pernet-Coudrier et al. 2011, Hsu et al. 2003). On

the other hand, OM coming from overland flow in stormwater from highly developed landscapes has high SUVA values (Luan et al. 2015). Allochthonous OM with high SUVA values may better protect against Cu and Pb toxicity than autochthonous OM with low SUVA values (Schwartz et al. 2004).. Thus the traditionally modeled metal binding using isolated OM sources from natural sources may differ significantly from OM in urban streams.

Large metal binding constants in effluent OM have been reported in previous studies, especially at low Cu concentrations (Sedlak et al. 1997, Sarathy et al. 2005, Matar et al. 2015). Synthetic chelating agents like EDTA detected in effluent could partially explain the higher metal binding affinity (Baken et al. 2011). A recent study comparing stormwater runoff and effluent OM stability constants by voltammetry suggests they both have similar conditional stability constants with Cu log K' of 7.2 and 7.4, respectively, but that effluent data fit better to a two-ligand model with a second log K' of 6.4 and with an overall 2-3 fold higher concentration of binding ligands compared to stormwater runoff (Chaminda, Nakajima et al., 2010). Our studies suggest that in one urban watershed with 3-fold higher OM concentrations in stormwater compared to effluent, there were similar binding capacities for Zn in stormwater and effluent, while stormwater runoff had about a 5-fold higher binding capacity for Cu than effluent (Luan and Vadas, 2015). These differences of stability constants and binding capacity in effluent or stormwater source OM have not been adapted into current biotic ligand models (BLM) The measurement of stability constants and binding ligand concentrations of source OM are important to predict free metal concentrations and uptake in aquatic environment.

In addition to metal transport into the algal cell, the interaction of metals and OM with cell surfaces is important due to its role in trophic transfer. OM interaction with cell surfaces can involve cation bridging, electrostatic, hydrogen-bonding or van der Waals attraction between the cell surface and different domains of the OM (Campbell et al. 1997). In experiments with humic acid associated metals, humic substances were found to promote the adsorption of OM associated metals to algae (Lamelas et al. 2008). However, in studies of effluent OM associated Cd and Pb, Worms et al. (2010) suggested that adsorption to cell surfaces

was insignificant due to both the relatively low SUVA values (i.e. low aromaticity) and the relatively high pH of 7.5. Organic matter from stormwater runoff generally has more humic content and higher aromaticity (Luan et al. 2015), and may adsorb to a greater extent to algal surfaces. Understanding both metal uptake and surface adsorption are necessary when considering algal cells and their role in trophic transfer.

The association of metal-OM species with cell surfaces may also be affected by its size. In contrast to smaller OM, larger OM could generate higher electrostatic fields and will have greater probability of forming multidentate metal binding sites than small molecules. A recent study showed the colloidal OM (>1 kDa and <0.45 μm) of the WWTP effluents reduced Cd uptake to algae but increased Pb uptake (Worms et al. 2010) compared to the uptake in the absence of OM. The failure of correlation of Pb uptake to ion-exchangeable Pb concentration in the presence of colloidal effluent OM suggests an effect of size or chemical composition heterogeneity that has to be considered for OM adsorption and metal uptake. Further experiments with different size fractions are needed to better understand the role of OM size on metal bioavailability and attachment.

The purpose of this study was to experimentally assess and model Cu biouptake including both internalization and attachment in the presence of different source OM and size fractions to the freshwater algae *Pseudokirchneriella subcapitata* in short-term exposure experiments. Conditional stability constants were determined using competitive ligand exchange-solid phase extraction (CLE-SPE). Binding ligand concentrations of source OM were measured using Cu titrations. Variations in source OM and size fractions were incorporated into an existing modeling framework to predict Cu accumulation within or on the surface of algae.

4.2. Materials and Methods

4.2.1. Sample collection and processing

WWTP effluent was collected from the Vernon, CT, USA water pollution control facility. Stormwater runoff was collected from a stormdrain outfall within the same watershed. Samples were collected in acid-

washed 20 L HDPE carboys. The samples of effluent and stormwater runoff were divided into total and dissolved fractions by filtration through a 0.45 μm pore size nitrocellulose membrane. A tangential flow ultrafiltration system (TFF, Millipore) was used to separate dissolved source water into three size fractionations based on observations from field samples (Luan et al. 2015): 1) truly dissolved (0-650 Da); 2) small colloids (650-50 kDa); 3) large colloids (50kDa-0.45 μm) using polyethersulfone filters (Pellicon, Millipore). All plastic containers and instruments were acid washed prior to use.

4.2.2. Growth and preparation of algae

Freshwater algae *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*; Aquatic Research Organisms Inc., New Hampshire, USA) was used. Algae was cultured in a standard growing medium (Bolea et al. 2006) under constant aeration and continuous illumination. Cell densities were determined by counting under a microscope on a hemocytometer. Cells in their mid-exponential growth phase where the cell density was about 2.5×10^6 cell mL^{-1} were harvested by filtration (3.0 μm nitrocellulose filters; Millipore), washed with culture medium, then resuspended in the experimental medium.

4.2.3. Short-term algal Cu uptake experiments

Metal uptake by the algae was quantified by determination of adsorbed and internalized metal content after a constant short-term exposure time of 35 min based on previous research. The short-term exposure was to limit the effect of efflux, exudate production, or variable cell sizes and numbers. The effects of different sources, concentrations and size fractions of OM on algal Cu uptake were examined in this study using short-term uptake experiments. Ionic strength of effluent and stormwater solutions was fixed at 5×10^{-3} M by addition of NaCl, MgSO_4 , MgCl_2 , CaCl_2 , KNO_3 , NaHCO_3 to match effluent background and minimize variation between the source waters. The pH was adjusted to 6.6 for all samples. In experiments comparing effluent and stormwater sources of OM, algae were exposed to raw and 0.45 μm filtered source waters to examine the effects of dissolved and particulate phases on metal uptake and adsorption. In experiments to compare dissolved Cu concentration was maintained at 20 ppb while DOC

was fixed at 3 ppm for the following experiments: i) Algal Cu uptake in the presence of effluent dissolved OM and stormwater dissolved OM was compared; ii) a comparison of algal uptake in the presence of the truly dissolved, small colloids, larger large colloids; iii) Effluent DOC varied from 1 to 10 mg/L to see the DOC concentrations influence on metal speciation and uptake. The selected Cu and DOC concentrations was within environment condition and allowed a detected different algal Cu contents.

4.2.5. Harvesting and processing of algae from metal exposure experiments

After metal exposure, the algae suspension was divided into two fractions. One fraction (40 mL) was collected through a 0.45 μm nitrocellulose membrane (Fisher Scientific) and rinsed twice with 5 mL of 0.01 M EDTA (ethylenediaminetetraacetic) to remove metals adsorbed on the cell for intracellular algal Cu content determination (Hassler et al. 2004). The other fraction was collected through a 0.45 μm nitrocellulose membrane as well but rinsed with experimental medium for total algal Cu content determination. The membranes with algae samples were dried in the oven at 50 $^{\circ}\text{C}$ for 15 hours to obtain the dry weight (dw). Then the filters were digested following standard method (EPA). Briefly, the filters were heated at 95 $^{\circ}\text{C}$ in 2 mL of concentrated nitric acid in a 15 mL digestion tube until no brown fumes subsided. Subsequently, hydrogen peroxide (30%) was added stepwise until the effervescence is minimal or until the general sample appearance was unchanged.

4.2.6. Determination of free Cu^{2+} , hydrophobic fraction and conditional stability constants.

Free Cu concentrations were measured by Cu^{2+} -selective electrodes (Thermo Orion 9629BNWP) in each experimental medium before algal exposure. The Cu-DOM conditional stability constants were determined following a CLE-SPE method (Craven et al. 2012). Briefly, a 1-cm diameter glass column (BioRad) packed with 0.5 g C_{18} resin (ENVI-18 resin). The column was cleaned following previous method (Hsu et al. 2003). Approximately 250 mL of sample was pumped through the acid-cleaned resin column at 4 mL/minute. OM complexes were divided into operationally defined hydrophobic OM, which were retained in the C_{18} resin, and hydrophilic OM fraction that passed through. The fraction of hydrophobic and hydrophilic for Cu complex and OM respectively were determined from the elute concentration without

competing ligand addition. Benzoylacetone was used as the hydrophobic competing ligand, and nitrilotriacetic acid was used as the hydrophilic competing ligand. The conditional stability constants were calculated by eq. (1) for hydrophilic comparing the eluted Cu concentration with and without competing ligand additions. The Cu:DOM ratio in effluent and stormwater samples were maintained at 0.003 mg Cu mg DOC⁻¹ during the determination of stability constants.

$$K_{\text{CuL}_{\text{DOM}}} = \frac{K_{\text{CuBz}_2} [\text{CuL}_{\text{DOM}}] [\text{Bz}^-]^2}{[\text{Cu}(\text{Bz})_2^0] [\text{L}_{\text{DOM}}]} \quad (1)$$

where $[\text{CuL}_{\text{DOM}}]$ was the concentration of Cu that passes through the C18 resin bound to hydrophilic DOM (M); $[\text{Bz}^-]$ was the concentration of the deprotonated form of benzoylacetone (M), which was calculated for pH 6.6 from the total concentration and the pKa of benzoylacetone; $[\text{Cu}(\text{Bz})_2^0]$ is the concentration of hydrophobic Cu–benzoylacetone complex (M); and $[\text{L}_{\text{DOM}}]$ was calculated by the difference in Cu concentration passing through the resin before and after benzoylacetone addition.

The concentration of $[\text{Cu}(\text{Bz})_2^0]$ was uncertain and calculated as a range. This uncertainty resulted in a range of values for the calculated Cu–DOM stability constants. The maximum value for $[\text{Cu}(\text{Bz})_2^0]$ was estimated assuming that all of the Cu retained on the resin after ligand addition was bound to benzoylacetone:

$$[\text{Cu}(\text{Bz})_2^0] = [\text{Cu}]_{\text{hydrophobic, Bz}} \quad (2)$$

The minimum value $[\text{Cu}(\text{Bz})_2^0]$ was estimated by assuming that the Cu retained on the resin before ligand addition ($[\text{Cu}]_{\text{hydrophobic, no Bz}}$) continues to be bound to the hydrophobic DOM.

$$[\text{Cu}(\text{Bz})_2^0] = [\text{Cu}]_{\text{hydrophobic, Bz}} - [\text{Cu}]_{\text{hydrophobic, No Bz}} \quad (3)$$

The same equations were applied for NTA.

4.2.7. Chemical analysis

Total and dissolved metal samples were acidified to 2% HNO₃ (trace metal grade). Total metal samples were digested according to EPA Method 200.8 (1994). An inductively coupled plasma mass spectrometer

(ICP-MS, Agilent 7700x with He collision cell, Agilent, Delaware, USA) was used for determination of Na, Mg, K, Ca, Cu, with Sc used as an internal standard. Standards and quality control checks were prepared from independent high purity standards (Spex-Certiprep, Metuchen, NJ, USA and VHG Labs, Inc., Manchester, NH, USA). Quality control samples were within 10% or better of expected values.

Organic matter concentrations in effluent and stormwater samples were determined with a Teledyne Tekmar combustion TOC analyzer. In experiments to determine OM sorption to algal cells, before and after algal uptake was determined using Sievers DOC analyzer due to its lower detection limit. The detection limit of combustion and portable DOC analyzer was 0.7 mg/L and 0.2 mg/L respectively.

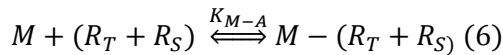
4.2.8. Modeling of Metal Uptake and OM Adsorption to Algae.

The uptake model assumes that only free metal, e.g. Cu^{2+} , interacts with the transport sites. The intracellular metal concentration, C_{int} , is described by the Michaelis-Menten saturation equation (Sunda et al. 1998):

$$C_{\text{int}} = \frac{C_{\text{max,int}}[M]K_M}{[M]K_M + 1} \quad (5)$$

Where $[M]$ is the concentration of either the free metal ion. K_M is an affinity constant for the binding of metal M to the transport site (which can compete with aqueous organic ligands for M), $C_{\text{max,int}}$ is the maximum metal concentration achieved at metal saturation of the transport sites.

Similarly, the total cellular metal concentration (internalized plus adsorbed metal, C_{cell}) can be described by the same saturation equation, assuming that metal adsorbs simultaneously to two types of binding sites, but only one is followed by internalization (Lamenas et al. 2008):



where R_T is the concentration of a physiologically active metal binding site on the surface of the organism (e.g. a transporter protein). R_S is the concentration of adsorption metal binding site on the surface of the organism (Slaveykova and Wilkinson 2005).

$$C_{cell} = \frac{C_{max,cell}[M]K_{M-A}}{[M]K_{M-A} + 1} \quad (7)$$

Where $C_{max, cell}$ is the max cellular metal concentration and K_{M-A} is the effective average binding constant of metal to algae, representing metal affinity for both adsorption and internalization.

In addition, it has been demonstrated that OM will adsorb to algal surfaces (Lamelas et al. 2007). Therefore, the total metal content in a system containing algae, metal, and OM needs to consider both the metal uptake by algae and metal bound to OM and adsorbed to algae.

$$C_{cell,tot} = C_{cell} + C_{OM, adsorbed} \quad (8)$$

$$C_{cell,tot} = \frac{C_{max,cell}[M]K_{M-A}}{[M]K_{M-A} + 1} + K_H^{OM}[OM][M]K_{M-OM} \quad (9)$$

Where K_H^{OM} is the linear regime adsorption coefficient.

The adsorption of OM to the algal surface was assessed at 3 and 10 mg C/L using short-term uptake experiments. No significant adsorption for both effluent and stormwater samples was observed at these two concentrations (below detection limit, i.e. <0.2 mg/L). Therefore, in our models, we disregarded the OM adsorption term.

4.2.3. Determination of algal binding parameters

Maximum binding sites (C_{max}) and Cu binding constants (K_M and K_{M-A}) for the transporter sites and total algal surface sites, respectively, were determined in the absence of DOM using short-term uptake (35-min) experiments with varying Cu concentrations from 2 to 200 µg/L. Intracellular and total algal Cu contents were used to obtain intracellular and total algal binding parameters respectively. The $C_{max,int}$ and K_M were calculated from a linear fitting equation (5) while $C_{max,cell}$ and K_{M-A} were from eq. (7). Maximum binding sites was obtained from the slope of linear line and binding constants was calculated from the intercept (Van den Berg et al. 1979).

Table 1. Initial water chemistry parameters in effluent and stormwater samples in mg/L

| Sample | Na | Mg | Ca | K | Cl ⁻ | NO ₃ ⁻ | SO ₄ ²⁻ | CO ₃ ²⁻ |
|------------|----|-----|----|----|-----------------|------------------------------|-------------------------------|-------------------------------|
| Effluent | 70 | 4 | 20 | 11 | 109.7 | 11.9 | 56.8 | 18.5 |
| Stormwater | 6 | 0.4 | 2 | 1 | 3.4 | 0.3 | 3.3 | 2.5 |

4.2.9. Modeling free Cu²⁺ with WHAM VII and Visual MINTEQ

The chemical equilibrium modeling programs WHAM VII and Visual MINTEQ 3.1 were used to model Cu speciation in order to compare to the electrode measured concentrations. Water quality parameters of effluent were used as input to both codes (Table 1). A hydrophobic and hydrophilic model ligand respectively with measured conditional stability constants and binding ligand concentrations in this study were added to the Visual MINTEQ database. Humic acid was assumed to account for 10% of total dissolved OM in WHAM VII. WHAM used binding constants of humic/fulvic acid and doesn't allow input of binding constants. The results of the Cu uptake model were compared using the Cu²⁺ concentration from the electrode, Visual MINTEQ and WHAM VII.

4.3. Results and Discussion

4.3.1 Conditional stability constants

Average conditional stability constants of the whole dissolved effluent OM were 0.5 to 1 log unit higher than those of stormwater OM for both hydrophilic and hydrophobic OM (Table 3). A little less than one order of magnitude higher conditional stability constant of effluent OM than stormwater OM resulted in 50% less measured free Cu concentration. The conditional stability constants of effluent and stormwater samples in this study were lower than those of natural OM isolates (log K=10.4-13.2) (Craven et al. 2012) measured in previous studies at the same pH. This could be due to the fact that conditional stability constants decrease with increasing Cu:OM ratio due to Cu binding to the stronger ligands present (Craven et al. 2012). The small difference of conditional stability constants between effluent and stormwater OM indicated stormwater OM had as strong of Cu binding capacity as effluent OM. Similar conditional stability constants

for effluent and stormwater OM was observed in a previous study (Chaminda et al. 2010). Sedlak (1997) also reported the presence of strongly complexed Cu in both effluent and stormwater runoff. However, the higher binding ligand concentration in stormwater OM was opposite to Chaminda's study, in which effluent had a higher binding ligand concentration. The stormwater OM in this study was collected from a suburban area with a mixture of development, lawn and forested areas and more direct interaction with soil and ground surface sources of OM likely contributed compared to the stormwater collected directly from a roadway. The stability constants of the source OM were all higher than those of the algae surface (Table 2), which were comparable to algal surface binding constants in other studies (Lamelas et al. 2007, Stoiber et al. 2012).

Table 2. Maximum total) and intracellular) metal concentration (M/g) and respective binding constant at pH = 6.6 and I = 5×10^{-3} M, obtained from the experiments in the absence of OM.

| | $C_{max,cell}$ (mole/g) | Log K_{M-A} (M^{-1}) | $C_{max,int}$ (mole/g) | Log K_M (M^{-1}) |
|--|----------------------------|-------------------------------|---------------------------|---------------------------|
| <i>Pseudokirchneriella subcapitata</i> | 5.03×10^{-6} | 6.10 | 1.34×10^{-6} | 6.60 |

Cu primarily bound hydrophilic OM (80%) in effluent while it primarily bound hydrophobic OM (77%) in stormwater. Although the conditional stability constants of hydrophilic and hydrophobic OM for both effluent and stormwater were similar, the 80% initially occupying hydrophilic sites in effluent OM indicated a possible presence of stronger binding sites which were not directly quantified in the conditional stability constant measurement. The strongest binding sites were likely located in the hydrophilic fraction of effluent and in the hydrophobic fraction in stormwater based on the initial Cu binding. This matched our expectation that hydrophilic OM in effluent was more important than hydrophobic OM. The fraction of hydrophobic and hydrophilic OM in source water was similar to Cu complexes. Hydrophilic OM accounted for approximately 67% in effluent OM and 30% in stormwater OM. This fraction matched with previous observations that effluent has a much higher hydrophilic fraction observed in the range of 41 – 80%, compared to natural organic matter sources of 8 – 50% (Pernet-coudrier et al. 2008, Quaranta et al. 2012, Matar et al. 2015). In spite of the large fraction of hydrophilic OM and the strong affinity of the-

Table 3. Conditional stability constants and binding ligand concentration in DOM of WWTP effluent, stormwater, and respective size fractions.

The range in log K values represents the variation of distribution of Cu bond to competing ligand and source OM retained on resin.

| Samples | | Hydrophobic | | Hydrophilic | | Binding ligand concentration |
|------------|---------------------------|-------------|------------|-------------|------------|---------------------------------|
| | | Log K | % Cu bound | Log K | % Cu Bound | ($\mu\text{M}/\text{mg}$) |
| Effluent | Total Dissolved | 8.4-9.3 | 20 | 8.0-8.4 | 80 | 0.119 |
| | 0-650 Da | 8.5-9.2 | 20 | 7.9-8.3 | 80 | 0.116 |
| | 650 Da-50 kDa | 8.5-9.2 | 23 | 8.0-8.4 | 77 | 0.118 |
| | 50 kDa-0.45 μm | 8.5-9.2 | 16 | 8.2-8.6 | 84 | 0.128 |
| Stormwater | Total Dissolved | 7.9-8.0 | 77 | 7.2-7.8 | 23 | 0.163 |
| | 0-650 Da | 7.5-7.6 | 74 | 5.0-5.6 | 26 | 0.087 |
| | 650 Da-50 kDa | 7.8-8.1 | 54 | 6.3-6.7 | 46 | 0.139 |
| | 50 kDa-0.45 μm | 8.0-8.3 | 59 | 6.7-7.3 | 41 | 0.198 |

hydrophilic fraction for metal ions in this and other studies (Sarathy et al. 2005, Buzier et al. 2006, Louis et al. 2014), the metal binding constant of hydrophilic DOM has not yet been taken into account in the widely used BLM (Di Toro et al. 2001). The variability in DOM stability constants is a critical parameter to predict metal uptake and toxicity, and therefore, the existing models such as BLM may be not reliable in streams with more anthropogenic inputs that differ significantly from the OM used to develop the models..

As for the differences in conditional stability constants with size fractions, all effluent OM size fractions showed a similar binding strength as the whole effluent sample while stormwater OM had an increasing stability constant value with increasing OM size fraction. This was surprising given the expectation that strong synthetic ligands are often present in effluent (Ledin et al. 1993, Sedlak et al. 1997, Nowack et al. 2005, Baken et al. 2012). Higher binding constants and binding capacity in the lower size fractions (< 3 kDa) have been reported (Hoffmann, Shafer et al. 2007) and are likely dominated by the synthetic or strong natural ligands. It is likely that the effluent used in the current study had low concentrations of strong synthetic ligands. The truly dissolved fraction of stormwater OM had the lowest conditional stability constant and the log K of hydrophilic fraction was even lower than the algal binding constants, suggesting that the small size stormwater OM may not be as competitive as the algal surface for Cu binding and therefore may not as effectively limit Cu uptake. The higher conditional stability constants of the small and large colloidal fractions of stormwater OM suggest Cu speciation and uptake to algae could be more strongly controlled by colloidal OM in stormwater. Our results agree with the previously observed opposite trend in natural water sources and some stormwater, with higher binding constants in larger size fractions. Given the differences of conditional stability constants in effluent and stormwater sources, and some OM size fractions, the addition and validation of unique binding constants for individual sources in metal binding models should be considered.

The dramatic difference of OM character between effluent and stormwater could result in different transport behavior, adsorption and uptake in streams. Hydrophobic OM is more easily adsorbed by

suspended particulate matter and sediment (Voice et al. 1983, Minshall et al. 2000) in streams, thus stormwater OM may be less likely to transport longer distances and instead be retained in particulate materials and sediments. The transport of stormwater OM is likely restrained within the discharge area and the effects of stormwater OM mixing with stream water on metal speciation is altered by the lower conditional stability constants. On the contrary, the majority hydrophilic effluent OM could travel further in streams and complex more free Cu concentrations due to their higher conditional stability constants.

In addition, in order to understand bioavailability from waterborne sources, and to set reasonable aquatic criteria for metals using tools such as the BLM, accurate modeling of solution speciation is required. The development of organic matter binding models has primarily been based on the use of humic or fulvic acids, which by their operational isolation (XAD resins) do not include the majority of hydrophilic molecules (Thurman et al. 1981). Similarly, the existing WHAM model divides OM into humic or fulvic acid, and into particulate or colloidal OM, and it uses the same binding constants for each size fraction. Including different stability constants for effluent and stormwater OM should improve the function of these models.

As for attachment to algal surfaces, stormwater OM promoted more accumulation of Cu on the algal surface than effluent OM (data not shown), but this accounted for a small percentage (9.2%) of the total algal Cu content. Due to the relatively low SUVA values and high hydrophilic fraction of effluent OM determined in previous studies, the low adsorption of effluent OM to algae was expected and should be minimum. Even for most hydrophobic humic acids, less than 25%-30% of the cellular Cu has been reported bound to the humic acid adsorbed to algae (Lamelas et al. 2007), though in our case the humic content was much lower. Although stormwater OM in the present study was highly hydrophobic, the adsorption of OM on algal surface was expected lower than pure humic acid. Therefore, the small percentage of stormwater OM adsorption on the algal surface was understandable. Also, to adsorb 20% of the total algal Cu content on the algal surface the loss of DOC from the experimental medium is only 0.3-0.5 mg/L through back calculation using proposed model. This is close to the detection limit of the TOC analyzer, which made it

difficult to determine the adsorbed OM complexes experimentally. Furthermore, the contribution of supplementary binding sites due to OM adsorption to algal surface (e.g., ternary complex formation) to metal uptake could be expected to be greater under the following conditions: (i) high quantity of humic acid adsorbed onto algal surface; (ii) relatively high affinity of the metal to humic acid; or (iii) relatively low affinity of the metal to algae (Lamelas et al. 2007). The present study satisfied the latter two conditions but failed for the first one. Therefore, the effect of adsorbed OM to algal surface on metal uptake was insufficient in this study and the prediction model was mainly considering the cellular uptake part.

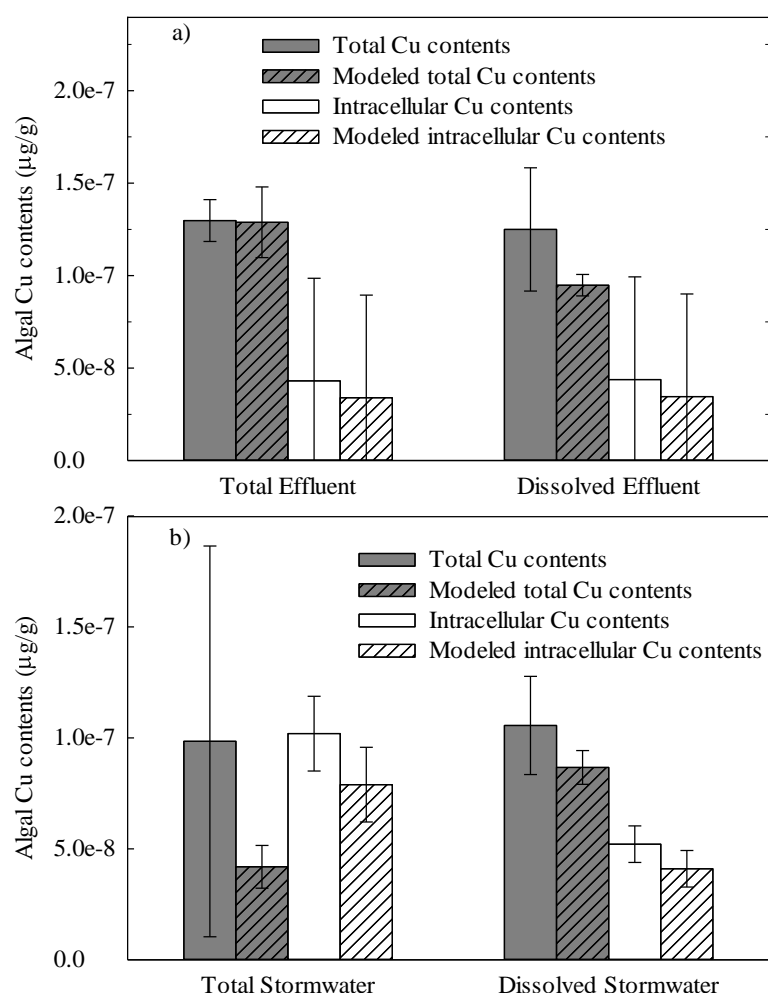


Figure 1. Experimental and MINTEQ modeled (based on Visual MINTEQ modeled Cu^{2+}) uptake of Cu in the presence of a) total and dissolved effluent and b) total and dissolved stormwater. The error bar was

one standard deviation for experimental data and range of Cu uptake in terms of range of measured conditional stability constants.

4.3.2. Algal Cu uptake in the presence of effluent and stormwater

Algal Cu uptake was similar in the presence of total and dissolved effluent while intracellular algal Cu content was higher in the presence of stormwater compared to dissolved stormwater (Figure 1). Similar algal Cu uptake in the presence of and dissolved effluent was expected because particulate Cu and OM only accounted for a small percentage, about 10% and 16.6%, respectively in effluent. While total algal Cu content was also similar in samples exposed to total and dissolved stormwater, the variability was much higher in the presence of total stormwater (Figure 1). Given the higher particulate matter and associated Cu (72.7%) in stormwater and the fact that a couple associated particles collected with the algae could dramatically alter concentrations, this is not surprising. Considering total Cu concentration (5.5 $\mu\text{g/L}$) was higher than dissolved Cu (1.5 $\mu\text{g/L}$) in stormwater, similar total algal Cu content in the presence of total and dissolved stormwater suggested particulate Cu was not efficiently adsorbed on the algal surface. Higher intracellular algal Cu content in the presence of total stormwater was either because of the higher Cu concentration or because attached Cu was more labile and bioavailable. The modeled Cu uptake based on the free Cu concentration calculated by MINTEQ (assuming the same binding constants for particulate and dissolved OM) generally matched the experimental data, with the exception of the total algal Cu in the presence of total stormwater. The underestimation of total algal Cu content in the presence of total stormwater was likely due to different binding properties of particulate OM in stormwater.

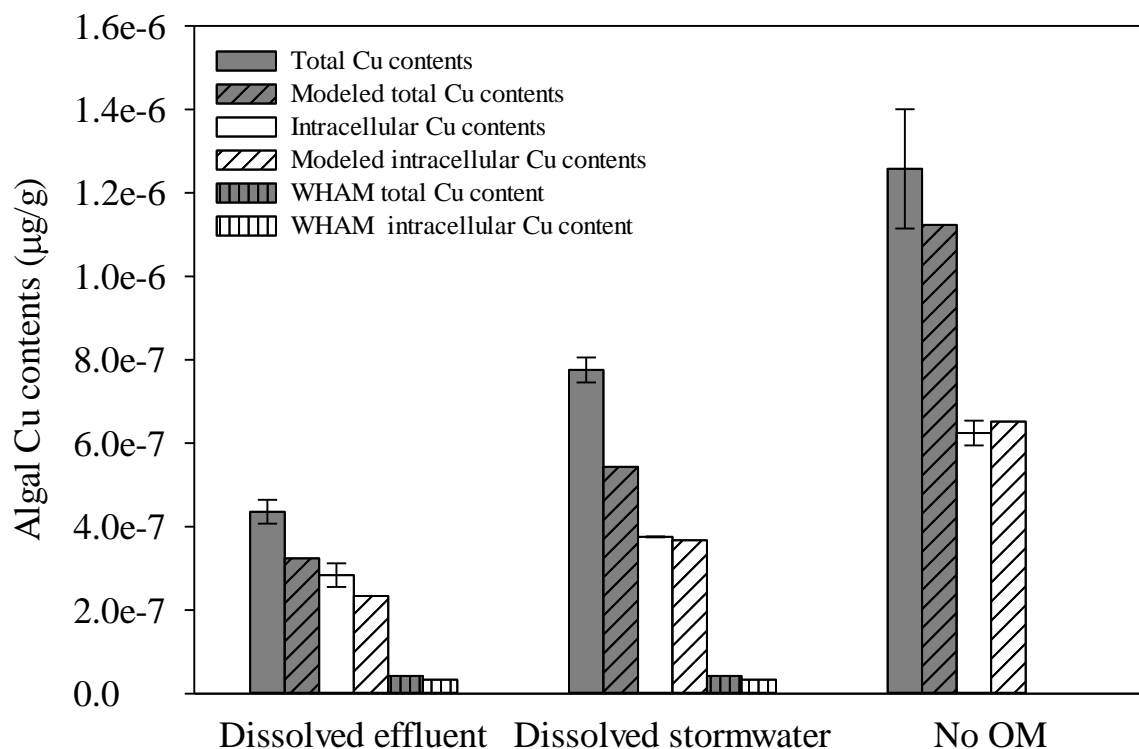


Figure 2. Experimental and modeled uptake (electrode or WHAM based free Cu^{2+}) of Cu in the presence of dissolved effluent, stormwater and No OM where total dissolved Cu concentration=20 ppb and dissolved organic carbon concentration = 3 ppm. Modeled uptake of Cu was calculated from the free Cu concentrations measured by Cu electrode.

In short-term algal uptake experiments exposed to different source waters with equal Cu and OM concentrations, there was 46% less total Cu uptake and 25% less intracellular Cu uptake in the presence of effluent OM compared to stormwater OM (Figure 2). The more pronounced reduction in uptake in the presence of effluent OM compared to stormwater was expected based on the higher conditional stability constants measured for effluent OM than stormwater OM (Table 3). Incorporating the measured free Cu^{2+} concentration into the algal uptake model, the prediction of Cu uptake was within 20% of the experimental data.

A comparison of free Cu concentrations determined in the current study with the WHAM VII model found that WHAM predicted free Cu concentration was 1-2 orders of magnitude less than electrode

measured values in each source samples. It has been reported either Visual MINTEQ incorporating NICA–Donnan or WHAM VI underestimates free Cu concentration by 2-5 or 1-3 orders of magnitude, respectively of magnitude in natural water samples (Unsworth et al. 2006), just as our WHAM prediction of free Cu concentration did compared to electrode measured Cu^{2+} . The underestimation may be due to the inappropriate use of generic binding constants representing an average of metal humic binding parameters determined in laboratory experiments with extracted humic substances. In a more complicated aquatic system, the more wide ranging binding properties of humic acid and more competition and interferences such as Fe(III) (Tipping et al. 2002) may result the inaccuracy of free Cu concentration prediction. WHAM predicts the same free Cu concentration and modeled algal uptake in the presence of either effluent or stormwater OM due to the uniform binding constants it uses, which certainly does not match our experimental data. If binding ligand concentrations are input as the dissolved organic matter concentrations in WHAM, the predicted free Cu concentration matches more closely with the measured free Cu concentration in the presence of stormwater OM. This is reasonable given that WHAM was trained based on natural samples. Overall, a specific conditional binding constants for anthropogenic source like effluent OM and binding ligand concentrations were necessary to incorporate into current bioavailability model to obtain a better prediction of metal uptake.

4.3.3. Size fractions effects

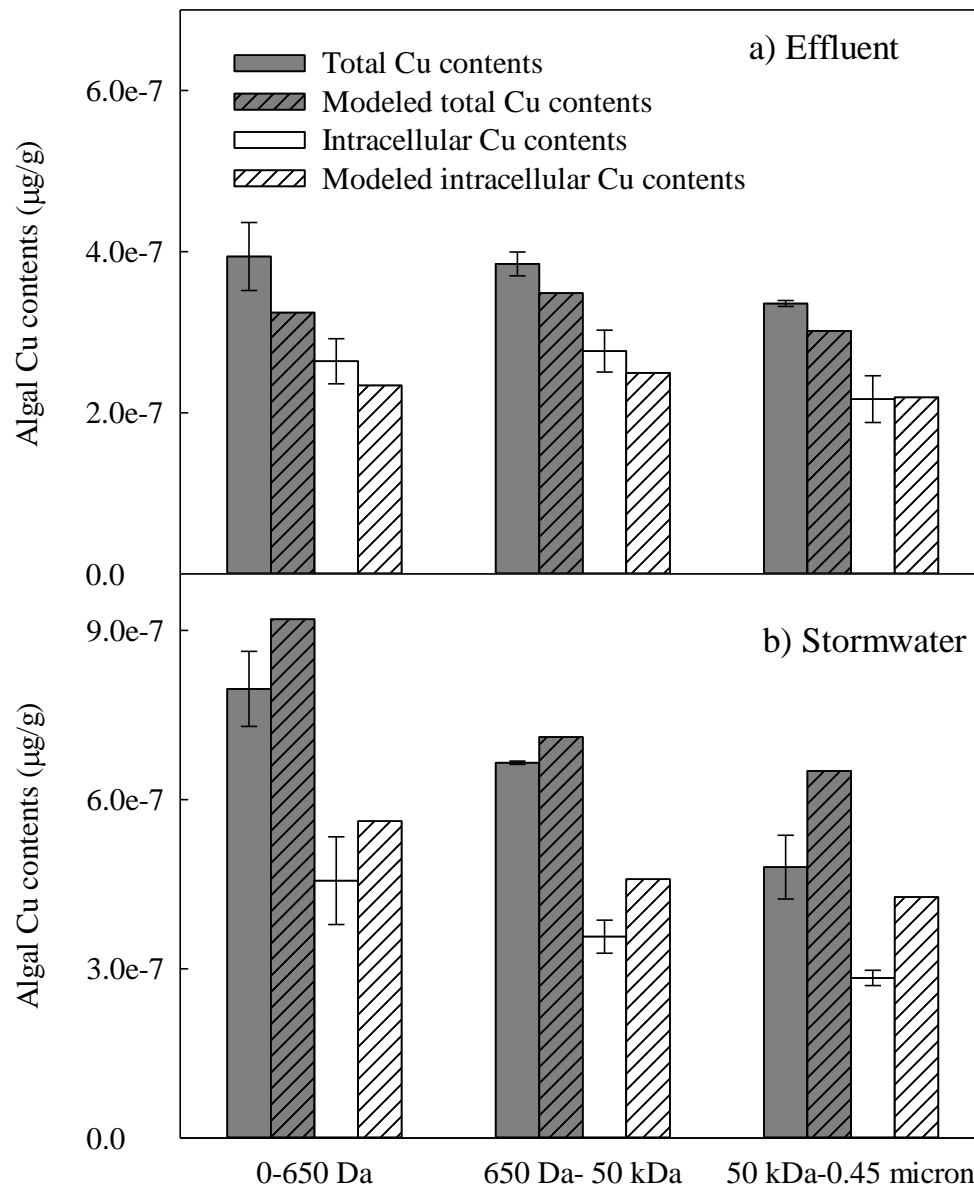


Figure 4. Experimental, modeled uptake of Cu in the presence of a) effluent size fractions; b) stormwater size fractions. Modeled uptake of Cu was calculated from the free Cu concentrations measured by Cu electrode.

Algal Cu uptake was similar in the presence of effluent size fractions while algal Cu uptake reduced with increasing stormwater size fractions, corresponding to the decreasing conditional stability constants as well as the dissolved source OM. In spite of the slightly lower Cu uptake in the presence of large size effluent OM, there was no significant difference between each size fraction of effluent OM and the algal

Cu uptake in the presence of each size fractions were similar ($p>0.05$) to that in the presence of whole dissolved effluent OM. These could be inferred from the similar conditional stability constants measurements for effluent. The predicted Cu uptake was slightly lower but within 20% of the experimental data. As for stormwater size fractions, the small size fraction had highest algal Cu uptake with lowest conditional stability constants. The uptake was lower when the OM size became larger. The algal Cu uptake in the presence of stormwater OM was a little over predicted but still within 30%. Although the absence of stronger binding ligands in effluent was not expected, the differences of conditional stability constants and algal Cu uptake between size fractions of stormwater OM suggested size dependence of metal binding have to be considered to better understand the effect on metal speciation and uptake within dissolved fraction.

Stormwater runoff could bring more particulate OM and Cu to streams. The concentrations of OM and Cu varies over a wide range but the effect of stormwater OM on metal speciation and uptake is temporally and spatially limited because storms usually occur over a short time period and both particulate and dissolved stormwater OM may transport shorter distances due to its high hydrophobicity. Also, the small size fraction was likely degradation products of larger size fractions like humic or fulvic acids used in WHAM or BLM. This small size fraction showed low binding constants and could not provide enough protection for Cu uptake to algae. Therefore, although large stormwater size fractions or whole stormwater had fairly high binding constants, they may lose this high metal binding capacity when degraded to smaller size fractions. Effluent was a more consistent source of OM and Cu. The evenly distributed metal binding properties across size fractions suggested a uniform composition of effluent OM. The effluent OM with higher Cu binding constants is continuously complexing free Cu concentrations in streams and protecting Cu uptake to algae or higher trophic organisms.

4.4. Environmental Implications

The stormwater is less protective of Cu uptake. Given the same Cu concentration, exposure to stormwater would result in at least a temporary increase in Cu uptake to algae. Depending on patterns of storms and feeding patterns of organisms, storms could increase grazer organism metal exposure following

the storm event. WHAM (or BLM) underestimated free Cu concentration and algal Cu uptake by several orders of magnitude using the default or uniform binding parameters compared to our experimental data. This prediction would result in a higher water quality criteria of Cu set by regulatory agencies, which would lead to more bioaccumulation in aquatic systems. A large portion of effluent OM was in hydrophilic form which has a higher metal binding constant. The hydrophilic fraction had a bigger effect on algal metal uptake and they could transport further in streams due to their unique chemical characteristic comparing to stormwater OM or natural OM. In a stream with more anthropogenic influence, the current bioavailability model like WHAM or BLM which were developed based on natural samples would generate biased prediction without considering the contribution of human released hydrophilic OM fraction.

In conclusion, the present study showed effluent OM reduced more Cu uptake to freshwater algae than stormwater OM. This more pronounced reduction was corresponding to the conditional stability constants of each source OM. The Cu uptake to algae was predicted well using proposed model when the conditional stability constants and binding ligand concentrations of each source OM were incorporated. Effluent OM exhibited similar metal binding properties and algal uptake throughout three size fractions while stormwater OM showed a decreasing conditional stability constants with size increasing then an increasing algal uptake. The results suggest specific conditional stability constants were necessary to obtain an accurate modeling of solution metal speciation, especially for an anthropogenic source like effluent which is often dominated by hydrophilic OM, and in particular can have a substantial fraction of strong metal binding ligands. The general use of humic and fulvic acid as binding ligands in existing bioavailability prediction model like WHAM is insufficient to predict free Cu concentration and metal speciation correctly then produce biased aquatic criteria for metals. Also, binding ligand concentration is another important factor in metal speciation prediction. The actual binding ligand concentration is much lower than the dissolved organic carbon concentration and different from each source OM. The free Cu concentration and algal uptake are underestimated in orders of magnitude without considering the binding ligand concentration. A further extension of WHAM by including specific conditional stability constants and

binding ligand concentrations would improve its site-specific predictive ability, particularly in the presence of a source OM which is very different from humic and fulvic acids. In addition, given the observed differences in stormwater size fractions, the addition and validation of unique binding constants for defined size fractions, will more accurately represent equilibrium metal speciation, and ultimately BLM models, in urban stream systems.

4.5. References

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Chapter 5 Conclusion and future work

The form or size fraction of metals in urban streams has major implications for both transport and bioavailability. Colloidal phase metals are transported downstream similarly to truly dissolved metals, but are less likely to be impeded by other processes such as sorption or precipitation reactions. Alternatively, they may be subject to removal via flocculation during major changes in water chemistry, such as ionic strength or pH that may occur as different source waters mix. Metals may also repartition between phases as component concentrations change and we saw some evidence for this when spiking mixtures with either metals or other cations. The issue of bioavailability is particularly important with respect to colloidal organic matter bound metals. While some researchers suggest colloidal phase metals are less bioavailable (Ross and Sherrell, 1999), it really depends on the lability of the metal and the fate of the organic colloid surrounding the metals, i.e. how it interacts with an organism and/or undergoes trophic transfer. In addition, the exposure patterns of organisms to these different size fractions varies in time scales, i.e. short durations over storm events, or constant exposure from effluent during baseflow. Different organic matter sources are unique with regard to spectral properties and functional groups, and have differences in metal lability as observed during the spiking reactions. The differences in hydrophilic or hydrophobic organic fractions will also alter the sorption to surfaces such as periphyton, which could alter the direct uptake by those organisms and trophic transfer to higher organisms, though we saw no significant sorption to periphyton surfaces. In addition, the differences observed in binding capacity and changes in metal binding due to competing elements imply differences in ambient exposure to organisms, as opposed to ingestion, due to exchangeability of metals to the organism surface. Understanding the dynamics of both altered transport processes as well as biological exposure in streams coupled to the lability of metals in these different source and size fractions would be valuable to targeting metal source management in impaired water bodies.

OM source and characteristics influence metal lability and bioavailability. Effluent OM increased periphyton Cu content but reduced DGT-labile measured Cu, possibly due to its higher hydrophilicity and smaller size. This contradiction limits the use of DGT as a bioavailability predictor, especially in the

presence of anthropogenic hydrophilic inputs or multiple source mixtures of metals and OM. The assumption that the DGT-labile Cu increased with increasing effluent percentage and the reason to explain the contradiction to this assumption needs to be explored in the future. Stormwater OM behaves more like natural OM but is less protective than effluent OM for periphyton uptake. The results of this study shows that different source OM influences metal lability and bioavailability to different extents, which is important to note when typical regulatory procedures use on set of natural OM separated into humic or fulvic acids in current bioavailability prediction models. In a stream with more anthropogenic influence, the current bioavailability model like WHAM or BLM which were developed based on natural samples would generate biased prediction without considering the contribution of human released hydrophilic OM fraction.

Although our results with regard to effluent or stormwater regulated Cu uptake conducted within the same period are comparable, the research out in the field at different Cu concentrations and different exposing time are difficult to compare considering the kinetic controls on metal uptake and sorption to algae and periphyton. An understanding of uptake kinetics with respect to speciation is necessary to interpret dynamic aquatic systems, especially with sporadic stormwater input. Storm events generate variable metal and OM loads in streams, and it is unclear to what extent they elevate and persist metal concentrations in periphyton communities. In our few observations of field exposure to periphyton, intracellular metal content was sometimes elevated and sometimes low. Extracellular metal content was more variable than intracellular content, which is to be expected due to the regulation of metal homeostasis by the organism and the fast sorption exchange processes that are likely to occur on organisms. Building on equilibrium studies of biofilm metal association, research on understanding the influence of dynamic metal and OM exposure on periphyton metal content is warranted. The kinetic information will provide a sense of the relative importance of changes in surface water speciation as different source waters mix for different periods of time.

Our results showed effluent OM reduced Cu uptake to freshwater algae more than stormwater OM. This more pronounced reduction was corresponding to the conditional stability constants of each source

OM. The Cu uptake to algae was predicted well using the proposed model. Effluent OM exhibited similar metal binding properties and algal uptake throughout three size fractions while stormwater OM showed decreasing conditional stability constants with size resulting in increasing algal Cu uptake. Given the same Cu concentration, exposure to stormwater would result in at least a temporary increase in Cu uptake to algae. Depending on patterns of storms and feeding patterns of organisms, storms could increase grazer organism metal exposure following the storm event. The results suggest that using specific conditional stability constants for different OM sizes or types will improve the prediction of solution metal speciation and therefore organism uptake that relies on free metal concentration.

The general use of humic and fulvic acid as binding ligands in existing bioavailability prediction models like WHAM is insufficient to predict free Cu concentration and metal speciation and produces biased aquatic criteria for metals. Also, binding ligand concentration is another important factor in metal speciation prediction. The actual binding ligand concentration is much lower than the dissolved organic carbon concentration and different from each source OM. The free Cu concentration and algal uptake are underestimated by orders of magnitude without considering the binding ligand concentration. WHAM (or BLM) underestimated free Cu concentration and algal Cu uptake by several orders of magnitudes using the default or uniform binding parameters when comparing to experimental data. This prediction would result in a higher water quality criteria of Cu and would be less protective of the algal species in the ecosystem. A further extension of WHAM by including specific conditional stability constants and binding ligand concentrations would improve its site-specific predictive ability, particularly in the presence of a source OM which is very different from humic and fulvic acids. In addition, given the observed differences in stormwater size fractions, the addition and validation of unique binding constants for defined size fractions, will more accurately represent equilibrium metal speciation, and ultimately BLM models, in urban stream systems.

The presumption right now in developed streams is to limit effluent metal contributions to below existing aquatic criterion, and to install BMPs to primarily reduce flow and remove particulate loads to

streams. As more knowledge is gained about the dissolved metal speciation entering streams in developed landscapes, assays could be designed to target toxicity and regulations and management strategies could target reductions in loads from specific sources that contribute more directly to organism impairment or toxicity in the system.