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DETECTING COPPER RESIDUES IN SEDIMENTS FROM AQUATIC COPPER-BASED PESTICIDE APPLICATIONS

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DETECTING COPPER RESIDUES IN SEDIMENTS FROM AQUATIC COPPER-BASED PESTICIDE APPLICATIONS

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In Partial Fulfillment
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Master of Science
Forest Resources

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by
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Accepted by:
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ABSTRACT

Copper-based algaecides and herbicides are commonly used to mitigate risks of problematic algae and weeds, however there are concerns regarding copper accumulation from treatments. Modeling and laboratory experiments can be used to predict copper accumulation and measurement in sediments, as well as benthic invertebrate responses following treatments. Modeling was used to estimate copper residual concentrations in sediments from algaecide and herbicide treatments. Reported and measured method detection limits (MDLs; minimum concentration of an analyte of interest that can be measured with 99 percent confidence in a specific matrix using an analytical method) were compared to model estimated copper residual concentrations in sediments to determine measurement of copper accumulation from treatments. Laboratory toxicity experiments were used to measure the bioavailability of copper in sediments. Comparisons of results from modeling and laboratory experiments to field measurements were used to determine the accuracy of site specific predictions. Site specific predictions of copper accumulation and responses of benthic invertebrates following treatments can be used to guide decisions regarding risk mitigation of algae and aquatic weeds with copper-based algaecides and herbicides.

DEDICATION

I would like to dedicate this thesis to my family and friends for their encouragement. I am fortunate for the support and guidance my family has committed to me throughout my education, and the continuous enthusiasm of my friends has made my college experience a wonderful adventure.

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CHAPTER ONE

INTRODUCTION

There are concerns regarding potential risks of copper accumulation in sediments due to algaecide and herbicide applications (Huggett *et al*. 1999; Gallagher *et al*. 2005; Jones et al. 2008). Copper-based algaecides and herbicides are widely used to restore water resource uses impeded by problematic algae and aquatic weed infestations (Sutton *et al*. 1970; Brooker and Edwards 1975; Button and Hostetter 1977; Nor 1987; Murray-Gulde *et al*. 2002), and have been used since the early 1900's (Fitzgerald and Faust 1963). Due to the lithic biogeochemical cycle of copper, the majority of this metal introduced to an aquatic system rapidly partitions to sediments (Gallagher *et al*. 2005).

Research delving into the analytical measurement and prediction of post-treatment copper concentrations in sediments would be advantageous for understanding copper exposures from algaecide and herbicide applications. In this research, a mathematical model was developed to estimate the measurement and concentration of residual copper in bottom sediments of water resources from copper-based algaecide and herbicide applications. The model variables were dynamic to adjust to site characteristics, mass of copper applied as an algaecide or herbicide, and improvements in analytical methods. Assumptions of this model were: copper applied as an algaecide or herbicide completely partitions to sediments in the area treated, and sediment accretion is insignificant during the time period of interest.

The bioavailability of copper in sediments is influenced by acid volatile sulfides (Allen et al. 1993), pH (Burton 1991), organic matter content and type (Besser et al.

2003; Milani et al. 2003), cation exchange capacity (Chapman et al. 1998), and particle size distribution (Hoss et al. 1997). The method detection limit (MDL; lowest concentration of an analyte that can be measured with 99% confidence with a specific analytical method and matrix; Creed et al. 1994; APHA 2005) of copper in sediments can differ by the analytical method, sediment, and "background" sediment copper concentration. Sediment characteristics that can influence the bioavailability and MDL of copper in sediment can differ from site to site (Suedel and Rodgers 1991). Sediment characteristics that influence the bioavailability and MDL could be similar and correlated. Therefore MDLs of copper in sediments could be potentially used to predict the bioavailability of copper in sediments, based on the hypothesis as MDLs of copper in sediment increases, copper bioavailability in sediments decreases.

Laboratory experiments and modeling could be used to predict the exposure and responses of organisms to residual copper in sediments from algaecide applications. Comparison of results from laboratory experiments and modeling to field measurements can be used to evaluate and refine (if needed) laboratory experiments and modeling to predict sediment copper concentrations and responses of benthic invertebrates following algaecide applications. Field measurements of accumulated copper in sediments and subsequent benthic invertebrate responses following a treatment can derive essential data for risk characterization of residual copper in sediments from algaecide applications.

To advance the understanding of the fate and effects of residual copper in sediments from copper-based algaecide and herbicide applications the following studies were conducted. The first study was to develop a model to estimate the sediment residual copper concentrations from algaecide and herbicide applications. This research provides data for understanding the potential sediment copper concentrations from algaecide and herbicide applications to guide laboratory and field experiments. The next study compares the bioavailability and MDL of copper in five sediments with divergent characteristics. This study is useful for understanding the relationship (or lack thereof) with analytical measurements and bioavailability of copper in sediments. The final study compares results of laboratory experiments and modeling to field measurements of post-treatment sediment copper concentrations and responses of benthic invertebrates following an algaecide application. Data derived from this investigation can be used to evaluate and refine (if needed) laboratory experiments and modeling to predict post-treatment sediment copper concentrations and responses of benthic invertebrates following algaecide applications to water resources.

Chapter Two Objectives

The purpose of this research was to develop a mathematical model for detectable additions of copper to bottom sediments, and compare those values with copper loadings from algaecide and herbicide applications. The objectives were to: 1) obtain data regarding the masses of copper applied from algaecide and herbicide applications based on label recommendations, 2) model copper residual accumulations in sediments from applications, 3) obtain “reported” detection limits for copper in sediments from peer reviewed literature, 4) measure the detection limits for copper residues in sediments for US EPA method 3050b using Inductively Coupled Plasma-Optical Emission

Spectroscopy, and 5) compare estimated sediment copper concentration increases from applications with measured detection limits of copper residues in sediments.

Chapter Three Objectives

The goal of this research was to compare sediment characteristics to the analytical and toxicological detection of a copper addition to five different sediments, and compare the difference between bioavailability and analytical detection of copper amendments to sediments. The objectives were to: 1) select and characterize five sediments with different characteristics, 2) amend and measure a range of copper concentrations in the five sediments to determine MDLs and bioavailability of copper amendments in those sediments, 3) discern relationships with sediment characteristics to MDLs and bioavailability of copper in the five sediments, and 4) compare MDLs and observed toxicity to *H. azteca* Saussure as an indicator of copper bioavailability in the five sediments.

Chapter Four Objectives

This research investigates the capability of a mathematical model and laboratory experiments to predict the number of algaecide applications required to detect an addition of copper to sediment at a pond. The objectives of this research were to: 1) determine the copper-based algaecide treatment for the pond necessary to control a targeted alga, 2) predict the post-treatment sediment copper concentration for a specific algaecide treatment in the pond based on laboratory experiments and a material balance model, 3) predict responses of benthic invertebrates to post-treatment copper in sediments from the algaecide treatment in the pond based on laboratory experiments, 4) repeat algaecide treatments until benthic invertebrates respond or an increase in sediment copper concentration is measured in the pond, 5) compare the predicted and measured post-treatment sediment copper concentrations in the pond, and 6) compare the predicted and measured responses of benthic invertebrates to post-treatment sediment copper concentrations in the pond.

Organization of Thesis

This thesis is arranged in subsequent chapters intended for publication in peer-reviewed journals. Therefore, chapters two through four are written and formatted for a specific journal, and some of the introductory information and materials and methods were repeated. Chapter two has been submitted for peer-review in the *Journal of Aquatic Plant Management*; chapter three has been submitted for peer-review in the journal *Water, Air, and Soil Pollution*; and chapter four is targeted for submission to the journal *Ecotoxicology and Environmental Safety*.

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CHAPTER TWO

PREDICTING AND MEASURING COPPER RESIDUES IN SEDIMENTS FROM ALGAECIDE AND HERBICIDE APPLICATIONS

Abstract

To make risk-based decisions regarding accumulation of copper residuals in sediments from algaecide and herbicide applications, water resource managers require information on concentrations of copper in sediments. The focus of this research was to develop a mathematical model to estimate concentrations of copper residuals in sediments from algaecide and herbicide applications and compare the estimated concentrations to analytical detection limits of copper in sediments. The conservative assumptions in the model were: 1) all of the copper from algaecide or herbicide applications partitions to sediments in the area treated, and 2) sediment accretion is insignificant during the time period of this analysis. Recommendations on labels and reported values of bioturbation depth and sediment dry bulk density of bottom sediments were used to estimate residual copper concentrations in sediments following algaecide and herbicide applications. A literature search was used to determine reported analytical detection limits, and empirical data were obtained to measure analytical detection limits. The model indicated applications of less than 0.5 mg Cu L^{-1} would not result in detectable copper residuals in sediments, unless the site has a relatively shallow bioturbation depth (less than 9.7 cm) and a relatively low sediment dry bulk density (less than 1 g cm^{-3}). Applications greater than 0.5 mg Cu L^{-1} could result in measurable copper

residuals in sediments depending on the analytical method used and the sediment accretion rate.

Key Words Risk, Accumulation, Concentration, Detection

Introduction

Investigations of copper residues in sediments from aquatic algaecide or herbicide applications are limited (Hullebusch *et al*. 2003; Liu *et al*. 2006). To understand and predict responses of non-target organisms to intermittent applications of copper-based algaecides or herbicides, accurate measurements of copper concentrations in sediments are needed. Copper from algaecide and herbicide applications has a propensity to bind and precipitate in sediments (Huggett *et al*. 1999; Gallagher *et al*. 2005).The subsequent bioavailability of accumulated copper in sediments from repeated applications is a concern for non-target species (Jones *et al*. 2008). Risk characterizations of effects on non-target species from accumulated copper in sediments require at least two accurate measurements: 1) copper accretion in sediments, and 2) bioavailability and toxicity of copper in sediments. This research focuses on modeling copper residues in sediments from applications of copper-based algaecides and herbicides to estimate the number of applications required to produce measurable residuals.

Measurements of residual copper in sediments after an algaecide or herbicide application depend on: 1) the analytical instrument and method used, 2) the mass of copper applied, 3) the matrix (i.e. sediment), and 4) the "background" copper concentration in the sediment. For measuring sediment copper concentrations, several analytical instruments with different detection limits are used. Depending on the instrument used [e.g. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Energy Dispersive X-ray Fluorescence (ED-XRF), or Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)], sediment samples can be prepared for analysis by digestion or directly analyzed. Sediments are digested for analytical techniques requiring a liquid sample, such as Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) or Flame Atomic Absorption Spectroscopy (FAAS). Sediment copper concentrations can also be measured directly (i.e. no digestion step) using three methods: 1) GFAAS using slurry sample analysis, 2) ED-XRF, and 3) Neutron Activation Analysis (Miller-Ihli 1993; Witkowska *et al*. 2005; Obhodas and Volkovic 2010). Detection limits for copper in sediments differ for the analytical approaches used (i.e. sample preparation and instrument analysis), as well as for the sediment matrix. The detection limit for a specific analytical approach and sediment matrix can be quantified as the method detection limit (MDL). An MDL is the minimum concentration of an analyte of interest (i.e. copper) that can be measured with 99 percent confidence in a specific matrix using an analytical method (i.e. sample preparation and analysis; Kimbrough and Wakakuwa 1993; Creed *et al*. 1994; APHA 2005).

Copper residues in sediments can be measured if the applied mass of copper is sufficient to increase sediment copper concentrations to a level greater than the MDL. However, if the background concentration of copper in a sediment sample is significantly greater than the mass of applied copper, then the likelihood of detecting residual copper in sediment decreases. Also, direct analysis methods and analytical methods that require digestion prior to sediment copper analysis can differ in percent recoveries (i.e. measured recovery of an analyte amended to a sample; Equation 1; Hoenig *et al*. 1989; Kimbrough and Wakakuwa 1989; Pai *et al*. 1993; Prichard *et al*. 2007) due to the sediment matrix. Consequently, the MDLs can also be influenced by the sediment matrix.

$$\%R = \frac{Csp\text{-}Cb}{Cs} \times 100 \qquad (1)$$

Where: %R = Percent Recovery

Csp = Mean value observed from repeated measurements of amended sample

Cb = Mean value observed from repeated measurements of unamended sample

Cs = Calculated increase in concentration from amendment

Copper-based algaecide or herbicide applications for control of nuisance algae or aquatic plants are currently governed in the United States by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and by the Clean Water Act. Recent judicial decisions have resulted in a requirement for a National Pollutant Discharge Elimination System permit for algaecide and herbicide applications to water resources (US EPA 2011). The United States Code of Federal Regulations states in Title 7 that pesticide applications must follow label recommendations (40 C.F.R. §136j 2010). These label recommendations specify the volume of water treated and the copper concentration of the algaecide or herbicide applied to a water resource. Applications of algaecides or herbicides can occur as frequently as every two days (e.g. aquaculture) or as infrequently as once a year depending on the specific problems and the desired results (Gallagher *et al*. 2005; Liu *et al*. 2006). Based on label recommendations, the mass of copper applied as algaecides and herbicides can be calculated, and site characteristics (i.e. surface area, bioturbation depth, and sediment dry bulk density) can be used to estimate the mass of sediment associated with the applied copper.

A simple model based on mass balance principles would be useful for predicting whether an application of copper-based algaecides or herbicides results in measurable copper residuals in a sediment. Further, this model could be used to derive the number of applications required to achieve a measurable copper residual in sediment for site-specific situations. This model can be developed with primary components including: 1) the mass of copper from an application or series of applications, 2) the mass of sediment sorbing the applied copper, and 3) the detection limits for analytical methods measuring sediment copper concentrations.

The purpose of this research was to develop a mathematical model that could be widely applied for determining if copper residues from algaecide and herbicide applications can be measured in sediments. The objectives were to: 1) obtain data regarding the masses of copper applied from algaecide and herbicide applications based on label recommendations, 2) model copper residual accumulations in sediments from applications, 3) obtain "reported" detection limits for copper in sediments from peer reviewed literature, 4) measure the detection limits for copper residues in sediments for US EPA method 3050b using Inductively Coupled Plasma-Optical Emission Spectroscopy, and 5) compare estimated sediment copper concentration increases from applications with measured detection limits of copper residues in sediments.

Materials and Methods

To estimate the masses of copper applied from liquid algaecide and herbicide applications, the range of copper concentrations applied [i.e. maximum label rate (MLR) and fractions of the MLR] and volume of water treated were used based on recommendations from copper-based herbicide and algaecide labels. These data were tabulated and used to define the range of volumes of water treated and concentrations of copper applied as algaecides and herbicides to water resources for subsequent modeling of copper accumulation in sediments. From the ranges of copper concentrations that are applied and volumes of water treated, the range of masses of copper applied to a water resource was estimated for algaecide and herbicide treatments. The mass of copper applied from a granular algaecide was also estimated from label recommendations.

A mathematical model was designed to estimate concentrations of copper residuals that accumulate in sediments from algaecide and herbicide applications, and to predict the number of algaecide or herbicide applications required to achieve measurable copper residuals in sediments. The conservative assumptions of this model were: 1) all of the applied copper from algaecide or herbicide applications partitions to sediments in the area treated, and 2) sediment accretion is insignificant during the time period of this analysis. This model was developed by comparing detection limits with the mass of copper applied from an application or series of applications of algaecides or herbicides divided by the mass of sediment associated with the applied copper (Eq. 2).

$$\mathrm{MDL}\ (\mathrm{mg\ Cu\ kg^{-1}}) \leq \frac{\mathrm{C}\ (\mathrm{mg\ Cu\ L^{-1}}) \times \mathrm{V}\ (\mathrm{L}) \times \mathrm{N} \times 1000\ (\mathrm{g\ kg^{-1}})}{\mathrm{S}\ (\mathrm{g/cm^{3}}) \times \mathrm{A}\ (\mathrm{cm^{2}}) \times \mathrm{Bio}\ (\mathrm{cm})} = \frac{\mathrm{MA}\ (\mathrm{mg})}{\mathrm{MS}\ (\mathrm{kg})} \qquad (2)$$

Where: MDL = Analytical detection limit for copper in sediment

C = Copper concentration applied

V = Volume of water treated

N = Number of applications

S = Sediment dry bulk density

A = Surface area treated

Bio = Bioturbation depth

MA = Mass of applied copper

MS = Mass of sediment

This model considers bioturbation depth and sediment dry bulk density as site specific parameters affecting the concentration of accumulated copper residuals in sediments. To account for influences of sediment dry bulk density on the measurement of copper residuals in sediments, a literature search for sediment dry bulk densities was performed to define the range expected in water resources to serve as an input in the model. To discern the influence of bioturbation depth on measurement of copper residuals from algaecide and herbicide applications, a range of bioturbation depths expected to be encountered in water resources was also input in the model. To model applications of chelated granular algaecide, the mass of copper applied is divided by the mass of sediment in the application area.

Initially, a literature search for MDLs for analytical methods for measuring copper in sediments was conducted to determine reported analytical limits. These data were sorted and tabulated by MDLs. Also, detection limits for copper residues in sediments were empirically measured using US EPA method 3050b with ICP-OES[1] (1995). To assess the potential range of detection limits for copper in sediments, five sediments were collected with divergent characteristics (e.g. sediment dry bulk density, particle size distribution, and background copper concentration) from different physiographic provinces of the contiguous United States (Table 1). The five sediments were collected from the Mississippi River in Mississippi, an aquaculture pond in South Carolina, an irrigation pond in Colorado, Lake John Hay in Indiana, and an urban pond in California.

Detection limits for copper in sediments were empirically derived through two bench-scale experiments involving a series of increasing copper sulfate pentahydrate[2] ($CuSO_4 \cdot 5H_2O$) amendments. An initial range finding experiment targeted sediment copper amendments of untreated control, 0.5, 1, 2, 4, and 8 mg Cu/kg. Based on the results from the range finding experiment, five treatments were defined for definitive experiments to determine the detection limits for copper residues in the sediments (Table 2). The experiments were conducted in 125 mL flasks containing wet sediment (moisture content was from extant pore water in the sediment sample) and the mass of sediment in each flask was 30 g dry sediment as estimated by percent solids. Sediment copper amendments were achieved by addition of one mL of stock solution with the appropriate mass of copper as copper sulfate pentahydrate to increase sediment copper concentrations. Stock solutions were made by diluting a 1,000 mg Cu L^{-1} solution in NANOpure water[3] (18Ω) to achieve the targeted mass of copper in one mL of solution.

The acid soluble copper concentrations of each stock solution were measured with ICP-OES by acidifying samples to pH < 2 with trace metal grade nitric acid[4] and filtering with a 0.45 µm filter (APHA 2005). Based on measured aqueous residence times of copper algaecides ranging from 2.6-5.7 days (Murray-Gulde *et al*. 2002), sediments amended with copper were allowed a contact period of two weeks before analysis of sediment copper concentrations. Three replicate measurements of sediment copper concentrations were obtained for each amendment. The analytical detection limits of accumulated copper residuals in sediments were defined as the lowest treatment with a measurable increase greater than the untreated control and were calculated using ANOVA and Dunnett's multiple range tests ($\alpha = 0.01$).

Results and Discussion

Algaecide and herbicide labels are primary sources of information for estimating masses of copper applied in treatments. Recommendations on algaecide and herbicide labels specify concentrations of copper for treatments at specific sites based on factors such as the anticipated sensitivity of targeted genera, degree of infestation, and water hardness, but applications must not exceed the MLR (i.e. 1 and 2 mg Cu L^{-1} for chelated copper formulations and copper sulfate, respectively; Table 3). The lowest recommended application concentrations contained on the labels of chelated copper formulations and copper sulfate are 0.06 and 0.25 mg Cu L^{-1}, respectively (Table 3). Algaecide and herbicide labels offer two approaches for refining the appropriate concentration range for a site: 1) taxonomic (genus-based), or 2) growth habit-based [e.g. filamentous (mat forming), planktonic (suspended), submerged plants, and floating plants]. Typically, water hardness and the degree of algal or vascular plant infestation are used to refine the concentration of copper applied at a site based on the range specified for a targeted genus or growth-habit. For instance, an application of the upper range of copper concentrations is recommended on the labels for a targeted genus in hard water (defined as greater than 50 mg L^{-1} as $CaCO_3$) and the lower portion of the range for a targeted genus growing in soft water (defined as less than 50 mg L^{-1} as $CaCO_3$). Recommendations on labels also suggest increased copper concentrations as the degree of infestation increases. These recommendations are summarized in a short narrative on the label (e.g. infestation designations: slight, moderate, heavy, severe, or similar adjectives). Clearly, the amount of algaecide or herbicide applied at a site is somewhat subject to the discretion of the applicator or water resource manager. Regardless, recommendations on algaecide and herbicide labels confine the concentrations of copper applied in a treatment from 0.06 to 1 mg Cu L^{-1} for chelated copper formulations [liquid and one granular herbicide (Harpoon®)] and 0.25 to 2 mg Cu L^{-1} for copper sulfate (Table 3). The recommendation on the chelated granular algaecide label confines the mass of copper applied from an application as 668 g (2.2 Lbs) copper per 0.4 hectares (1 acre).

The volume of water treated in a water resource is the average depth of infestation multiplied by surface area according to recommendations on copper-based herbicide and algaecide labels. More specifically limiting the volume treated in a water resource, two algaecide labels (i.e. K-Tea* and Symmetry™) recommend not to apply algaecide to a depth greater than 1.82 m (6 ft) when the water body is stratified (Table 3). Since most applications are expected to occur during summer months while water resources are stratified, those applications would treat up to 0.74 hectare-meters (6 acre-ft) of water in a water resource of 0.4 hectares (1 acre). By delimiting the applied copper concentration (0.06 to 2 mg L^{-1}) and volume of water treated [0.12 to 0.74 hectare-meters (1 to 6 acre-ft)], based on recommendations from labels, the expected mass of copper per application to 0.4 hectares (1 acre) of a water resource ranges from 74 to 14,800 g (0.16 to 32.6 lbs). Following an application of algaecide or herbicide, the copper residues partition relatively rapidly (~ 2 weeks or less; Haughey *et al*. 2000; Murray-Gulde 2002) from the water column to the sediment (Gallagher *et al*. 2005).

To estimate residual copper concentrations in sediments from algaecide and herbicide applications, the mass of sediment associated with the applied copper must be determined and can be estimated from the sediment dry bulk density, surface area, and bioturbation depth (i.e. the well mixed layer; US EPA 2000; Equation 2). The ranges of sediment dry bulk densities and bioturbation depths expected for bottom sediments in water resources in the US are 0.2 to 1.8 g cm^{-3} (Avnimelech *et al*. 2001) and 3 to 20 cm (Boudreau 1998), respectively. Delimiting sediment dry bulk densities (0.2 to 1.8 g cm^{-3}) and bioturbation depths (3 to 20 cm) while maintaining the surface area constant [0.4 hectares (1 acre)], the estimated mass of sediment associated with the applied copper from a treatment can range from 24,282 to 1,456,868 kg (53, 532 to 3,211,844 lbs).

Using the parameters defined above, the range of residual copper concentrations in sediments from applications of copper sulfate and chelated copper formulations can be estimated. The estimated lowest copper residual concentration in sediment from a copper sulfate application was 0.21 mg Cu kg^{-1} [model inputs were 0.25 mg Cu L^{-1}, 0.12 hectare-meters (1 acre-ft), 1 application, 0.4 hectares (1 surface acre), 1.8 g cm^{-3} dry bulk density, and 20 cm bioturbation depth]. The estimated highest copper residual concentration in sediment from a copper sulfate application was 610 mg Cu kg^{-1} [model inputs were 2 mg Cu/L, 0.74 hectare-meter (6 acre-ft), 1 application, 0.4 hectares (1 surface acre), 0.2 g cm^{-3} dry bulk density, and 3 cm bioturbation depth]. Thus, the estimated range of copper residues in sediment from copper sulfate applications was 0.21 to 610 mg Cu kg^{-1} (Figure 1). The estimated lowest residual copper concentration in sediment from an application of chelated copper formulations [liquid and one granular herbicide (Harpoon®)] of algaecides and herbicides was 0.05 mg Cu kg^{-1} [model inputs were 0.06 mg Cu L^{-1}, 0.12 hectare-meters (1 acre-ft), 1 application, 0.4 hectares (1 surface acre), 1.8 g cm^{-3} dry bulk density, and 20 cm bioturbation depth]. The highest estimated residual copper concentration in sediment from an application of chelated copper formulations [liquid and one granular herbicide (Harpoon®)] was 305 mg Cu kg^{-1} [model inputs were 1 mg Cu L^{-1}, 0.74 hectare-meter (6 acre-ft), 1 application, 0.4 hectares (1 surface acre), 0.2 g cm^{-3} dry bulk density, and 3 cm bioturbation depth]. The estimated range of residual copper concentrations in sediments from applications of chelated copper algaecide and herbicide formulations [liquid and one granular herbicide (Harpoon®)] was 0.05 to 305 mg Cu kg^{-1} (Figure 2). The estimated range of residual copper concentrations in sediments from applications of the chelated granular algaecide (Cutrine®-Plus Granular) was 0.45 to 28 mg Cu/kg. The range of estimated sediment residual copper concentrations from Cutrine®-Plus Granular applications was the lower 10 percent and 5 percent of the estimated range of residual copper concentrations from other chelated and copper sulfate algaecides and herbicide applications, respectively. To predict potential detection of post-treatment copper residues in sediment, detection limits were obtained from peer reviewed literature (Table 4).

For this study, the analytical instruments of interest for measuring copper residuals in sediments were ICP-MS, GFAAS, FAAS, ICP-OES, and ED-XRF. Based on reported MDLs, detection limits for each instrument from lowest to highest were GFAAS (0.032 to 1.6 mg Cu kg^{-1}), ICP-MS (0.04 to 2.5 mg Cukg^{-1}), FAAS (0.1 to 2.0 mg Cu kg^{-1}), ICP-OES (0.7 to 1 mg Cu kg^{-1}), and ED-XRF (3 to 13 mg Cu kg^{-1}; Table 4). The range of reported MDLs for analytical techniques investigated in this study was from 0.032 mg Cu kg^{-1} (GFAAS; Acar 2006) to 13 mg Cu kg^{-1} (Energy Dispersive X-ray Fluorescence; Enzweiler and Vendemiatto 2004). Since the MDL of copper in sediments can be affected by the sediment matrix as well as the analytical technique, the detection limit for copper residues in sediments using ICP-OES (US EPA method 3050b) was experimentally determined using five different sediments (Table 1). Further, measured MDLs could be compared with the reported MDL for the method of choice (i.e. ICP-OES).

Measured detection limits for copper residues in sediments using ICP-OES (US EPA method 3050b) ranged from 1.5 to 6 mg Cu kg^{-1} (Table 2). The experimentally derived detection limits were greater than the reported MDLs for ICP-OES used for measuring copper in sediments (Table 4). This difference is attributed to the sediment matrices and methods used to estimate the detection limits. For example, two of the reported MDLs for copper in sediment using ICP-OES were derived from river sediment

(Bettinelli et al. 2000a; Yang and Low 2009). The river sediment (i.e. Mississippi River) in the present study had the lowest measured detection limit (1.5 mg Cu kg^{-1}; Table 2). The reported MDLs for ICP-OES were estimated based on the standard deviation of replicate measurements (Bettellia 2000b; Lewis *et al*. 2000). For the present study, an empirical method was used to determine the detection limits of copper accumulation in sediment samples. Based on the comparison between reported MDLs (i.e. 0.7 to 1.0 mg Cu kg^{-1}) and empirically measured MDLs (1.5-6 mg Cu kg^{-1}) for ICP-OES, the reported detection limits can provide guidance within one order of magnitude regarding the detection limits of copper residues in sediments. However, an experimentally derived MDL is more accurate for a specific analytical method and matrix.

Considering the empirically measured detection limits obtained from this study, copper residues from a relatively low mass of applied copper (based on label recommendations) would not be measured in sediment. For example, an application of 0.25 mg Cu L^{-1} to a volume of 0.12 hectare-meters (1 acre-ft) in a water resource that has a surface area of 0.4 hectares (1 acre), a bioturbation depth of 20 cm, and a sediment dry bulk density of 1.8 g cm^{-3} would have an estimated sediment copper residue of 0.21 mg Cu kg^{-1}. The number of applications required to achieve analytical detection of copper residuals in sediment from the scenario above, based on the measured detection limits, would be 8 to 32 applications assuming there was no sediment accretion (Figure 3). However, if the sediment accretion was 1 cm year^{-1} (the sediment accretion rate for reservoirs ranges from less than 1 to 22 cm year^{-1}; Mulholland and Elwood 1982) and assuming four applications of herbicide or algaecide each year, 12 to 40 applications (or

3 to 10 years of applications) would be required before copper residues could be detected in the sediment. For situations where the ICP-OES (US EPA method 3050b) may not detect copper residues in sediments from algaecide and herbicide applications, other methods that have a greater reported sensitivity (e.g. GFAAS or ICP-MS) could be applicable to measure copper residues.

Since the differences of the lowest reported MDLs for the two most sensitive analytical methods in this study (GFAAS 0.032 Cu kg^{-1} and ICP-MS 0.04 mg Cu kg^{-1}) and the lowest estimated sediment residual copper concentration (0.05 mg Cu/kg) were minimal (GFAAS 0.018 mg Cu kg^{-1} and ICP-MS 0.01 mg Cu kg^{-1}), analytical measurement of copper residuals in sediments may not be feasible for every algaecide and herbicide application. The model developed in this study can aid in determining the practicality and the appropriate analytical methods for measuring copper residuals in sediments for specific herbicide or algaecide applications. For instance, the copper sulfate application reported by Button and Hostetter (1977) at Hoover Reservoir in OH, U.S.A. to control algae would have produced an estimated sediment residual copper concentration of 1.36 mg Cu kg^{-1} [model inputs: applied copper concentration = 0.66 mg Cu L^{-1}, bioturbation depth = 9.7 cm (average bioturbation depth; Boudreau 1998), and sediment dry bulk density = 1 g cm^{-3} (mid range value of sediment dry bulk density; Avnimelech *et al*. 2001)]. Therefore, based on reported detection limits and the estimated sediment residual copper concentration from the Hoover Reservoir copper sulfate application (1.36 mg Cu kg^{-1}), GFAAS, ICP-MS, FAAS, and ICP-OES could be viable methods for measuring the copper residues in the sediment. If more definitive information is required, experiments with the reservoir sediment could confirm the analytical methods that have the sensitivity to measure the sediment copper residues.

Analytical detection may be possible for applications of algaecides or herbicides using more than half of the MLR recommended on labels. For example, an application of 0.7 mg Cu L^{-1} to a volume of 0.36 hectare-meters (3 acre-ft) in a water resource with a surface area of 0.4 hectare (1 acre), a bioturbation depth of 9.7 cm (average bioturbation depth), and a sediment dry bulk density of 1 g cm^{-3} (mid range value of sediment dry bulk density; Avnimelech *et al*. 2001) would have an estimated sediment copper residue of 6.6 mg Cu kg^{-1}. However, analytical detection of copper residues in sediment could be impractical for applications of algaecide or herbicide of less than half of the concentration of copper recommended on labels for 0.4 hectare (1 acre). For example, an application of 0.3 mg Cu L^{-1} to a volume of 0.12 hectare-meters (1 acre-ft) in a water resource with a surface area of 0.4 hectare (1 acre), a bioturbation depth of 9.7 cm (average bioturbation depth), and a sediment dry bulk density of 1 g cm^{-3} (mid-range value of sediment dry bulk density) would produce an estimated sediment copper residue of 0.94 mg Cu/kg. Similarly, the analytical detection of copper residues in sediment may not be possible from an application of half of the recommended concentration of granular copper algaecide. For example, an application of 334 g Cu (1.1 Lbs. Cu) in a water resource with a surface area of 0.4 hectare (1 acre), a bioturbation depth of 9.7 cm (average bioturbation depth), and a sediment dry bulk density of 1 g cm^{-3} (mid range value of sediment dry bulk density) would produce an estimated sediment copper residue of 0.85 mg Cu/kg.

The mass of copper from a single application or a series of applications and the mass of sediment associated with the applied copper can differ from site to site, and can influence the copper residual concentrations in sediments. The model described in this study can assist in discerning an analytical method that could be used to measure copper residues in sediments from algaecide and herbicide applications, or determining the likelihood that copper residues can be measured in sediment from specific site applications. Based on the conservative assumptions in the model (Equation 2), copper residues from applications of more than half the recommended concentration of copper per 0.4 hectares (1 acre) could be detected in sediment depending on the analytical method selected and the sediment accretion rate. Applications of less than half of the concentration of copper recommended on labels per 0.4 hectares (1 acre) will likely not result in detectable copper residuals in sediment, unless the site has a relatively shallow bioturbation depth (less than 9.7 cm) and a low sediment dry bulk density (less than 1 g cm^{-3}).

Sources of Materials

[1] Inductively Coupled Plasma Optical Emission Spectroscopy, Perkin-Elmer Optima 3100RL, Perkin-Elmer Inc., Waltham, MA, USA

[2] Copper sulfate pentahydrate, Acros® Organics, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA

[3] NANOpure water, Barnstead, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA

[4] Trace metal grade nitric acid, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA

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Table 2.1 Characteristics of sediments used to experimentally determine the analytical detection limits for copper amendments. N.D. represents non-detect. %OM, CEC, AVS, and SEM represent percent organic matter, cation exchange capacity, acid volatile sulfides, and simultaneously extracted metals, respectively.

Physical/chemical characteristics	**Mississippi River**	**Aquaculture Pond**	**Lake John Hay**	**Irrigation Pond**	**Urban Pond**
Background Cu concentration (mg Cu kg^{-1})	N.D.	10	12	18	150
AVS (umol g^{-1}) ± standard deviation	N.D.	14.8 ± 1.5	7.4 ± 1.9	27.8 ± 4.1	3.315 ± 0.24
SEM (umol g^{-1}) ± standard deviation	0.0083 ± 0.0001	0.066 ± 0.011	0.068 ± 0.019	0.9 ±0.13	10.24 ± 1.91
CEC (meq 100 g^{-1}) ± standard deviation	5.71 ± 3.46	16.93 ± 1.92	18.19 ± 0.044	53.79 ± 9.73	25.18 ± 6.37
pH	7.15	6.88	6.7	7.45	7.80
Percent solids (%)	81	45	76	71	80
Sediment dry bulk density (g cm^{-3})	1.60	0.631	1.51	1.18	1.62
% Sand	98	26	66	5	86
% Silt	2	41	27	55	11
% Clay	N.D.	33	7	34	3
Surface area (square cm/g)	53	2628023	542788	2720253	230734

Table 2.2 Analytical detection limits for copper amendments in five sediments using ICP-OES (US EPA method 3050b).

Sediment Source	**Sediment Copper Amendments**	**Detection Limit for Copper Amendments**
Mississippi River	1, 1.5, 2, 2.5, and 3 mg Cu/kg	1.5 mg Cu/kg
Aquaculture pond	3, 4, 5, 6, and 7 mg Cu/kg	4 mg Cu/kg
Lake John Hay	4, 5, 6, 7, and 8 mg Cu/kg	6 mg Cu/kg
Irrigation pond	3, 4, 5, 6, and 7 mg Cu/kg	4 mg Cu/kg
Urban pond	5, 6, 7, 8, and 9 mg Cu/kg	6 mg Cu/kg

Table 2.3 Recommendations from copper-based algaecide and herbicide labels for copper concentrations and the volumes of water to treat. N/A represents no reported description of volume treated for an application.

Algaecide and Herbicide Formulation	Algaecide/ Herbicide Designation	Minimum - Maximum Recommended Copper Concentrations (mg Cu L^{-1})	Volume Treated Recommendations	Information Source
AB Brand Copper Sulfate Crystals	Algaecide	0.25-2.0	N/A	Applied Biochemists 2009a
Algimycin®-PWF	Algaecide Cyanobacteriocide	0.06-1.0	Treat to the average depth of algal growth	Applied Biochemists 2010a
Captain*	Algaecide	0.2-0.8	Treat to a depth of 3-4 ft for suspended and free floating filamentous mats of algae	SePRO 2006a
Clearigate®	Algaecide Herbicide	0.1-1.0	Treat to a depth of 1-4 ft for surface applications	Applied Biochemists 2010b
Copper Sulfate Fine Crystals	Algaecide	0.25-2.0	N/A	Old Bridge Chemicals Inc. n.d.
Cutrine®- Plus	Algaecide	0.2-1.0	Treat to the average depth of algal growth	Applied Biochemists 2009b
Cutrine®- Plus granular	Algaecide	668*	N/A	Applied Biochemists n.d.
Cutrine® -Ultra	Algaecide Herbicide Cyanobacteriocide	0.5-1.0	Treat to the average depth of algal growth	Applied Biochemists 2002

Table 2.3 Continued

Algaecide and Herbicide Formulation	Algaecide/ Herbicide Designation	Minimum - Maximum Recommended Copper Concentrations (mg Cu L^{-1})	Volume Treated Recommendations	Information Source
Formula F-30®	Algaecide	0.2-1.0	Treat to the average depth of infestation	Diversified Waterscapes n.d.
Komeen*	Herbicide	0.5-1.0	N/A	SePRO 2005a
Harpoon® Granular	Herbicide	0.5-1.0	N/A	Applied Biochemists 2011
K-Tea*	Algaecide	0.2-1.0	Apply 1-6 ft in stratified water bodies and the entire depth in unstratified water bodies	SePRO 2005b
Nautique*	Herbicide	0.5-1.0	N/A	SePRO 2006b
Symmetry™	Algaecide	0.2-1.0	Apply 1-6 ft in stratified water bodies and the entire depth in unstratified water bodies	Phoenix Environmental Care b

* Units are g Cu $acre^{-1}$ as specified on algaecide label

Table 2.4 Reported method detection limits (MDLs) for copper in sediments from peer-reviewed literature, and a brief summary of each method employed.

Instrument	**MDL**	**Method Description**	**Source**
Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)	1.6 mg/kg	HF, HNO_3, and HCl Microwave digestion	(Acar 2006)
	0.032-0.056 mg/kg	HNO_3, HF, and $HClO_4$ Microwave digestion	(Lima *et al*. 2001)
	0.05 mg/kg	HNO_3, HF, and $HClO_4$ Microwave digestion	(Fang and Wang 2006)
Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)	0.04 mg/kg	HNO_3, HCl, and H_2O_2 Digestion block	(Yang and Low 2009)
	~0.5-2.5 mg/kg	HF/ HNO_3/HCl Microwave digestion	(Bettinelli *et al*. 2000b)
Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)	1 mg/kg	HNO_3, HCl, and H_2O_2 Digestion block	(Yang and Low 2009)
	0.7 mg/kg	HNO3 with microwave digestion	(Lewis *et al*. 2001)
	~1 mg/kg	Two methods Aqua regia and HF/ HNO_3/HCl Microwave digestion	(Bettinelli *et al*. 2000a)
Flame Atomic Absorption Spectroscopy (FAAS)	2.0 mg/kg	HNO_3 and $HClO_4$ 80ºC water bath	(Aardt and Erdmann 2004)
	0.1 mg/kg	Hot acid mixture of HNO_3 and HCl	(Lu *et al*. 2008)
Energy Dispersive X-ray Fluorescence (ED-XRF)	3 mg/kg	Direct measurement	(Obhodas and Volkovic 2010)
	13 mg/kg	Direct measurement	(Enzweiler and Vendemiatto 2004)

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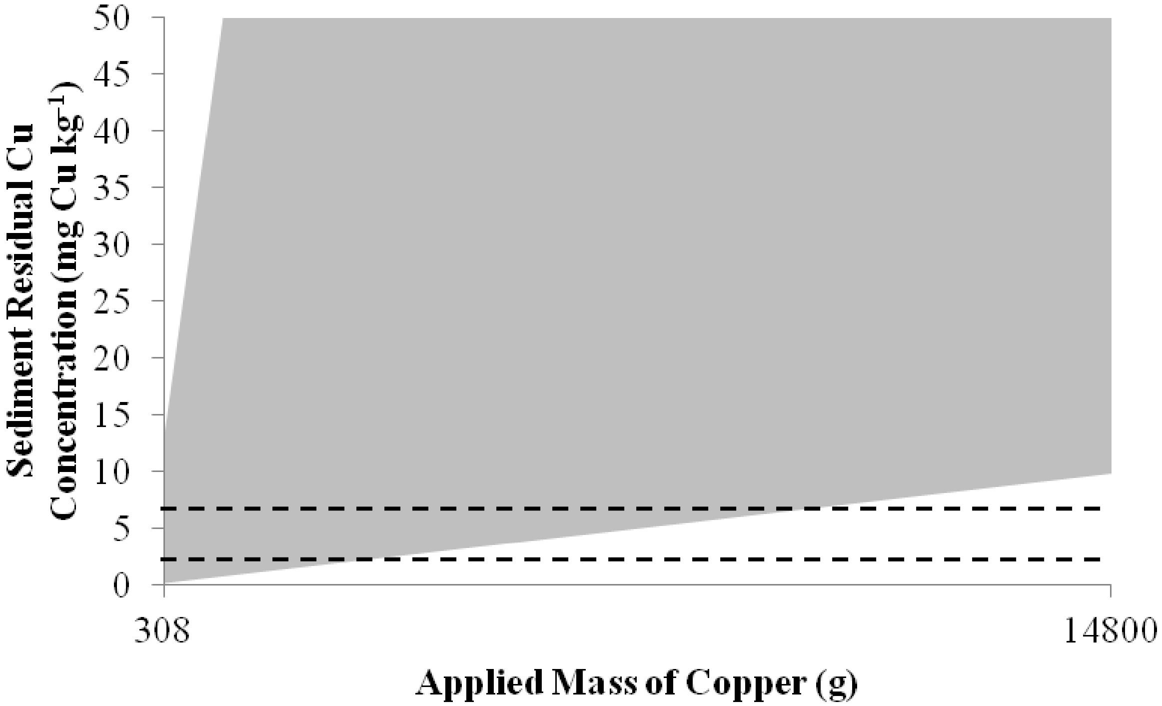
Fig. 2.1 The range (displayed in gray) of sediment residual copper concentrations for one application of copper sulfate algaecide or herbicide in 0.4 hectares (1 acre). The estimated range was based on: copper concentration applied (0.25 to 2 mg L^{-1}), volume treated [0.12 to 0.74 hectare-meters (1 to 6 acre-ft), sediment dry bulk density (0.2-1.8 g cm^{-3}), and bioturbation depth (3 to 20 cm). Dashed lines indicate the range of empirically measured MDLs for copper residues in five sediments.

Fig. 2.2 The range (displayed in gray) of sediment residual copper concentrations for one application of chelated copper algaecide or herbicide in 0.4 hectares (1 acre). The estimated range of copper residue concentrations in sediments was based on: copper concentration applied (0.06 to1 mg/L), volume treated [0.12 to 0.74 hectare-meters (1 to 6 acre-ft)], sediment dry bulk density (0.2 to 1.8 g cm^{-3}), and bioturbation depth (3 to 20cm). Dashed lines indicate the range of measured detection limits for copper residues in five sediments.

Fig. 2.3 The estimated copper residual concentration in sediments following multiple applications of copper-based algaecide or herbicide to a 0.4 hectare (1 acre) water resource. The residual sediment copper concentrations were estimated based on the model parameters of: 1 to 36 applications, 0.25 mg Cu L^{-1}, 0.12 hectare-meters (1 acre-ft), 1.8 g/cm^3, and 20 cm. Dashed lines represent the empirically measured MDLs of copper in five sediments, and dotted lines represent the number of applications required to detect copper residues in sediments using ICP-OES (US EPA method 3050b).


Sediment Residual Cu Concentration (mg Cu kg^{-1})
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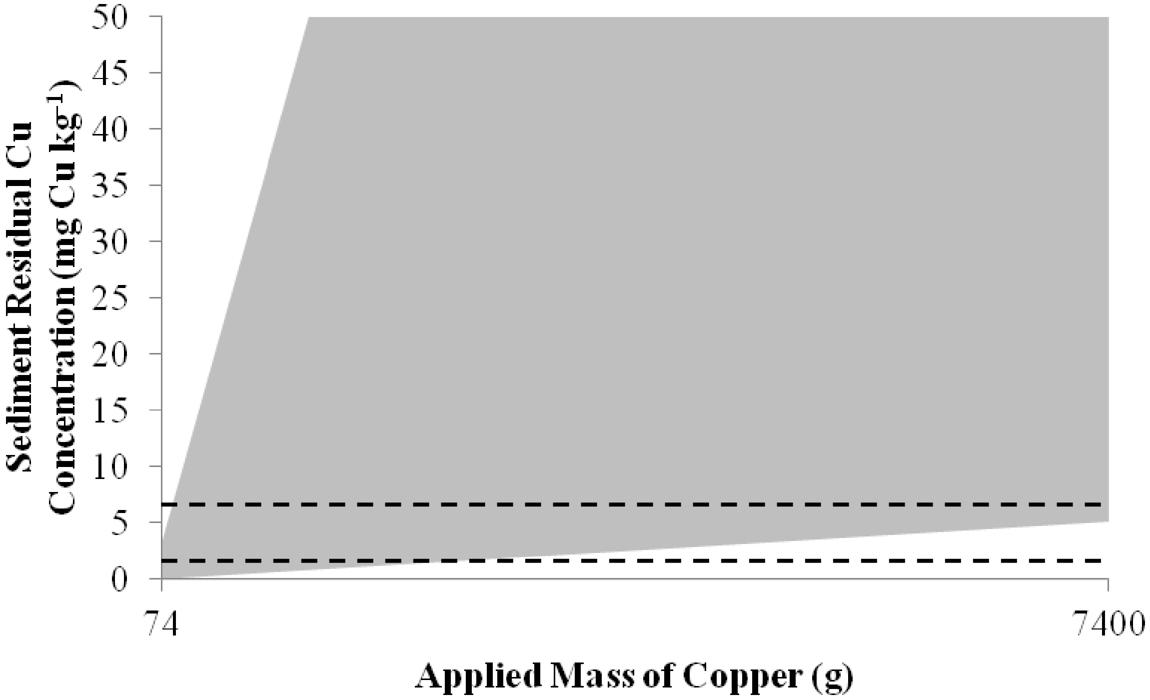
40
35
30
25
20
15
10
5
0
308
14800
Applied Mass of Copper (g)

Fig. 2.1


50
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25
20
15
10
5
0
Sediment Residual Cu Concentration (mg Cu kg^{-1})
74
7400
Applied Mass of Copper (g)

Fig. 2.2

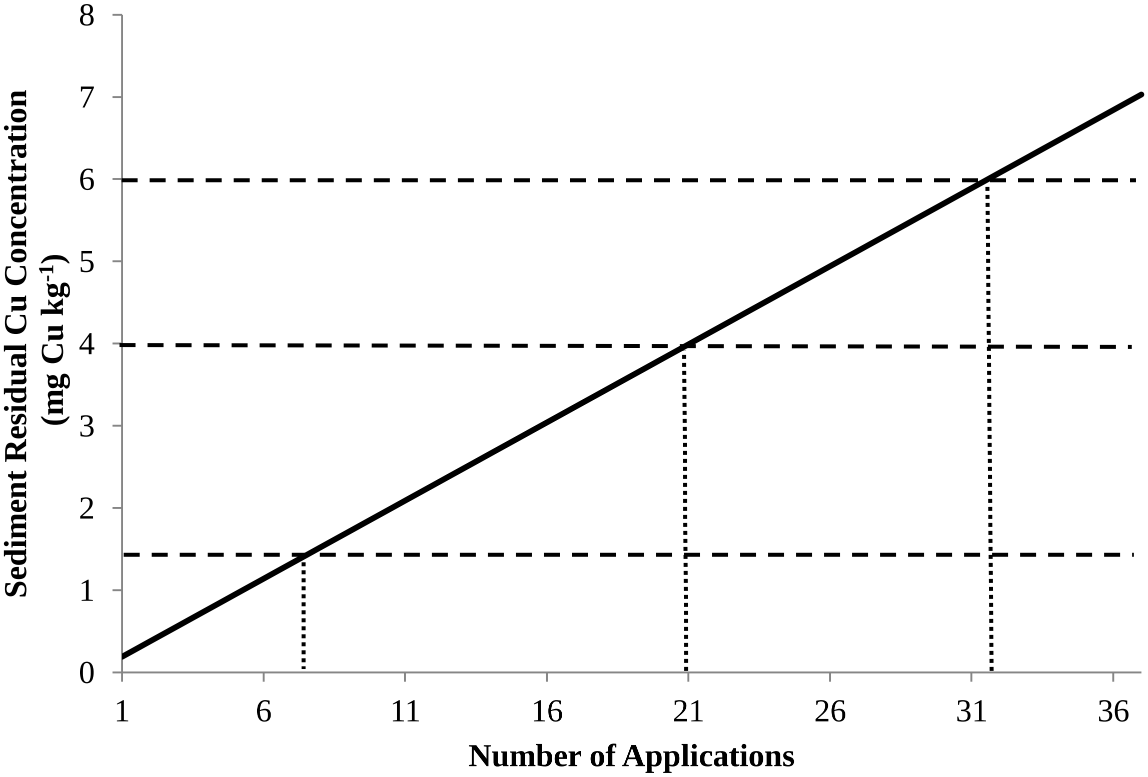
8
7
6
5
4
3
2
1
0
Sediment Residual Cu Concentration (mg Cu kg-1)
1
6
11
16
21
26
31
36
Number of Applications

Fig. 2.3

CHAPTER THREE

BIOAVIALABILITY AND ANALYTICAL MEASUREMENT OF COPPER RESIDUALS IN SEDIMENTS

Abstract

Analytical measurements are commonly used to screen for toxicity or lack of toxicity from sediment associated copper. Comparisons of analytical measurements with toxicological responses can be useful for determining the practicality of analytical measurements for assessing the toxicity of copper in sediments. The purpose of this research was to determine the utility of method detection limits (MDLs; i.e. minimum concentration of an analyte such as copper that can be measured with 99% confidence with a specific analytical method and matrix) to predict the bioavailability of copper in five different sediments. The specific objectives of this research were to 1) select and characterize five sediments with different characteristics, 2) amend and measure a range of copper concentrations in the five sediments to determine MDLs and bioavailability of copper amendments in those sediments, 3) discern relationships with sediment characteristics to MDLs and bioavailability of copper in the five sediments, and 4) compare MDLs and observed toxicity to *Hyalella azteca* Saussure as an indicator of copper bioavailability in the five sediments. The lowest copper concentrations that elicited an observable adverse effect (LOECs) ranged from 15-550 mg Cu/kg, and the MDLs ranged from 1.5-6 mg Cu/kg. The MDLs and measured copper concentrations were not adequately predictive of the bioavailability and toxicity of copper in the five sediments. Since the LOECs of copper in the five sediments ranged two orders of magnitude, the National Oceanic and Atmospheric Administration screening values for the lack of toxicity (threshold effect level) and toxicity (probable effect level) were not predictive of the lack of toxicity or toxicity of copper amended to the sediments.

Keywords Toxicity, Method Detection Limit, Accumulation, Risks

Introduction

There are numerous studies of copper in sediments, but questions still remain regarding measurement and bioavailability of copper residues that accumulate in sediments. There is concern that copper may accumulate in sediments from anthropogenic activities such as parking lot stormwater runoff, mining activities, industrial processes, as well as algaecide and herbicide applications (Teasdale et al. 2003; Gillis and Birch 2006; Jones et al. 2008). To monitor copper accumulation in sediments through time as well as the consequences of those residuals, information is needed regarding the sensitivity of analytical measurements and the bioavailability of accumulated copper in sediments.

Analytical measurements of copper residues in sediments can be accomplished if the sediment copper concentration is in excess of a method detection limit (MDL). An MDL is the minimum concentration of an analyte of interest (i.e. copper in this case) that can be measured with 99 percent confidence for a specific matrix and analytical method (Kimbrough and Wakakuwa 1993; Creed et al. 1994; APHA 2005). MDLs for copper in sediments can differ due to the sediment matrix and the analytical method used. Reported MDLs for copper in sediments can range from 0.032 to 13 mg Cu/kg depending on the analytical method and sediment matrix (Acar 2006; Enzweiler and Vendemiatto 2004).

Because sediment characteristics often range widely from site to site (Suedel and Rodgers 1991), we would expect MDLs for copper residues in sediment to concomitantly differ from site to site. There are apparently no published data that would indicate how sediment characteristics can influence MDLs for copper in sediments. Due to copper's strong affinity for organic matter and fines in sediments (i.e. clay and silt; Laing et al. 2009), one can hypothesize that organic matter content and particle size, as well as other sediment characteristics, could influence MDLs for copper in sediments. Further, if background sediment copper concentrations are substantially greater than the increase of copper concentration attributed to copper residues in effluents or pesticide applications over time, the likelihood of measuring copper residues would decrease.

The influence of sediment characteristics on the bioavailability of copper sorbed to sediment has been the subject of intense study. Differences in the bioavailability of sediment sorbed copper have been attributed to organic matter type and content (Besser et al. 2003; Milani et al. 2003), acid-volatile sulfides (Allen et al. 1993; DiToro et al. 1990), cation exchange capacity (CEC; Chapman et al. 1998), pH (Burton 1991), and particle size distribution (Hoss et al. 1997). To measure the bioavailability of sediment sorbed copper, an organism is needed that can thrive in sediments with diverse characteristics. *Hyalella azteca* Saussure has been used in sediment toxicity experiments and tolerates a wide range of organic matter contents and particle size regimes. *H. azteca* is commonly used to discern influences of sediment characteristics on the bioavailability of sediment-sorbed copper (Cairns et al. 1984; Suedel et al. 1996; Deaver et al. 1996; Gallagher et al. 2005).

Because the bioavailability and MDLs of sediment sorbed copper differ among sediments, analytical measures may be inadequate to assess exposures of copper residuals in sediments that could elicit adverse effects to benthic organisms. Comparisons of MDLs and toxicological responses [i.e. lowest observed effect concentrations (LOECs), LC50s, and potency slopes] to copper residuals in sediments could provide information regarding the adequacy of analytical methods for measuring sediment copper exposures that may elicit adverse effects to benthic fauna. Further, comparisons between the bioavailability and MDLs of copper amendments to sediments could be useful for determining if analytical measurements (e.g. MDLs) and toxicological responses are related. We hypothesized that there could be an inverse relationship between MDLs and the bioavailability of copper in sediments (i.e. lower MDLs are proportional to more bioavailable copper in sediments; higher MDLs are proportional to less bioavailable copper in sediments).

Monitoring programs to assess risks associated with copper accumulation in sediments may be restricted by analytical methods for measuring copper exposures and methods to detect adverse effects from copper residues in sediments. The purpose of this research was to measure MDLs for copper in sediments with divergent characteristics, and discern relationships for MDLs and bioavailability of copper amendments in sediments. Specific objectives of this study were to: 1) select and characterize five sediments with different characteristics, 2) amend and measure a range of copper concentrations in the five sediments to determine MDLs and bioavailability of copper amendments in those sediments, 3) discern relationships with sediment characteristics to

MDLs and bioavailability of copper in the five sediments, and 4) compare MDLs and observed toxicity to *H. azteca* Saussure as an indicator of copper bioavailability in the five sediments.

Materials and Methods

Sediment Sampling and Characterization

Five sediments with divergent characteristics were collected from the Mississippi River in Mississippi (32º 07 N, 91º 01 W), an aquaculture pond in South Carolina (34º 68 N, 82º 81 W), an irrigation pond in Colorado (39º 16 N, 104º 53 W), Lake John Hay in Salem, Indiana (38º 61 N, 86º 1 E), and an urban pond in Buena Park, California (33º 87 N, 117º 98 W). Sediment samples were collected from different physiographic provinces of the contiguous United States based on the ranges of sediment characteristics found in freshwater bottom sediments by Suedel and Rodgers (1991). Approximately twenty liters of surficial sediments were collected from each site with a polypropylene scoop acquiring the top ten cm of sediment. The sediment samples were placed in polyethylene bags pre-washed with 10% technical grade nitric acid (Thermo Fisher Scientific, Inc.). Twenty liters of corresponding surface water were also collected from each site. All samples were shipped on wet ice to a laboratory at Clemson University for analysis. Sediment handling and storage methods followed those specified by Plumb (1981). Surface water samples were stored at approximately 4ºC until analysis. Each sediment sample was placed in a separate container and gently homogenized. Three replicates of each sediment were characterized for particle size distribution (hydrometer method; Gee and Bauder 1986), particle surface area (calculated based on particle size classes; Suedel and Rodgers 1991), organic matter (loss on ignition; Nelson and Sommers 1986), cation exchange capacity (displacement after washing ammonia probe analysis; CEC), pH (probe analysis), percent solids (gravimetric; Plumb 1981), dry bulk density (gravimetric; Blake 1965), acid volatile sulfides (AVS), and simultaneously extractable metals (SEM; purge and trap; Leonard et al. 1996).

Measurement of MDLs for Copper in Sediments

The MDLs for copper in the five sediment samples were empirically derived from two bench scale experiments with a series of copper sulfate pentahydrate ($CuSO_4•5H_2O$; Thermo Fisher Scientific, Inc.) amendments. Flasks (125 mL) containing wet sediment (i.e. 30 g dry sediment estimated by percent solids) were used for the experiments to determine MDLs for copper in sediments. Stock solutions were made by diluting a 1,000 mg Cu/L stock solution in 100 mL of nanopure water (18Ω). The acid soluble copper concentrations in the stock solutions were measured by acidifying the samples with trace metal grade nitric acid (Thermo Fisher Scientific, Inc.) to a pH $\leq$ 2 for 24 hours then filtering samples through a 0.45 µm filter before analysis by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES; Perkin-Elmer Optima 3100RL; APHA 2005). For sediment copper amendments, one mL of stock solution with the targeted mass of copper was added to each flask. Sediment amendments were allowed a contact period of two weeks before sediment copper concentration analysis based on a previous study by Murray-Gulde et al. (2002) indicating that copper sulfate partitioned to sediment within two weeks. An initial range finding experiment targeted sediment copper amendments of untreated control, 0.5, 1, 2, 4, and 8 mg Cu/kg was used to determine copper amendments for definitive MDL experiments. Based on the results from range finding experiments, different copper amendments (i.e. treatments) were used for each sediment sample for definitive MDL experiments. Targeted copper amendments for definitive experiments determining copper MDLs were: 1) untreated control, 1, 1.5, 2, 2.5, and 3 mg Cu/kg for the Mississippi River sediment sample, 2) untreated control, 3, 4, 5, 6, and 7 mg Cu/kg for the South Carolina aquaculture pond sediment sample, 3) untreated control, 4, 5, 6, 7, and 8 mg Cu/kg for the Lake John Hay (Indiana) sediment sample, 4) untreated control, 3, 4, 5, 6, and 7 mg Cu/kg for the Colorado irrigation pond sediment sample, and 5) untreated control, 5, 6, 7, 8, and 9 mg Cu/kg for the California (Buena Park) urban pond sediment sample.

Sediment copper concentrations were measured three times for each amended sediment sample using a digestion block and ICP-OES (US EPA Method 3050b). The MDLs of copper in sediments were defined as the lowest amendment with a measured sediment copper concentration greater than unamended control, discerned by ANOVA and Dunnett's multiple range tests ($\alpha = 0.01$) with copper amended treatments compared to untreated controls. Regression analyses were used to determine correlations of sediment characteristics and MDLs of copper residues in sediments (SAS 9.2 2010).

Sediment Copper Bioavailability Experiments

Bioavailability of copper in the five sediment samples was measured using copper sulfate pentahydrate amended sediments in toxicity experiments. Sediments were amended using the method outlined by Huggett et al. (1999), and allowed a contact time of two weeks prior to organism introduction based on a previous study by Murray-Gulde et al. (2002) indicating that copper sulfate partitions to sediments within 2 weeks. The targeted sediment copper amendments for definitive toxicity experiments were: 1) untreated control, 10, 15, 25, 35, 50, 60, and 70 mg Cu/kg for the Mississippi River sediment sample, 2) untreated control, 75, 100, 125, 175, 250, 450, and 550 mg Cu/kg for the South Carolina aquaculture pond sediment sample, 3) untreated control, 50, 75, 100, 150, 250, 450, and 550 mg Cu/kg for the Indiana Lake John Hay sediment sample, 4) untreated control, 350, 400, 450, 550, 600, 650, and 750 mg Cu/kg for the Colorado irrigation pond sediment sample, and 5) untreated control, 400, 550, 650, 800, 1000, 1200, 1400 mg Cu/kg for the California urban pond sediment sample. Bioavailability of copper in the five amended sediment samples was measured using 10-day static non-renewal sediment toxicity experiments and second instar *H. azteca* (approximately 2 to 3 weeks old, collected using a sieving method; Deaver and Rodgers 1996) cultured at Clemson University following the procedures of de March (1981). Sediment toxicity experiments were conducted at 23 ± 1°C under a 16 hour light/8 hour dark photoperiod (US EPA 2000). Experiments were initiated with 10 *H. azteca* in three replicate 250 borosilicate beakers, with 160 mL of overlying site water and 40 mL of sediment (Suedel et al. 1996). *H. azteca* were fed three 7 mm *Acer rubrum* discs at test initiation (Huggett et al. 1999). Water and sediment samples were collected at the termination of the experiments for measurement of soluble copper concentrations in the overlying water and sediment copper concentrations. Soluble copper concentrations in the overlying water were analyzed by filtering each sample through a 0.45 µm filter, and the filtrates were acidified to a pH $\leq$ 2 with trace metal grade nitric acid before analysis by ICP-OES

(Thermo Fisher Scientific Inc.; APHA 2005). Sediment copper concentrations were measured using ICP-OES (US EPA method 3050b). Water characteristics (i.e. hardness, alkalinity, pH, conductivity, and temperature) were measured at the initiation and termination of the toxicity experiments to insure the environmental tolerances of *H. azteca* were satisfied (APHA 2005).

The probit method was used to calculate LC50s with 95% confidence intervals (SAS 9.2 2010). Analysis of variance (ANOVA) and Dunnett's multiple range tests were used to discern differences between treatments and untreated control to estimate the No Observed Effect Concentrations (NOECs) and Lowest Observed Effect Concentrations (LOECs; $\alpha = 0.05$; Suedel et al. 1996; SAS 9.2 2010). Potency slopes were calculated for the linear portion of the exposure-response curves (Johnson et al. 2008) to discriminate differences of copper potency with different sediments, and determine relationships with the LOECs and LC50s to the potency of copper amended sediments (SAS 9.2 2010).

Relationships of MDLs and Bioavailability of Copper in Sediments

The MDLs for copper in sediments and the observed responses (i.e. LOECs, LC50s, and potency slopes) were used to test for relationships between the MDLs and bioavailability of copper in sediments. Regression analyses were used to determine relationships with the MDLs of copper in sediments and the respective LOECs, LC50s, and potency slopes for the five sediment samples (SAS 9.2 2010).

Results and Discussion

Sediment Characteristics

Characteristics of the five sediments collected from a variety of locations differed widely (Table 1). In particular, the pre-amendment copper concentrations in the five sediment samples ranged from < 1.5 - 150 mg Cu/kg (Table 1). Reported background copper concentrations in sediments for physiographic provinces throughout the United States range from 0.8 - 50 mg Cu/kg (Flemming and Trevors 1989). The original purpose for collecting sediments for these experiments was to capture a sufficient range of characteristics so the results of these experiments may be widely applicable. The percent sand ranged from 5 - 98%, percent silt ranged from 2 - 55%, and percent clay ranged from <1 - 34% (Table 1). Particle surface area ranged from 53 - 2,700,00 cm^2/g. Sediment dry bulk density ranged from 0.63 - 1.62 g/cm^3, and the organic matter content ranged from 0.26 - 5.39% . The CEC ranged from 5.71 - 53.79 meq/100 g, and pH ranged from 6.7 - 7.8 SU. AVS and SEM ranged from <0.2 - 27.8 µmol/g and 0.008 - 10.24 µmol/g, respectively.

MDLs and Bioavailability of Copper in Sediments

The MDLs (standard deviation) for copper in the five sediments (in sequence from lowest to highest) were 1.5 (0.3) mg Cu/kg (Mississippi River), 4 (0.5) mg Cu/kg (aquaculture pond), 4 (0.6) mg Cu/kg (irrigation pond), 6 (1.9) mg Cu/kg (Lake John Hay), and 6 (3.2) mg Cu/kg (urban pond). The percent recoveries for copper in the five sediments (in sequence from lowest to highest) were, 111% (Mississippi River), 99% (aquaculture pond), 93% (irrigation pond), 95% (Lake John Hay), and 85% (urban pond). Based on the

MDLs for these diverse sediments, the range of MDLs would likely not differ more than one order of magnitude when ICP-OES (US EPA method 3050b) is used. Based on results from these sediments, the analytical method used (ICP-OES with US EPA method 3050b) is relatively sensitive and can detect changes in sediment copper concentrations of approximately 1.5 - 6 mg Cu/kg.

The toxicity experiments using *H. azteca* indicated that the bioavailability of copper also ranged widely in these sediments. The 10-d LC50s ranged from 26 - 592 mg Cu/kg (a difference of 566 mg Cu/kg; Table 2). Based on measured sediment copper concentrations, the 10-d LOECs for the copper-amended sediments ranged from 15 mg Cu/kg (Mississippi River) to 550 mg Cu/kg (urban pond), and these 10-d LOECs differed by 535 mg Cu/kg (15 - 550 mg Cu/kg; Table 2). For these copper amended sediments, 10-d potency slopes ranged from 0.071- 1.7 % mortality/mg Cu/kg (a difference of 1.629 % mortality/mg Cu/kg; Table 2). Previous studies have noted that toxicity observed from sediments containing copper was related more to copper concentrations in overlying water than to sediment copper concentrations (Cairns et al. 1984; Suedel et al. 1996; Deaver and Rodgers 1996). When the 10-d LC50s and 10-d LOECs were calculated based on soluble copper concentrations in the overlying water, the range was 13 - 160 µg Cu/L (a difference of 147 µg Cu/L), and the range for 10-d LC50s was 43-167 µg Cu/L (a difference of 124 µg Cu/L). The 10-d potency slopes calculated based on soluble copper concentrations in the overlying water, ranged from 0.095 - 0.48 % mortality/µg/L (a difference of 0.385 % mortality/µg/L). The wide range of 10-d LOECs, 10-d LC50s, and 10-d potency slopes based on sediment copper concentrations compared to the range observed from soluble copper concentrations measured in the overlying water, supports the notion that soluble copper in the overlying water is a more accurate measure of the bioavailable fraction of copper for *H. azteca* in these laboratory sediment toxicity experiments than the bulk sediment copper concentrations.

Based on measured sediment copper concentrations, two negative logarithmic trends were observed with 10-d potency slopes and 10-d LOECs (R^2 = 0.77; potency slope = -0.425 ln (10-d LOEC) + 2.564) and the 10-d potency slopes and 10-d LC50s (R^2 = 0.78; potency slope = -0.497 ln (10 - d LC50) + 3.044) for the five copper-amended sediments (Fig. 1 and 2). The observed relationships of 10-d potency slopes and 10-d LOECs and 10-d LC50s demonstrates that the 10-d LOEC or 10-d LC50 increases as the potency of copper decreases in sediments. These relationships are likely due to different binding mechanisms that affect bioavailability of copper in these sediments. Based on the copper amendments required to elicit observable adverse effects on *H. azteca* in these sediments, increases in sediment copper concentrations of 14 - 400 mg Cu/kg would be required to measure adverse effects.

Relationships with Bioavailability and MDLs of Copper in Sediments and Sediment Characteristics

There were no significant linear correlations ($\alpha = 0.05$) of MDLs of copper in the five sediments with the measured or calculated sediment characteristics in this study (Table 3). Organic matter content had the highest correlation coefficient, but the relationship was not sufficient to be predictive ($P = 0.153$, $R^2 = 0.547$; Table 3). Sediments with the highest and lowest pre-amendment copper concentrations had the highest and lowest measured MDLs, respectively (Fig. 3). A logarithmic relationship was observed for MDLs of copper in the sediments and pre-amendment sediment copper concentrations (R^2 =0.712, y=0.8781 ln(x) +

2.0717; Fig. 3) with higher copper concentrations in sediments associated with higher MDLs for copper.

Several sediment characteristics influence the bioavailability of copper in sediments (Jones et al. 2008). In this study, pH had the greatest correlation with the 10-d NOECs, 10-d LOECs, and 10-d LC50s (Table 4). An AVS/SEM ratio greater than one has been proposed as a predictor of the lack of sediment toxicity due to divalent metals (Ankley et al. 1994; DiToro et al. 1990). In this study, the urban pond sediment had an AVS/SEM of 0.3 (data taken from Table 1) and, according to the AVS/SEM hypothesis, divalent metals in this sediment may cause toxicity. Results from the toxicity experiment with the urban pond sediment indicated no “background” toxicity, and a greater amount of amended copper was required before toxicity could be detected compared to the other sediments in this study. Because the bioavailability of copper in sediments is influenced by several sediment characteristics (e.g. organic matter, CEC, and pH; Jones et al. 2008), the utility of a single sediment characteristic (such as AVS/SEM) to predict the bioavailability and toxicity of copper in sediment is limited (Ankley et al. 1993; Huggett et al. 1999; Jones et al. 2008).

Although sediment characteristics can differ widely, sediment characteristics can be correlated. For example, the percent organic carbon and organic matter are positively correlated with CEC, and primarily associated with fine particle sized sediments (Bailey and White 1964; Suedel and Rodgers 1991). As the CEC and organic matter increase bioavailability of copper in sediments decreases (Besser et al. 2003; Milani et al. 2003; Cairns et al. 1984). Characteristics correlated with increased bioavailability of copper in sediments such as percent sand (Hoss et al. 1997) decrease as the organic matter content and

CEC increase (Suedel and Rodgers 1991). Due to the wide range of sediment characteristics expected in the various physiographic provinces, copper concentrations that elicit adverse effects can span at least two orders of magnitude based on the estimated LOECs from this study. Results of this study indicated that sediment toxicity experiments or other sediment data such as benthic fauna analyses are required to accurately assess the toxicity and bioavailability of copper in sediments.

Comparisons of MDLs and Bioavailability of Copper in Sediments

The range of MDLs (1.5-6 mg Cu/kg) was less than the range of LOECs (15-550 mg Cu/kg) by approximately two orders of magnitude. The LOECs were greater than the MDLs for each of the sediments. Because the range and values of MDLs were less than the LOECs (toxicological detection limits), the concentrations of copper in sediments eliciting adverse effects to *H. azteca* could be readily measured. Further, *H. azteca* is a sensitive sentinel species recommended by the US EPA to measure the toxicity and bioavailability of sediment associated copper (Suedel et al. 1996; Kubitz et al. 1995; US EPA 1994). A comparison of LC50s demonstrates that *H. azteca* is more sensitive than the fish *Pimephales promelas* to copper sulfate, and is within two orders of magnitude of sensitivity compared to other sentinel species (i.e. *Ceriodaphnia dubia*, *Daphnia magna*, and *Daphnia pulex*) exposed to copper sulfate (Murray-Gulde et al. 2002). MDLs of copper in these sediments and the measured LOECs were related, however, MDLs were not adequate to predict the LOECs for the five copper amended sediments (Fig. 4). A logarithmic relationship was observed with the potency slopes and the MDLs [R^2 = 0.913, % mortality/mg Cu/kg = -1.128 ln (mg Cu/kg)

+ 2.04; Fig. 5]. The logarithmic relationship illustrated that the accuracy of sediment copper measurements increases as the potency of copper in the five sediments increases.

Because MDLs for copper were one to two orders of magnitude less than the 10-d LOECs for the five sediment samples, sediment copper concentrations that elicited *H. azteca* mortality were readily measured. Analytical measurements have been used to screen sediments for toxicity and predict the toxicological status of sediments (MacDonald et al. 2000; Buchman 2008). The National Oceanic and Atmospheric Administration (NOAA) screening quick reference tables (SQuiRTs) indicate sediment copper concentrations less than 35.7 mg Cu/kg are likely not toxic (TEL = Threshold Effect Level) and sediment concentrations greater than 197 mg Cu/kg would have probable adverse effects (PEL = Probable Effect Level; Buchman 2008). For this experiment, one of the five sediments had an LOEC less than the TEL (Buchman 2008), and two of the sediments had LOECs approximately two times greater than the PEL. For some sediments, copper concentrations less than the TEL may be toxic, while some sediments with copper concentrations greater than the PEL may not be toxic. To accurately assess the bioavailability and risks of copper accumulation in sediments for a site, toxicity experiments or other sediment data such as benthic fauna analyses are needed. The analytical method in this study was sufficiently sensitive for measuring the lowest sediment copper concentrations that elicited adverse effects for *H. azteca* exposed in the five sediment samples. However, analytical measurements were not predictive of the bioavailability and toxicity of copper for the five sediment samples.

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Table 3.1 Characteristics of sediments used in bioavailability experiments.

Physical/chemical characteristics	Mississippi River	Aquaculture Pond	Lake John Hay	Irrigation Pond	Urban Pond
Sediment					
Percent Solids (%)	81	45	76	71	80
Sediment Dry Bulk Density (g/cm^3)	1.60	0.63	1.51	1.18	1.62
pH	7.15	6.88	6.70	7.45	7.80
%OM ± SD	0.26 ± 0.11	5.39 ± 0.08	2.52 ± 0.07	0.34 ± 0.34	3.04 ± 0.61
CEC (meq/100 g)± SD	5.71 ± 3.46	16.93 ± 1.92	18.19 ± 0.04	53.79 ± 9.73	25.18 ± 6.37
% Sand	98	26	66	5	86
% Silt	2	41	27	55	11
% Clay	<1	33	7	34	3
Surface area (square cm/g)	53	2600000	540000	2700000	230000
Pre-amendment Cu concentration (mg/kg)	< 1.5	10	12	18	150
AVS (umol/g) ± SD	< 0.2	14.8 ± 1.5	7.4 ± 1.9	27.8 ± 4.1	3.32 ± 0.2
SEM (umol/g) ± SD	0.008 ± 0.0001	0.066 ± 0.011	0.068 ± 0.019	0.066 ± 0.010	10.24 ± 1.91

Average of three replicates ± SD

Table 3.2 Measured 10-d NOECs, 10-d LOECs, 10-d LC50s (95% confidence interval), and 10-d potency slopes based on measured sediment copper concentrations and soluble copper concentrations in the overlying water for *H. azteca* exposed to five copper amended sediments.

Parameter	Mississippi River	Aquaculture Pond	Lake John Hay	Irrigation Pond	Urban Pond
10-d NOEC					
Sediment (mg Cu/kg)	9	57	58	354	395
Overlying Water (ug Cu/L)	68	8	26	132	143
10-d LOEC					
Sediment (mg Cu/kg)	15	85	141	394	550
Overlying Water (ug Cu/L)	109	13	33	147	160
10-d LC50 (95% CI)					
Sediment (mg Cu/kg)	26 (22-29)	125 (82-184)	179 (150-208)	426 (262-688)	592 (526-653)
Overlying Water (ug Cu/L)	141 (126-156)	91 (18-162)	43 (33-54)	151 (118-160)	167 (155-177)
10-d Potency Slopes					
Sediment **(%** Mortality/mg Cu/kg)	1.7	0.75	0.2	0.25	0.071
Overlying Water **(%**Mortality/µg Cu/L)	0.48	0.095	0.88	0.24	0.45

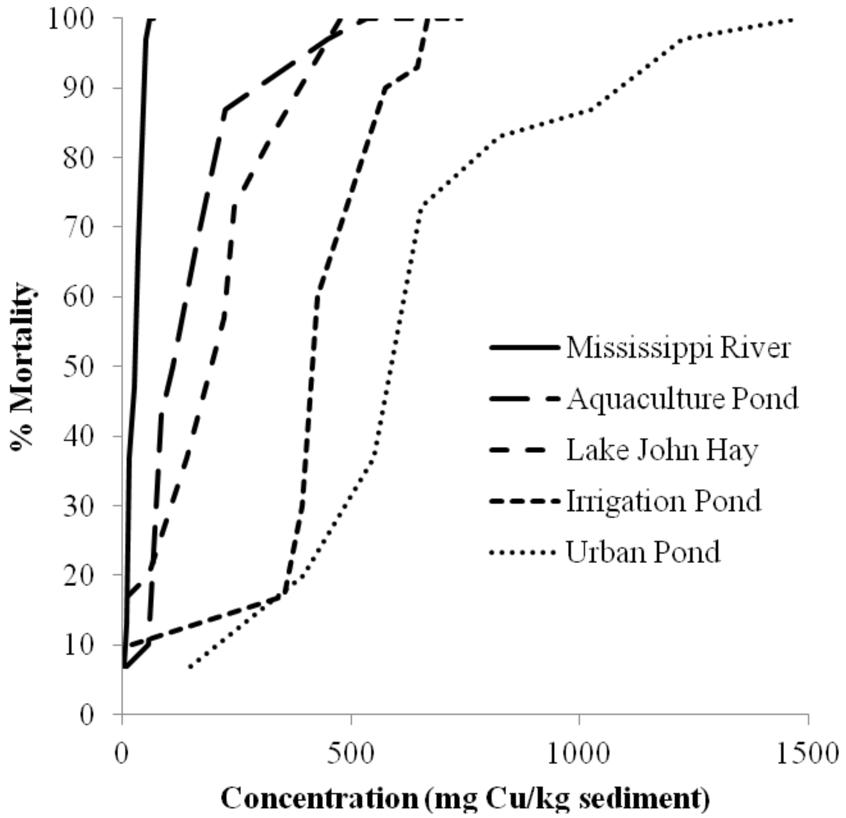
Table 3.3 Correlation coefficients and significance of linear relationships for sediment characteristics and MDLs for copper in sediments.

Sediment Characteristics	**P-value**	**R^2**
%OM	0.153	0.547
Percent Solid (%)	0.283	0.363
Sediment Dry Bulk Density (g/cm3)	0.346	0.293
Percent Sand	0.397	0.244
Percent Clay	0.438	0.210
Percent Silt	0.762	0.035
CEC (me/100 g)	0.811	0.022
pH	0.872	0.010
Surface area (square cm/g)	0.963	< 0.001
Pre-amendment sediment copper concentration	0.998	< 0.001

Table 3.4 Correlation coefficients of linear regressions for sediment characteristics and the 10-d NOECs, 10-d LOECs, 10-d LC50s, and 10-d potency slopes (based on measured sediment copper concentrations).

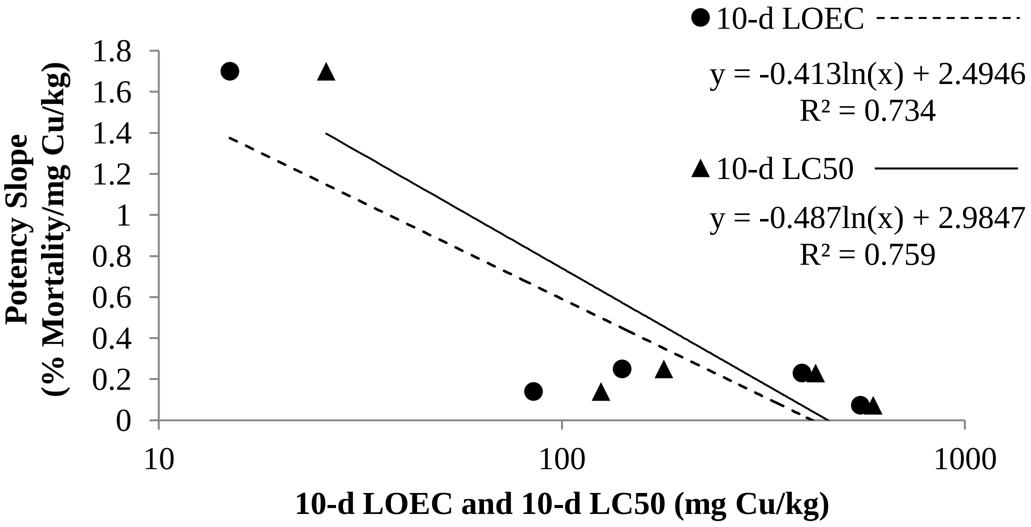
Sediment Characteristics	10-d NOEC	10-d LOEC	10-d LC50	10-d Potency Slope
%OM	0.023	0.004	<0.001	0.250
Percent Sand	0.060	0.015	0.019	0.262
Percent Clay	0.028	0.002	0.003	0.185
Percent Silt	0.063	0.021	0.027	0.331
CEC (me/100 g)	0.430	0.432	0.580	0.329
pH	0.751	0.706	0.671	0.029
Surface area (square cm/g)	0.029	0.002	0.003	0.182
Pre-amendment sediment copper concentration	0.526	0.682	0.685	0.202
AVS	0.146	0.060	0.064	0.230

List of Figures



% Mortality
100
90
80
70
60
50
40
30
20
10
0
0
500
1000
1500
Concentration (mg Cu/kg sediment)
Mississippi River
Aquaculture Pond
Lake John Hay
Irrigation Pond
Urban Pond

Fig. 3.1



● 10-d LOEC
y = -0.413ln(x) + 2.4946
R² = 0.734
▲ 10-d LC50
y = -0.487ln(x) + 2.9847
R² = 0.759
Potency Slope
(% Mortality/mg Cu/kg)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
100
1000
10-d LOEC and 10-d LC50 (mg Cu/kg)

Fig. 3.2

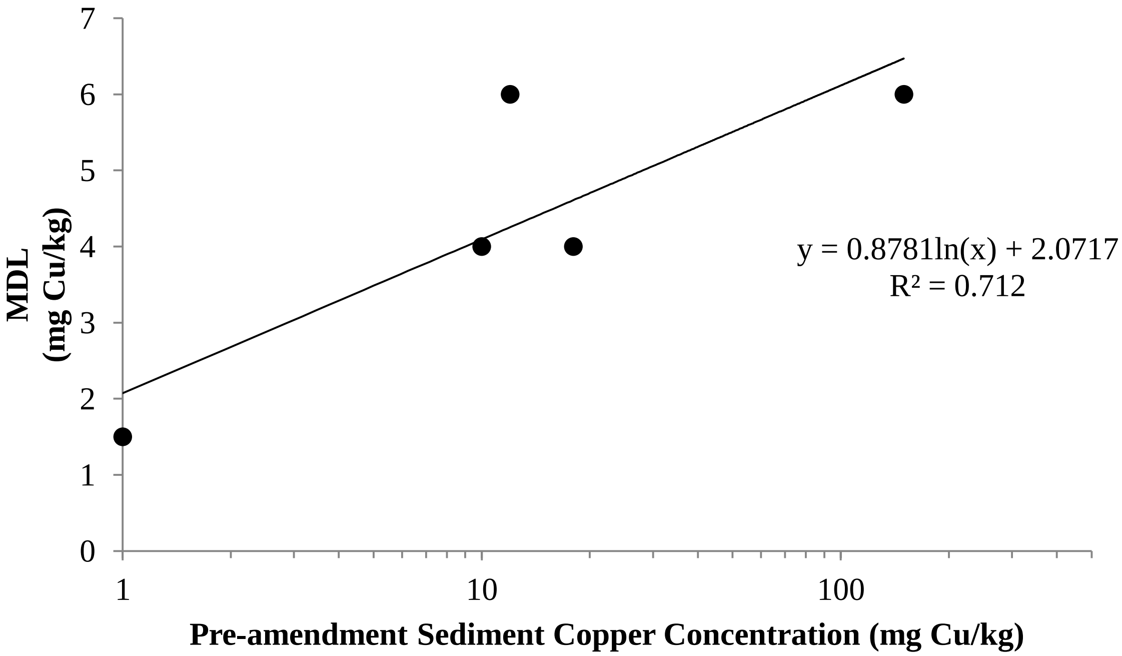
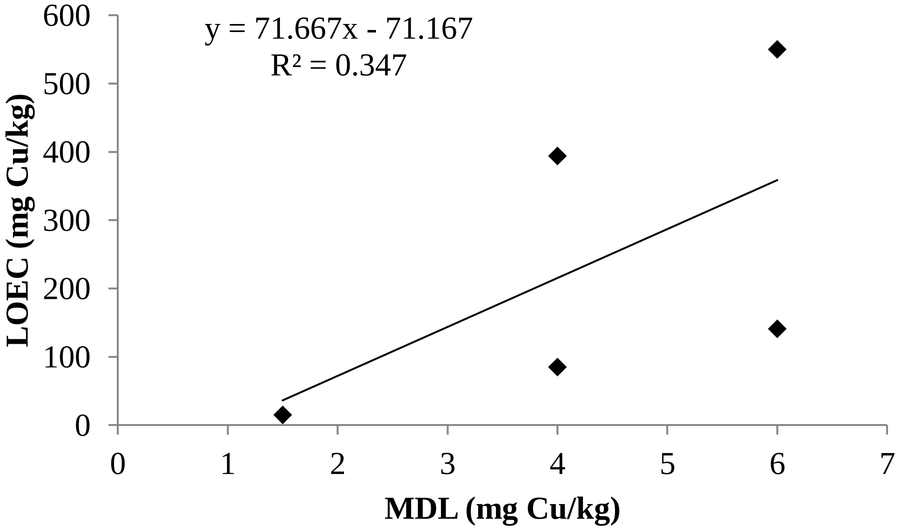
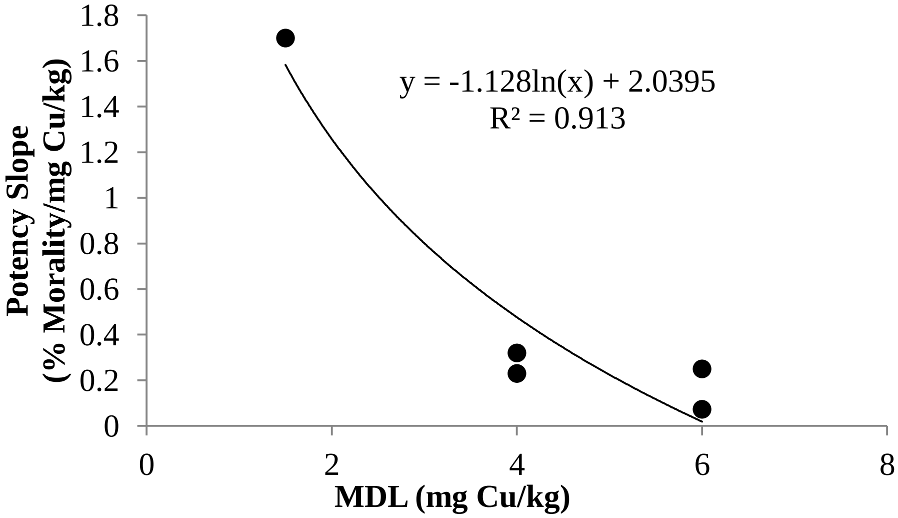

7
6
5
4
3
2
1
0
MDL
(mg Cu/kg)
1
10
100
Pre-amendment Sediment Copper Concentration (mg Cu/kg)
y = 0.8781ln(x) + 2.0717
R² = 0.712

Fig. 3.3



y = 71.667x - 71.167
R² = 0.347
600
500
400
300
200
100
0
LOEC (mg Cu/kg)
0
1
2
3
4
5
6
7
MDL (mg Cu/kg)

Fig. 3.4



1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Potency Slope
(% Morality/mg Cu/kg)
y = -1.128ln(x) + 2.0395
R² = 0.913
0
2
4
6
8
MDL (mg Cu/kg)

Fig. 3.5

CHAPTER FOUR

LABORATORY AND FIELD EVALUATIONS OF COPPER ACCUMULATION IN A POND SEDIMENT FROM ALGAECIDE APPLICATIONS

Abstract

Copper-based algaecide treatments are important in risk mitigation strategies for problematic algal blooms and prediction of the consequences of those treatments is crucial for informed management of impaired water resources. The purpose of this research was to compare laboratory derived site-specific predictions to field measurements of *Spirogyra* responses, sediment copper concentrations, and benthic invertebrate responses following an algaecide treatment in a 0.1 hectare pond. Results from laboratory algal toxicity experiments indicated that Clearigate® at 0.25 mg Cu/L should significantly decrease the biomass of *Spirogyra* in the pond, and the lack of *Spirogyra* mats one week after treatment confirmed the predicted response. Based on the mass of copper introduced from the algaecide treatment (275 g Cu) and measured pre-treatment copper concentrations in the pond, post-treatment sediment copper concentrations were predicted to range from 16-23 mg Cu/kg. Measured post-treatment sediment copper concentrations were 20 and 17 mg Cu/kg in the top 3 and 8 cm of surficial sediment in the pond, respectively. Sediment toxicity experiments using *Hyalella azteca* exposed to pond collected sediment and water amended with copper indicated benthic invertebrates in the pond were not likely to be adversely affected from accumulated copper in the pond sediment following treatment. Measured *in situ* responses of benthic invertebrates and screening level toxicity experiments exposing

Hyalella azteca for 10-days to samples of post-treatment sediment from the pond confirmed the predicted benthic invertebrate responses. This study demonstrated that the laboratory experiments and modeling could be used to predict algal responses to an algaecide treatment, post-treatment sediment copper concentrations, and responses of benthic invertebrates following the algaecide treatment in the pond. Further, data from this study showed that although copper accumulation in sediment from algaecide treatments can be measured, it may be insufficient to elicit adverse effects on benthic invertebrates.

Introduction

Copper introduced to aquatic systems can accumulate in sediments from a variety of sources including algaecide applications (Gallagher et al. 2005). Due to concerns regarding adverse effects of accumulated copper in sediments from algaecide treatments (Anderson et al. 2001; Siemering et al. 2008), data are needed that include measurements of residual copper, as well as potential adverse effects on non-target organisms following a treatment. Laboratory experiments can provide predictions of exposures and adverse effects of copper residues in sediments for a site specific algaecide treatment, as well as responses of a targeted alga to that treatment.

Responses of a targeted alga to a copper-based algaecide depend on the algaecide formulation (i.e. form of copper), algaecide concentration, susceptibility of the target alga, and water characteristics (e.g. hardness, pH, alkalinity, and ionic strength; Murray Gulde et al. 2002). Laboratory experiments can be used to identify efficacious algaecide treatments for a site (i.e. algaecide formulation and concentration; Fitzgerald 1964;

Fitzgerald and Jackson 1979; Bishop and Rodgers 2011). Data collected from laboratory experiments to support decisions regarding treatment of algae with an algaecide can reduce time and capital required to identify an effective treatment compared to trial-and-error methods (Bishop and Rodgers 2011). The mass and form of copper containing algaecides introduced to an aquatic system can differ from site to site depending on the desired results, targeted alga, and site characteristics. Consequently, the mass of copper partitioning to sediments following an algaecide treatment can also differ.

A material balance model incorporating site characteristics [i.e. surface area of the water body treated with algaecide, mean mixing depth of surficial sediment (defined as the depth sediments are bioturbated; Burns et al. 2000), and sediment dry bulk density] and the mass of copper introduced as an algaecide could be used to predict the increase of sediment copper concentration following an algaecide treatment (Equation 1). Assumptions implicit in this modeling are complete partitioning of the copper applied in a treatment to sediments in the area treated and sediment accretion is insignificant during the time period of analysis. The increase in sediment copper concentration following a treatment may not be analytically measurable depending on the mass of copper introduced as algaecide, site characteristics, and analytical method used (Willis and Rodgers 2013). Method detection limits (MDL; lowest concentration of an analyte that can be measured with 99% confidence with a specific analytical method and matrix; Creed et al. 1994; APHA 2005) for copper in site-collected sediment coupled with a material balance model could be used to predict the detection of copper residue in sediment from an algaecide treatment.

Equation 1

$$E\ (\text{mg Cu/kg}) = \frac{MA\ (\text{mg}) \times 1000\ (\text{g/kg})}{S\ (\text{g/cm}^3) \times A\ (\text{cm}^2) \times B\ (\text{cm})}$$

Where: E = Concentration of copper residual in sediment

MA = Mass of copper used to treat algae

S = Sediment dry bulk density

A = Surface area treated

B = Bioturbation depth

Laboratory sediment toxicity experiments using site collected sediment can be used to predict responses of benthic invertebrates to copper residues in sediment for the site (Huggett et al. 1999). Bioavailability of copper in sediments depends on sediment characteristics [e.g. acid volatile sulfides (Di Toro et al. 1990), organic carbon type and content (Besser et al. 2003; Milani et al. 2003), pH (Burton 1991), cation exchange capacity (Chapman et al. 1998), and particle size distribution (Hoss et al. 1997)]. Laboratory sediment toxicity experiments using site collected sediment amended with estimated post-treatment sediment copper concentrations could provide predictions regarding responses of benthic invertebrates to accumulated copper in sediment for a site specific treatment (Gallagher et al. 2005).

Field measurements can be used to evaluate the accuracy of laboratory-based predictions of benthic invertebrate responses to post-treatment sediment copper concentrations for a specific site. This study was designed to compare laboratory predictions of exposures and consequent responses of benthic invertebrates to copper algaecide residues in sediment for a specific site (Fig. 1). Knowledge gained by comparing laboratory results to field observations can be used to refine laboratory experiments and material balance modeling to predict concentrations and analytical measurements of copper residues in sediments, as well as responses of benthic invertebrates to copper residues from algaecide treatments for site specific situations.

The focus of this study was to evaluate laboratory experiments and material balance modeling for predictions of measurement and concentrations of copper residues in sediment, as well as responses from benthic invertebrates to accumulated copper in sediments following an algaecide treatment in a 0.1 hectare pond. Specific objectives of this research were to: 1) determine the copper-based algaecide treatment for the pond necessary to control a targeted alga, 2) predict the post-treatment sediment copper concentration for a specific algaecide treatment in the pond based on laboratory experiments and a material balance model, 3) predict responses of benthic invertebrates to post-treatment copper in sediments from the algaecide treatment in the pond based on laboratory experiments, 4) repeat algaecide treatments until benthic invertebrates respond or an increase in sediment copper concentration is measured in the pond, 5) compare the predicted and measured post-treatment sediment copper concentrations in the pond, and 6) compare the predicted and measured responses of benthic invertebrates to post-treatment sediment copper concentrations in the pond.

Materials and Methods

Algaecide efficacy experiment

The pond used in this study had a surface area of approximately 0.1 hectares, an average water depth of 1 m, and was located at Clemson University, Clemson, SC, USA

(34º 68 N, 82º 81 W). The pond water had a conductivity of 83 µs/cm, a pH of 7 SU, an alkalinity of 34 mg/L as $CaCO_3$, and a hardness of 23 mg/L as $CaCO_3$. Frequent *Spirogyra* "blooms" occur in the pond and could be treated with a copper-based algaecide. A laboratory experiment was initiated with *Spirogyra* and water collected from the pond to identify an efficacious copper-based algaecide treatment for the targeted filamentous alga (Bishop and Rodgers 2011). In the laboratory, *Spirogyra* (0.1 g wet weight) was exposed to four copper-based algaecides (Cutrine-Plus®, Cutrine-Ultra®, Clearigate®, and Algimycin-PWF®; Table 1) at concentrations of background (i.e. untreated control), 0.625, 1.25, 2.5, 5, and 10 mg Cu/ g algae, representing applications of 0.03- 0.5 mg Cu/L in the pond (Bishop and Rodgers 2011). Copper concentrations were measured at experiment initiation using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES; Perkin-Elmer Optima 3100RL; APHA 2005). Algal biomass and chlorophyll-*a* were measured 10 days after treatment following the procedure outlined by Bishop and Rodgers (2011) and *Standard Methods* (2005), respectively. The lowest algaecide concentration that resulted in the greatest decrease in algal biomass and chlorophyll-*a* based on graphical interpolation was used in the subsequent experiment for treating *Spirogyra* in the pond.

Predictions of post-treatment sediment copper concentrations and measurement of copper residues

The post-treatment sediment copper concentrations in the study pond were predicted based on two scenarios. One scenario assumed complete partitioning of applied copper to the bioturbated sediment using the mass of algaecide applied, the pond surface area, bioturbation depth, the mean sediment dry bulk density of bioturbated sediment, and the mean pre-treatment sediment copper concentration in that sediment. An alternative scenario assumed complete partitioning of applied copper to the top 3 cm sediment in the pond using the mass of algaecide applied, the pond surface area, 3 cm copper residual mixing depth, the mean dry bulk density of the top 3 cm of sediment, and the mean pre-treatment copper concentration in the top 3 cm of sediment. The 3 cm copper residual mixing depth in the alternative scenario was chosen based on greater measured accumulation copper applied as an algaecide in the top 3 cm of bottom sediments in a pond following algaecide treatments reported by Liu et al. (2006). Post-treatment sediment copper concentrations were estimated by adding the predicted increases in sediment copper concentrations following treatment (Equation 1) and pre-treatment sediment copper concentrations in the pond. The bioturbation depth in the pond sediment was estimated based on the maximum depth that invertebrates were observed in sediment cores (5 cm diameter; n = 9) collected from two sampling sites located at water depths of 0.5 and 1.5 m. To estimate dry bulk density and background copper concentration of sediment in the pond, the mean dry bulk densities and copper concentrations in 3 and 8 cm depth sediment cores (2.5 cm diameter; n = 16) were used from 16 sampling sites along four transects across the pond. Sediment dry bulk density was measured using the method described by Blake (1965), and sediment copper concentrations were measured using ICP-OES (US EPA method 3050b). To predict the number of treatments required to measure copper residues in the pond sediment, a laboratory-estimated MDL for copper in the pond sediment was divided by the predicted sediment residual copper concentration (i.e. increase in sediment copper concentration relative to pre-treatment). In the laboratory, the MDL was estimated by a series of copper amendments to pond sediment to account for matrix effects and background copper concentration. For the MDL experiment, targeted sediment copper amendments were untreated control, 3, 4, 5, 6, and 7 mg Cu/kg using the experimental and analytical procedures outlined by Willis and Rodgers (2013). The MDL was defined as the lowest treatment with a measured sediment copper concentration greater than untreated control discerned by an ANOVA and Dunnett's multiple range tests ($\alpha = 0.01$).

Prediction of benthic invertebrate responses to post-treatment copper in sediments

The responses of benthic invertebrates to post-treatment copper in pond sediments were predicted from laboratory toxicity experiments using *Hyalella azteca* Saussure. *H. azteca* were cultured for toxicity experiments at Clemson University following the procedures of de March (1981). The sediment toxicity experiment followed the procedure of Suedel et al. (1999) using sediment from the pond amended with copper as Clearigate®. For sediment toxicity experiments copper amendments were untreated control, 100, 150, 225, 300, 325, and 400 mg Cu/kg (dry weight). The sediment copper amendments followed the procedure of Huggett et al. (1999). Based on the aqueous half-life of copper applied as an algaecide (Murray-Gulde et al. 2002), sediment amendments were allowed two weeks of contact time before experiment initiation. Sediment copper concentrations were measured at experiment termination using ICP-OES (US EPA 3050b). The lowest observed effect concentration (LOEC) was defined as the lowest treatment (i.e. lowest copper amendment) with a statistically significant difference in *H.*

azteca survival relative to untreated control. ANOVA and Dunnett's multiple range tests were used to discern significant differences (α = 0.05; Suedel *et al*. 1996). The laboratory estimated LOEC was used to predict the lowest sediment copper concentration in the pond that would be expected to elicit a detectable adverse response from benthic invertebrates. Responses of benthic invertebrates to post-treatment copper in the pond sediment were predicted by contrasting the predicted post-treatment sediment copper concentration with the laboratory estimated LOEC.

Field Experiment

Clearigate® (~0.25 mg Cu/L) was applied to the pond until an increase in the sediment copper concentration was analytically or toxicologically detected. Toxicological detection was defined as either a change in the density of benthic invertebrates after treatment or observed sediment toxicity with *H. azteca* in screening level toxicity experiments using post-treatment sediment samples.

To discern analytical detection of an increase in sediment copper concentration, post-treatment sediment samples were collected after the copper concentration in the water column returned to pre-treatment concentrations (i.e. background). Sixteen vertical composite samples of the water column and sediment samples from 3 and 8 cm sediment cores were collected along four transects distributed across the pond. Sediment samples were collected prior to the first treatment and following each treatment to measure the increase in sediment copper concentration. Acid soluble copper concentrations of water samples were measured using ICP-OES according to *Standard Methods* (APHA 2005). The return of the copper concentration in the water column to background following treatment was defined as no significant difference in acid soluble copper concentrations before and after treatment. Significant differences of the copper concentrations in the water before and after treatment were discerned with a Student's T-test ($\alpha = 0.05$). Copper accumulation in the pond sediment was discerned with a paired Student's T-tests comparing the pre-treatment and post-treatment sediment copper concentrations in the 3 cm cores and the pre-treatment and post-treatment sediment copper concentrations in the 8 cm cores ($\alpha = 0.01$).

To measure responses of benthic invertebrates to post-treatment copper in sediment, nine sediment cores (5.4 cm diameter and 8 cm deep) were collected from two sampling areas with water depths of approximately 1.5 and 0.5 m. Invertebrates were separated from the cores with a 250 µm (no. 60) sieve, and identified to genus (New 1998). A detectable response of benthic invertebrates to post-treatment copper in sediment was determined by a significant difference in the density of benthic invertebrates before and after algaecide treatment, discerned with Student's T-tests ($\alpha = 0.05$).

To compare responses of *H. azteca* in the laboratory and benthic invertebrates in the pond to post-treatment copper concentrations, screening level toxicity experiments were conducted with sediment collected before and after algaecide treatment. Sediments used in the screening level toxicity experiments were collected from the top 3 cm of sediment in the pond. Toxicity screening experiments were conducted under the same conditions as stated for the laboratory experiment using *H. azteca* with one treatment (i.e. sediment collected post-treatment) compared to the control (i.e. sediment collect prior to treatment). Toxicological detection of post-treatment copper in sediment with the screening level toxicity experiments was defined as a significant difference in *H. azteca* survival after 10 d exposures in a treatment compared to results from the untreated control. Significant differences in *H. azteca* survival for the controls and treatments were discerned with a Student's T-test ($\alpha = 0.05$).

Comparison of predictions and field observations of post-treatment sediment copper concentration and benthic invertebrate responses

The laboratory-based predictions of post-treatment sediment copper concentrations were compared to the measured sediment copper concentrations from 3 and 8 cm cores. Significant differences in the predicted and measured sediment copper concentrations were discerned using Student's T-tests ($\alpha = 0.05$). Predicted benthic invertebrate responses to post-treatment sediment were compared with measured responses of benthic invertebrates following algaecide treatment. Post-treatment responses of benthic invertebrates were compared with responses of *H. azteca* in screening level toxicity experiments using post-treatment sediment.

Results

Responses of Spirogyra to Four Copper-based Algaecides in the Laboratory

Results from laboratory exposures of Clearigate®, Cutrine-Plus®, Cutrine-Ultra®, and Algimycin-PWF® illustrated that Clearigate ® was the most efficacious of the algaecides tested for treatment of *Spirogyra*. Ten days after treatment (10 DAT) in the laboratory, Clearigate ® at 5 mg Cu/g of algae (0.25 mg Cu/L as an aqueous treatment in the pond) decreased chlorophyll-*a* and biomass (wet weight) of *Spirogyra* (Figs. 2 and 3) by 74% and 95%, respectively. Based on the results from these laboratory experiments, Clearigate® at 5 mg Cu/g algae (0.25 mg Cu/L *in situ*) was chosen as the algaecide treatment for the pond study.

Predicted post-treatment sediment copper concentration

The predicted post-treatment sediment copper concentration in the study pond was 16 mg Cu/kg. This prediction was based on the pre-treatment ("background") copper concentration in 8 cm sediment cores ($\bar{X}$ = 11 mg Cu/kg; n = 16) and the estimated residual copper concentration in the top 8 cm of sediment in the study pond (= 5 mg Cu/kg). The estimated residual copper concentration in the top 8 cm of sediment in the study pond was generated based on material balance model inputs of: 275 g Cu (mass of copper as Clearigate® applied to the study pond), 0.68 g/cm^3 (mean sediment dry bulk density from 8 cm sediment core samples from the study pond; n= 16), 0.1 hectares (surface area of the study pond), and 8 cm (sediment bioturbation depth). The estimated post-treatment sediment copper concentration resulting from complete partitioning of copper from the algaecide treatment to the top 3 cm of sediment in the study pond was 23 mg Cu/kg. This estimate was based on the pre-treatment ("background") sediment copper concentration in 3 cm sediment cores ($\bar{X}$ = 8 mg Cu/kg) and the estimated sediment residual copper concentration in the top 3 cm in the study pond (= 15 mg Cu/kg). The estimated residual copper concentration in the top 3 cm of sediment was generated based on material balance model inputs of: 275 g Cu (mass of copper as Clearigate® applied to the study pond), 0.60 g/cm^3 (mean sediment dry bulk density from 3 cm sediment core samples from the study pond; n= 16), 0.1 hectares (surface area of the study pond), and 3 cm (depth copper residual partitioned in sediment).

Due to the magnitude of the predicted increase of copper concentration in the top 8 cm of sediment (5 mg Cu/kg) and the estimated MDL for copper in the pond sediment (4 mg Cu/kg; Fig.4), measurement of residual copper in the top 8 cm sediment in the study pond from one treatment is unlikely. The residual copper concentration following two treatments (=10 mg Cu/kg) should be measurable in the top 8 cm of pond sediment. The predicted increase in copper concentration in the top 8 cm sediment in the study pond from two treatments is more than two times the estimated MDL for copper in the pond sediment (= 4 mg Cu/kg) and would approximately double the background sediment copper concentration (from 11 to 21 mg Cu/kg). As an alternative scenario, complete partitioning of applied copper from one treatment to the top 3 cm of sediment in the study pond should be measurable (= 15 mg Cu/kg). The estimated residual copper concentration in the top 3 cm of sediment (= 15 mg Cu/kg) is more than three times greater than the estimated MDL for copper in the pond sediment and would result in more than double the background sediment copper concentration in the 3 cm cores (from 8 to 23 mg Cu/kg).

Predicted responses of benthic invertebrates to post-treatment sediment copper concentrations

Based on responses of *H. azteca* to copper (as Clearigate$^{®}$) amended pond sediment in the laboratory, the 10-day LOEC was 232 mg Cu/kg (Fig. 5). The estimated 10-day LOEC for *H. azteca* exposed to copper-amended pond sediment was more than 10 times greater than the predicted copper concentration in the study pond sediment following one treatment. Therefore, benthic invertebrates in the pond were not likely to be adversely affected by one treatment. Assumptions implicit in this prediction include:

similar copper bioavailability in the laboratory-amended sediments, *H. azteca* are similar in sensitivity or more sensitive than extant benthic invertebrates in the pond, sediment accretion in the pond over the time of interest in this study is insignificant, and copper applied as Clearigate® partitions completely to the pond sediments. Based on the minimum time interval between Clearigate® applications permitted by the algaecide label (1 week; Applied Biochemists 2012) and the period of treatment from June to July (i.e. 9 weeks growing season for *Spirogyra* in SC, USA; Smith et al. 1976), we predicted that one year of weekly treatments for *Spirogyra* using Clearigate® at 0.25 mg Cu/L would not elicit adverse effects for the benthic invertebrates in the pond. This prediction is based on the estimated sediment copper concentrations in the top 8 cm of sediment in the study pond following nine treatments (= 56 mg Cu/kg) and the estimated 10-day LOEC for *H. azteca* exposed to copper amended pond sediment in the laboratory (232 mg Cu/kg). Assumptions in this prediction are: copper bioavailability in sediment does not change over time, sediment accretion is insignificant during the time period that nine treatments were applied, the sensitivity of *H. azteca* to copper exposures is similar or more sensitive than benthic invertebrates in the pond, and copper applied as Clearigate® partitions completely to the pond sediments.

Field Observations: Algaecide Treatment in Study Pond

Prior to treatment of the study pond with Clearigate®, the mean (± standard deviation) acid soluble copper concentration in pond water samples was 3 (± 2) µg Cu/L (n=16). Thirty minutes following treatment, the mean (± standard deviation) acid soluble copper concentration in pond water samples was 0.38 (± 0.3) mg Cu/L (n = 16) with a targeted concentration of 0.25 mg Cu/L. Variance in aqueous copper concentrations in samples from across the pond was attributed to horizontal dispersion of the algaecide from an easterly wind immediately after treatment. The mean (± standard deviation) post-treatment acid soluble copper concentrations in the pond water samples were 15 (± 2) μg Cu/L 7 DAT, 15 (± 3) μg Cu/L 14 DAT, and 5 (± 2) μg Cu/L 21 DAT (n = 16). There were no significant differences in measured copper concentrations in the water samples collected prior to treatment and 21 DAT ($\alpha = 0.05$).

Visual observations of *Spirogyra* 1 DAT confirmed the initial responses to algaecide exposure (i.e. algal mats changing color from green to yellow). *Spirogyra* biomass notably decreased from 4 DAT until 7 DAT when there were no visually observable mats. Two benthic *Spirogyra* mats (~0.5 m^2) were observed and measured 24 DAT, however, *Spirogyra* growth was relatively static until the following year (11 months after treatment). Approximately 11 months after treatment, *Spirogyra* “bloomed” and returned to pre-treatment biomass. Based on the observed regrowth of *Spirogyra* almost a year after treatment, annual treatments with Clearigate® would likely control the density of *Spirogyra* in the pond.

The mean (± standard deviation) sediment copper concentrations 21 DAT in the 3 and 8 cm core samples were 20 (±7) and 17 (± 5) mg Cu/kg, respectively. Mean (± standard deviation) pre-treatment (“background”) sediment copper concentrations in the 3 and 8 cm sediment core samples were 8 (± 3) and 11 (± 2) mg Cu/kg. The mean (± standard deviation) increase in sediment copper concentrations 21 DAT in the 3 and 8 cm sediment cores were 12 (± 3) and 6 (± 5) mg Cu/kg, respectively (n = 16). Based on the mean measured residual copper concentrations in the 3 and 8 cm sediment cores 21 DAT, ~ 65% and ~35% of the residual copper partitioned to the top 3 cm and to the 3 - 8 cm depth interval in the pond sediment, respectively.

Chironomus was the sole benthic invertebrate observed in both pre- and post-treatment sediment cores. There were no significant decreases in densities of *Chironomus* 21 DAT relative to pre-treatment densities ($\alpha = 0.05$; Fig. 6). In laboratory screening level toxicity experiments, there were no observed differences in survival of *H. azteca* exposed to pre-treatment and post-treatment sediments ($\alpha = 0.05$; Fig 6).

Comparison of predicted and measured post-treatment sediment copper concentrations in the study pond

The predicted post-treatment sediment copper concentration based on the algaecide application to the study pond (= 16 mg Cu/kg) did not differ significantly from the measured sediment copper concentrations in the 8 cm cores 21 DAT ($\bar{X} = 17$ mg Cu/kg; $\alpha = 0.05$; Fig.7). The estimated post-treatment sediment copper concentration based on complete partitioning of residual copper to the top 3 cm of sediment in the study pond (= 23 mg Cu/kg) did not differ significantly from the measured sediment copper concentrations in the 3 cm cores 21 DAT ($\bar{X} = 20$ mg Cu/kg; $\alpha = 0.05$; Fig.7). The predicted post-treatment increase in sediment copper concentration in the top 8 cm of sediment (= 5 mg Cu/kg) was not significantly different from the measured sediment residual copper in the 8 cm sediment cores from the pond ($\bar{X} = 6$ mg Cu/kg; $n = 16$; $\alpha = 0.05$). The estimated residual copper concentration in the top 3 cm of sediment in the pond (= 15 mg Cu/kg, assuming complete partitioning of Clearigate® residual copper to the top 3 cm of pond sediment) was not significantly different from the measured sediment copper concentration increase in the 3 cm cores 21 DAT ($\overline{X}$ = 12 mg Cu/kg; n = 16; α = 0.05).

Comparison of predicted and measured responses of benthic invertebrate to post-treatment sediment copper concentration

The 10-day LOEC for *H. azteca* exposed to copper amended (as Clearigate$^{®}$) sediments in the laboratory was predictive of the no observed adverse effects for *Chironomus* exposed to post-treatment sediment copper concentrations in the pond (Fig.6). The laboratory estimated 10-day LOEC for *H. azteca* exposed to copper amended sediments was more than 11 times greater than the measured sediment copper concentrations in the pond 21 DAT. Exposures to post-treatment sediments containing residual copper did not adversely affect *H. azteca* in 10 day laboratory screening level toxicity experiments or *Chironomus* in the pond 21 DAT (Fig. 6).

Discussion

Responses of pond collected *Spirogyra* differed by the chelated copper-based algaecide exposure in the laboratory 10 DAT. Based on the measured responses (biomass and chlorophyll-*a*) of *Spirogyra* to measured algaecide exposures in the laboratory, Clearigate® at 0.25 mg Cu/L *in situ* (5 mg Cu/kg) was predicted to be efficacious for treating *Spirogyra* in the study pond. Algal responses to copper-based algaecides are influenced by water characteristics (e.g. pH, hardness, conductivity) and the targeted alga (Murray-Gulde et al., 2002). By incorporating site collected water and *Spirogyra* in laboratory toxicity experiments, responses of algae *in situ* to algaecide treatments can be accurately predicted (Fitzgerald and Jackson 1979; Bishop and Rodgers, 2011). Because algal responses to algaecides differ, approaches for efficient use of algaecides are advantageous for reducing the amount of algaecides applied to water resources concomitantly decreasing risks to non-target species.

Predictions of post-treatment sediment copper concentrations and responses of benthic invertebrates can be used to evaluate potential risks of an algaecide treatment. Using conservative assumptions in a material balance model, the predicted post-treatment sediment copper concentrations represent the maximum range expected. Due to the sensitivity of *H. azteca* to copper in sediments compared to the benthic invertebrates present in the study pond (Chironomid larvae; Suedel et al. 1996; Milani et al. 2002), the predicted concentration of copper in the study pond sediment at which effects may be initially observed (232 mg Cu/kg) was likely conservative. Data derived from laboratory experiments exposing sensitive sentinel species to copper amended sediments can be used to conservatively predict effects and evaluate risks of copper accumulations in sediments from site specific algaecide treatments (Huggett et al. 1999; Gallagher et al. 2005).

Comparisons of results from laboratory experiments and modeling with field measurements can be useful for evaluating the accuracy (or lack thereof) for predicting outcomes in field situations (Cairns 1986). Further, there is a pressing need for field studies evaluating management strategies for water resources (Osgood 2007). As data from field studies accrue, our ability to predict outcomes of management actions (or the “no action” alternative) can improve. Results from this study demonstrated that laboratory algal toxicity experiments using site collected water and targeted alga identified an effective algaecide treatment for this pond that was less than the maximum copper concentration that could be applied (= 1 mg Cu/L according to recommendations on the algaecide label). Therefore the laboratory algal toxicity experiment was beneficial for determining the minimal algaecide concentration required to achieve desired results, as well as concurrently reducing risks for non-target organisms and post-treatment sediment copper concentrations in the pond. The results of laboratory experiments and modeling were predictive of both sediment copper concentrations and benthic invertebrate responses to accumulated copper in sediments following the specific algaecide treatment to the study pond. Copper accumulation from the algaecide application was measurable in sediments, however was insufficient to elicit adverse responses from extant benthic invertebrates. Measurements of copper accumulations in sediments can provide crucial data for monitoring fate and effects of residual copper from algaecide applications.

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Table 4.1 Physical properties of Algimycin®-PWF, Cutrine®-Plus, Cutrine®-Ultra. and Clearigate®.

Algaecide	**Algimycin®-PWF**	**Cutrine®-Plus**	**Cutrine®-Ultra**	**Clearigate®**
% Elemental copper[a]	5.0	9.0	9.0	3.8
Maximum application label rate[a]	1.0 mg Cu/L	1.0 mg Cu/L	1.0 mg Cu/L	1.0 mg Cu/L
Formulation[a,b]	copper-citrate and copper-gluconate	copper-ethanolamine	copper-ethanolamine and D-limonene	copper-ethanolamine and D-limonene
Appearance[b]	Blue liquid	Blue viscous liquid	Blue viscous liquid	Blue viscous liquid
Water Solubility[a,b]	Miscible	Miscible	Miscible	Miscible
Specific gravity (g/cm^3)[a]	1.229	1.22-1.23	1.220-1.225	1.0-1.1
pH (SU)[a]	1.8	10.3-10.5	[c]10.2-10.3	9.5-10
Vapor pressure (mm Hg)[a,b]	Non-volatile	Non-volatile	[c]No data available	Non-volatile

[a] Kamrin (1997)

[b] Applied Biochemists (2011)

List of Figures

There was no variance in survival (90%) of *H. azteca* exposed to pond sediment sampled 21 DAT (n = 30).

Fig. 4.7 Predicted post-treatment sediment copper concentrations in the top 3 and 8 cm of sediment in the study pond and measured sediment copper concentrations in 3 and 8 cm sediment cores from the pond before treatment and 21 DAT. Error bars are 2 standard deviations (n=16).

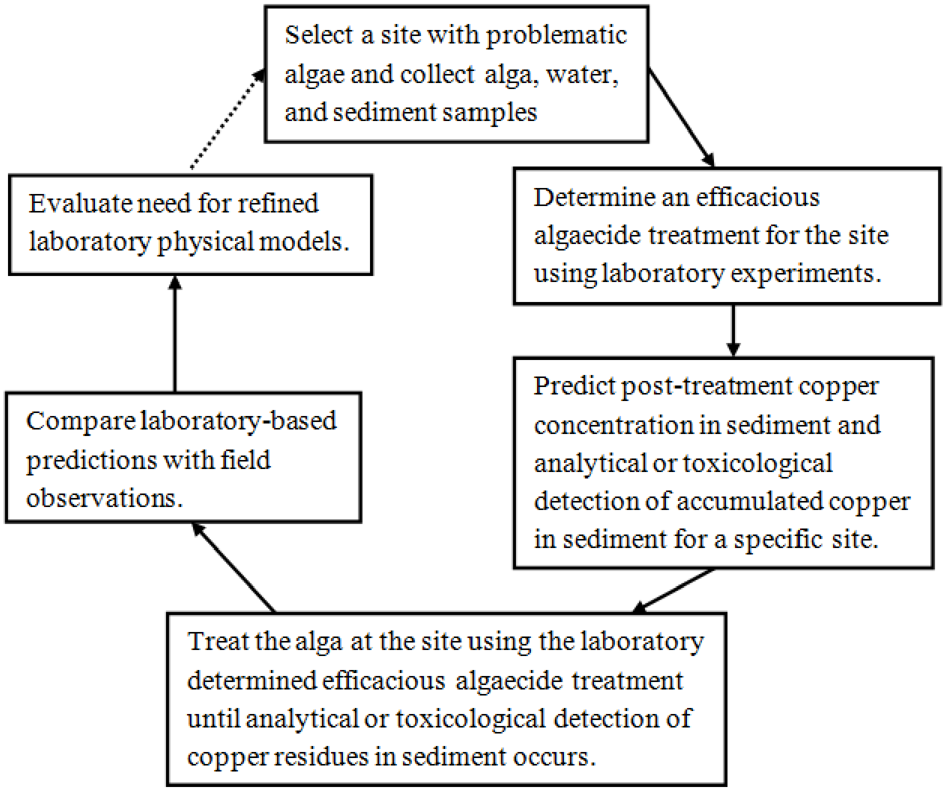

Select a site with problematic algae and collect alga, water, and sediment samples
Determine an efficacious algaecide treatment for the site using laboratory experiments.
Predict post-treatment copper concentration in sediment and analytical or toxicological detection of accumulated copper in sediment for a specific site.
Treat the alga at the site using the laboratory determined efficacious algaecide treatment until analytical or toxicological detection of copper residues in sediment occurs.
Compare laboratory-based predictions with field observations.
Evaluate need for refined laboratory physical models.

Fig. 1

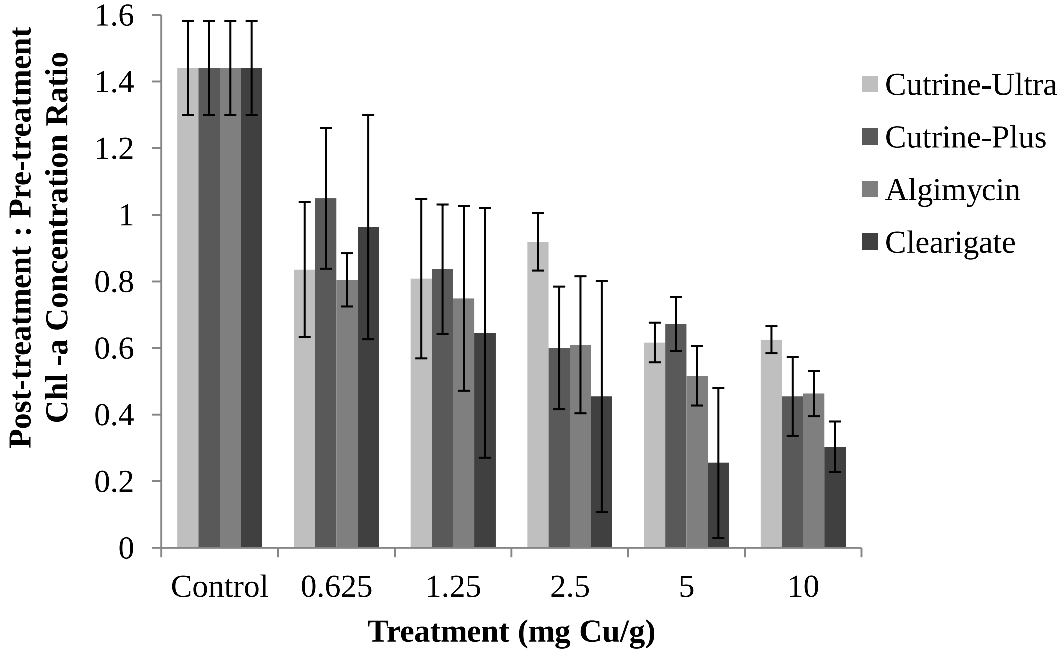

Post-treatment : Pre-treatment
Chl -a Concentration Ratio
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
0.625
1.25
2.5
5
10
Treatment (mg Cu/g)
Cutrine-Ultra
Cutrine-Plus
Algimycin
Clearigate

Fig. 2

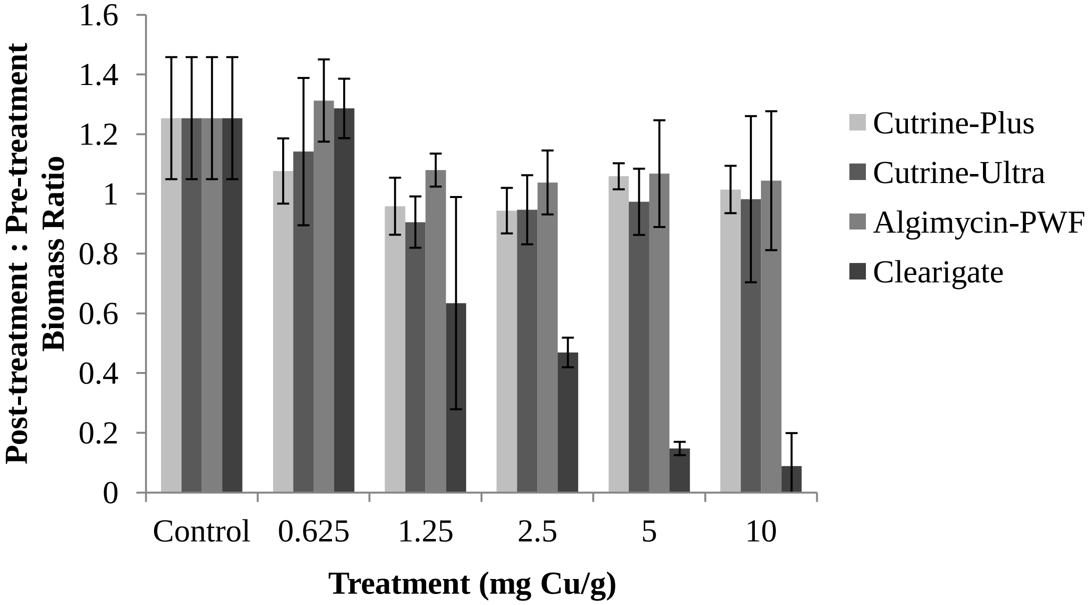

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Post-treatment : Pre-treatment Biomass Ratio
Control
0.625
1.25
2.5
5
10
Treatment (mg Cu/g)
Cutrine-Plus
Cutrine-Ultra
Algimycin-PWF
Clearigate

Fig. 3

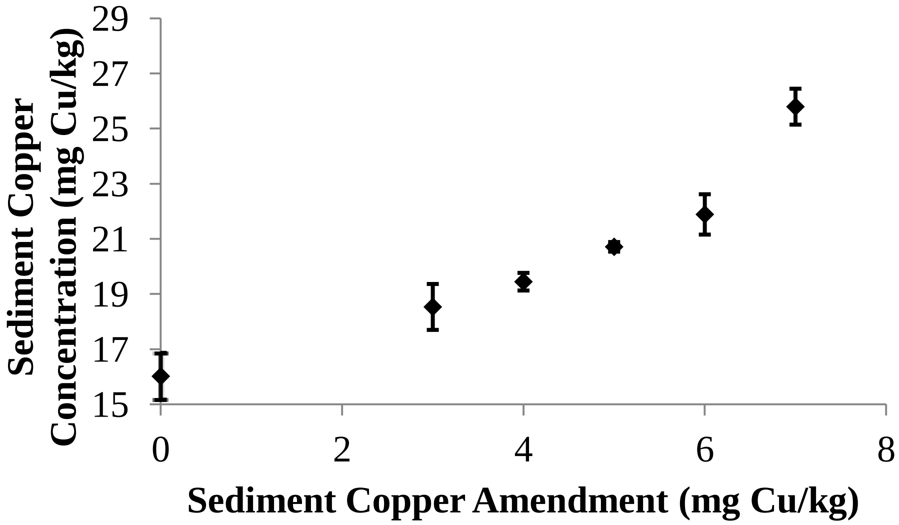
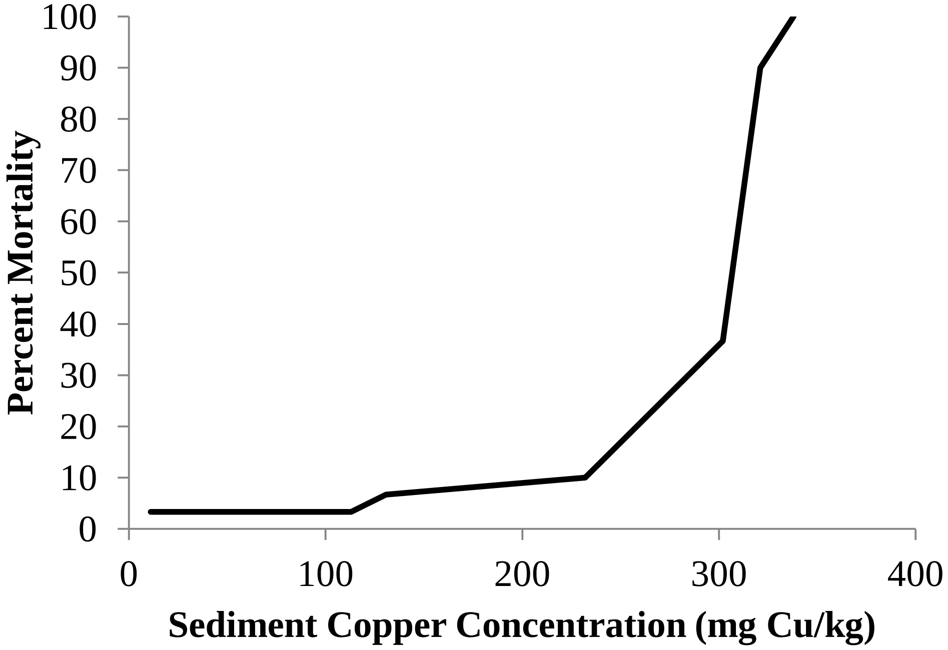

Sediment Copper Concentration (mg Cu/kg)
29
27
25
23
21
19
17
15
0
2
4
6
8
Sediment Copper Amendment (mg Cu/kg)

Fig. 4



Percent Mortality
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
Sediment Copper Concentration (mg Cu/kg)

Fig. 5

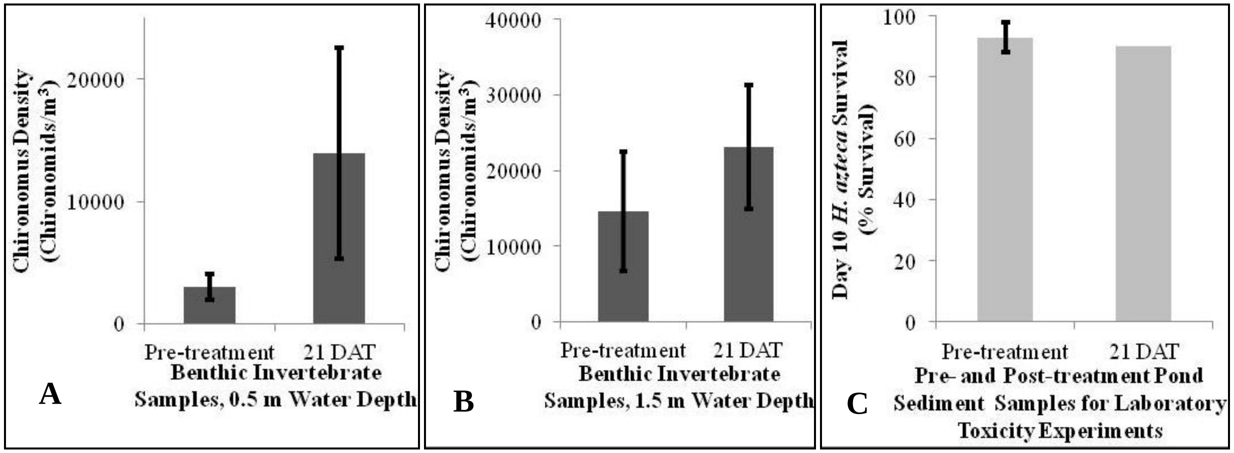

Chironomus Density
(Chironomids/m3)
20000
10000
0
Pre-treatment
21 DAT
Benthic Invertebrate
Samples, 0.5 m Water Depth
A
Chironomus Density
(Chironomids/m3)
40000
30000
20000
10000
0
Pre-treatment
21 DAT
Benthic Invertebrate
Samples, 1.5 m Water Depth
B
Day 10 H. azteca Survival
(% Survival)
100
80
60
40
20
0
Pre-treatment
21 DAT
Pre- and Post-treatment Pond
Sediment Samples for Laboratory
Toxicity Experiments
C

Fig. 6

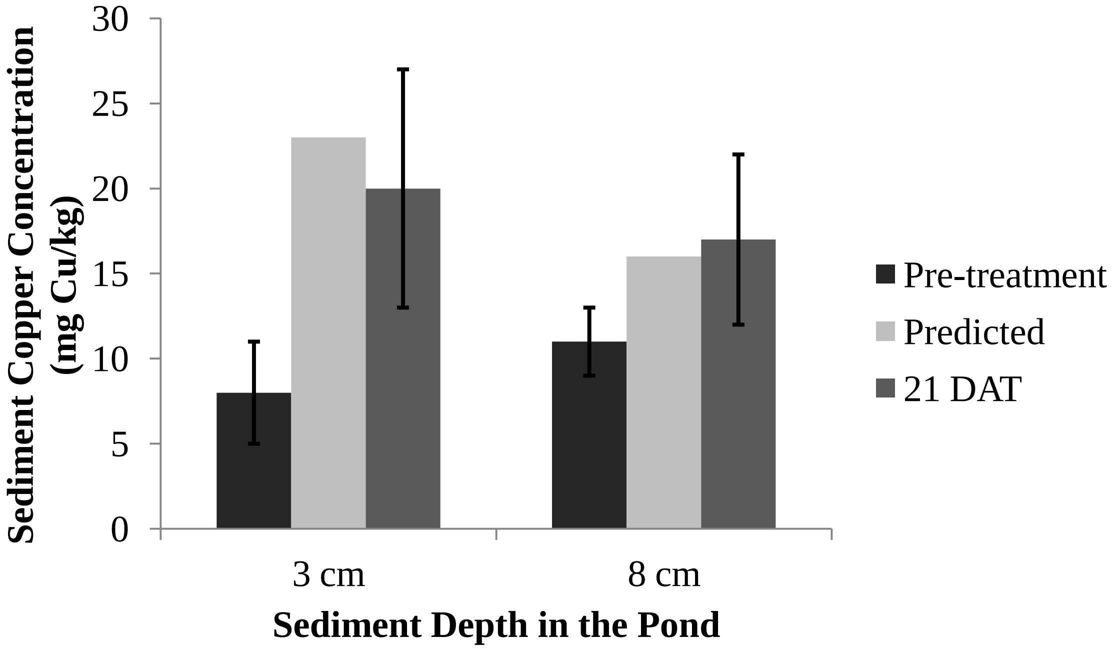

30
25
20
15
10
5
0
Sediment Copper Concentration (mg Cu/kg)
3 cm
8 cm
Sediment Depth in the Pond
Pre-treatment
Predicted
21 DAT

Fig. 7

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

Copper applied to aquatic systems as an algaecide or herbicide can accumulate in bottom sediments of water resources (Hullebusch et al. 2003; Liu et al. 2007), and there are concerns regarding those accumulations from treatments (Huggett et al. 1999; Han et al. 2001; Gallagher et al. 2005; Jones et al. 2008). The concentration and bioavailability of copper residuals in sediments from algaecide or herbicide treatments can differ depending on the site specific situation. Predictions of copper residual concentrations in sediments and responses of benthic invertebrates following site specific treatments can be beneficial for water resource managers to make informed decisions regarding risk mitigation of algae and aquatic weeds with copper-based algaecides or herbicides.

To predict the concentrations of copper residuals in sediments from algaecide or herbicide applications, a model was developed that can provide conservative estimates of copper residual concentrations in sediments from site specific treatments. The estimated range of residual copper concentrations in sediment from different possible scenarios of algaecide or herbicide treatments (based on recommendations from algaecide labels) indicated the accumulated copper concentration in sediment can be less than reported method detection limits (MDLs; lowest concentration of an analyte that can be measured with 99% confidence with a specific analytical method and matrix; Creed et al. 1994; APHA 2005) for copper in sediments. Importantly, laboratory experiments demonstrated that MDLs of copper for an analytical technique can range from 1.5 to 6 mg Cu/kg depending on the sediment. Model analysis, laboratory experiments, and field measurements indicated the mass of copper applied to a water resource, analytical technique, and sediment sampling depth influence the measurement of copper residuals in sediments from treatments. The magnitude of influence for these three parameters on measurement of copper residuals from an algaecide or herbicide treatments can be estimated with modeling and laboratory experiments. Predicted residual copper concentrations and measured MDLs of copper in sediment can provide crucial data to guide development of monitoring programs for residual copper in sediments for site specific algaecide or herbicide treatments.

Data from the laboratory toxicity experiments indicated that the bioavailability of copper residuals from an algaecide or herbicide treatment depends on characteristics of bottom sediments in a water resource. Because sediments differ in water resources (Suedel and Rodgers 1991), site specific predictions are needed to characterize the risks of copper residues in sediments. Exposing sensitive benthic invertebrates (relative to indigenous invertebrates of a water resource) to site collected sediments amended with copper algaecides or herbicides can be used for conservative predictions of benthic invertebrate responses to post-treatment sediment copper concentrations. The conservative assumptions increase the likelihood that site specific predictions provide prudent data to guide decisions for risk mitigation of algae or aquatic weeds with copper-based algaecides or herbicides. Comparisons of results from laboratory experiments and modeling to field measurements demonstrated that the methods presented in this thesis were accurate for predicting sediment copper concentrations and benthic invertebrate responses following an algaecide application to a pond. This research has increased the understanding of copper accumulation in sediments and responses of benthic invertebrates following algaecide and herbicide treatments, as well as development of laboratory experiments and modeling to predict sediment copper concentrations and benthic invertebrate responses following site specific algaecide or herbicide applications.

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