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LABORATORY AND FIELD RESPONSES OF TARGET AND NON-TARGET SPECIES TO ALGAECIDE EXPOSURES

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LABORATORY AND FIELD RESPONSES OF TARGET AND NON-TARGET SPECIES TO ALGAECIDE EXPOSURES

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by
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ABSTRACT

In order for water resource managers to effectively and efficiently react to algal growths that are prohibiting use of a lake, reservoir or stream, information must be obtained on the response of the specific algae in site waters to algaecide exposures. In the absence of this information, ineffective algaecides or excessive treatments may be implemented. Research on development of an efficient algaecide assay using site specific samples could contribute to better decisions regarding algaecide applications and increase margins of safety for non-target species. Since these laboratory experiments provide predictions of responses of algae in site waters to algaecide exposures, these predictions need to be confirmed in the field.

Confirmation of laboratory responses with results from field applications of algaecides will increase confidence in the laboratory assay. Additional research on the responses of non-target species such as sensitive fish and invertebrates to algaecide exposures will permit better decisions by water resource managers regarding potential risks associated with a site specific application of algaecide. Research is needed to clarify this situation. Objectives of this research were to 1) develop a planktonic algal bioassay using site water and copper-containing algaecides, 2) measure responses of *Lyngbya* to algaecide exposures in the laboratory and the field, and 3) contrast responses of *Pimephales promelas* and *Ceriodaphnia dubia* to laboratory and simulated field exposures of copper sulfate and Cutrine®-Plus. Results of this research indicated that a smaller

volume (≥ 100ml) and shorter duration (≥72 h) of exposure can be used in planktonic algal bioassays using cell density and water from the site. Also, this research demonstrated that algaecide effectiveness in the laboratory, when site algae and water were used, can be observed at field sites. Confirmation of laboratory responses with results from field application of algaecides increases confidence in the laboratory assay. Results also indicated that less toxicity was observed in realistic declining exposures when compared to typical constant laboratory exposures. Laboratory studies that are predictive of responses of problematic algae to exposures of algaecides in field situations, and have been verified through field studies, can provide critical information for water resource managers to take effective actions.

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I would like to thank Applied Biochemists Inc. for funding this research and assisting with sample collection.

DEDICATION

This dissertation is dedicated to my parents Conwell and Bobbie Duke, Jr., my sister, Van E. Ross and my nephew, Sherman J. Ross for their unlimited support. I would also like to dedicate this dissertation to my grandmother, Lydia Lyons and to the memory of my grandparents, Conwell and Elizabeth Duke, Sr. and my grandfather Andrew L. Lyons. My only regret is that they are not here to share in the pride and accomplishment of attaining this degree.

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CHAPTER 1

PREFACE

As the human population increases and as people move to locations adjacent to water resources, their awareness of algae and problematic growths of algae becomes more acute. With population growth comes changes in land use within watersheds and concomitant increases in nutrients and other materials in these aquatic systems (WHO 2003; Figueiredo et al. 2004). As a consequence, algal growths or "blooms" have become more prevalent. These blooms can cause problems including: 1) altered aesthetics and decreased adjacent property values (WHO 2003; Figueiredo et al. 2004); 2) interference with recreational activities such as fishing, boating, and swimming (Brown et al. 1982; WHO 2003; Figueiredo et al. 2004); 3) adverse effects on drinking water including production of taste and odor compounds (e.g. geosmin and 2-methylisoborneal) (Mastin et al. 2002); 4) the presence of off flavor compounds in fish and other vertebrates (WHO 2003), and 5) production of toxins directly impacting invertebrates, fish, avian species, and mammals including humans (Behm 2003; WHO 2003; Wilde et al. 2005). Since these water resources have become used for more purposes, as well as more extensively, control is required of algal growths causing adverse impacts on water resource usages when critical usages are interrupted or prohibited by the algae. It is important for water resource managers to evaluate all options when developing a control strategy for problematic algal growths.

Options for controlling algal growths include physical, biological, mechanical, and chemical. Physical tactics may include benthic barriers or dyes that attenuate photosynthetically active radiation. These approaches are of limited utility due to their costs and specific environmental requirements for successful performance. Mechanical devices such as harvesters and cutters may be used, but these tactics are usually very costly, have limited areas of applicability, and cause severe collateral environmental damage. Biological control may include phytophagous fish such as a grass carp (*Ctenopharyngodon idella*). However, there is a concern that grass carp extirpate native fish, consume native plants, do not eat the targeted algae, and there is concern that the grass carp may actually serve as a vector for avian vacuolar myelinopathy (AVM) to fish-eating birds (Wilde et al. 2005; Bowerman-personal communication 2006). Clearly, chemical tactics involving algaecides need to be carefully evaluated for use in critical situations where cost, ability to produce a rapid response, and the lack of toxicity to non-target species are critical (Quimby 1981; Kay et al. 1983; Kay et al. 1984; Mastin and Rodgers 2000; Mastin et al. 2002).

In order for water resource managers to effectively and efficiently react to algal growths that are prohibiting use of a lake, reservoir, or stream, information must be obtained on the response of the specific algae in site waters to algaecide exposures. In the absence of this information, ineffective algaecide or excessive treatments may be implemented. Research on development of an efficient algaecide assay using site specific samples could contribute to better decisions regarding algaecide applications and increase margins of safety for non-target

species. Since these laboratory assays or experiments provide predictions of responses of algae in site waters to algaecide exposures, these predictions need to be confirmed in the field. Confirmation of laboratory responses with results from field application of algaecides will increase confidence in the laboratory assay. Additional research on the responses of non-target species such as sensitive fish and invertebrates to algaecide exposures will permit better decisions by water resource managers when evaluating the risks associated with a site specific application of algaecide. Laboratory toxicity data for non-target species are typically developed using laboratory waters and relatively constant or non-varying exposures. In field applications of algaecides, the exposures rapidly decline with time. Fundamentally, the responses of non-target species to these exposures should differ significantly. If water resource managers use laboratory data to directly predict responses of non-target species in the field to algaecide exposures, the risks of an application may be greatly overestimated. Research is needed to clarify this situation.

An efficient planktonic algal bioassay using site water and copper-containing algaecides

In order to predict responses of problematic algal species in site waters to algaecide exposures, representative samples of algae in site water are shipped to the laboratory and responses of the algae to algaecide exposures can be measured. Obviously, algal species vary in their responses to an algaecide (Murray-Gulde et al. 2002), and algaecides vary in their potency for a given algal species (Heatley 2002). Importantly, site water characteristics may also influence the

bioavailability of an algaecide resulting in a strong influence on the responses of an algal species to an exposure. Current procedures for evaluating responses of algal species in site waters to algaecide exposures involve collection and shipping of considerable volumes of water (8-16 L). If the volume of water could be reduced and the same results could be obtained, then considerable savings in terms of resources would be achieved. The current laboratory procedures require approximately 10 to 14 days to predict responses of the algae at a field site to an algaecide exposure. If the time required to obtain predictive results regarding algal responses to algaecide exposures could be reduced to a few days, then field applications could be implemented in a timely fashion. In critical situations (e.g. when potent toxins are being produced in vital water resources), initiation of treatment as soon as possible may be critical to mitigate risks.

Responses of Lyngbya to algaecide exposures in the laboratory and field

Application of laboratory results to field situations has been an area of interest particularly in the case of problematic algal species. Laboratory tests have been developed to predict responses of algae to nutrient exposures (US EPA 1985). These laboratory experiments were confirmed to some extent in field studies (Auer et al. 1986). Similarly, we need to confirm, through *in situ* studies, the accuracy of predictions from laboratory studies of responses of algal species to algaecides. The fundamental question is: can similar responses be expected if laboratory exposures are essentially duplicated in the field?

Responses of Pimephales promelas Rafinesque and Ceriodaphnia dubia Richard to laboratory and simulated field exposures of copper-sulfate pentahydrate and Cutrine®-Plus

Water resource managers evaluate responses of both target and non-target species to potential treatment chemicals (i.e. algaecides) prior to application. Typically, data from laboratory studies of responses of sensitive invertebrates and fish (USEPA 2002) are contrasted with the necessary treatment concentrations of algaecides used to control problematic algae. The result of this comparison is often called the "margin of safety" for the non-target species where the lowest observed effects concentration for the non-target species is divided by the concentration of the algaecide that is required to control the algae. If the ratio is greater than one, there is a margin of safety associated with the algaecide use. Such a simple calculation and evaluation can greatly overestimate the risk to non-target species associated with an algaecide application if the laboratory results do not accurately predict the responses of the non-target species in the field situation. Field observations indicate that the laboratory results developed from toxicity testing utilizing continuous (essentially non-varying) exposures, may over estimate the toxicity observed in the field due to a declining (i.e. pulse) exposure in an algaecide application. Additional data are needed to determine whether or not the laboratory exposures are predictive of field responses of non-target species. More accurate predictions of the responses of non-target species to algaecide exposures will permit water resource managers to make more defensible decisions regarding mitigation strategies for problematic algae.

Research Objectives and Rationale

With the foregoing discussion in mind, this research was initiated to address some pressing questions regarding the utilization of algaecides to control growths of problematic algae in water resources. Three issues were addressed in this research:

1. An efficient planktonic algal bioassay using site water and copper-containing algaecides;
2. Responses of *Lyngbya* to algaecide exposures in the laboratory and the field;
3. Responses of *Pimephales promelas* Rafinesque and *Ceriodaphnia dubia* Richard to laboratory and simulated field exposures of copper sulfate pentahydrate and Cutrine®-Plus.

The initial research focused on decreasing the volume, time, and resources expended in laboratory experiments to predict responses of problematic algae to algaecide exposures. The objective of this research effort was to measure the responses of problematic algae to algaecide exposures in a series of volumes of site waters. The time to response was also measured to determine the minimum time required to discern responses of the algae to the algaecide exposures.

The second objective of this research involved comparison of laboratory predictions of responses of problematic algal species to algaecide exposures with responses observed in the field using site waters. The fidelity of the laboratory predictions should be confirmed in the field to increase confidence in using the

necessary amount of algaecide to gain control of problematic algae even if the required amount is less than the recommended concentration or maximum allowable amount that could be applied. Using as much of an effective algaecide as needed, but not using excess algaecide, should increased the margin of safety for non-target species and decrease costs of applications or treatments.

To accomplish the third objective of this research, laboratory responses of sensitive non-target species (vertebrates: *Pimephales promelas* and invertebrates: *Ceriodaphnia dubia*;) to algaecide exposures were compared with simulated field algaecide exposures. The essentially constant or non-varying traditional exposures were contrasted with pulse or declining exposures. The pulse and declining exposures are more representative of an algaecide application in the field. If the non-target species respond differently to these divergent exposures, then water resource managers need to reconsider their estimates or predictions of risks when considering algaecide applications in the field.

Organization of this Dissertation

This dissertation consists of five chapters, including the Preface (Chapter 1) and Summary (Chapter 5). The body of this dissertation is comprised of three independent manuscripts formatted for publication in a scientific journal. Therefore, some redundancy of material was necessary. These manuscripts and their targeted journal are:

Chapter II: An efficient planktonic algal bioassay using site water and copper-containing algaecides, prepared

for submission to the *Journal of Aquatic Plant Management*.

Chapter III. Responses of *Lyngbya* to algaecide exposures in the laboratory and the field, prepared for submission to the *Journal of Aquatic Plant Management*.

Chapter IV. Responses of *Pimephales promelas* Rafinesque and *Ceriodaphnia dubia* Richard to laboratory and simulated field exposures of copper sulfate pentahydrate and Cutrine®-Plus, prepared for submission to the *Journal of Aquatic Plant Management*.

Together, these manuscripts contribute to enhanced understanding of toxicity of algaecides to target and non-target species in waters that are maintained by water resource managers.

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CHAPTER 2

AN EFFICIENT PLANKTONIC ALGAL BIOASSAY USING SITE WATER AND COPPER-CONTAINING ALGAECIDES.

Prepared for submission to the

Journal of Aquatic Plant Management

Abstract

Algaecides are applied in field situations when problematic algae interfere with water resource usages. Prediction of algal responses using laboratory exposures of algaecides is necessary in order to efficaciously treat the algae in field sites. Current laboratory testing requires relatively large volumes of water (~20 L) and as long as 14 days between receipt of the sample and reporting of test results. The primary objective of this laboratory research was to contrast responses of target algae using standard testing parameters (i.e. 200 ml; 96 h) with lesser volumes and shorter exposure durations. Algaecide exposures in the laboratory ranged from 0.2-1.0 mg Cu / L as Cutrine®-Ultra, Cutrine®-Plus, Algimycin® PWF, and copper sulfate pentahydrate. Laboratory exposure volumes were 25, 50, 100, and 200 ml. Responses of algae were measured as chlorophyll *a* concentrations and cell density at sampling intervals of 24, 48, 72, and 96 h. Following a 72 h exposure duration in a 100 ml exposure volume, no algae were observed in the Aquaculture Pond experiments and the Pawnee Reservoir experiments at concentrations of 0.8 and 0.4 mg Cu / L as Cutrine®-Ultra , respectively. These data support using an exposure volume of no less than 100 ml with an exposure duration of at least 72 hours to predict field responses. Therefore, if a representative site water sample contains algae that are sensitive to algaecide application, algae will respond more rapidly (24 to 48 h) after treatment. However, if the water sample is not representative, or contains algae which are tolerant to algaecides, then larger sample volumes and longer durations (≥ 96 h) of exposures may be required.

Introduction

The incidence of problematic algae may become more prevalent with the onset of global climate change (Hallegraeff et al. 1993; Behm 2003; Haines et al. 2000). When these nuisance algal species interfere with critical water resources usages such as domestic water supply, livestock watering, subsistence fishing, and irrigation, immediate control actions by water resource managers may be required (Brown and Boyd 1982; Figueiredo et al. 2004). Often, chemical control, such as strategic application of an algaecide, is a cost-effective and environmentally sound response to pressing algal problems such as extreme densities or blooms of planktonic algae and production of taste and odor compounds or toxins (Fitzgerald and Jackson 1979; Murray-Gulde et al. 2002; Franklin et al. 2002). However, numerous field trials may be required to discern an effective algaecide for a particular species at a specific site. These field trials often involve applications at the maximum label concentration (e.g. 1 mg Cu / L) even if an exposure of that magnitude is not required to control the algae. Field trials are also costly because they are performed on a larger scale and may adversely affect non-target species at maximum label rates. A laboratory screening approach evaluating algaecide exposures to control problematic algae in site waters could be useful to discern treatments that would be efficient and effective in the field (Murray-Gulde et al. 2002; Duke et al. 2007, Chapter 3; Tedrow 2007).

Laboratory algal toxicity experiments have been effective predictors of field responses to algaecide applications (Duke et al. 2007, chapter 4; Tedrow 2007). However, laboratory testing currently requires relatively large volumes

(20 L) of water and more than a week (~14 days) to complete the experiment and report the results to water resource managers. Meanwhile algal problems may become more acute and may approach densities requiring multiple treatments. If communication of results from the screening tests is delayed, algal densities or populations may drift so the indicated treatment based on laboratory data will be unreliable.

The standard algal toxicity test, US EPA (2002) method (1003), recommends at least five experimental concentrations of test material, with a volume of 100 ml per experimental chamber and a minimum of four replicates. US EPA also suggests an initial algal cell density of 10,000 cells / ml and a mean control cell density of 1.0×10^6 cells / ml at experiment conclusion. According to the US EPA, endpoints considered are cell counts, absorbance, biomass, and chlorophyll *a* fluorescence. Experimental chambers are placed on a rotary or oscillatory shaker at 100 rpm continuously for the experimental period or swirled twice daily by hand. US EPA also recommends that experiments should be conducted using controlled temperature (25 ±1 ºC), and continuous light.

This research was prompted by the need for a laboratory algaecide screening approach producing the same useful information as the current method, but requiring a lesser volume of site water and shorter test duration. Therefore intervention in the field could occur sooner. It would be important to verify that the same information could be obtained with lesser volume and decreased exposure time. The objectives of these experiments were to: 1) determine if a lesser volume (100 ml, 50 ml, or 25 ml) and a shorter duration of exposure (72 h,

48 h, or 24 h) produce the same results (e.g. not significantly different) as a larger volume (200 ml) and longer duration exposure (96 h) using copper-containing algaecides (Table 2.1) in laboratory toxicity tests with site water containing problematic algae, and 2) determine if algal responses (chlorophyll *a*, and cell density) to exposures of algaecides change with decreases in volume and time.

Materials and Methods

Characteristics of Site Waters

Site waters used for these experiments were obtained from an aquaculture pond at Clemson University located in Pickens County, South Carolina, (Figure 2.1) and from Pawnee Reservoir, a recreational reservoir, located in Lancaster County, Nebraska (Figure 2.2; Table 2.2). Algal taxa in the aquaculture pond included *Chlorella* sp., *Chroococcus* sp., *Ulothrix* sp., *Scenedesmus* sp., *Staurastrum* sp., *Microcystis* sp., *Mallamonas* sp., and *Aphanizomenon* sp. (Table 2.3). Site water from Pawnee Reservoir contained *Microsytis aeruginosa* (Table 2.3). Characteristics of site waters that were measured included pH (standard units), dissolved oxygen (mg O_2 / L), conductivity (µS / cm^2), alkalinity (mg / L as $CaCO_3$), hardness (mg / L as $CaCO_3$), and temperature (°C) and these parameters were measured according to APHA (1998).

Table 2.1. Physical properties and characteristics of Cutrine®-Ultra, Cutrine®-Plus, Algimycin®-PWF, and Copper Sulfate.

	Cutrine®-Ultra	Cutrine®-Plus	Algimycin®-PWF	Copper Sulfate
% of Cu as elemental[b]	9.0	9.0	5.0	25.4
Application Rate (mg Cu/L)	0.2-0.8	0.2-1.0[b]	0.06-0.5[b]	0.05-0.5[c]
Formulation[a,b]	Copper-ethanolamine in an emulsified complex	Copper-ethanolamine Complex	Chelates of copper Citrate and copper gluconate	$CuSO_4 \bullet 5H_2O$
Chemical class[a,b]	Chelated elemental copper (Cu_2CO_3)	Chelated elemental copper (Cu_2CO_3)	Chelated elemental copper (Cu_2CO_3)	Copper salt
Mode of action[a,b]	cell toxicant[a]	Cell toxicant[a]	Cell toxicant[a]	Cell toxicant[a]
Appearance[a,b]	Blue viscous liquid	Blue viscous liquid	Blue viscous liquid	Blue crystalline
Odor[b]	Orange	Slight amine	Slight amine	N/A
Water solubility (mg/L)[a,b]	Complete	Complete	Complete	316,000
Boiling point (^oC)[b]	100	100	Not determined	N/A
Melting point (^oC)[a,b]	N / A	N/A	N/A	110
Specific gravity (g/cm^3)[b]	1.22	1.21	1.20	N/A
pH[b]	10.0-10.5	10.0-11.0	1.5-2.0	N/A

Karmin 1997

Table 2.2. Experimental water characteristics for water from Aquaculture Pond, Pickens County, South Carolina and Pawnee Reservoir, Lancaster County, Nebraska.

Site water (Algaecide)	pH (S. U.)	Hardness mg / L (as $CaCO_3$)	Alkalinity mg / L (as $CaCO_3$)	Conductivity ($\mu S / cm^2$)	Dissolved Oxygen (mg Oxygen / L)
Aquaculture Pond (Cutrine®-Ultra)	7.07 - 8.11	50 – 52	50 – 52	155 - 173	6.7 - 8.9
Aquaculture Pond (Cutrine®-Plus)	7.34.- 8.75	50 – 52	50 – 54	172 - 175	7.9 - 8.5
Aquaculture Pond (Algimycin®-PWF)	7.45 - 8.65	52 – 54	50 – 54	188 - 209	7.4 - 12.9
Aquaculture Pond ($CuSO_4*5H_2O$)	7.35 - 8.71	54 – 56	50 – 54	169 - 174	6.7 - 10.8
Pawnee Reservoir (Cutrine®-Ultra)	8.0 - 9.0	164 – 176	150 – 168	703 - 789	6.7 - 10.1

Table 2.3. Average cell densities of algae in site waters from Aquaculture Pond, Pickens County, South Carolina and Pawnee Reservoir, Lancaster County, Nebraska at the initiation of experiments.

Sample Location	Taxa Present	Average Cells / ml (±std. dev.)
Pawnee Reservoir	*Microcystis* sp.	3.16×10^5 ($\pm 1.09 \times 10^4$)
Aquaculture Pond	*Chlorella* sp.	1.74×10^5 ($\pm 1.08 \times 10^4$)
Aquaculture Pond	*Chroococcus* sp.	1.47×10^5 ($\pm 11.02 \times 10^4$)
Aquaculture Pond	*Ulothrix* sp.	1.64×10^5 ($\pm 12.59 \times 10^4$)
Aquaculture Pond	*Scenedesmus* sp.	5.06×10^4 ($\pm 7.6 \times 10^3$)
Aquaculture Pond	*Staurastrum* sp.	3.33×10^4 ($\pm 1.19 \times 10^3$)
Aquaculture Pond	*Microcystis* sp.	3.19×10^5 ($\pm 2.73 \times 10^4$)
Aquaculture Pond	*Mallamonus* sp.	1.13×10^4 ($\pm 2.74 \times 10^4$)
Aquaculture Pond	*Aphanizomenon* sp.	2.49×10^5 ($\pm 3.70 \times 10^4$)

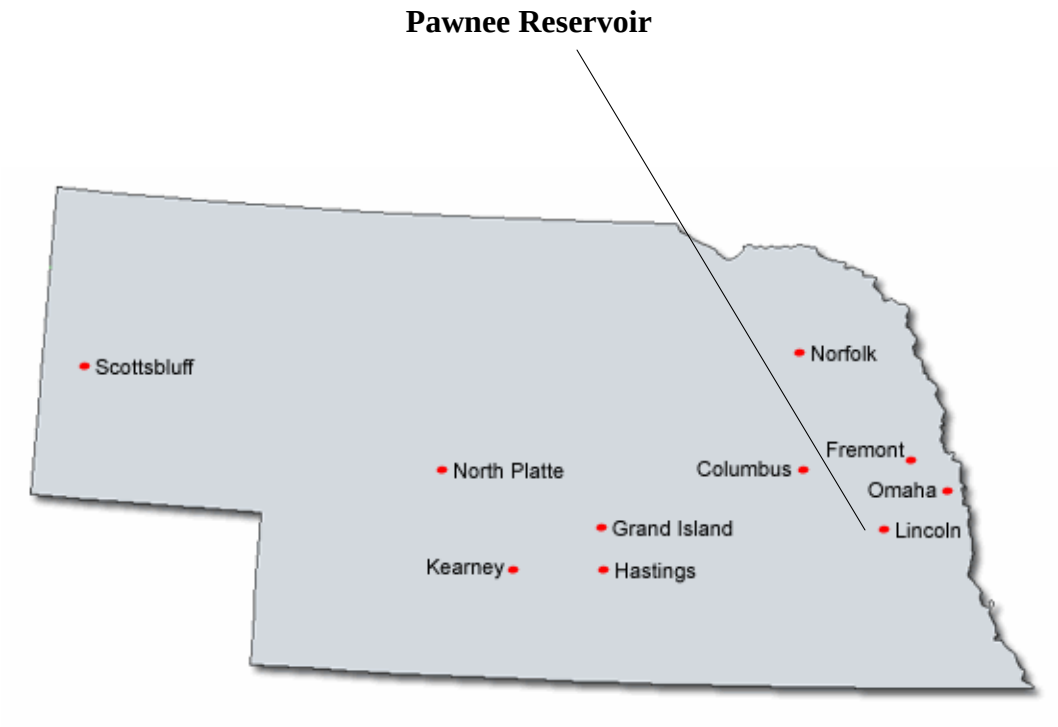


Figure 2.1. Site location map of Pawnee Reservoir, Lancaster County, Nebraska.

Figure 2.2. Site location map of Aquaculture Pond, Clemson, Pickens County, South Carolina.

Experimental Procedure

Treatment concentrations of these algaecides consisted of 0.2, 0.4, 0.6, 0.8 and 1.0 mg Cu / L as algaecide, and each treatment contained four replicates. Treatment concentrations were prepared by adding stock algaecide solution (stock = 100 mg Cu / L) to site water and diluting to volume in a 1-L volumetric flask using site water. Algal test exposures were conducted for 96 hours. All experiments were conducted in light-and- temperature controlled settings at 21-25 ºC with a 16 h light / 8 h dark photoperiod using "cool white" fluorescent lighting (US EPA 2002). Experimental chambers were borosilicate Erlenmeyer flasks (US EPA 1978; US EPA 2002), and exposure volumes consisted of 200 ml (US EPA 2002), 100 ml, 50 ml, and 25 ml. Site waters in Erlenmeyer flasks were mixed by swirling twice daily by hand (US EPA 2002). To confirm algaecide exposures in the test chambers, acid extractable (US EPA 1983) and soluble (US EPA 1985) copper concentrations were measured using a Perkin-Elmer AAnalyst 5100 flame and graphite furnace atomic absorption (AA) spectrometer (APHA 1998).

Toxicity Testing Response Parameters and Data Analysis

Chlorophyll *a* was measured fluorometrically using a SpectraMax® 190 Gemini spectrofluormeter (Molecular Devices Corporation, Sunnyvale, California 94089) with an excitation wavelength of 430 nm and an emission wavelength of 663 nm (APHA 1998). An Improved Neubauer Hemacytometer was used to measure cell density (APHA 1998). Chlorophyll *a* and cell density were measured at experiment initiation, 24 h, 48 h, 72 h, and 96 h (experiment

conclusion) for each treatment. At the conclusion of each experiment, statistical analyses of response parameters were accomplished using Sigma Stat® software and included analysis of variance (ANOVA; ά =0.05) followed by Bonferroni's T-test if data were normally distributed and variances were homogenous (ά =0.05). If the data did not meet the assumptions for parametric testing, then non-parametric ANOVA Dunn's test on ranked data were used (Ott 1993).

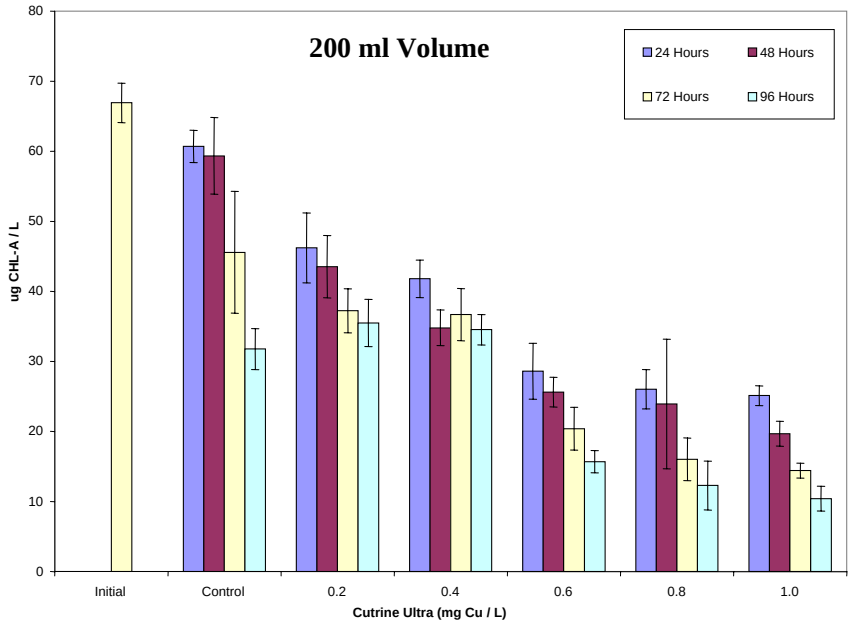
Results

Experimental Water Chemistry Parameters of Site Waters

Water chemistry parameters were measured from samples of water from the Aquaculture Pond and Pawnee Reservoir (Table 2.2). The pH of water from the Aquaculture Pond ranged from 7.07 - 8.71. Hardness and alkalinity ranged from 50-56 mg / L as $CaCO_3$ and 50-54 mg / L as $CaCO_3$, respectively. Conductivity and dissolved oxygen ranged from 155 - 209 µS / cm^2 and 6.7 - 12.9 mg O_2 / L, respectively. Pretreatment acid extractable and soluble copper concentrations, and associated standard deviations, were 6.2 µg Cu / L (± 0.01) and 4.3 µg Cu / L (±0.03). The pH of water from Pawnee Reservoir ranged from 8.0-9.0 SU. Hardness and alkalinity ranged from 164-176 mg / L as $CaCO_3$, and 150-168 mg / L as $CaCO_3$, respectively. Conductivity and dissolved oxygen ranged from 703-789 µS / cm^2 and 6.7-10.1 mg O_2 / L. Pretreatment acid extractable and soluble copper concentrations were 23.1 µg (± 2.8) and 17.8 µg Cu / L (±2.3), respectively.

Experimental Volume and Duration

Based upon the laboratory experiments with these two waters, a lesser volume (≥ 100 ml per replicate) and a shorter duration of exposure (≥ 72 h) can provide the same results in algal toxicity tests as the current method which involves 200 ml per replicate and 96 h of exposure. There were no statistically significant differences in the responses of algae to algaecide exposures ≥ 72 h (Figures 1-10) when cell density was the endpoint measured. Further, there were no significant differences in terms of cell densities in the algaecide exposures in volumes of 100 and 200 ml (Figures 1-10). Lesser volumes (e.g. 25 ml to 50 ml) and shorter durations of algaecide exposures (24h and 48h) produced results that differed significantly from results obtained from the "standard" 200 ml volume and 96 h exposure duration.



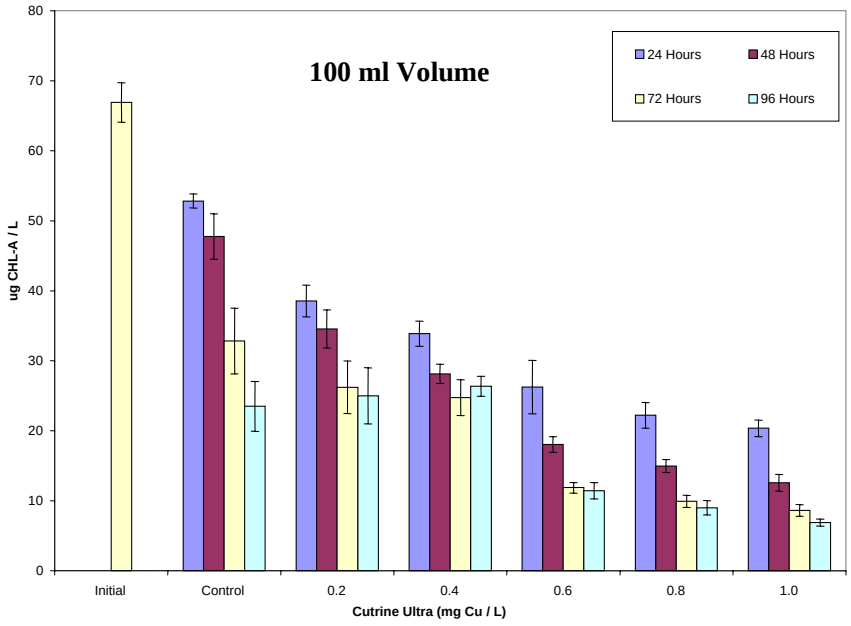
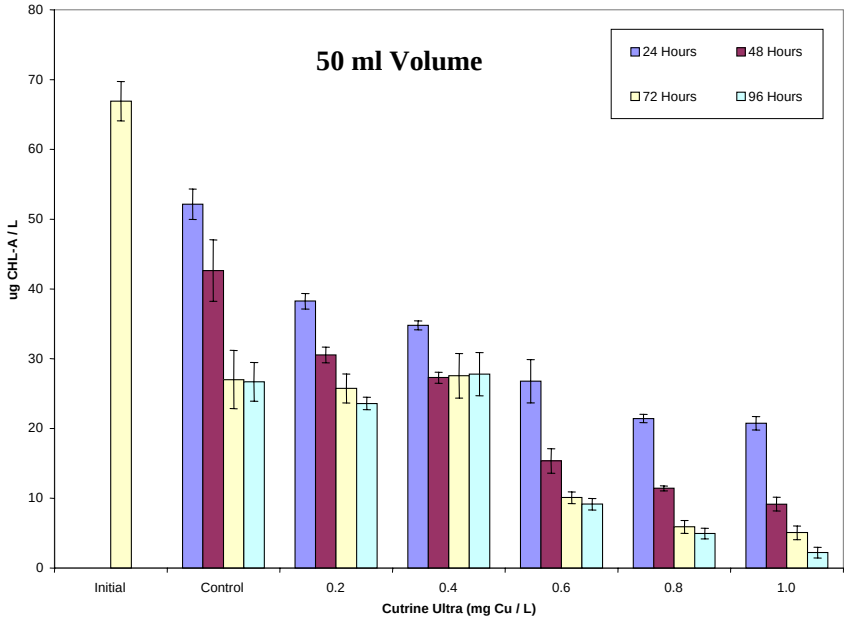


Figure 2.3. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations.



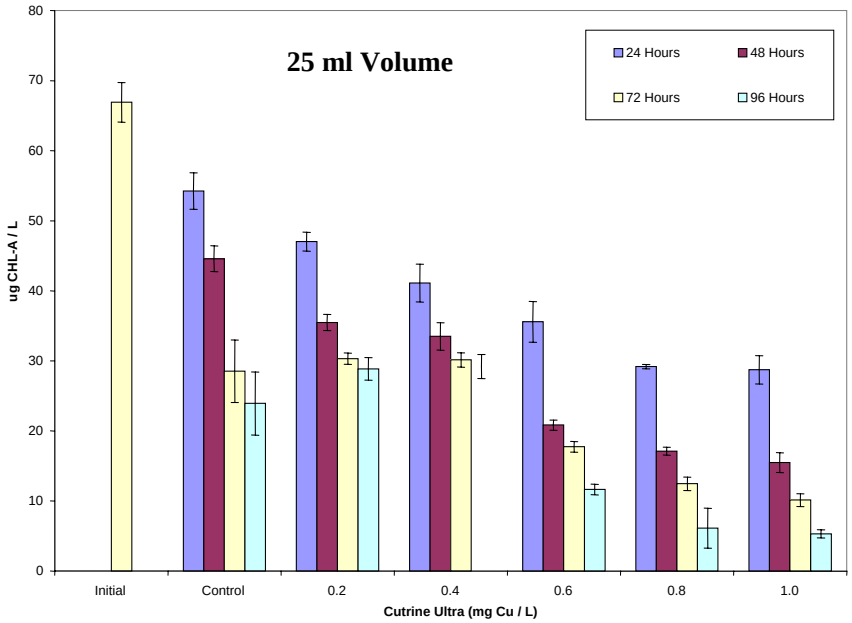
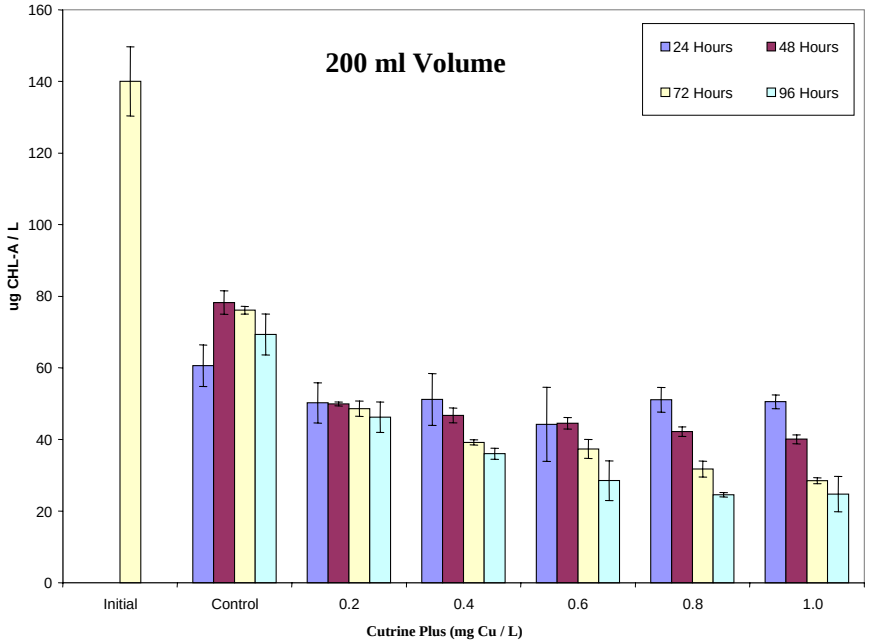


Figure 2.3. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations (Continued).



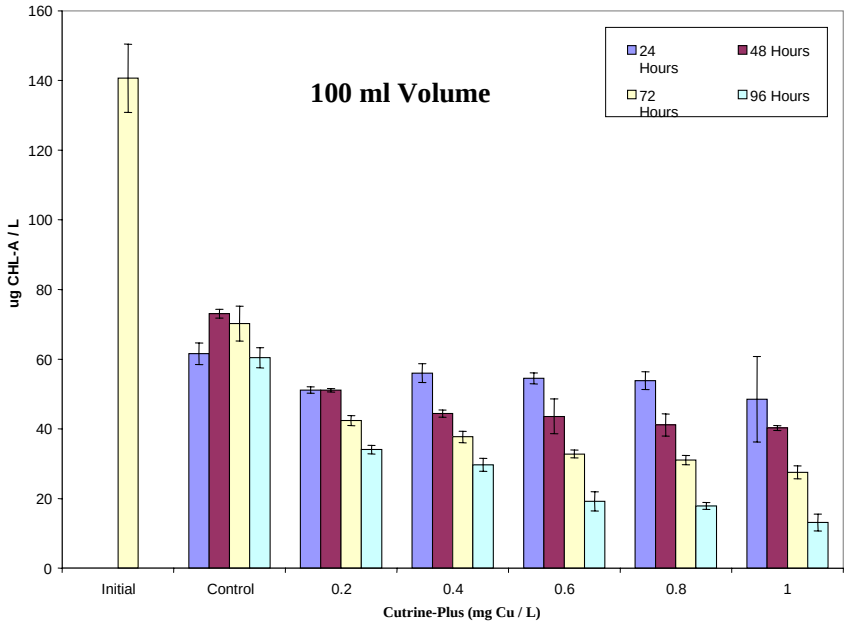


Figure 2.4. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Plus exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations.

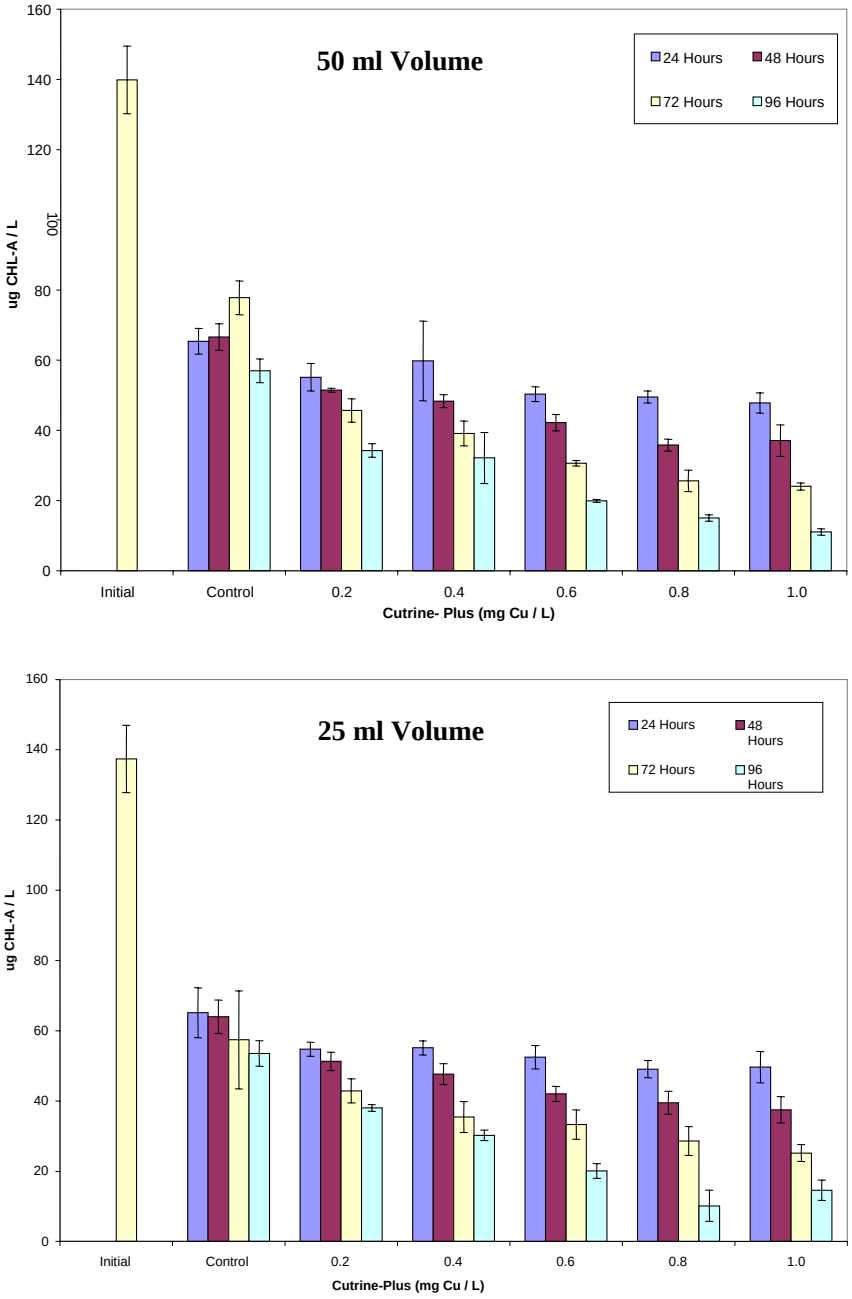


Figure 2.4. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Plus exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations (Continued).

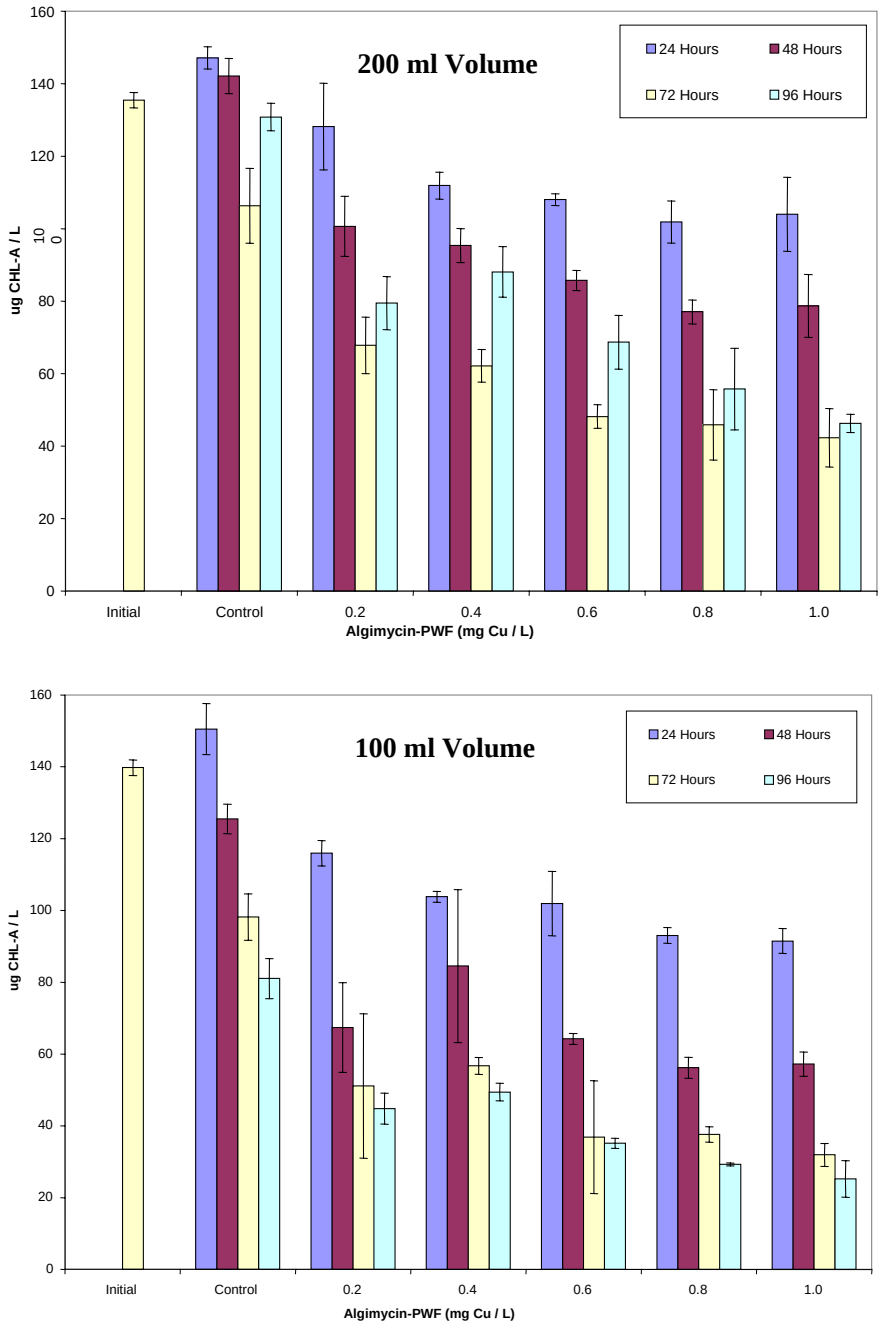


Figure 2.5. Responses of algae in samples from the Aquaculture Pond to Algimycin®-PWF exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations.

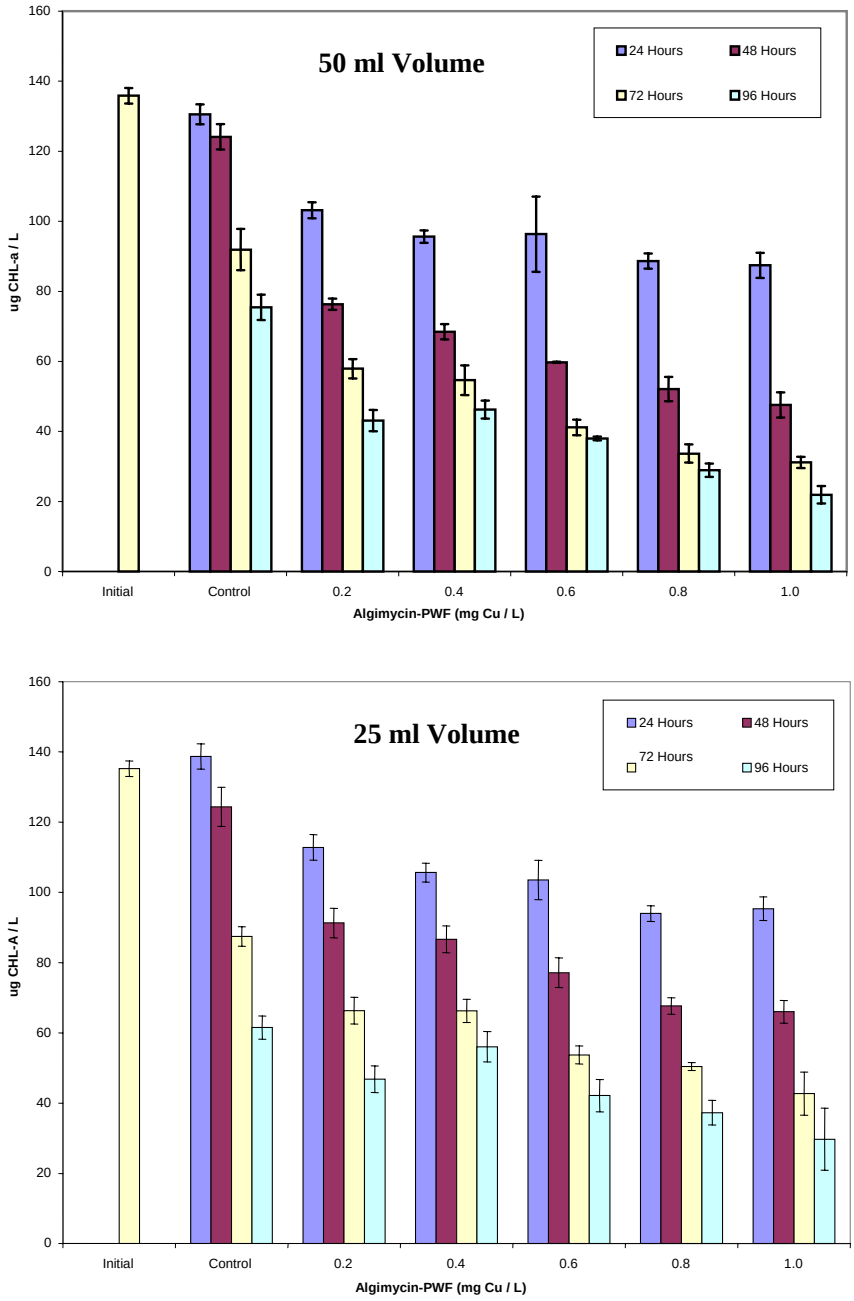


Figure 2.5. Responses of algae in samples from the Aquaculture Pond to Algimycin®-PWF exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations (Continued).

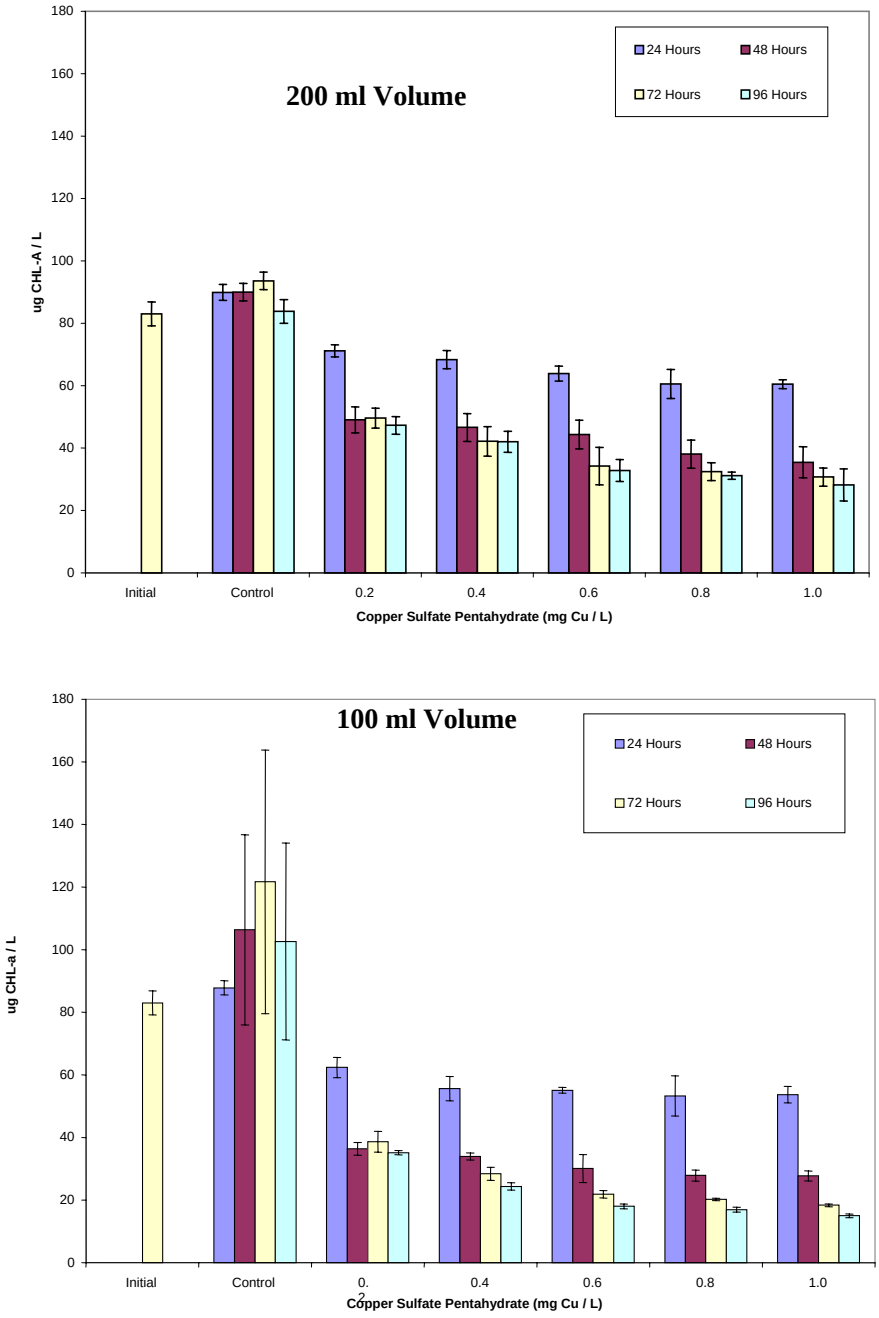
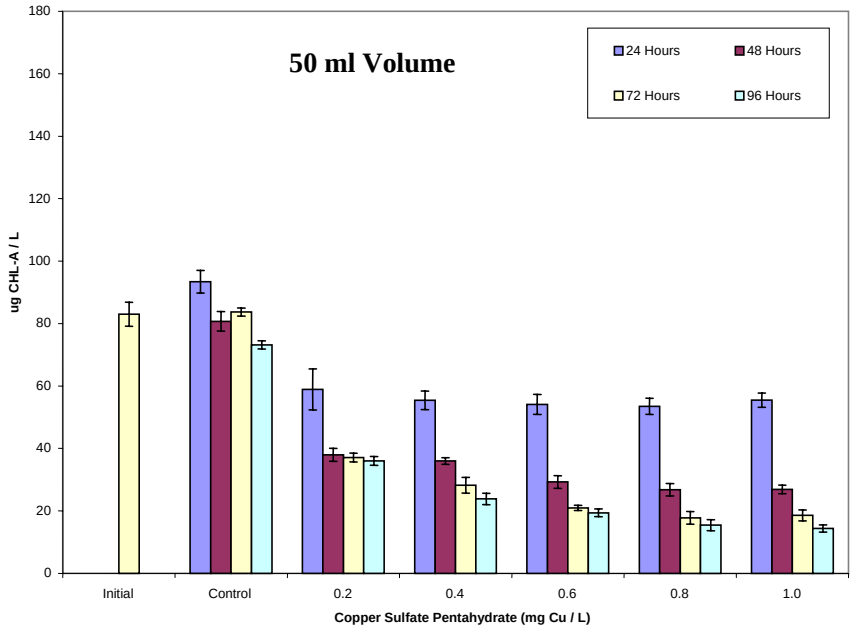


Figure 2.6. Responses of algae in samples from the Aquaculture Pond to copper sulfate pentahydrate exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of chlorophyll *a* concentrations and associated standard deviations.



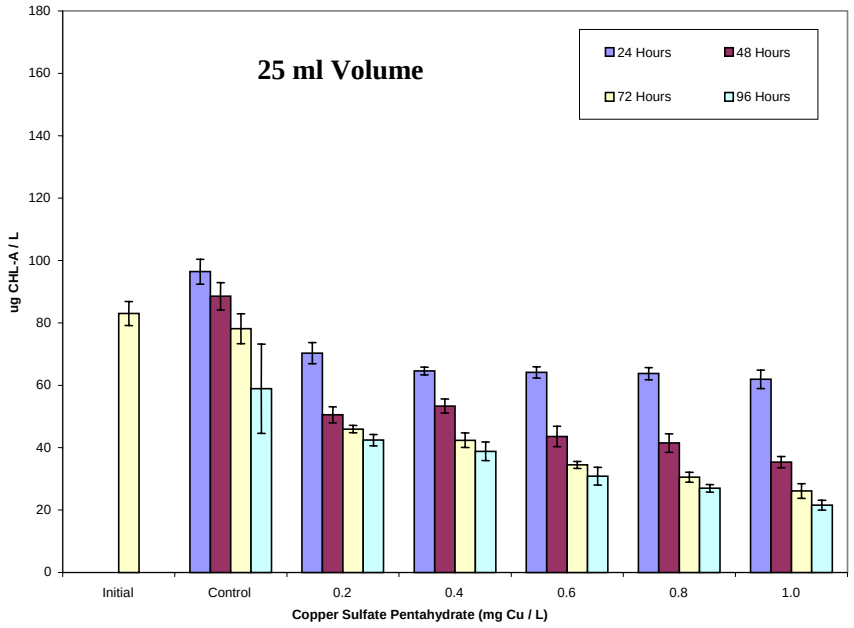


Figure 2.6. Responses of algae in samples from the Aquaculture Pond to copper sulfate pentahydrate exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of Chlorophyll *a* concentrations and associated standard deviations (Continued).

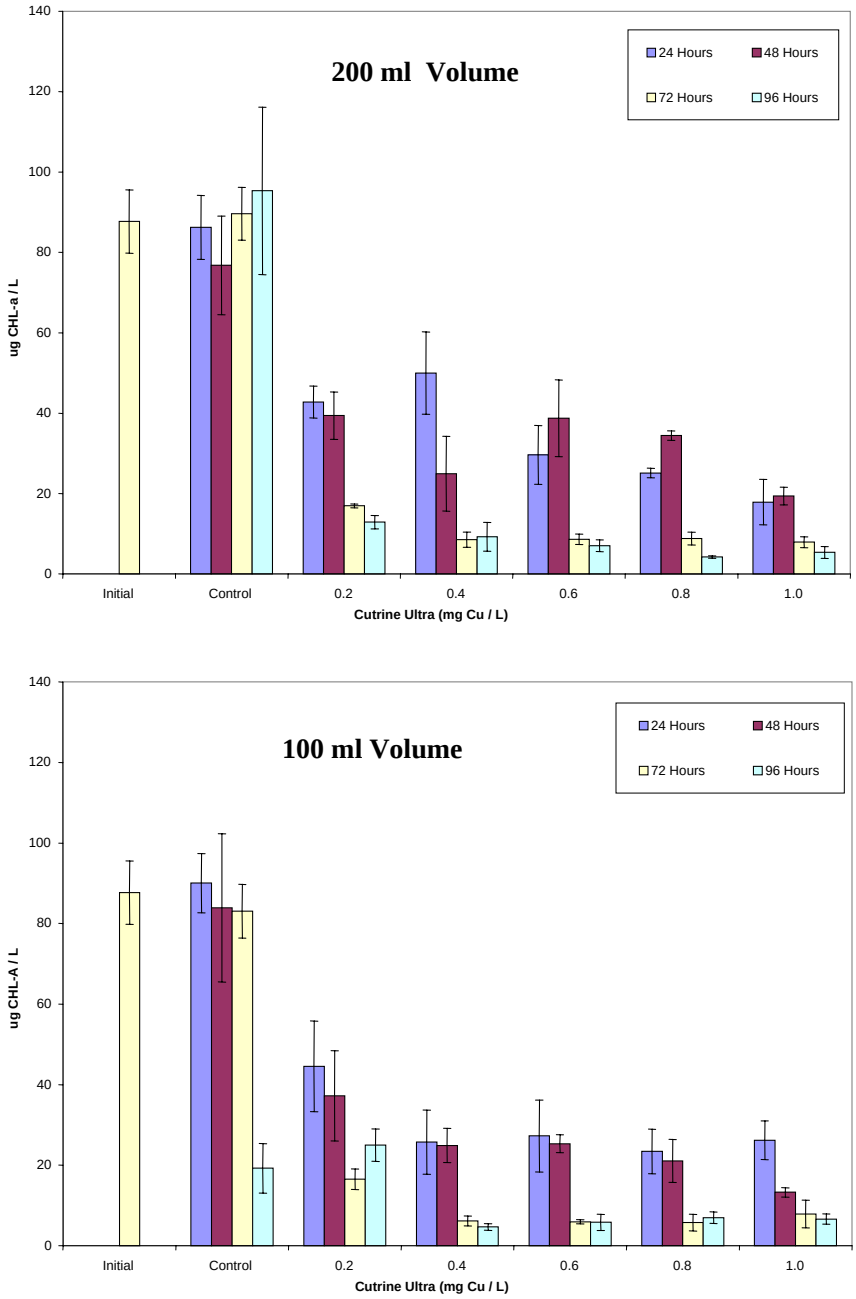


Figure 2.7. Responses of algae in samples from Pawnee Reservoir to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of Chlorophyll *a* concentrations and associated standard deviations.

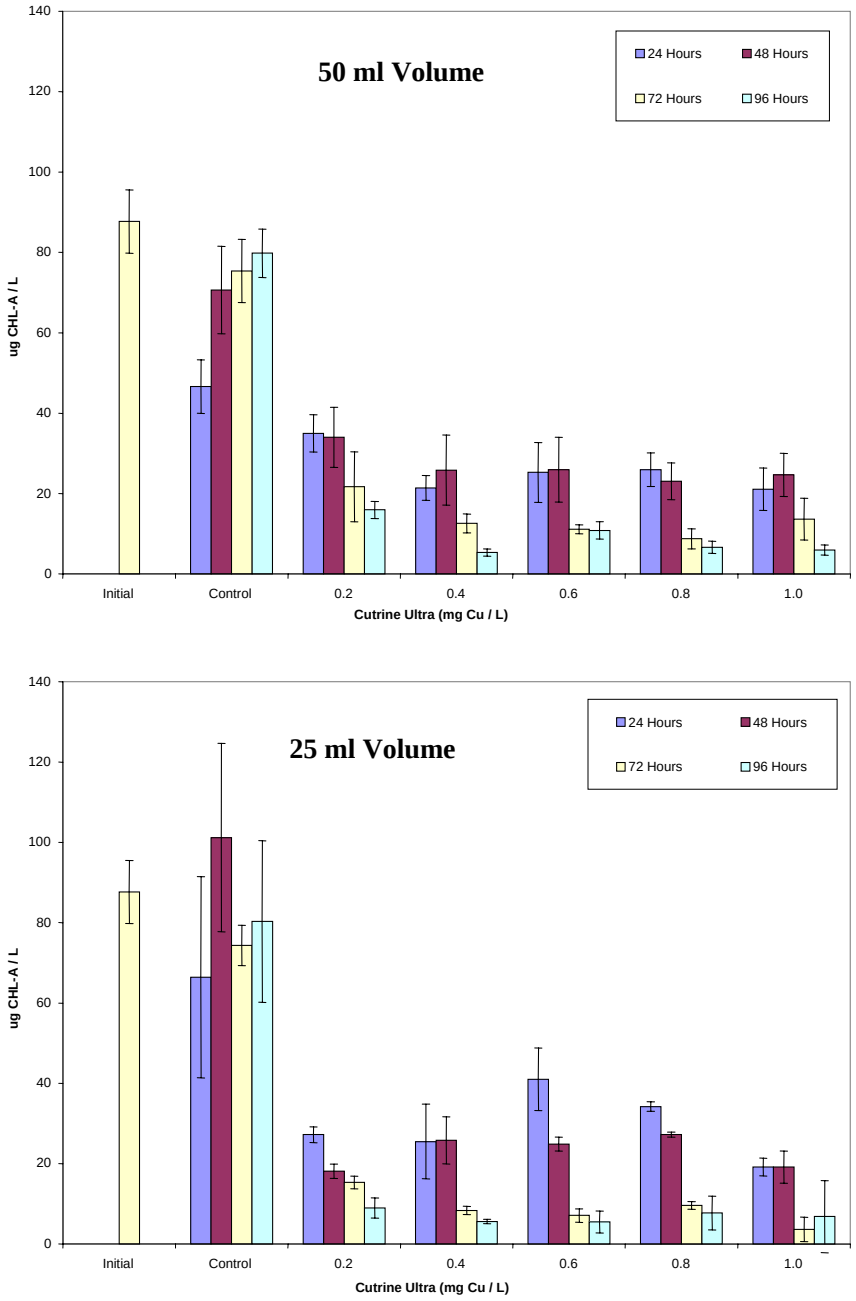


Figure 2.7. Responses of algae in samples from Pawnee Reservoir to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of Chlorophyll *a* concentrations and associated standard deviations (Continued).

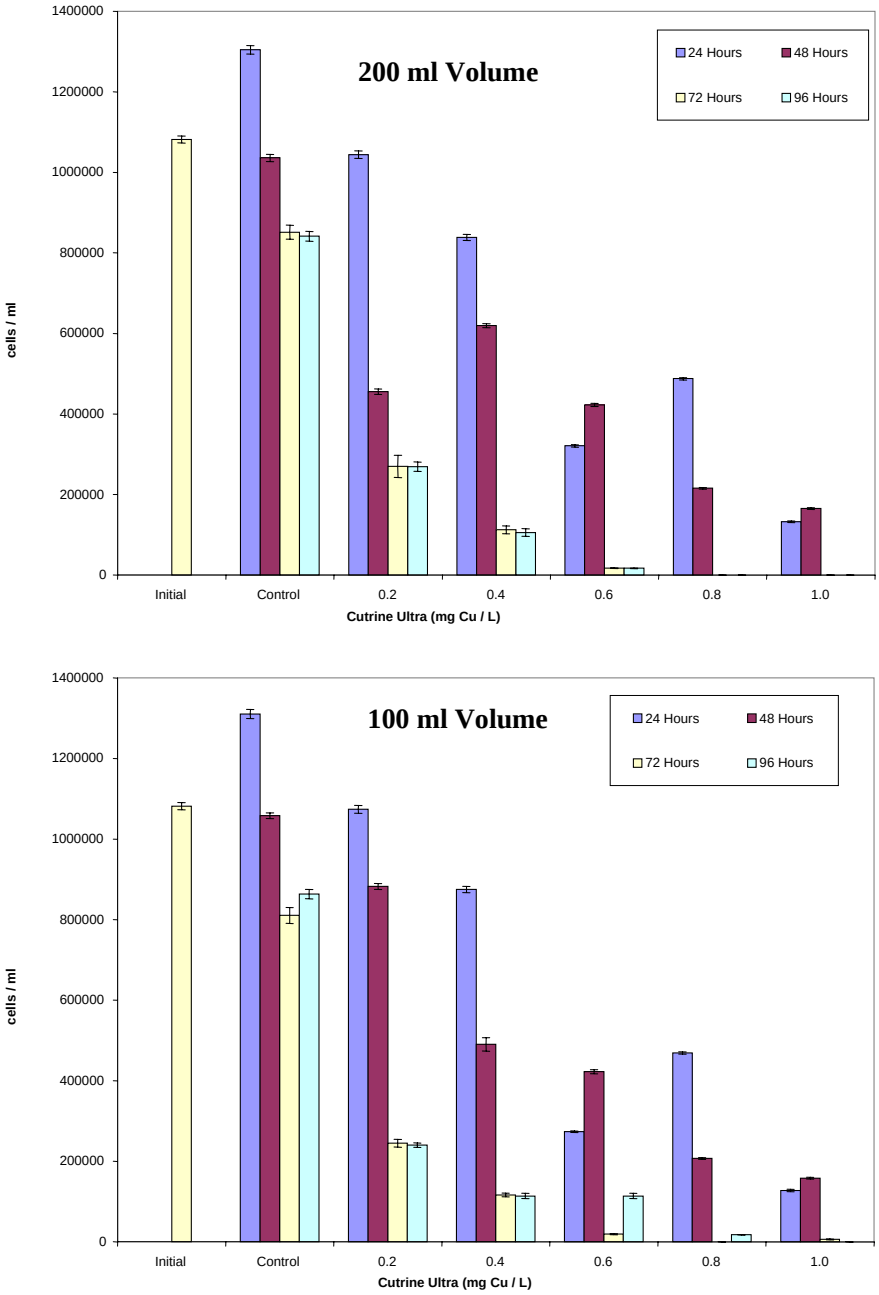


Figure 2.8. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations.

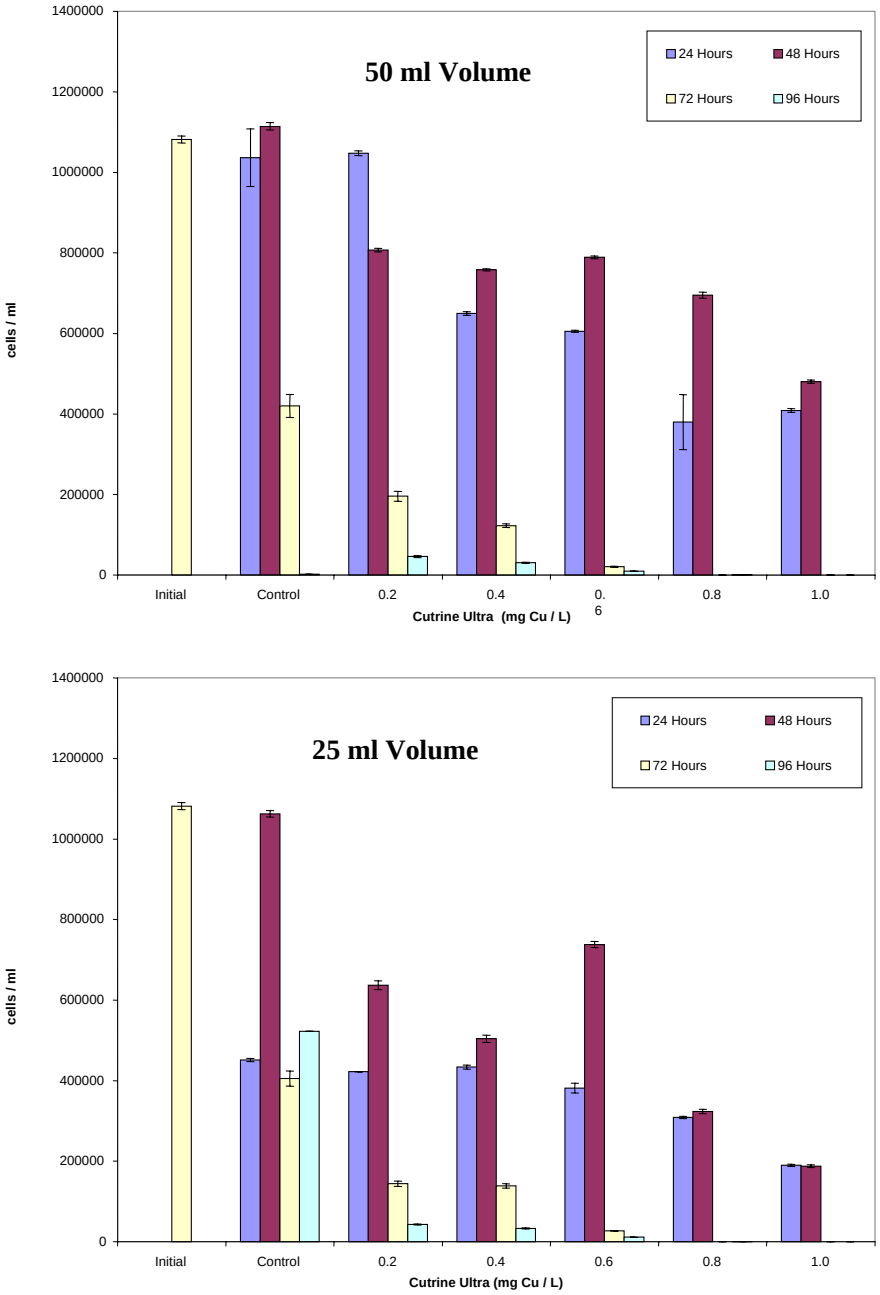


Figure 2.8. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations (Continued).

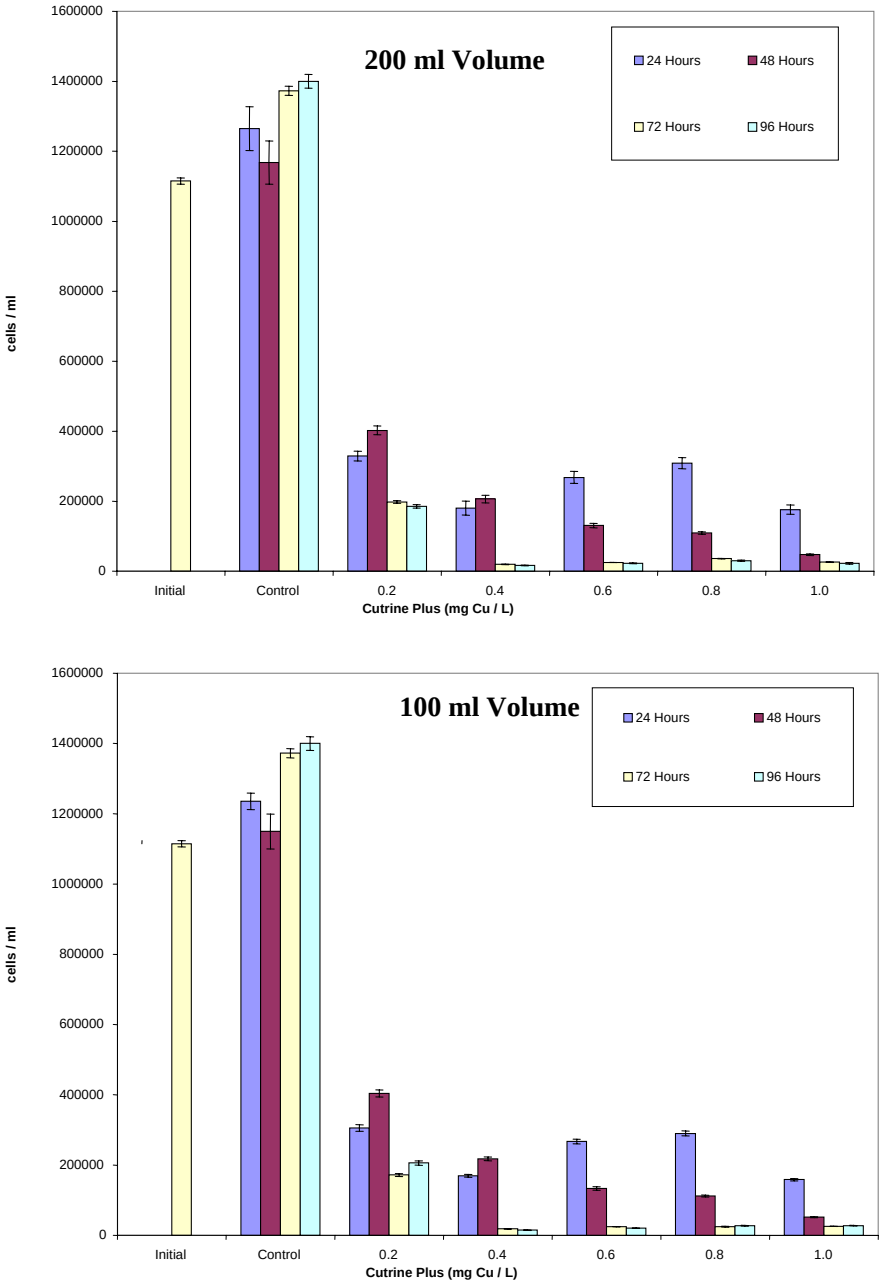
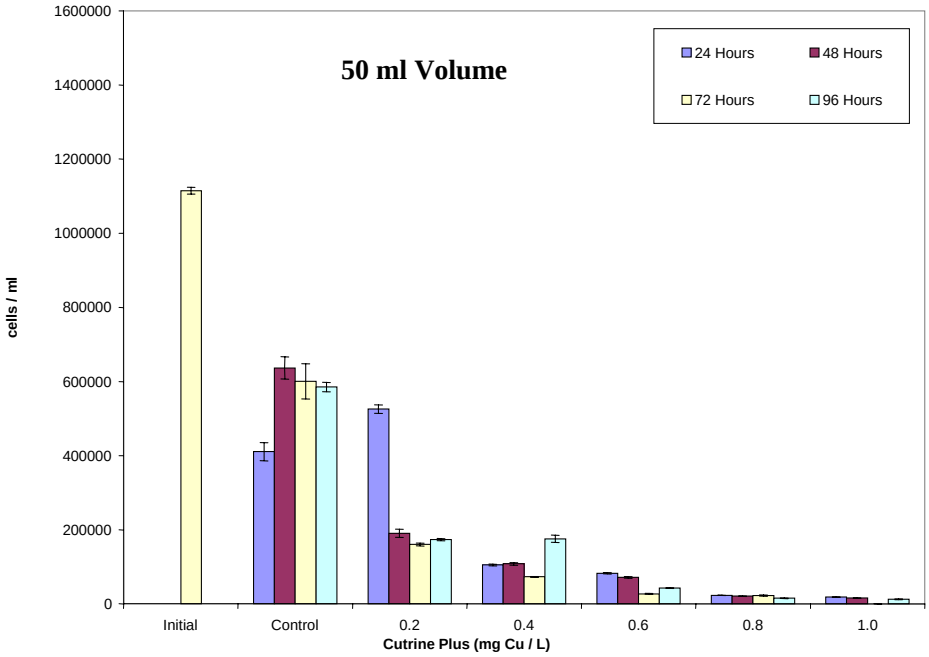


Figure 2.9. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Plus exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations.



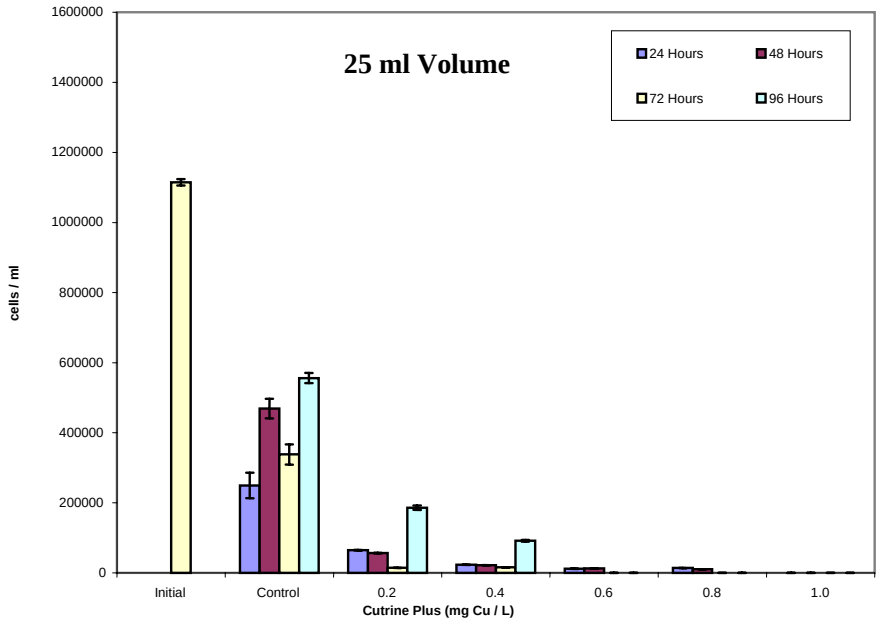


Figure 2.9. Responses of algae in samples from the Aquaculture Pond to Cutrine®-Plus exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations (Continued).

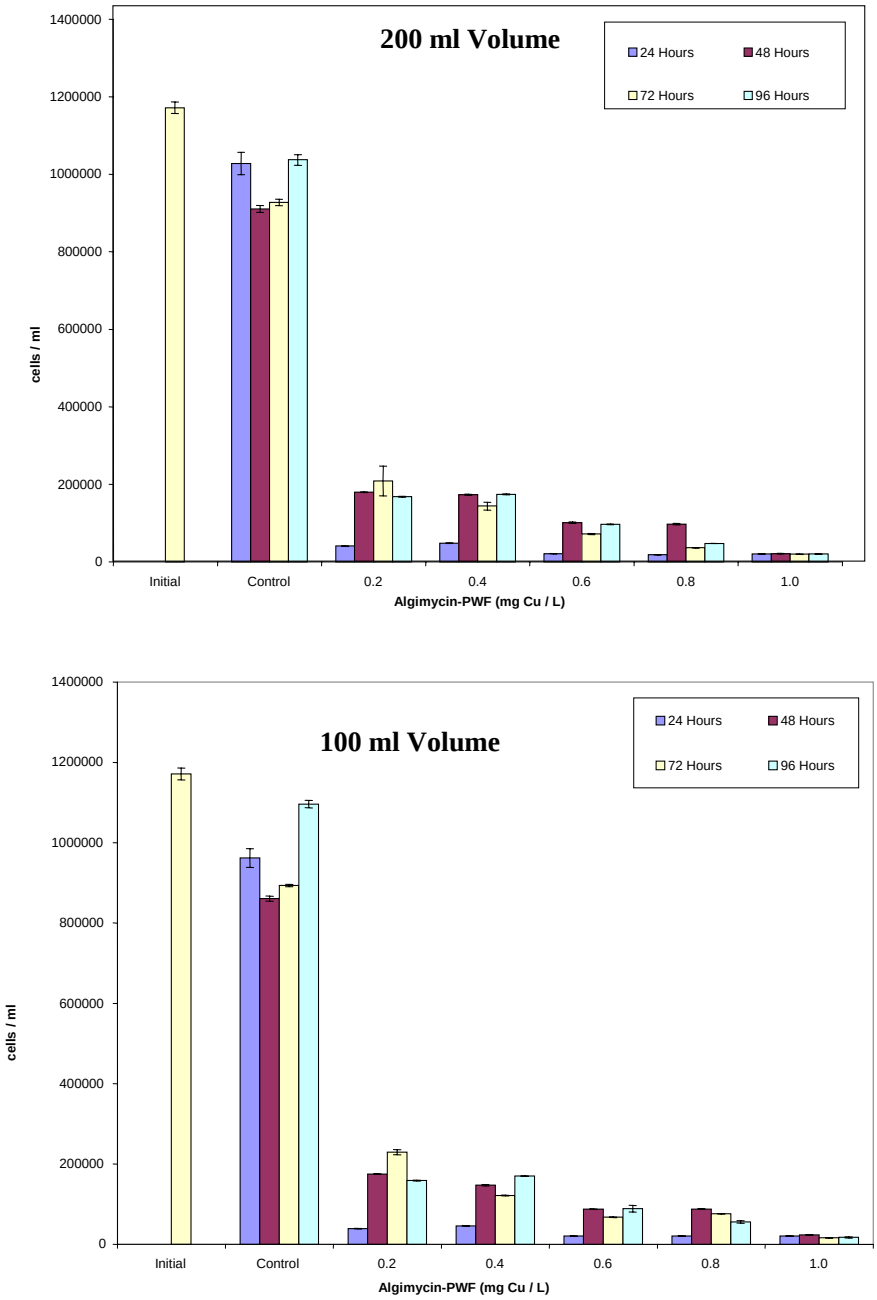


Figure 2.10. Responses of algae in samples from the Aquaculture Pond to Algimycin®-PWF exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations.

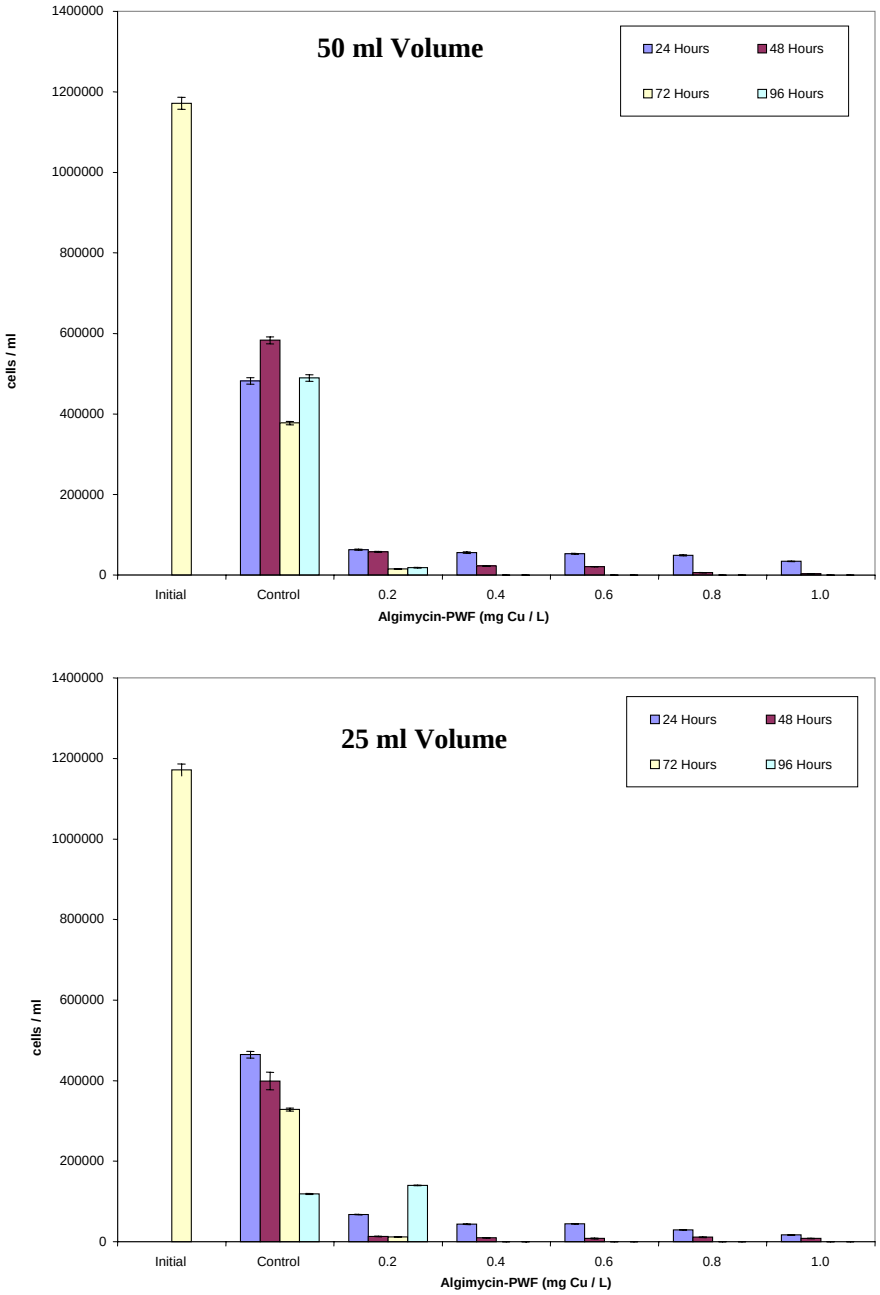


Figure 2.10. Responses of algae in samples from the Aquaculture Pond to Algimycin®-PWF exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cell / ml and associated standard deviations (Continued).

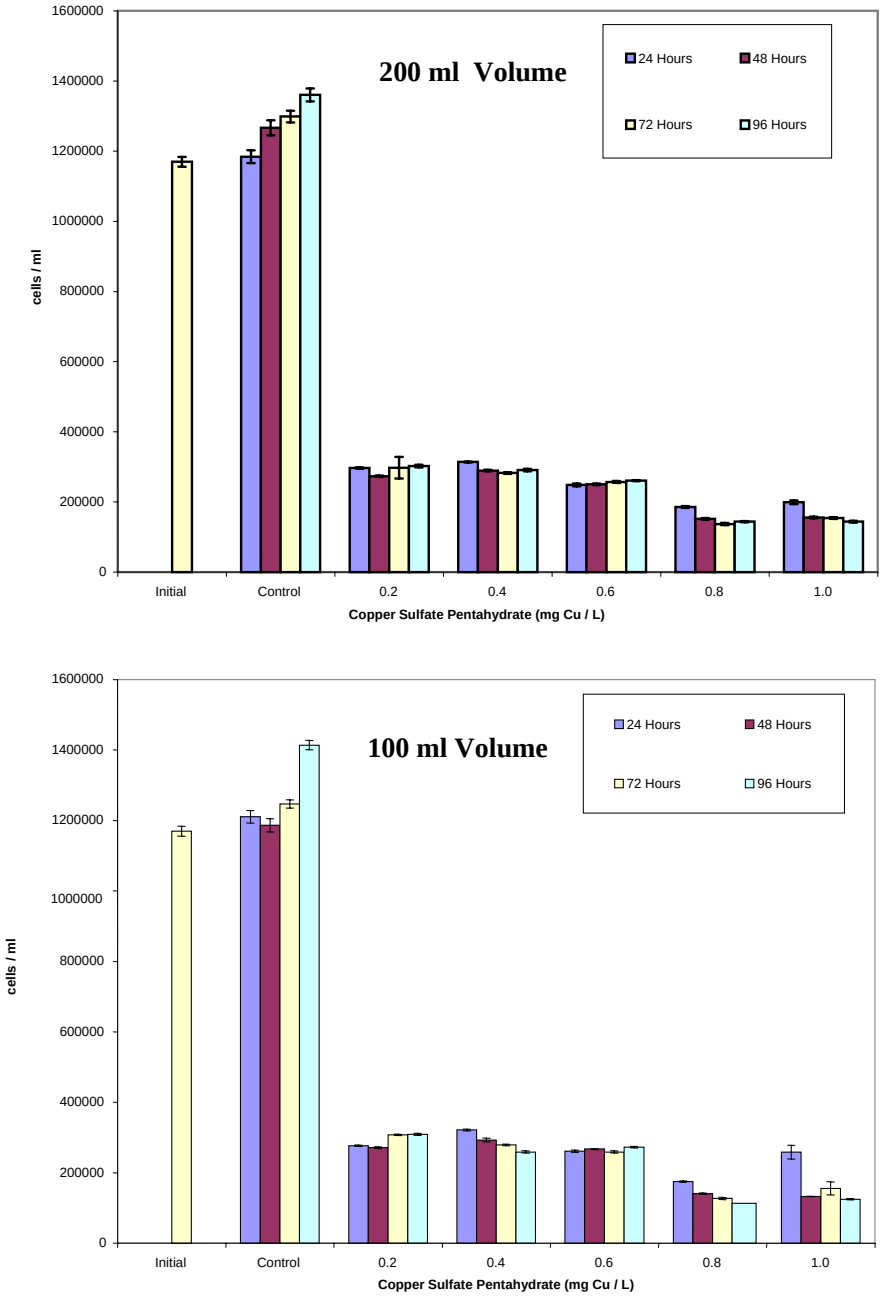


Figure 2.11. Responses of algae in samples from the Aquaculture Pond to copper sulfate pentahydrate exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations.

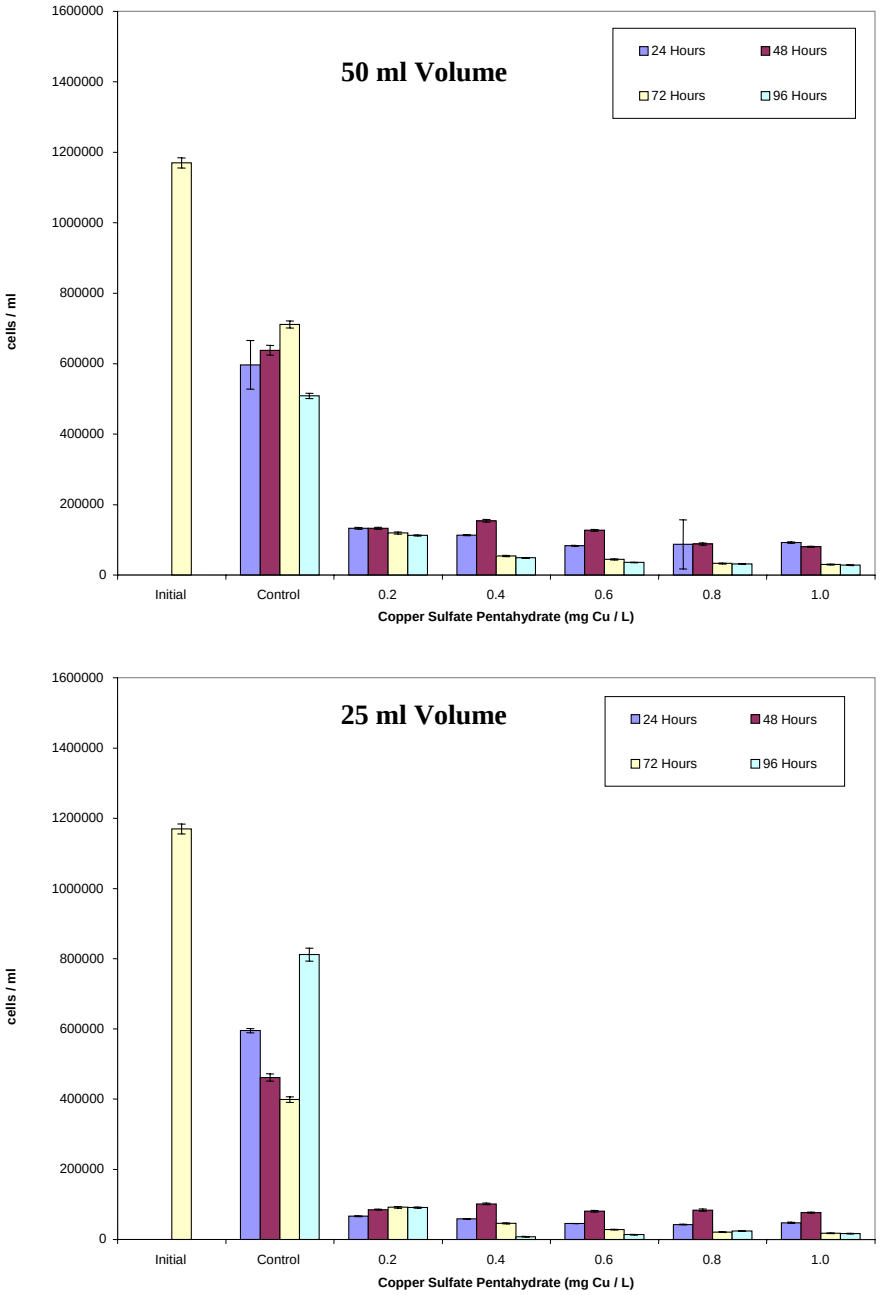


Figure 2.11. Responses of algae in samples from the Aquaculture Pond to copper sulfate pentahydrate exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations (Continued).

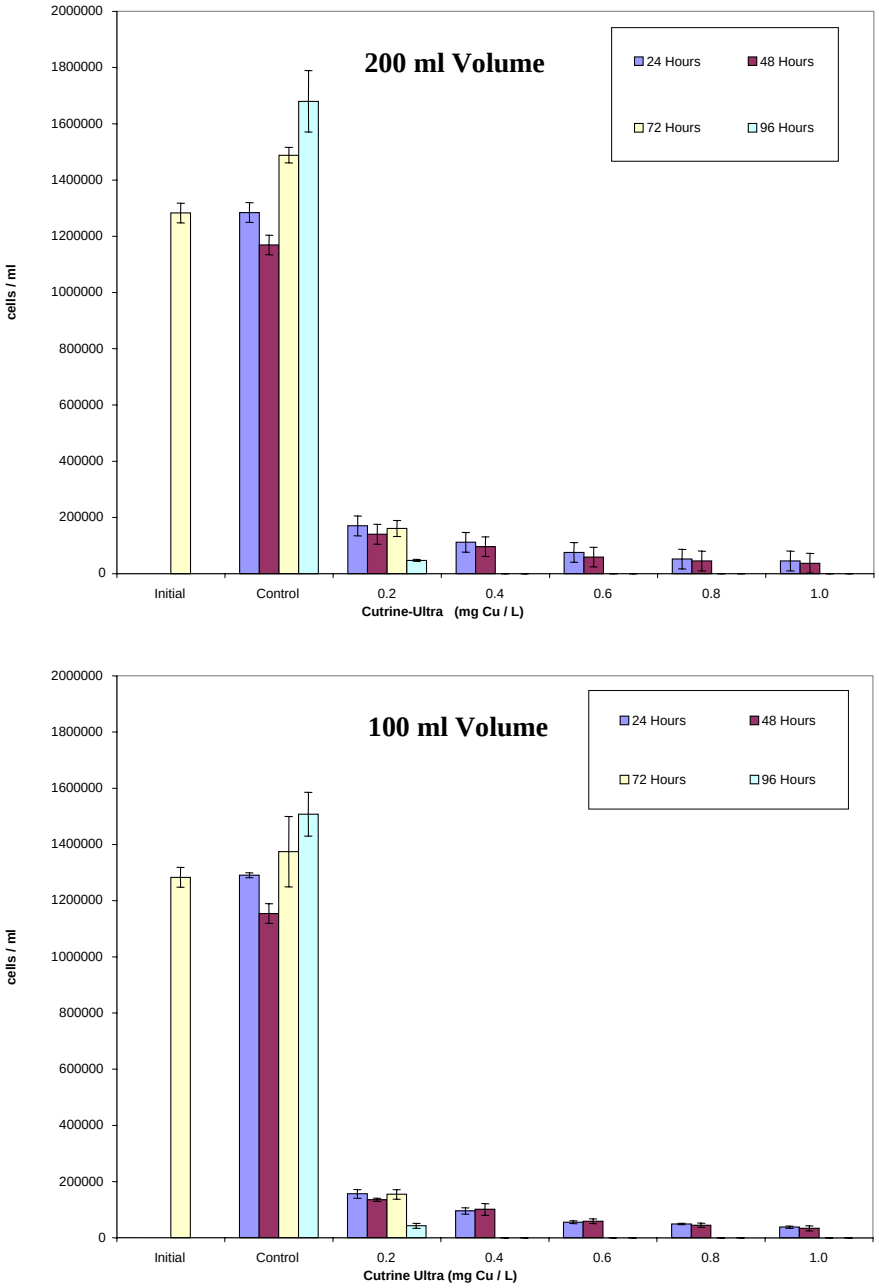


Figure 2.12. Responses of algae in samples from Pawnee Reservoir to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations.

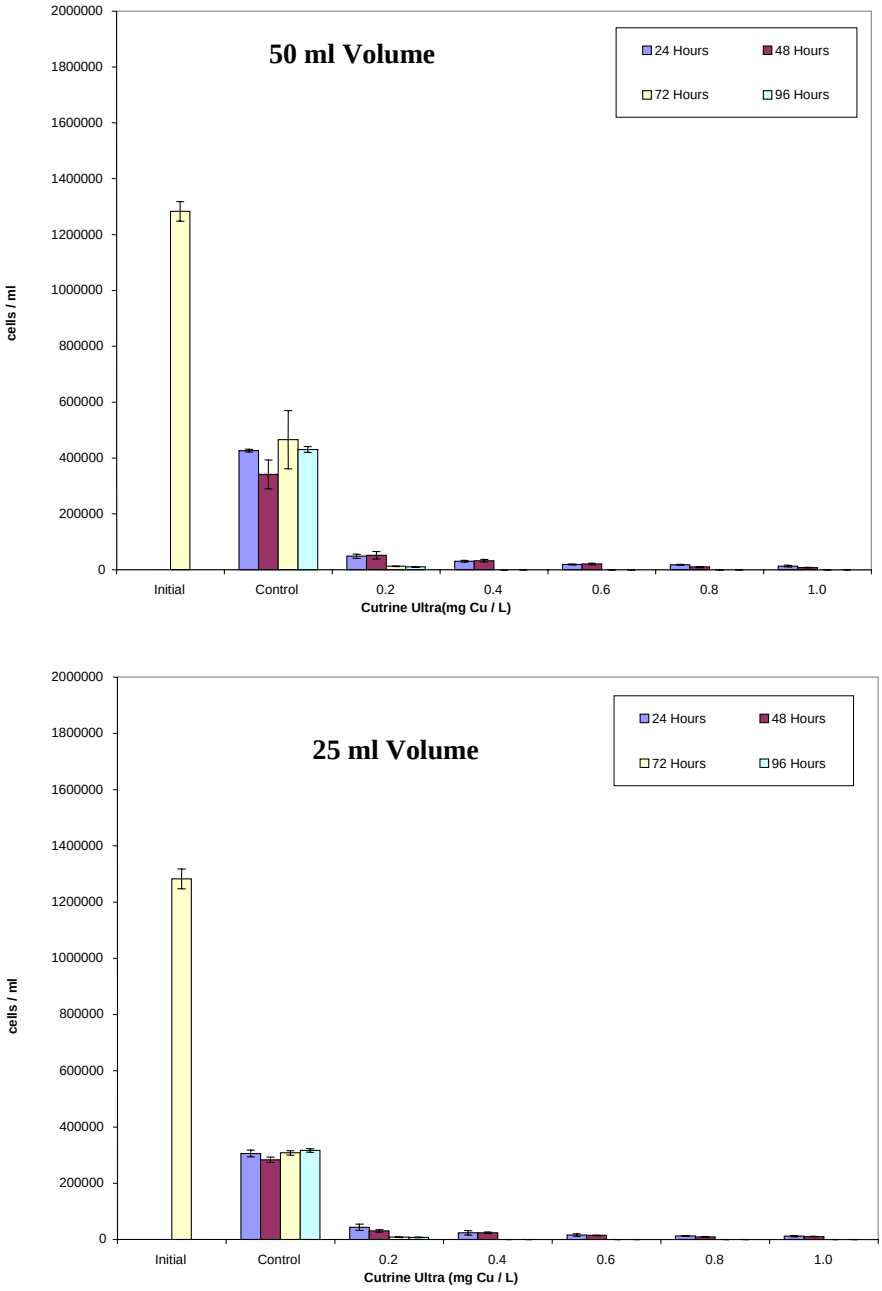


Figure 2.12. Responses of algae in samples from Pawnee Reservoir to Cutrine®-Ultra exposures in 200 ml, 100 ml, 50 ml, and 25 ml volumes in terms of cells / ml and associated standard deviations (Continued).

Chlorophyll a and Cell Density Response Parameters

Chlorophyll *a* in these experiments tended to underestimate the toxicity of copper to algae (Figures 2.1 - 2.10) when compared with cell density measurements (Figures 2.11 - 2.20). Chlorophyll *a* was present in all treatments at conclusion of the experiment for all of the algaecides, however, algal cells were not detected for some treatments of Cutrine®-Ultra for both site waters.

In the Aquaculture Pond water, algal cells were present in all treatments of Cutrine®-Plus, Algimycin®-PWF and copper sulfate pentahydrate at the conclusion of the experiment. However, no algal cells were observed in exposures at ≥ 0.8 mg Cu / L, as Cutrine®-Ultra. In Pawnee Reservoir water samples, no algal cells were observed in exposures at ≥ 0.4 mg Cu / L, as Cutrine®-Ultra.

Discussion

Site water

These site waters have different chemical characteristics (e.g. pH and conductivity), therefore, these waters provide a test of the hypothesis that algae may respond differently to different forms of copper. The copper formulations in these experiments range from copper containing salts (e.g. copper sulfate pentahydrate) to thoroughly chelated triethanolamine compounds (e.g. Cutrine®-Ultra and Cutrine®-Plus; Table 2.1). Likewise, the form of copper can affect the responses of algae to an algaecide exposure. Chelated copper formulations are used because they typically remain in the water column longer than non-chelated

formulations, which increases the duration of exposure (Masuda and Boyd 1993). These copper chelated algaecides also have an affinity for algae (Murray-Gulde et al. 2002). Cutrine®-Ultra was the most efficacious algaecide used to treat algae in water from the Aquaculture Pond, however, this does not mean that Cutrine®-Ultra will always be the most efficacious. Therefore, different sites that contain the same algal species must be evaluated independently to determine the most efficacious treatment, since the bioavailability of copper algaecide can be influenced by water characteristics.

Experimental Volume and Duration

Smaller experimental volumes (25 and 50 ml) of site waters may not be sufficient to contain representative quantities of algae that are present in sample volumes ≥ 100 ml. Algal cells that are not homogeneously distributed may be captured in a larger volume (≥100 ml) and missed entirely in a smaller volume (≤ 50 ml).

Several factors may affect chlorophyll *a* including temperature, cell integrity, photoperiod and the quantity of nutrients present (WHO 2003). Chlorophyll *a* may degrade within a few minutes to a few days after exposure to an algaecide (WHO 2003). Algal cells often settle from the water column and degrade within a few days (Murray-Gulde 2002). Therefore, chlorophyll *a* may not be detected in smaller volumes.

Chlorophyll a and Cell Density Response Parameters

For these algal toxicity experiments, cell density was a reliable indicator of algaecidal efficacy. However, chlorophyll *a* did not always accurately indicate the effectiveness of an algaecidal exposure. According to Cullen (1982), Desortova (1981), and Grandberg and Harujula (1982), chlorophyll *a* measurements must be used cautiously as an estimate of phytoplankton biomass. A significant number of algal cells in the water column are not viable, and actually consist of particulate detritus in various stages of decomposition (e.g., Wetzel and Likens 2000).

Conclusions

As the human population grows and as people move to locations adjacent to water resources, their awareness of algae and problematic growths of algae becomes more acute. With population growth comes changes in land use within watersheds, and concomitant increases in nutrients and other materials in these aquatic systems (WHO 2003; Figueiredo et al. 2004). As a consequence, algal growths or "blooms" have become more prevalent. These blooms can cause problems including: 1) altered aesthetics and decline in adjacent property values (WHO 2003; Figueiredo et al. 2004); 2) interference with recreational activities such as fishing, boating, and swimming (Brown et al. 1982; WHO 2003; Figueiredo et al. 2004); 3) adverse effects on drinking water including production of taste and odor compounds (e.g., geosmin and 2-methylisoborneal) (Mastin et al. 2002); and 4) production of toxins directly impacting invertebrates, fish

(Figueiredo 2004), avian species (e.g. Avian Vacuolar Myelinopathy; Wilde et al. 2005) and mammals including humans (Behm 2003; WHO 2003).

Since water resources have become used for more purposes, as well as more extensively, control is required of algal growths causing adverse impacts on water resource usages when critical usages are interrupted or prohibited by the algae. Therefore, if time (96 h) and volume (200 ml) required to obtain predictive results can be decreased (i. e. 72 h, 100 ml), then field applications can be implemented in a more timely fashion (~7-10 days). In critical situations (e.g. when potent toxins are being produced in vital water resources) initiation of treatment as soon as possible may be necessary to mitigate risks. Algae can grow exponentially and cell doubling time may range from 24 - 48 h (WHO 2003). Therefore, it is important to treat early with the appropriate algaecide, so that smaller amounts of algaecide may be required. In critical situations, the ability to quickly and accurately determine the most efficacious algaecide and treatment allows the greatest economic impact to be realized by water resource managers. Therefore, a more rapid response (24 to 48 h) will be observed after treatment if a representative water sample is obtained and if algae are sensitive to algaecides. However, if the water sample is not uniformly distributed and not representative, or contains algae which are tolerant to algaecides, then larger sample volumes and longer durations ($\geq$ 96 h) of exposures may be required.

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CHAPTER 3

RESPONSES OF *LYNGBYA* TO ALGAECIDE EXPOSURES IN THE LABORATORY AND FIELD

Prepared for submission to the
Journal of Aquatic Plant Management

Abstract

Laboratory experiments to determine efficacious approaches for treatment of *Lyngbya* can decrease time, effort and expense relative to field-scale treatments seeking a viable approach for managing benthic algae. Confirmation is needed of results from the laboratory that are applied directly to field situations. The specific objectives of this research were to: 1) contrast responses of *Lyngbya* to laboratory exposures of algaecides and adjuvants in a laboratory study to determine efficient and efficacious treatment options, 2) measure field responses of *Lyngbya* to exposures of algaecides and an adjuvant, and 3) contrast responses of *Lyngbya* in laboratory exposures of algaecide and adjuvant with responses to field exposures. To accomplish the initial objective, field-collected *Lyngbya* was exposed to selected algaecides in laboratory algal toxicity tests with site water. Of the algaecide applications tested, the laboratory treatment that was most efficacious for controlling *Lyngbya* growth was 10 mg PAK™-27 and 0.5 mg Cu, as Algimycin®-PWF, with 0.1 ml of Cide-Kick II® / 0.1 g of *Lyngbya*. Field treatments based on the laboratory recommendation were applied in two Alabama reservoirs (Lay and Jordan Lakes) and the algae responded as predicted from the laboratory results. Use of these water resources was restored. The laboratory information on sensitivity to algaecides, coupled with early detection of growth leading to development of extreme *Lyngbya* densities, can assist implementation of an effective management strategy that can restore and maintain critical water resource usages.

Introduction

Lyngbya is a cyanobacterium that can grow to densities that are problematic in aquatic systems (Speziale and Dyck 1992). Extensive growths of *Lyngbya* can produce taste and odor compounds (2 methyl-isoborneol and geosmin) which cause off-flavor in catfish (Brown and Boyd 1982) and other edible fish. Toxins, which cause dermal irritation for mammals and avoidance behavior by macroinvertebrates and fish, are also produced by *Lyngbya* (Falconer et al. 1999; Mastin et al. 2002). Due to production of toxins, extensive algal growths may also limit water resource uses such as recreational activities (e.g. swimming and fishing) and potable water for humans and other animals (Falconer 1999; Mastin et al. 2002).

When *Lyngbya* interferes with usages of critical water resources such as domestic water supply, livestock watering, and irrigation, immediate control actions by water resource managers may be required (Figueiredo et al. 2004). Excessive growths of *Lyngbya* in two Alabama reservoirs (Lay Lake and Lake Jordan) provided an opportunity to test hypotheses regarding responses to algaecide exposures in the laboratory as well as responses after field applications. Using the minimum amount of algaecide required to achieve control, laboratory studies have successfully predicted responses of noxious algae in the field (Fitzgerald and Jackson 1979; Murray-Gulde et al. 2002). Algaecides currently registered by the U.S. Environmental Protection Agency that may be used to manage *Lyngbya* include chelated copper algaecides (e.g., Cutrine®-Plus, Cutrine®-Ultra, Clearigate®, and Algimycin®-PWF), peroxide based algaecides

(e.g. Phycomycin™), endothall (e.g. Hydrothol191®;), and diquat (Reward®). Some adjuvants that may also be used to enhance algaecide activity include Poly An®, Poly Control 2, Cide-Kick II®, Big Sur 90®, Sil Energy, Silnet 200, and Big Wet®. The efficacy of individual algaecides and potential synergy with adjuvants can be evaluated in the laboratory using site water and algae to determine a viable approach for managing benthic algae such as *Lyngbya*.

Laboratory screening approaches are efficient and effective for identifying efficacious approaches for controlling benthic algae (Fitzgerald and Jackson 1979; Mastin et al. 2002; Murray-Gulde et al. 2002; Tedrow 2007; Duke et al. 2007, Chapter 2 of this dissertation). Laboratory experiments to determine effective treatments for *Lyngbya* decrease time, effort and expense relative to field-scale treatments seeking a viable approach for managing benthic algae. Confirmation is needed of results from the laboratory that are applied directly to field situations. With the forgoing in mind, the specific objectives of this research were to: 1) compare responses of *Lyngbya* to exposures of algaecides and adjuvants in a laboratory study to determine efficient and efficacious treatment options; 2) measure field responses of *Lyngbya* to exposures of algaecides and an adjuvant, and 3) contrast responses of *Lyngbya* in laboratory exposures of algaecide and adjuvant with responses to field exposures.

Materials and Methods

Laboratory Algal Toxicity Tests

Site waters and *Lyngbya* used for these experiments were obtained from sloughs (inlets of reservoirs) at Lay Lake and Lake Jordan located in Shelby County and Chilton County, Alabama, respectively (Figure 3.1). Field-collected *Lyngbya* from Lay Lake and Lake Jordan was exposed to selected algaecides using methods modified from Murray-Gulde et al. (2002). Algal samples (0.1 g ± 0.01) were exposed to algaecides in 250 ml beakers with 200 ml of site water. All experiments were conducted in light-and-temperature controlled settings at 21 - 25 ºC with a 16 h light / 8 h dark photoperiod using "cool white" fluorescent lighting (400 ±40 ft-c) and 4 replicates (U.S. EPA 2002). Beakers were swirled once daily by hand during the 7 day exposure period. Chlorophyll *a* (µg chlorophyll a / g of *Lyngbya*) was measured at experiment initiation and at 7 d (experiment conclusion) for each treatment replicate. Algae replicates weighing 0.1 g (±0.01) were frozen at -20 ºC for a minimum of 24 hours. Frozen algal samples were ground thoroughly using a mortar and pestle, and 20 ml of 90 % acetone buffered with magnesium carbonate was added directly to the ground algal replicate (APHA 1998). The sample was stored at -20 ºC for a minimum of 24 hours before chlorophyll *a* analyses. Chlorophyll *a* was measured fluorometrically using a SpectraMax® 190 Gemini spectrofluorometer (Molecular Devices Corporation, Sunnyvale, CA 94089) with an excitation wavelength of 430 nm and an emission wavelength of 663 nm (APHA 1998). To confirm copper algaecide exposures in the test chambers, acid extractable (U.S. EPA

1983) copper concentrations were measured using a Perkin-Elmer AAnalyst 5100 flame and graphite furnace atomic absorption (AA) spectrometer (APHA 1998). Algaecide exposures consisted of applications of single algaecides, as well as combinations of compatible algaecides and adjuvants. The algaecides and adjuvants evaluated for effectiveness against *Lyngbya* in this study are listed in Tables 3.1 and 3.2, respectively.

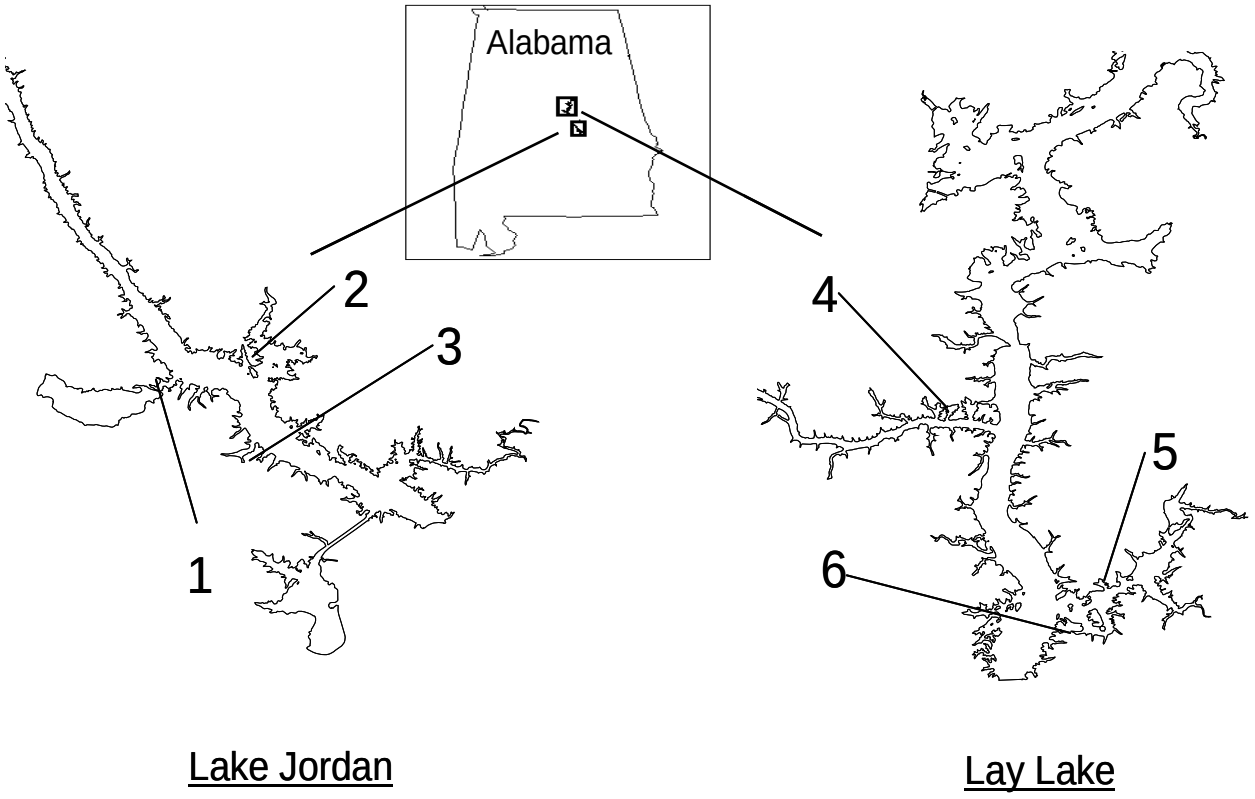

Figure 3.1. Sample sites located in Lake Jordan: 1) Blackwell's Bridge (untreated reference site), 2) Kelly's Slough, 3) Nelson Slough; and Lay Lake 4) Powerline Slough, 5) Flat Top Slough, 6) Powerline South (untreated reference site).

Table 3.1. Algaecides evaluated for effectiveness for controlling *Lyngbya* in Lay Lake and Lake Jordan, AL.

	% Active ingredient	pH (S. U.)	Water solubility (mg / L)	Specific gravity (g / cm^3)	References
Cutrine®-Ultra	9 % Copper	10.0 - 10.5	Complete	1.22	Applied Biochemists 2005 (a)
Cutrine®-Plus	9 % Copper	10.0 - 11.0	Complete	1.21	Applied Biochemists 2004
Clearigate®	3.8 % Copper	9.5 - 10.0	Complete	1.10	Applied Biochemists 2000
Algimycin®-PWF	5 % Copper	1.5 - 2.0	Complete	1.20	Applied Biochemists 2005 (b)
Hydrothol®191	53 % Endothall	NA	>500,000	1.44	Cerexagri, Inc. 2003
Reward®	37 % Diquat	4 – 6	718,000	1.20	Syngenta, Inc. 2005
PAK™ -27	85 % Sodium Carbonate Peroxyhydrate	10.4 - 10.6	140,000	ND	Solvay Chemical, Inc. 2005
Green Clean-Pro®	85 % Sodium Carbonate Peroxyhydrate	10.4 - 10.6	140,000	ND	BioSafe Systems, Inc. 2005

Table 3.2. Adjuvants evaluated for effectiveness for controlling *Lyngbya* in Lay Lake and Lake Jordan, AL.

	Appearance	Odor	Water solubility	Specific gravity (g / cm^3)	References
Cide-Kick II®	Clear	Pine	Yes	0.87	Brewer 2000 (a)
Poly An®	White liquid	Hydrocarbon	Limited	1.0	Brewer 2000 (b)
Poly Control 2®	White	Chemical	Yes	1.0	Brewer 2006
Big Wet®	Clear White	Chemical	Yes	1.05	Brewer 1999 (b)
SilEnergy®	Clear	Chemical	Yes	1.06	Brewer 2001 (b)
Silnet 200®	Clear	Chemical	Yes	1.06	Brewer 1999 (a)
Big Sur 90®	Clear Yellow	Chemical	Yes	1.1	Brewer 2001 (a)

Laboratory algal toxicity experiments using (copper chelated algaecides) *Cutrine®-Ultra, Cutrine®-Plus, Clearigate®, Algimycin®-PWF, and (sodium carbonate peroxyhydrate algaecides*) *PAK-27™, Green Clean Pro®, and (a diquat dibromide algaecide) Reward® and (a Endothall algaecide)Hydrothol-191®*

Exposures of copper containing algaecides were 14, 28, and 56 mg Cu / g of algae, as Cutrine®-Ultra, Cutrine®-Plus, Clearigate®, and Algimycin®-PWF (Table 3.1). Exposures of sodium carbonate peroxyhydrate were 1200, 1267, and 1300 mg of sodium carbonate peroxyhydrate / g of *Lyngbya*, as PAK-27 and Green Clean Pro® (Table 3.1). Exposures of Reward® were 4.0, 13, and 19 mg as Reward®/ g of *Lyngbya* (Table 3.1). Hydrothol-191® exposures were 30, 50, and 70 mg Hydrothol-191® / g of *Lyngbya* (Table 3.1).

Laboratory algal toxicity experiments using adjuvants and Algimycin®-PWF

Exposures of 14, 28, and 56 mg Cu, as Algimycin®-PWF with 1 ml of an adjuvant (Silnet 200®, Sil Energy®, Big Sur 90®, Poly An®, Big Wet®, Poly Control 2®, and Cide-Kick II® (Table 3.2).

Laboratory algal toxicity experiments using combinations of PAK™-27 and Algimycin®-PWF with Cide-Kick II®

Results of algaecide and adjuvant exposures indicated that the treatment of PAK™-27 followed by Algimycin®-PWF with Cide-Kick II® was the most efficacious treatment tested (Tables 3.1 and 3.2). Therefore, combinations of these algaecides exposures are: 1) 100 mg sodium carbonate peroxyhydrate, 3.0 mg Cu, as Algimycin®-PWF, and 1.0 ml of Cide-Kick II® / g of *Lyngbya*; 2) 100

mg sodium carbonate peroxyhydrate, 5.0 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® / g of *Lyngbya*; 3) 100 mg sodium carbonate peroxyhydrate, 7.0 mg Cu, as Algimycin®-PWF, and 1.0 ml of Cide-Kick II® was / g of *Lyngbya*; 4) 100 mg sodium carbonate peroxyhydrate, 3.0 mg Cu, as Algimycin®-PWF, and 0.5 ml of Cide-Kick II® g of *Lyngbya*; 5) 100 mg sodium carbonate peroxyhydrate, 7.0 mg Cu as Algimycin®-PWF and 1.5 ml of Cide-Kick II® / g of *Lyngbya*; 6) 50 mg sodium carbonate peroxyhydrate, 0.3 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® / g of *Lyngbya*; 7) 150 mg sodium carbonate peroxyhydrate, 0.7 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® / g of *Lyngbya*. The most efficacious treatment was 100 mg sodium carbonate peroxyhydrate, followed by 5.0 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® / g of *Lyngbya* (Table 3.6).

Lay Lake and Lake Jordan: Algaecide Application Study

Selected field sites for treatment in Lay Lake included Flat Top Slough (6.1 acres; 2.47 hectares) and Powerline Slough (10.9 acres; 4.43 hectares, Figure 3.1), and selected treatment sites in Lake Jordan were Nelson Slough (3.52 acres; 1.43 hectares) and Kelly Slough (7.0 acres; 2.83 hectares, Figure 3.1). Untreated reference sites for Lay Lake and Lake Jordan were Powerline South and Blackwell Bridge, respectively. Measured characteristics of site waters included pH (standard units), dissolved oxygen (mg O_2 / L), conductivity (μS / cm^2), alkalinity (mg / L as $CaCO_3$), hardness (mg / L as $CaCO_3$), and temperature (°C) [APHA 1998].

Three line transects from each designated sampling site in Lay Lake and Lake Jordan (Figure 3.1; Table 3.3) were used to collect *Lyngbya* and water samples. Using 6.25 and 12.5 cm coring devices, dry weight (APHA 1998) of *Lyngbya* mats was determined, prior to algaecide treatment and after the final algaecide treatment. Water samples (20 L / transect) were also collected from each site and taken to the laboratory at Clemson University where total copper, pH, hardness, and alkalinity and conductivity and dissolved oxygen (mg O_2 / L) were measured. Field applications or treatments consisted of 5.3 mg sodium carbonate peroxyhydrate / L (PAK™-27) followed by (24 h later) 1 mg Cu / L, as chelated copper (Algimycin®-PWF) with 1.9 ml / m^2 of adjuvant (Cide Kick II®). This application regimen was repeated three times, totaling three treatments for each treated site. A minimum of two weeks was allowed between treatments at each site in accordance with the Algimycin® -PWF label instructions (Table 3.3).

Table 3.3. Sites selected for treatment and untreated reference sites in Lay Lake and Lake Jordan, Alabama.

Reservoir	Sample site	Coordinates	Treated / untreated	Treatment dates	Sampling Dates
Lay Lake	Flat Top	32° 59' 9.0" N 86° 29' 55.4" W	Treated	19 May 2005 9 June 2005 23 June 2005	19 May 2005 9 June 2005 23 June 2005 15 August 2005
	Powerline Slough	33° 1' 39.6" N 86° 31' 46.0" W	Treated	19 May 2005 9 June 2005 23 June 2005	19 May 2005 9 June 2005 23 June 2005 15 August 2005
	Powerline South	32° 58' 29.0" N 86° 30' 22.5" W	Untreated		19 May 2005 9 June 2005 23 June 2005 15 August 2005
Lake Jordan	Nelson Slough	32° 38' 9.4" N 86° 18' 6.4" W	Treated	16 June 2005 29 June 2005 19 July 2005	16 June 2005 29 June 2005 19 July 2005 15 August 2005
	Kelly Slough	32° 39' 56.6" N 86° 18' 6.4" W	Treated	16 June 2005 29 June 2005 19 July 2005	16 June 2005 29 June 2005 19 July 2005 15 August 2005
	Blackwell's Bridge	32° 39' 19.6" N 86° 19' 55.1" W	Untreated		16 June 2005 29 June 2005 19 July 2005 15 August 2005

Statistical Analysis

Statistical analysis was performed using Sigma Stat® software and included analysis of variance (ANOVA; alpha = 0.05) followed by Dunnett's test if the data were normally distributed and variances were homogenous (Ott 1993). If the data did not meet the assumptions for parametric testing, then non-parametric ANOVA (alpha = 0.05) with a Dunn's test on ranked data were used (Ott 1993). To further determine algaecide and adjuvant effectiveness average chlorophyll a concentration were used. Average chlorophyll *a*, biomass and copper concentrations were calculated with associated standard deviation.

Results and Discussion

Laboratory algal toxicity tests

The primary purpose of the laboratory algal toxicity tests was to identify an efficacious algaecide and treatment (concentration and duration of exposure) that would control the growth of *Lyngbya* in Lay Lake and Lake Jordan water. Results from exposures of algaecides and adjuvants in the laboratory algal toxicity tests were reported as nominal exposure concentrations (Tables 3.4, 3.5 and 3.6). For these laboratory exposures, Lay Lake and Lake Jordan water characteristics (i.e. pH, alkalinity, hardness, and conductivity) were indicative f soft water (Table 3.7; Sawyer et al. 1994). Average chlorophyll *a* concentrations and associated standard deviations were reported. Chlorophyll *a* concentrations of all treatments were statistically significant from the chlorophyll *a* concentrations of the control, therefore, to further determine algaecide and

adjuvant effectiveness average chlorophyll *a* concentration were used. The average pre-treatment chlorophyll *a* concentration for these laboratory algal toxicity experiments was 18,815 (±1,153) µg chlorophyll *a* / g *Lyngbya*.

Table 3.4. Algaecides evaluated for the effectiveness for controlling *Lyngbya* in Lay Lake and Lake Jordan, Alabama, and average chlorophyll *a* concentrations with associated standard deviation values. Initial chlorophyll *a* concentration was 17,922 (±1140) µg / g of *Lyngbya*.

Algaecide	Active Ingredient	Algaecide concentration based on Active Ingredients (mg / g of *Lyngbya*) unless otherwise noted	Final Chlorophyll *a* concentration (µg / g)
Cutrine®-Ultra	Chelated copper	14	1719 (± 120)
		28	1390 (± 109)
		56	1241 (± 88)
Cutrine®-Plus	Chelated copper	14	1628 (± 86)
		28	1278 (± 79)
		56	1182 (± 97)
Clearigate®	Chelated copper	14	1234 (± 94)
		28	1067 (± 81)
		56	887 (± 75)
Algimycin®-PWF	Chelated copper	14	232 (± 16.7)
		28	124 (± 14.2)
		56	2.69 (± 1.26)
Hydrothall® 191	Endothall	30*	27.8 (± 4.74)
		50*	9.35 (± 1.49)
		70*	7.30 (± 0.88)
Reward®	Diquat	4.0*	39.3 (± 4.03)
		13*	15.0 (± 2.16)
		19*	4.65 (± 0.67)
PAK™-27	Sodium carbonate	1200	17.5 (± 3.70)
	peroxyhydrate	1267	13.3 (± 1.71)
		1333	4.28 (± 0.35)
Green Clean-Pro®	Sodium carbonate	1200	19.0 (± 4.97)
	peroxyhydrate	1267	15.3 (± 1.71)
		1333	4.45 (± 0.44)

*based on concentration of Algaecide

Table 3.5. Adjuvants and Algimycin®-PWF evaluated for the effectiveness for controlling *Lyngbya* in Lay Lake and Lake Jordan, Alabama, and average chlorophyll *a* concentrations with associated standard deviation values. Initial chlorophyll *a* concentration was 17,922 (±1140) µg / g of *Lyngbya*. Initial chlorophyll *a* concentration was 18671 (± 1272) µg / g of *Lyngbya*.

	Adjuvant (ml adjuvant / g of *Lyngbya*)	Concentration of Algimycin®-PWF (mg / g of *Lyngbya*)	Final Chlorophyll *a* concentration (µg / g)
Silnet 200®	1.0	14	81.2 (± 6.43)
	1.0	28	56.3 (± 5.91)
	1.0	56	21.9 (± 3.04)
Sil Energy®	1.0	14	94.8 (± 6.44)
	1.0	28	53.4 (± 3.88)
	1.0	56	34.2 (± 2.15)
Big Sur 90®	1.0	14	47.6 (± 5.39)
	1.0	28	28.7 (± 2.28)
	1.0	56	11.8 (± 1.24)
Poly An®	1.0	14	103 (± 6.92)
	1.0	28	63.7 (± 6.81)
	1.0	56	36.6 (± 2.99)
Big Wet®	1.0	14	77.2 (± 4.08)
	1.0	28	55.1 (± 6.16)
	1.0	56	32.4 (± 4.93)
Poly Control 2®	1.0	14	65.4 (± 5.62)
	1.0	28	42.2 (± 5.41)
	1.0	56	24.4 (± 3.34)
Cide-Kick II®	1.0	3.0	34.3 (± 3.35)
	1.0	5.0	17.3 (± 4.36)
	1.0	10.0	10.0 (± 0.79)
	1.0	14.0	6.88 (± 0.49)
	1.0	28.0	2.38 (±.0.25)
	1.0	56.0	2.2 (± 0.28)

Table 3.6. Combinations of PAK™-27, Algimycin®-PWF and Cide-Kick II® evaluated for the effectiveness for controlling *Lyngbya* in Lay Lake and Lake Jordan, Alabama, and average chlorophyll *a* concentrations with associated standard deviation values. Initial chlorophyll *a* concentration was 17,922 (±1140) µg / g of *Lyngbya*. Initial chlorophyll *a* concentration was 17852 (± 1048) µg / g of *Lyngbya*.

Concentration of PAK-27™ (mg sodium carbonate peroxyhydrate / g of *Lyngbya*)	Concentration of Algimycin®-PWF (mg Cu / g of *Lyngbya*)	Concentration of Cide-Kick II® (ml Cide-Kick II® / g of *Lyngbya*)	Final Chlorophyll *a* concentrations (µg / g of *Lyngbya*)
100	3.0	1.0	14.2 (± 2.51)
100	5.0	1.0	1.68 (± 0.46)
100	7.0	1.0	1.53 (± 0.36)
100	3.0	0.5	28.0 (± 3.64)
100	7.0	1.5	2.15 (± 0.61)
50	3.0	1.0	24.8 (± 1.09)
150	7.0	1.0	2.05 (± 0.69)

Table 3.7 Average measured acid extractable copper concentrations 4 h post algaecide application at sample sites in Lay Lake and Lake Jordan and associated standard deviation values.

Reservoir	Sample site	mg Cu/L (19 May 2005)	mg Cu/L (9 June 2005)	mg Cu/L (23 June 2005)
Lay Lake	Flat Top Slough	0.971 (±0.008)	0.893 (±0.060)	0.876 (±0.028)
	Powerline Slough	0.922 (±0.001)	0.898 (±0.020)	0.883 (±0.009)
		mg Cu/L (16 June 2005)	mg Cu/L (29 June 2005)	mg Cu/L (19 July 2005)
Lake Jordan	Nelson Slough	0.802 (±0.057)	0.779 (±0.020)	0.848 (±0.018)
	Kelly Slough	0.826 (±0.027)	0.835 (±0.035)	0.891 (± 0.013)

Laboratory algal toxicity experiments using copper chelated algaecides

Measured chlorophyll *a* concentrations decreased significantly after exposures of 14, 28, and 56 mg Cu / g of algae, as Cutrine®-Ultra, Cutrine®-Plus, Clearigate®, and Algimycin®-PWF (Table 3.4). Chlorophyll *a* concentrations after exposures of Cutrine®-Ultra and Cutrine®-Plus were 1719 (±120) µg chlorophyll *a* / g of *Lyngbya,* 1390 (±109) µg chlorophyll *a* / g of *Lyngbya*, 1241 (±88) µg chlorophyll *a* / g of *Lyngbya* and 1628 (±86) µg chlorophyll *a* / g of *Lyngbya*, 1278 (±79) µg chlorophyll *a* / g of *Lyngbya*, 1182 (±97) µg chlorophyll *a* / g of *Lyngbya*, respectively. Measured chlorophyll *a* concentrations after Clearigate® exposures were 1,234 (±94) µg chlorophyll *a* / g of *Lyngbya*, 1067 (±81) µg chlorophyll *a* / g of *Lyngbya*, 887 (±75) µg chlorophyll *a* / g of *Lyngbya*. However, *Lyngbya* was most responsive to Algimycin®-PWF exposures with chlorophyll *a* concentrations of 232 (±16.7) µg chlorophyll *a* / g of *Lyngbya*, 124 (±14.2) µg chlorophyll *a* of / g *Lyngbya*, and 2.69 (±1.26) µg chlorophyll *a* / g of *Lyngbya*, respectively (Table 3.4).

Laboratory algal toxicity experiments using sodium carbonate peroxyhydrate algaecides

After exposures of 1200, 1267, and 1300 mg of sodium carbonate peroxyhydrate / g of *Lyngbya* (Table 3.4), as PAK-27 and Green Clean Pro®, measured chlorophyll *a* concentrations were 17.5 (±3.70) µg chlorophyll *a* / g of *Lyngbya*, 13.25 (±1.71) µg chlorophyll *a* / g of *Lyngbya*, 4.28 (±0.35) µg chlorophyll *a* / g of *Lyngbya* and 19.0 (±4.97) µg chlorophyll *a* / g of *Lyngbya*,

15.3 (±1.71) µg chlorophyll *a* / g of *Lyngbya*, and 4.45 (±0.44) µg chlorophyll *a* / g of *Lyngbya*, respectively.

Laboratory algal toxicity experiments using Reward®

Measured chlorophyll *a* concentrations after exposures of 4.0, 13, and 19 mg as Reward®/ g of *Lyngbya* were 39.3 (±4.03) µg chlorophyll *a* / g of *Lyngbya*, 15.0 (±2.16) µg chlorophyll *a* / g of *Lyngbya*, and 4.65 (±0.67) µg chlorophyll *a* / g of *Lyngbya*, respectively.

Laboratory Algal Toxicity Experiments using Hydrothol-191®

Measured chlorophyll *a* concentrations after exposures of 30, 50, and 70 mg Hydrothol-191® / g of *Lyngbya* were 27.8 (±4.74) µg chlorophyll *a* / g of *Lyngbya*, 9.35 (±1.49) µg chlorophyll *a* / g of *Lyngbya*, and 7.3 (±0.88) µg chlorophyll *a* / g of *Lyngbya*, respectively.

Laboratory algal toxicity experiments using adjuvants and Algimycin®-PWF

Measured chlorophyll *a* concentrations decreased significantly after exposures of 14, 28, and 56 mg Cu, as Algimycin®-PWF with 1 ml of an adjuvant (Silnet 200®, Sil Energy®, Big Sur 90®, Poly An®, Big Wet®, Poly Control 2®, and Cide-Kick II®; Table 3.5). Measured chlorophyll *a* concentrations after exposures using Silnet 200® were 81.2 (± 6.43) µg chlorophyll *a* / g of *Lyngbya*, 56.3 (± 5.91) µg chlorophyll *a* / g of *Lyngbya*, and 21.9 (± 3.04) µg chlorophyll *a* / g of *Lyngbya*, respectively. Measured chlorophyll *a* concentrations after exposures using Sil Energy® were 94.8 (±6.44) µg chlorophyll *a* / g of *Lyngbya*,

53.4 (±3.88) µg chlorophyll *a* / g of *Lyngbya*, and 34.2 (±2.15) µg chlorophyll *a* / g of *Lyngbya*, respectively.

Measured chlorophyll *a* concentrations after exposures using Big Sur 90® were 47.6 (±5.39) µg chlorophyll *a* / g *Lyngbya*, 28.7 (±2.28) µg chlorophyll *a* / g of *Lyngbya*, and 11.8 (±1.24) µg chlorophyll *a* / g of *Lyngbya*, respectively. Measured chlorophyll *a* concentrations after exposures using Poly An® were 103 (±6.92) µg chlorophyll *a* / g *Lyngbya*, 63.7 (±6.81) µg chlorophyll *a* / g *Lyngbya*, and 36.6 (±2.99) µg chlorophyll *a* / g *Lyngbya*, respectively. Measured chlorophyll *a* concentrations after exposures using Big Wet® were 77.2 (±4.08) µg chlorophyll *a* / g of *Lyngbya*, 55.1 (±6.16) µg chlorophyll *a* / g of *Lyngbya*, and 32.4 (±4.93) µg chlorophyll *a* / g of *Lyngbya*, respectively. Measured chlorophyll *a* concentrations after exposures using Poly Control® were 65.4 (±5.62) µg chlorophyll *a* / g of *Lyngbya*, 42.2 (±5.41) µg chlorophyll *a* / g of *Lyngbya*, and 24.4 (±3.34) µg chlorophyll *a* / g of *Lyngbya*, respectively. Measured chlorophyll *a* concentrations after exposures of 3.0, 5.0, 10, 14, 28 and 56 mg Cu, as Algimycin®-PWF with 1 ml of Cide-Kick II® were 34.3 (±3.35) µg chlorophyll *a* / g of *Lyngbya*, 17.3 (±4.36) µg chlorophyll *a* / g of *Lyngbya*, and 10.0 (±0.79) µg chlorophyll *a* / g of *Lyngbya*, 6.88 (±0.49) µg chlorophyll *a* / g of *Lyngbya*, 2.38 (±0.25) µg chlorophyll *a* / g of *Lyngbya*, and 2.2 (±0.28) µg chlorophyll *a* / g of *Lyngbya*, respectively.

Laboratory algal toxicity experiments using combinations of PAK™-27 and Algimycin®-PWF with Cide-Kick II®

Results of algaecide and adjuvant exposures indicated that the treatment of PAK™-27 followed by Algimycin®-PWF with Cide-Kick II® was the most efficacious treatment tested. Therefore, chlorophyll *a* measurement after exposures of 1) 100 mg sodium carbonate peroxyhydrate, 3.0 mg Cu, as Algimycin®-PWF, and 1.0 ml of Cide-Kick II® was 14.2 (±2.51) µg chlorophyll *a* / g of *Lyngbya*; 2) 100 mg sodium carbonate peroxyhydrate, 5.0 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® was 1.68 (±0.46) µg chlorophyll *a* / g of *Lyngbya*; 3) 100 mg sodium carbonate peroxyhydrate, 7.0 mg Cu, as Algimycin®-PWF, and 1.0 ml of Cide-Kick II® was 1.53 (±0.36) µg chlorophyll *a* / g of *Lyngbya*; 4) 100 mg sodium carbonate peroxyhydrate, 3.0 mg Cu, as Algimycin®-PWF, and 0.5 ml of Cide-Kick II® was 28.0 (± 3.64) µg chlorophyll *a* / g of *Lyngbya*; 5) 100 mg sodium carbonate peroxyhydrate, 7.0 mg Cu as Algimycin®-PWF and 1.5 ml of Cide-Kick II® averaged 2.15 (±0.61) µg chlorophyll *a* / g of *Lyngbya*; 6) 50 mg sodium carbonate peroxyhydrate, 0.3 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® was 24.8 (±1.09) µg chlorophyll *a* / g of *Lyngbya*; 7) 150 mg sodium carbonate peroxyhydrate, 0.7 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® was 2.05 (±0.69) µg chlorophyll *a* / g of *Lyngbya*. The most efficacious treatment was 100 mg sodium carbonate peroxyhydrate, followed by 5.0 mg Cu as Algimycin®-PWF and 1.0 ml of Cide-Kick II® / g of *Lyngbya* (Table 3.6).

Lay Lake and Lake Jordan: Algaecide Application Study

The specific approach chosen for field trials included three treatments of maximum label applications of PAK™27 (5.29 mg SCP / L) followed in 24-hours by a combination of Algimycin® PWF (1 mg Cu / L) and Cide-Kick II® (7.6 L / 4047 m^2) in Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough (Table 3.3).

Field Exposures of Copper as Algimycin®-PWF

Samples were collected after treatment for measurement of acid extractable copper (May 19, 2005 - Flat Top and Powerline Slough; June 19, 2005 - Nelson Slough and Kelly Slough; Table 3.3) and returned to the laboratory at Clemson University. Copper concentrations for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 0.971 (±0.008), 0.922 (±0.001), 0.826 (±0.027), and 0.802 (±0.057) mg Cu / L, as Algimycin®PWF, respectively. Measured acid extractable copper concentrations four hours after the second treatment (June 9, 2005, Flat Top and Powerline Slough; June 29, 2005, Nelson Slough and Kelly Slough; Table 3.3) for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 0.893 (±0.060), 0.898 (±0.020), 0.779 (±0.020), and 0.835 (±0.035) mg Cu / L, as Algimycin®-PWF, respectively. Measured acid extractable copper concentrations in samples collected four hours after the third treatment (June 23, 2005, Flat Top and Powerline Slough; July 19, 2005, Nelson Slough and Kelly Slough) (Table 3.3) for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 0.876 (±0.028), 0.883 (±0.009), 0.891 (± 0.013), and 0.848 (±0.018) mg Cu / L, as Algimycin®-PWF,

respectively. (Table 3.7) Total acid extractable copper concentrations in the treatment sites (Flat Top and Powerline Slough, Nelson Slough and Kelly Slough) returned to background by the last sampling period (August 15, 2005; Table 3.3).

Biomass measurements from Lay Lake and Lake Jordan

Algaecide applications at Flat Top Slough resulted in a statistically significant decrease of *Lyngbya* biomass from an average of 4.36 (±3.7) kg / m^2 to 0.308 (± 0.39) kg / m^2. Algaecide applications at Powerline Slough resulted in a statistically significant decrease of *Lyngbya* biomass from an average of 4.34 (±3.66) kg / m^2 to 0.703 (± 0.480) kg / m^2 (Figures 3.2 and 3.3). Algaecide applications at Kelly Slough resulted in *Lyngbya* biomass decreasing significantly, from an average of 3.87 (± 5.247) kg / m^2 to 1.29 (± 1.66) kg / m^2. Similarly, algaecide applications at Nelson Slough resulted in *Lyngbya* biomass decreasing significantly from an average of 1.68 (± 2.18) kg / m^2 to 0.36 (± 0.513) kg / m^2 (Figures 3.2 and 3.3).

Chlorophyll a measurements from Lay Lake and Lake Jordan

Pretreatment and post-treatment samples were collected for measurement of chlorophyll *a* analyses (May 19, 2005, Flat Top and Powerline Slough; June 19, 2005, Nelson Slough and Kelly Slough; Tables 3.3 and 3.8) and returned to the laboratory at Clemson University. Pretreatment chlorophyll *a* concentrations for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 20,998 (±580), 17,460 (±2,524), 21,028 (±3,962), 21,779 (±4,686) µg chlorophyll *a* / g of *Lyngbya*, respectively. Two weeks after the first application (June 9, 2005

at Flat Top and Powerline Slough; June 29, 2005, Nelson Slough and Kelly Slough; Tables 3.3 and 3.8), chlorophyll *a* concentrations for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 11,841 (±2,429), 12,241 (±2,683), 11,132 (±4,070), 8,605 (±4,104) µg chlorophyll *a* / g of *Lyngbya*, respectively. Two weeks after the second application (June 23, 2005, Flat Top and Powerline Slough; July 19, 2005, Nelson Slough and Kelly Slough), chlorophyll *a* concentrations for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 9,305 (±1,346), 10,671 (±3,505), 12,673 (±3,748), 12,556 (±1,675) µg chlorophyll *a* / g of *Lyngbya*, respectively (Table 3.8). On August 15, 2005, approximately six weeks (Flat Top and Powerline Slough) and four weeks (Nelson Slough and Kelly Slough) after the third application chlorophyll *a* concentrations for Flat Top Slough, Powerline Slough, Kelly Slough, and Nelson Slough were 12,527 (± 5,890), 17,392 (± 2,765), 17,392 (± 2,765), and 12,527 (± 5,890) µg chlorophyll *a* / g of *Lyngbya*, respectively (Table 3.8). Two weeks after the first application (June 29, 2005; Tables 3.3 and 3.8), chlorophyll *a* concentrations for Nelson Slough decreased significantly to 8,605 (±4,104) µg chlorophyll *a* / g of *Lyngbya*.

Table 3.8 Average measured chlorophyll *a* concentrations pre and post algaecide application at sample sites in Lay Lake and Lake Jordan, Alabama, with associated standard deviation values.

Reservoir	Sample site	µg Chl-*a* /g of *Lyngbya* (19 May 2005)	µg Chl-*a* /g of *Lyngbya* (9 June 2005)	µg Chl-*a* /g of *Lyngbya* (23 June 2005)	µg Chl-*a* /g of *Lyngbya* (15 August 2005)
Lay Lake	Flat Top Slough	20,998 (±580)	11,841 (±2,429)	9,305 (±1,346)	12,780 (±1,146)
	Powerline Slough	17,460 (±2,524)	12,241 (±2,683)	10,671 (±3,505)	16,988 (±5,024)
		µg Chl-*a* /g of *Lyngbya* (16 June 2005)	µg Chl-*a* /g of *Lyngbya* (29 June 2005)	µg Chl-*a* /g of *Lyngbya* (19 July 2005)	µg Chl-*a* /g of *Lyngbya* (15 August 2005)
Lake Jordan	Nelson Slough	21,779 (±4,686)	8,605 (±4,104)*	12,673 (±3,748)	17,392 (± 2,765)
	Kelly Slough	21,028 (±3,962)	11,132 (±4,070)	12,556 (±1,675)	12,527 (± 5,890)

* Statistically significant from initial chlorophyll *a* concentration

Lay Lake Reference Site: *Powerline South*

In the untreated reference site for Lay Lake, *Lyngbya* biomass statistically significantly increased by 89 % from an average of 7.86 (± 2.85) to 14.84 (± 1.614) kg / m^2 (August 15, 2005; Figures 3.2 and 3.3). Samples of *Lyngbya* from Powerline South were collected on May 19, June 9, June 23, and August 15, 2005 (Table 3.3), and chlorophyll *a* concentrations were 15,013 (±1,190), 20,799 (±5,383), 19,226 (±2,663), and 20,645 (±3,341) µg chlorophyll *a* / g of *Lyngbya*, respectively. The chlorophyll *a* concentration at Powerline South increased by 38% during the algaecide application period (May 19, 2005 and August 15, 2005), however, there were no statistically significant differences.

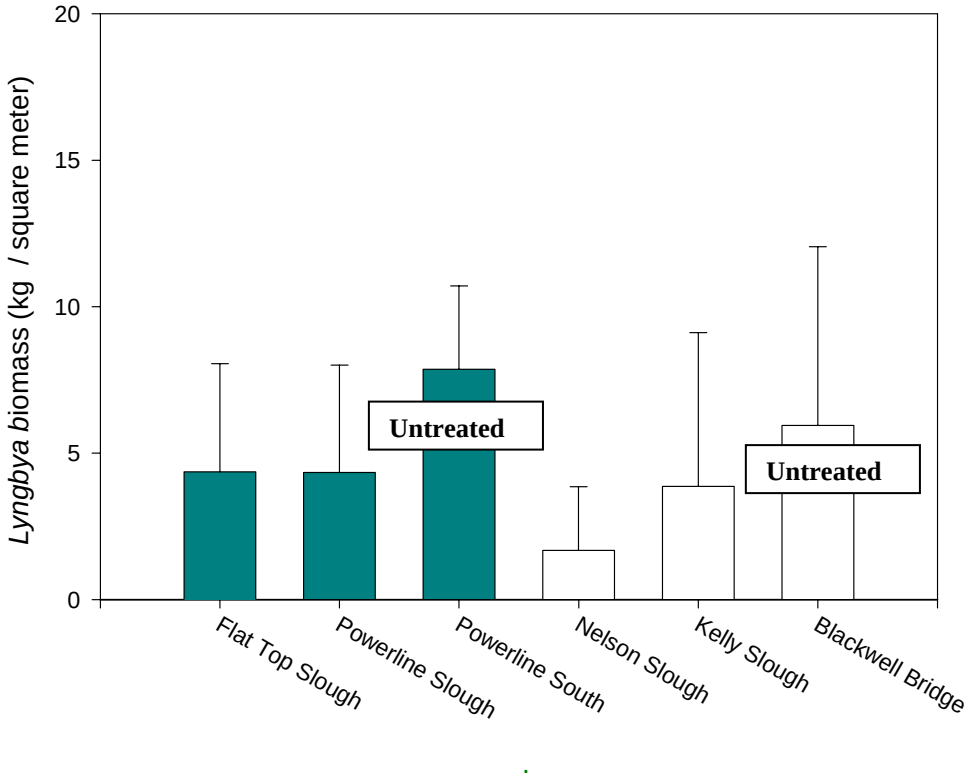

Figure 3.2 Pretreatment dry weight measurements of *Lyngbya* algal mat biomass, and associated standard deviations, from sites in Lay Lake and Lake Jordan, Alabama.

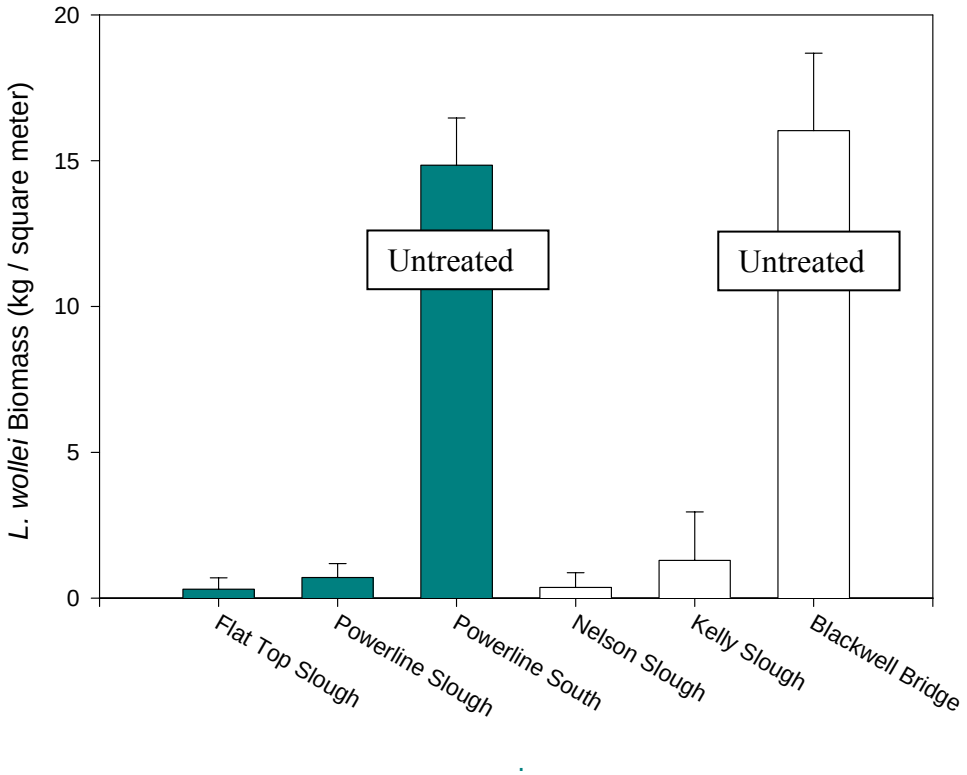

Figure 3.3 Post-treatment dry weight measurements of *Lyngbya* algal mat biomass, and associated standard deviations, from sites in Lay Lake and Lake Jordan, Alabama after 3 treatments.

Lake Jordan Reference Site: Blackwell Bridge Slough

In the untreated reference site for Lake Jordan, *Lyngbya* biomass statistically significantly increased by 169% from an average of 5.95 (±6.10) kg / m^2 (June 16, 2005) to 16.02 (±2.66) kg / m^2 (August 15, 2005; Figures 3.2 and 3.3). *Lyngbya* samples from Blackwell Bridge were collected June 16, June 29, July 19, and August 15, 2005, (Table 3.3), and chlorophyll *a* concentrations were 17,324 (±518), 16,721 (±735), 15,026 (±3,103), and 26,453 (±3,148) µg chlorophyll *a* / g of *Lyngbya,* respectively. The chlorophyll *a* concentration at Blackwell Bridge increased by 53 % during the algaecide application period (June 16, 2005 and August 15, 2005), and the final chlorophyll a concentration of 26,453 (±3,148) was statistically significantly different from the initial chlorophyll concentration of 26,453 (±3,148)

Contrasting Laboratory and Field Results

Laboratory Algal Toxicity Test Results

Results of algaecide and adjuvant exposures indicated that control of *Lyngbya* was obtained using PAK™-27 and Algimycin®-PWF with Cide-Kick II®. The laboratory treatment regimen included applications of 10.0 mg PAK™-27 / 0.1 g of *Lyngbya* followed approximately twenty-four hours later by a combination of 2.5 mg Cu /L as Algimycin® PWF / 0.1 g of *Lyngbya* and 0.1 ml of Cide-Kick II® / 0.1g of *Lyngbya* (potential burden ~5.0 mg Cu / g of *Lyngbya*). Initial chlorophyll *a* concentrations of 17, 852 (± 1048) and significantly decreased to 1.68 (±0.46) µg chlorophyll *a*. However, three field applications using the maximum field label rate (1 mg Cu / L) at Flat Top Slough, Powerline

Slough, Nelson Slough and Kelly Slough resulted in a potential burden (mg of copper / g of *Lyngbya*) of 2.29 (±1.13) mg Cu / g of *Lyngbya*.

Lay Lake and Lake Jordan: Algaecide Application Study Field Results

Lay Lake

Algaecide applications at Flat Top Slough resulted in a statistically significant decrease in *Lyngbya* biomass by decreasing 93 %, from an average of 4.36 (±3.7) kg / m^2 to 0.308 (± 0.39) kg / m^2. Algaecide applications at Powerline Slough resulted in a statistically significant decrease in *Lyngbya* biomass by decreasing by 84 %, from an average of 4.34 (±3.66) kg / m^2 to 0.703 (± 0.480) kg / m^2 (Figures 3.2 and 3.3).

Lake Jordan

Algaecide applications at Kelly Slough resulted in *Lyngbya* biomass decreasing (not statistically significant) by 67 %, from an average of 3.87 (± 5.247) kg / m^2 to 1.29 (± 1.66) kg / m^2. Similarly algaecide applications at Nelson Slough resulted in *Lyngbya* biomass decreasing (not statistically significant) by 79 %, from an average of 1.68 (± 2.18) kg / m^2 to 0.36 (± 0.513) kg / m^2 (Figures 3.2 and 3.3).

Table 3.9. Water characteristics for field waters from Lay Lake and Lake Jordan, Alabama.

Lake	**Slough**	**pH (S.U.)**	**Hardness (mg / L as $CaCO_3$)**	**Alkalinity (mg / L as $CaCO_3$)**	**Conductivity (μmhos / cm)**	**D.O. mg / L**	**Temperature° C**	**Background acid extractable aqueous Cu concentrations μg Cu / L**
Lay Lake	Flat Top	8.0 - 8.8	52 - 56	54 - 56	133-134	9.3 - 10.5	23.3-28.3	5.6 (±0.001)
	Powerline	7.3 - 7.8	52 - 54	54 - 56	135 - 154	9.9 - 10.0	28.5-29.7	10.6 (±0.004)
	Powerline South	7.1 - 7.9	54 - 56	54 - 56	129 - 137	7.0 - 7.2	22.5-28.7	12.9 (±0.0002)
Lake Jordan	Nelson	7.3 - 8.4	54 - 56	52 - 56	115 - 131	9.2 - 10.4	22.2-28.7	5.6 (±0.0007)
	Kelly	8.1 - 8.4	56 - 58	54 - 56	139 - 168	9.5 - 10.5	29.1-30.5	5.0 (±0.0003)
	Blackwell Bridge	7.7 - 7.9	56 - 58	54 - 56	130 - 139	9.4 - 10.3	28.2-31.6	9.1 (±0.0004)

Conclusions

Lyngbya, from Lay Lake and Lake Jordan, Alabama, in site water was sensitive to PAK™-27 and Algimycin®-PWF with Cide-Kick II® after a 7 d exposure (pre-treatment chlorophyll *a* concentration 17,852 µg / chlorophyll *a* / g of *Lyngbya*) and a treatment strategy for *Lyngbya* in Lay Lake and Lake Jordan was developed using PAK™-27 and Algimycin®-PWF with Cide-Kick II®. These laboratory data emphasize the value and utility of using site water and site algae for laboratory algal toxicity experiments. Using this approach for predicting field responses of algae to algaecide applications permits more efficient use of effective algaecides and minimizes risks for non-target species.

Field treatments were initiated on 19 May, 2005. Responses of *Lyngbya* to treatments of PAK™-27, and Algimycin®-PWF with Cide-Kick II® in Lay Lake and Lake Jordan closely resembled responses observed following laboratory exposures to PAK™-27, Algimycin®-PWF with Cide-Kick II®. Laboratory exposures used a predetermined volume of water in an exposure chamber and contact between the algaecide and *Lyngbya* was maximized. During field treatments, contact between the algaecide and *Lyngbya* was maximized by applying the algaecide below the water's surface. Algaecide application techniques included treatment of the infested area from the shoreline to deeper water and only partial treatment of the infested area. These application techniques decreased potential exposures for non-target species, increased potential margins of safety for fish and invertebrates, and provided refugia during treatment.

Mastin et al. (2002) measured responses of a benthic alga (*Lyngbya*) from a North Louisiana reservoir to laboratory exposures of Clearigate® in an aqueous culture medium with a mean pH, alkalinity, and hardness of 7.4, 176 mg / L as $CaCO_3$, and 92 mg / L as $CaCO_3$, respectively. *Lyngbya* used for this study responded to exposures of 0.3 and 0.6 mg Cu / L as Clearigate®, with a 75 and 78 % decrease in *Lyngbya* population after treatment. Tedrow (2007) sampled *Lyngbya* from Highpoint City Lake (NC) in site water with a mean pH, alkalinity, and hardness of 7.4, 50 mg / L as $CaCO_3$, and 44 mg / L as $CaCO_3$, respectively, responded to exposures of Algimycin®-PWF with a significant decrease in chlorophyll *a*. Repeat applications of Algimycin®-PWF were recommended due to the density of *Lyngbya* observed in City Lake, and *Lyngbya* biomass decreased significantly (~ 77 %) after field algaecide treatments. In the present experiment responses of *Lyngbya* sampled from two Alabama reservoirs (Lay Lake and Lake Jordan) to laboratory algaecide exposures in site waters with a mean pH, alkalinity, and hardness of 8.2, 54 mg / L as $CaCO_3$, and 60 mg / L as $CaCO_3$, respectively. *Lyngbya* used in this study responded to a sequential algaecide technique (using Algimycin® PWF) with decreases (2.7 to 40 %) in chlorophyll *a* concentration. Multiple applications of this sequential algaecide technique were required due to the density of *Lyngbya* observed in the treatment areas of Lay and Jordan reservoirs. After treatments, algal biomass decreased (67 - 93 %) in Lay and Jordan Reservoirs. It is apparent from these studies that responses of *Lyngbya* to algaecide exposures vary widely. Although genetic, physiological and morphological variation can explain some of the differences observed, water

characteristics that influence speciation and bioavailability can significantly alter exposures of copper-containing algaecides and subsequent responses of target algae. Water characteristics that influence copper speciation and form are pH, alkalinity and hardness. Solubility of copper and its speciation are mostly dependent on the pH of the aquatic environment. The stability of various copper complexes (both inorganic and organic) is pH dependent, influencing the forms and therefore the toxicity of copper to an organism (Flemming and Trevor 1989). As the pH of the water decreases, the associated toxicity generally increases. This increase in toxicity is the result of an increase in free bioavailable species (i.e. Cu^{+1} and Cu^{+2}) as pH decreases. However, hardness and alkalinity refers principally to calcium and magnesium ions present in the water. Therefore, the effect of hardness and alkalinity on copper speciation may be limited to competition with these ions for the same ligand (Erikson et al. 1996). In soft waters (i.e. alkalinity and hardness < 20 mg/L as $CaCO_3$) copper is generally more toxic (i.e. the LC_{50} is lower) than in moderately hard to hard waters (i.e. alkalinity and hardness > 80 mg/L as $CaCO_3$) (Suedel et al. 1996). This further emphasizes the importance and utility of using laboratory algal toxicity tests with site water and site algae to predict responses of target algae in field-scale applications. The laboratory information on sensitivity to algaecides, coupled with early detection of growth leading to development of extreme *Lyngbya* densities, can assist implementation of an effective management strategy that can restore and maintain critical water resource usages.

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CHAPTER 4

RESPONSES OF *PIMEPHALES PROMELAS* RAFINESQUE AND *CERIODAPHNIA DUBIA* RICHARD TO LABORATORY AND SIMULATED FIELD EXPOSURES OF COPPER SULFATE PENTAHYDRATE AND CUTRINE®- PLUS

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Abstract

The primary purpose of this research was to compare responses of two sensitive non-target species to typical laboratory exposures of copper-containing algaecides with responses to declining exposures which simulate "field" exposures. The exposures in this study encompass typical exposures during laboratory testing situations (e.g. "typical exposures") and declining exposures simulating field conditions after an application of an algaecide. Specific objectives of this research were to contrast: 1) responses of *Pimephales promelas* in typical exposures of copper sulfate pentahydrate versus declining exposures; 2) responses of *P. promelas* in typical exposures of Cutrine®-Plus versus declining exposures; 3) responses of *Ceriodaphnia dubia* in typical exposures of copper sulfate pentahydrate versus declining exposures; 4) responses of *C. dubia* in typical exposures of Cutrine®-Plus versus declining exposures, and to compare 5) responses of these two species to copper sulfate pentahydrate exposures and Cutrine®-Plus exposures to determine the relative risk of these algaecides. Declining exposures were less toxic than typical laboratory exposures when responses of *P. promelas* and *C. dubia* were measured using copper sulfate pentahydrate and Cutrine®-Plus. Using *C. dubia* and *P. promelas*, the declining exposures were 33 - 47% less toxic for copper sulfate pentahydrate and 30 - 49 % less toxic for Cutrine®-Plus than the typical laboratory exposures. Therefore, typical laboratory exposures may overestimate risks relative to declining exposures. Since concentrations of copper-containing algaecides rapidly decline after treatment, use of laboratory data with typical exposures to estimate risks in

field is likely very conservative given the propensity of copper to interact with a variety of ligands and decrease bioavailability.

Introduction

Applications of copper-containing algaecides or herbicides to aquatic systems often exceed concentrations that cause effects in laboratory toxicity testing of non-target species (US EPA 1985; US EPA 2002). The assumption based upon first order ecological risk assessment (US EPA 1992; Reinert et al. 1998) is that sensitive non-target species will be adversely affected by an application of algaecide since the predicted no-effect level based on laboratory data may be exceeded in field applications. However, the initial copper concentration declines over time after an algaecide or herbicide application in the field yielding a declining or episodic exposure (Button et al. 1976; Whitaker et al. 1978). Typical experimental exposures in the laboratory, which are relatively constant, are not reflective of declining environmental concentrations of copper (e.g., US EPA 1985). Water quality data for copper, that are intended to be protective of aquatic life, are derived from laboratory experiments and often do not reflect responses to exposures in the field, particularly declining or episodic exposures such as algaecide or herbicide applications. Declining exposures may consist of single or multiple exposures. If there are multiple declining exposures, as in the case of repeated applications of algaecides or herbicides, then relatively large time intervals occur between applications (weeks to months). In order to better understand potential risks of algaecide applications, we can contrast responses of sensitive, sentinel, non-target species to typical laboratory exposures

of copper-containing algaecides with responses to simulated declining "field" exposures.

Sentinel species of fresh water invertebrates and vertebrates, such as *Ceriodaphnia dubia* Richard and *Pimephales promelas* Rafinesque have been widely used to measure the potency of elements (Denton et al. 1996) and compounds (e.g., pesticides), as well as complex mixtures (e.g. effluents) (Norberg-King and Schmidt 1993). Both *C. dubia* and *P. promelas* are relatively sensitive to copper with reported 96h LC_{50}s of 60 and 675 µg Cu / L, in formulated moderately hard water, respectively (Murray-Gulde et al. 2002). The present experiments were designed to compare responses of these sensitive non-target species to typical laboratory exposures of copper-containing algaecides with responses to declining exposures which simulate "field" exposures. Typically, laboratory toxicity tests with these species involve exposures of 7-d duration with essentially constant concentrations maintained by renewal or flow-through systems (US EPA 1995; US EPA 2002; ASTM 1993a, 1993b). After applications of copper-containing algaecides in the field, concentrations of an environmentally active metal such as copper will decline over time due to several factors including: 1) the initial concentration of copper applied, 2) the form (or formulation) of copper, and 3) characteristics of the aquatic system, such as pH, hardness, alkalinity, and ionic strength or conductivity (Baudo et al. 1990; Breault et al. 1996; Deaver and Rodgers 1996).

The primary purpose of this research is to contrast responses of a sentinel aquatic invertebrate and vertebrate to exposures of copper-containing algaecides.

The exposures in this study encompass typical exposures during laboratory testing situations (e.g. "typical exposures") and declining exposures simulating field conditions after an application. Specific objectives of this research are to compare: 1) responses of *P. promelas* in typical exposures of copper sulfate pentahydrate versus declining exposures; 2) responses of *P. promelas* in typical exposures of Cutrine®-Plus versus declining exposures; 3) responses of *C. dubia* in typical exposures of copper sulfate pentahydrate versus declining exposures; 4) responses of *C. dubia* in typical exposures of Cutrine®-Plus versus declining exposures, and 5) responses of these two species to copper sulfate pentahydrate exposures and Cutrine®-Plus exposures.

Materials and Methods

Pimephales promelas and Ceriodaphnia dubia toxicity tests using copper sulfate pentahydrate (typical laboratory exposures)

All experiments were performed according to U.S. EPA protocol (Lewis et al. 1994). At test initiation, a solution of 1000 µg Cu / L, as copper sulfate pentahydrate (Table 4.1) was prepared using a 100 mg Cu /L, as copper sulfate pentahydrate, stock solution and formulated moderately hard water (Table 4.2). This solution was diluted daily using formulated moderately hard water (Table 4.2) to prepare static renewal concentrations of 200, 400, 600, and 800 µg Cu / L for *P. promelas* and concentrations of 10, 20, 30, 40, 50, and 75 µg Cu / L for *C. dubia*. *P. promelas* exposure chambers for each treatment consisted of four 250 ml beakers, which contained ten animals per beaker and 40 animals per exposure concentration. *C. dubia* exposure chambers for each treatment consisted of 20 ml

shell vials, which contained one animal per vial and 10 animals per exposure concentration. Experiments were conducted in a light-and-temperature controlled incubator at 25 ± 1 ºC with a 16-h light / 8-h dark photoperiod using "cool white" fluorescent lighting and a light intensity of 50-100 ft-c. Exposure solutions were renewed daily to avoid accumulation of excess detritus and food that may influence copper bioavailability. All test exposures were conducted for seven days. To confirm algaecide exposures in the test chambers, acid extractable copper concentrations (U.S. EPA 1983) were measured using a Perkin-Elmer AAnalyst 5100 flame and graphite furnace atomic absorption (AA) spectrometer (APHA 1998).

Table 4.1. Physical properties and fate characteristics of Cutrine®-Plus and copper sulfate pentahydrate

Properties or Characteristics	Copper Sulfate Pentahydrate	Cutrine®-Plus
% Cu as elemental	25.4	9.0
Formulation	$CuSO_4 \bullet 5H_2O$	Copper-ethanolamine Complex
Chemical class	Copper salt	Chelated elemental copper (Cu_2CO_3)
Mode of action	Cell toxicant	Cell toxicant
Appearance	Blue crystalline	Blue viscous liquid
Odor	NA	Slight amine
Water solubility (mg/L)	316,000	NA
Boiling point (^oC)	NA	100
Melting point (^oC)	110	NA
Specific gravity (g / cm^3)	NA	1.21
pH	NA	10.0 - 11.0

Kamrin 1997
NA, Not Available

Table 4.2. Water characteristics of formulated moderately hard test water for *Pimephales promelas* and *Ceriodaphnia dubia* toxicity tests.

Water Sample Parameters	**Units**	**Range for *Pimephales promelas* Exposures**	**Range for *Ceriodaphnia dubia* Exposures**
pH	SU	7.57 - 8.05	7.64 – 8.15
Alkalinity	mg / L (as $CaCO_3$)	72 - 76	72 - 76
Hardness	mg / L (as $CaCO_3$)	74 - 75	74 - 75
Conductivity	µS / cm^2	298 - 315	292 - 301
Dissolved Oxygen	mg O_2 / L	7.6 - 8.0	7.7 – 8.1
Temperature	ºC	23.5 - 25.0	23.2 - 25.0

Pimephales promelas and Ceriodaphnia dubia toxicity tests using copper sulfate pentahydrate (declining laboratory exposures)

A solution of copper sulfate pentahydrate was prepared at test initiation as described previously for typical laboratory exposures. The solution was diluted daily using formulated moderately hard water (Table 4.2) to prepare declining concentrations using the water column residence time (half-life = 3.5 d) of copper as copper sulfate pentahydrate based on mesocosm studies by Murray-Gulde et al. (2002). Initial exposure concentrations for *P. promelas* were 200, 400, 600, 800, and 1000 µg Cu / L and declined to concentrations of 64.3, 128.6, 192.9, 257.1, and 321.4 µg Cu / L as copper sulfate pentahydrate, respectively, at experimental conclusion (day 7). Initial concentrations of 10, 20, 30, 40, 50, and 75 µg Cu / L for *C. dubia* declined to concentrations of 3.2, 6.4, 9.6, 12.9, 16.1, and 24.1 µg Cu / L, as copper sulfate pentahydrate, respectively, at experimental conclusion (day 7).

Pimephales promelas and Ceriodaphnia dubia toxicity tests using Cutrine®-Plus (typical laboratory exposures)

At test initiation, a solution of 1000 µg Cu / L, as Cutrine®-Plus (Table 4.1) was prepared using a 100 mg Cu /L, Cutrine®-Plus, stock solution and formulated moderately hard water (Table 4.2). This solution was diluted daily using moderately hard water (Table 4.2) to prepare static renewal concentrations of 200, 400, 600, 800, and 1000 µg Cu / L, as Cutrine®- Plus, for *P. promelas* and concentrations of 10, 20, 30, 40, 50, and 75 µg Cu / L, as Cutrine®- Plus, for *C. dubia*. Experimental techniques are described previously in the *P. promelas* and *C. dubia* toxicity tests using copper sulfate pentahydrate declining laboratory

exposures section for the typical laboratory experiment using copper sulfate pentahydrate.

Pimephales promelas and Ceriodaphnia dubia toxicity tests using Cutrine®- Plus (declining laboratory exposures)

At test initiation, a solution of 1000 µg Cu / L, as Cutrine®-Plus (Table 4.1) was prepared using a 100 mg Cu /L, Cutrine®-Plus, stock solution and formulated moderately hard water (Table 4.2). The stock solution was diluted daily using formulated moderately hard water (Table 4.2) to prepare declining concentrations using the water column residence time (half-life = 2.8 d) of copper as Cutrine®-Plus were based on mesocosm studies by Murray-Gulde et al. (2002). Initial exposure concentrations for *P. promelas* were 200, 400, 600, 800, and 1000 µg Cu / L and declined to concentrations of 46.4, 92.9, 139.3, 185.7, and 232.1 µg Cu / L as Cutrine®-Plus, respectively, at experimental conclusion (day seven). Initial concentrations of 10, 20, 30, 40, 50, and 75µg Cu / L for *C. dubia* declined to concentrations of 2.3, 4.6, 6.9, 9.2, 11.5, and 17.4 µg Cu / L as Cutrine®-Plus, respectively, at experimental conclusion (day seven).

Statistical Analysis

No observed effects concentrations (NOECs and associated standard deviations) and lowest observed effects (LOECs and associated standard deviations) for *P. promelas* and *C. dubia* and each algaecide were determined by statistically significant differences relative to controls (Ott 1993). One-way analysis of variance (ANOVA) was performed with Dunnett's multiple range test (Ott 1993) to test for significance when comparing results from static renewal and

declining static renewal experiments ($p \leq 0.05$). Four day and 7-day lethal concentrations (4-d and 7-d LC_{50}s, with associated 95% confidence intervals were calculated) were calculated using Trimmed Spearman-Karber (Hamilton et al. 1977). Four day potency slopes were calculated by dividing percent mortality by µg Cu / L. Statistical calculations for declining laboratory exposure experiments were based on initial measured copper concentrations for copper sulfate pentahydrate and Cutrine®-Plus.

Results and Discussion

P. promelas and C. dubia toxicity tests using copper sulfate pentahydrate (typical laboratory exposures)

The copper sulfate pentahydrate 4-day and 7-day LC_{50} values for *P. promelas* in typical laboratory exposure experiments were 519 (95 % confidence intervals 440-612) and 282 (174-458) µg Cu / L as copper sulfate pentahydrate, respectively (Table 4.3). An NOEC of 169 (± 4.24) µg Cu / L, as copper sulfate pentahydrate, was estimated for the 4-day typical laboratory exposure experiment, an LOEC of 355 (± 2.83) µg Cu / L, as copper sulfate pentahydrate and a potency slope (% mortality / µg Cu / L) of 0.111 (Confidence intervals 0.124 - 0.098) was calculated (Table 4.4; Figures 4.1 and 4.3).

The copper sulfate pentahydrate 4-day and 7-day LC_{50} values for *C. dubia* in typical laboratory exposure experiments were 41 (29-57) and 23.5 (26-39) µg Cu / L, respectively (Table 4.5). NOECs of 28 (± 2.8) and 18 (±2.1) µg Cu / L as copper sulfate pentahydrate were estimated in the 4- and 7-day static renewal experiments. LOECs of 38 (±1.4) and 28 (± 2.8) µg Cu / L as $CuSO4*5H_2O$ were

estimated in the 4-day and-7-day typical laboratory exposure experiments. A 7-d NOEC of 10 (± 2.1) µg Cu / L, as $CuSO4*5H_2O$ was estimated for *C. dubia* reproduction in a typical laboratory exposure experiment with a 7-d LOEC of 18 (± 2.1) µg Cu / L, as $CuSO4*5H_2O$ and a potency slope (% mortality / µg Cu / L) of 1.11 (0.894 -1.32) was calculated (Table 4.5; Figures 4.2 and 4.4).

Table 4.3. Responses of *P. promelas* to exposures of copper sulfate pentahydrate and Cutrine®-Plus in typical laboratory and declining exposure scenarios. Both 4-day and 7-day LC_{50}s and associated 95 % confidence intervals were calculated.

Chemical	Typical Laboratory Exposures		Declining Laboratory Exposures	
	4-day LC_{50} [a] and C.I. [b] (µg Cu / L)	7-day LC_{50} [a] and C.I. [b] (µg Cu / L)	4-day LC_{50} [a] and C.I. [b] (µg Cu / L)	7-day LC_{50} [a] and C.I. [b] (µg Cu / L)
Copper Sulfate Pentahydrate	519 (440 - 612)	282 (174 - 458)	634 (513 - 782)	418 (356 - 490)
Cutrine®-Plus	731 (627 - 854)	485 (397 - 592)	973 (804 - 1176)	690 (574 – 831)

[a] Estimated concentration at which mortality was observed in 50% of the test population.
[b] 95 % confidence intervals.

Table 4.4. Four day no observed effects concentrations (NOECs) and lowest observed effects concentrations (LOECs) and potency slopes for *P. promelas* and *C. dubia* using copper sulfate pentahydrate and Cutrine®-Plus.

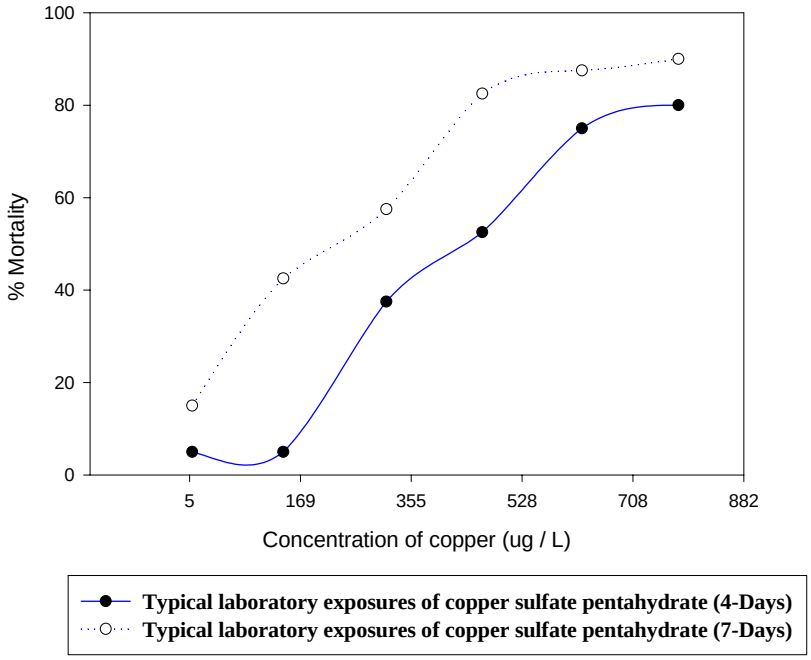
Chemical	Typical Laboratory Exposures			Declining Laboratory Exposures		
	NOEC [a] µg Cu / L (4-day)	LOEC [b] µg Cu / L (4-day)	Potency Slope % mortality/ µg Cu / L	NOEC [a] µg Cu / L (4-day)	LOEC [b] µg Cu / L (4-day)	Potency Slope % mortality/ µg Cu / L
	P. promelas			***P. promelas***		
Copper Sulfate Pentahydrate	169	355	0.111	169	355	0.076
Cutrine®-Plus	598	793	0.057	793	882	0.049
	C. dubia			***C. dubia***		
Copper Sulfate Pentahydrate	28	38	1.11	38	47	1.11
Cutrine®-Plus	41	51	0.491	51	72	0.349

[a] NOEC – No observed effect concentration. [b] LOEC – Lowest observed effect concentration.

Table 4.5. Responses of *C. dubia* to exposures of copper sulfate pentahydrate and Cutrine®-Plus in typical laboratory and declining exposure scenarios. Both 4-day and 7-day LC_{50}s and associated 95 % confidence intervals were calculated.

Chemical	Typical Laboratory Exposures		Declining Laboratory Exposures	
	4-day LC_{50}[a] and C.I. [b] (µg Cu / L)	7-day LC_{50}[a] and C.I. [b] (µg Cu / L)	4-day LC_{50}[a] and C.I. [b] (µg Cu / L)	7-day LC_{50}[a] and C.I. [b] (µg Cu / L)
Copper Sulfate Pentahydrate	41 (29 - 57)	24 (16 – 34)	56 (47 - 68)	35 (27 - 44)
Cutrine®-Plus	54 (43 - 68)	32 (26 – 39)	70 (55 - 90)	48 (33 - 68)

[a] Estimated concentration at which mortality was observed in 50% of the test population.
[b] 95 % confidence intervals.



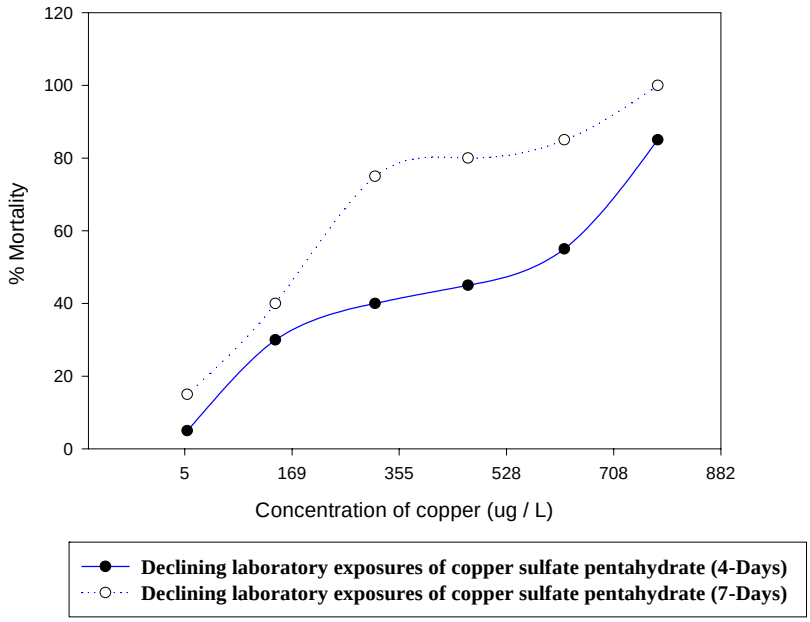
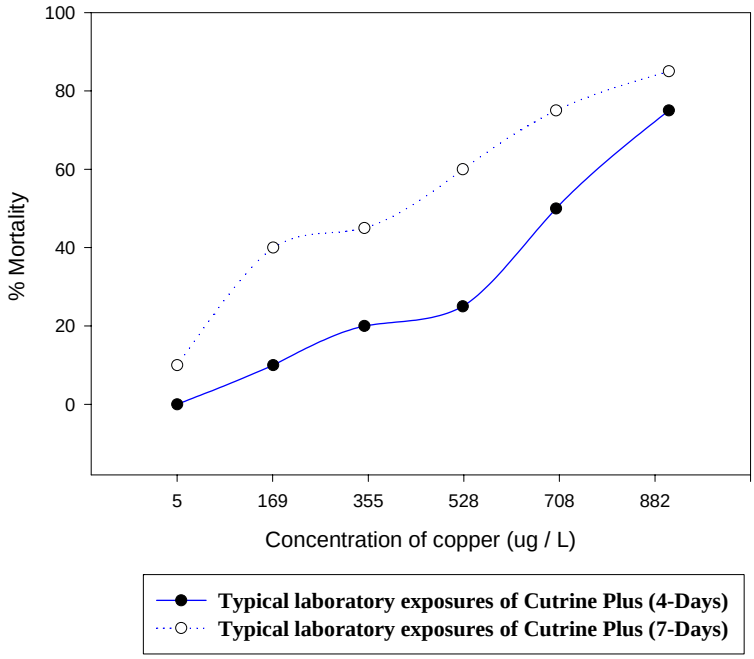


Figure 4.1. Responses of *Pimephales promelas* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus for 4-d and 7-d durations of exposure.



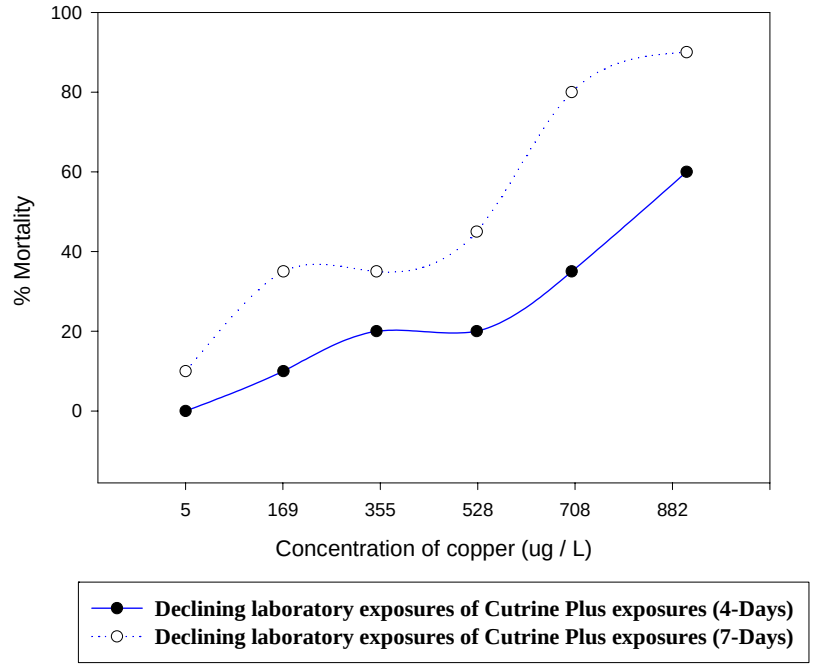
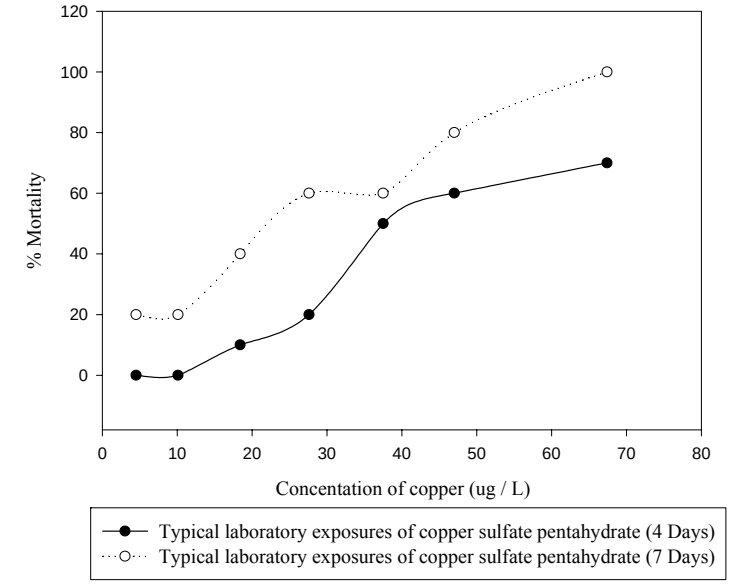


Figure 4.1. Responses of *Pimephales promelas* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus for 4-d and 7-d durations of exposure (Continued).



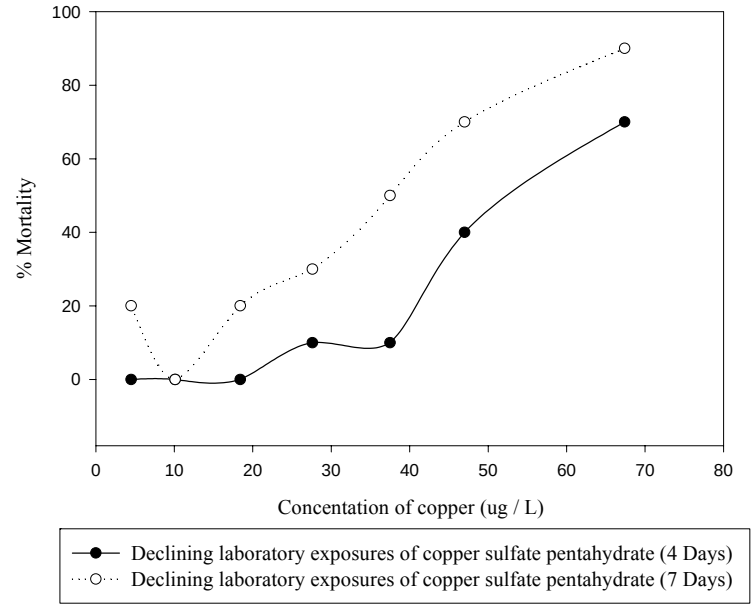


Figure 4.2. Responses of *Ceriodaphnia dubia* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus 4-d and 7-d durations of exposure.

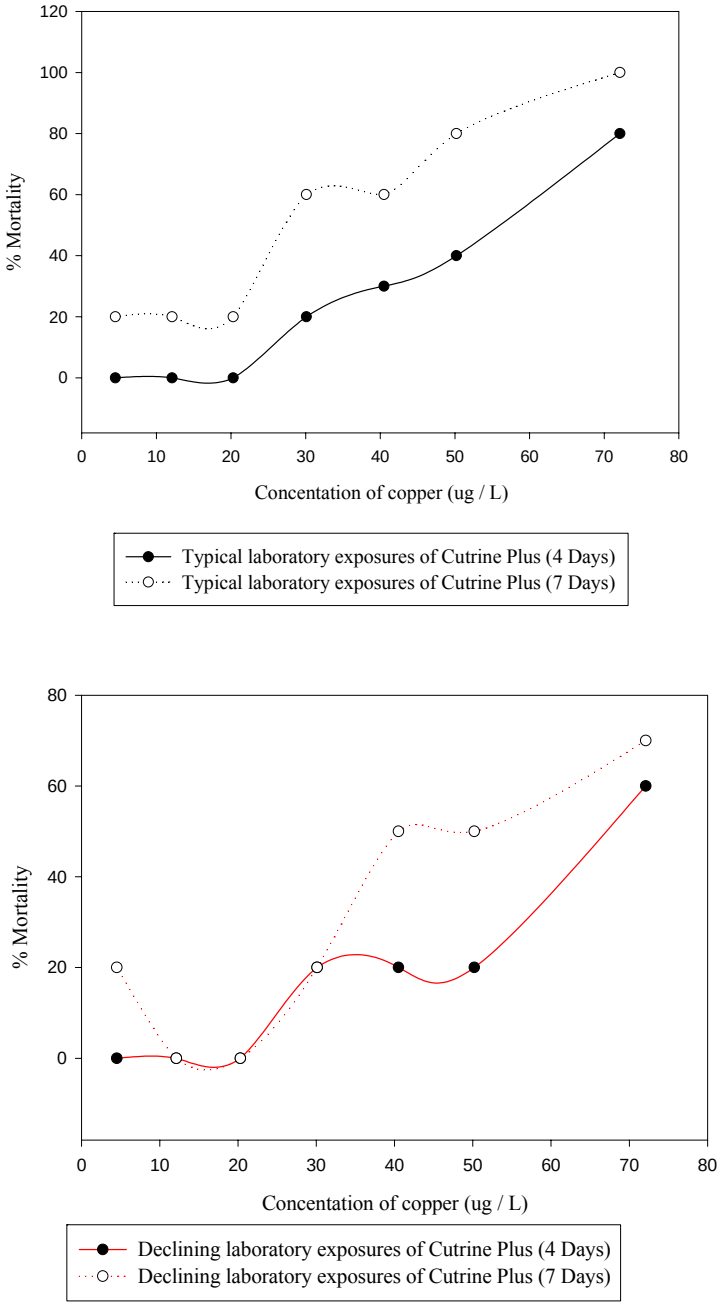
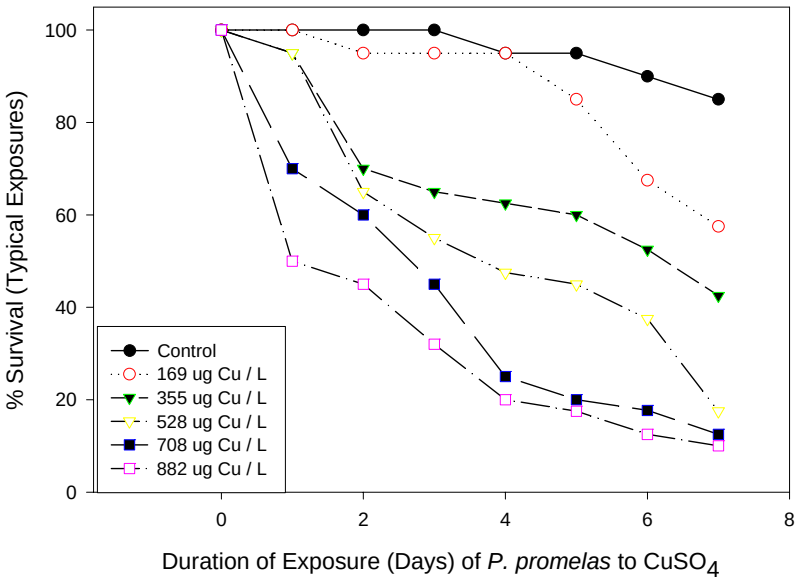


Figure 4.2. Responses of *Ceriodaphnia dubia* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus 4-d and 7-d durations of exposure (Continued).



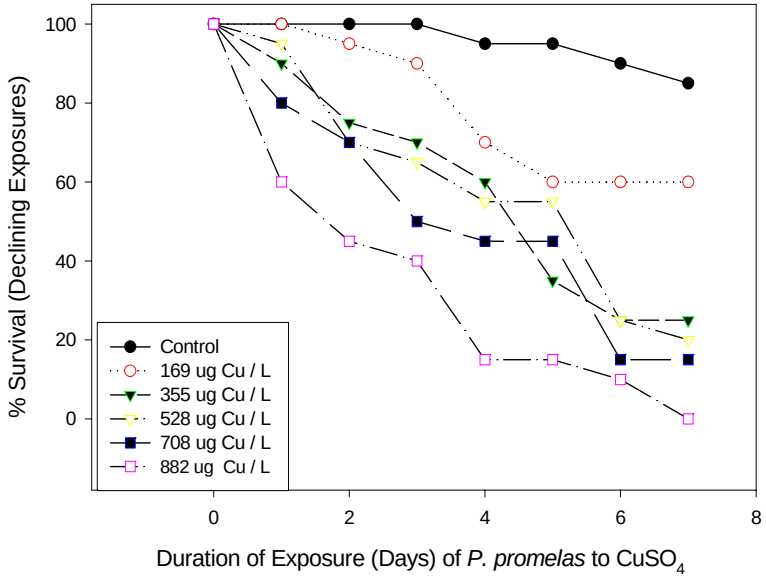
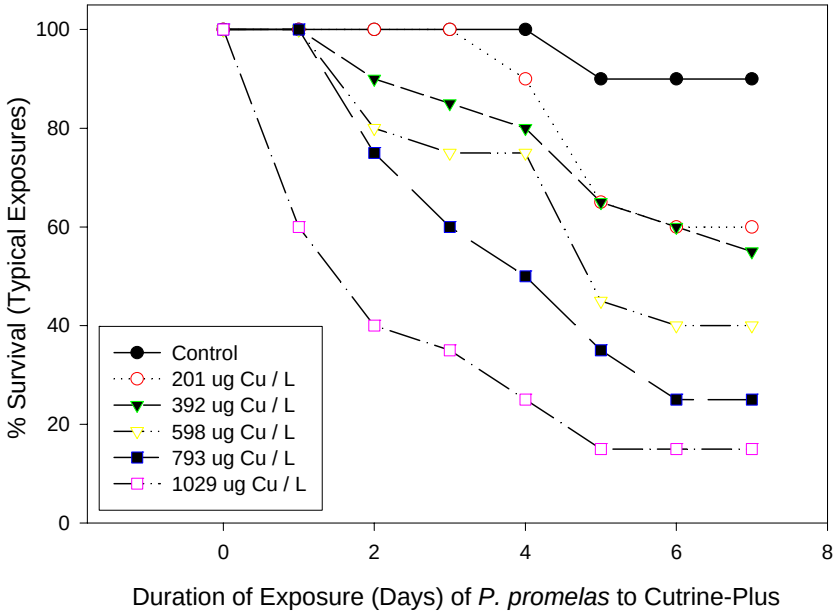


Figure 4.3. Responses of *Pimephales promelas* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus indicating time to mortality.



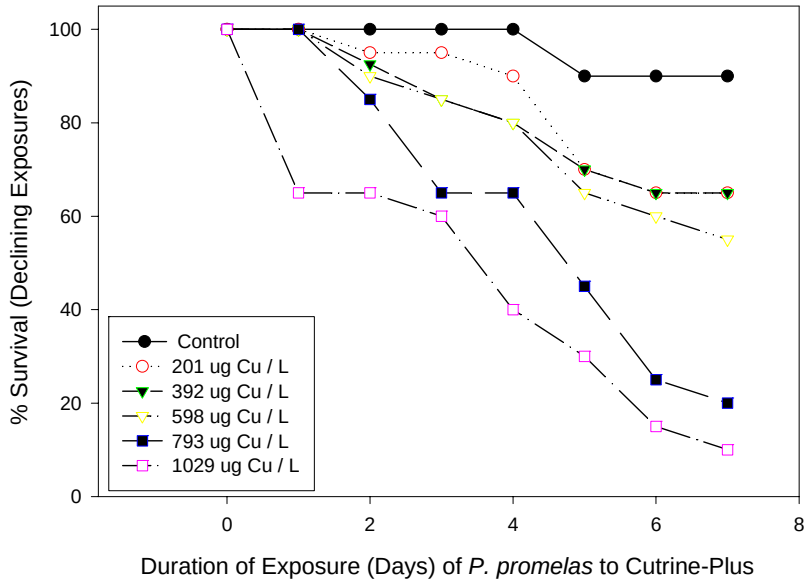
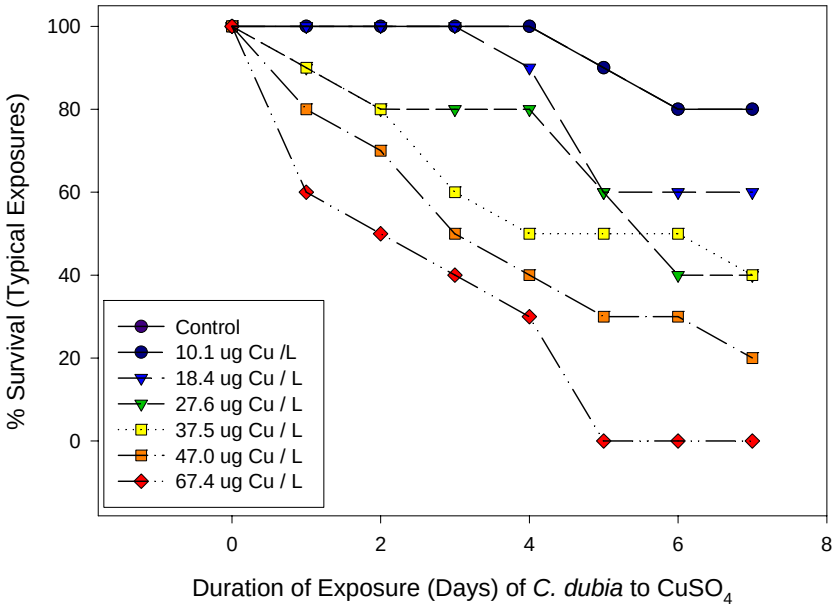


Figure 4.3. Responses of *Pimephales promelas* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus indicating time to mortality (Continued).



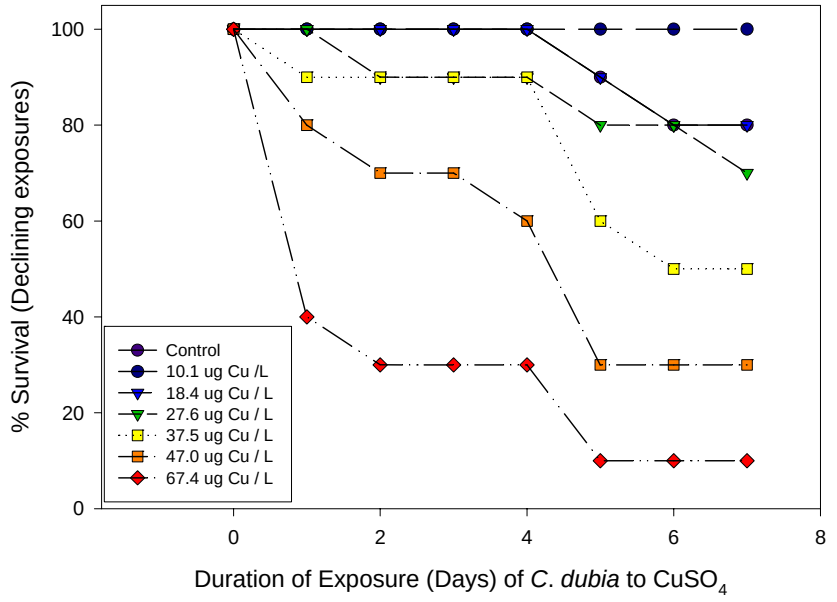
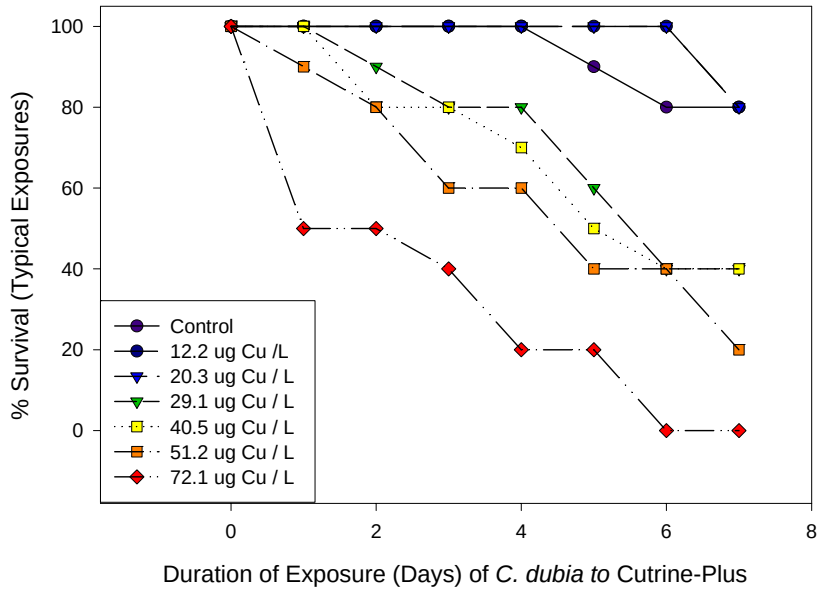


Figure 4.4. Responses of *Ceriodaphnia dubia* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus indicating time to mortality.



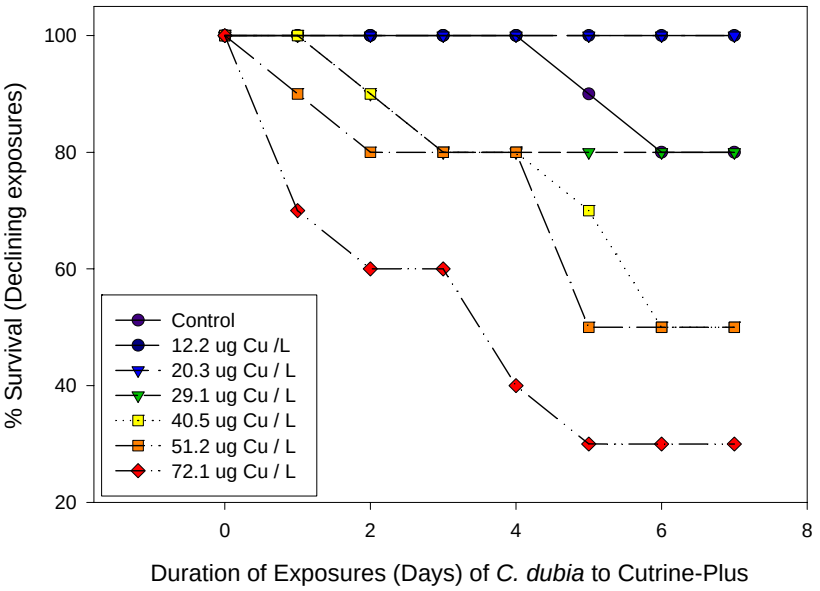


Figure 4.4. Responses of *Ceriodaphnia dubia* to typical and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus indicating time to mortality (Continued).

P. promelas and C. dubia toxicity tests using copper sulfate pentahydrate (declining laboratory exposures)

The copper sulfate pentahydrate 4-day and 7-day LC_{50} values for *P. promelas* in declining laboratory exposure experiments were 634 (513-782) and 418 (356 – 490) µg Cu / L, respectively (Table 4.3). An NOEC of 169 (± 4.24) µg Cu / L, as copper sulfate pentahydrate, was estimated for the 4-day declining laboratory exposures and an LOEC of 355 (± 2.83) µg Cu / L, as $CuSO_4*5H_2O$, was observed (Table 4.5), and a potency slope (% mortality / µg Cu / L) of 0.076 (0.071 - 0.081) was calculated (Figures 4.1 and 4.3).

The copper sulfate pentahydrate 4-day and 7-day LC_{50} values for *C. dubia* declining laboratory exposure experiments were 56 (47-68) and 35 (27-44) µg Cu / L, respectively, as copper sulfate pentahydrate (Table 4.4). NOECs of 28 (± 2.8) and 18 (± 2.1) µg Cu / L as copper sulfate pentahydrate were estimated in the 4 and 7-day static declining exposure experiments. The 4-day LOEC in declining laboratory exposures and the 7-day LOEC in declining laboratory exposures for *C. dubia* was 47 (± 1.8) µg Cu / L as copper sulfate pentahydrate (Table 5), and a potency slope (% mortality / µg Cu / L) of 1.11 (0.975 -1.24) was calculated (Figures 4.2 and 4.4).

Pimephales promelas and Ceriodaphnia dubia toxicity tests using Cutrine®-Plus (typical laboratory exposures)

The 4-day and 7-day LC_{50} values for *P. promelas* in typical laboratory exposure experiments were 731 (627-854) and 485 (397-592) µg Cu / L, respectively, as Cutrine®-Plus (Table 4.3). The 4-day and 7-day NOEC in typical laboratory exposure experiments for *P. promelas* were estimated to be 598

(± 4.95) and 201 (±1.4) µg Cu / L for Cutrine®-Plus, respectively (Table 4.5). However, the 4-day and 7-day LOECs in typical laboratory exposure experiments with for *P. promelas* were estimated to be 793 (±4.95) and 392 (± 3.54) µg Cu / L for Cutrine®-Plus, respectively (Table 4.5), and a potency slope (% mortality / µg Cu / L) of 0.057 (0.054 – 0.061) was calculated (Figures 4.1 and 4.3).

The 4-day and 7-day LC_{50} values for *C. dubia* typical laboratory exposure experiments were 54 (43-68) and 32 (26-39) µg Cu / L, respectively, as Cutrine®-Plus (Table 4.4). The 4-day and 7-day NOEC typical laboratory exposure concentrations for *C. dubia* was 41 (± 2.26) µg Cu / L as Cutrine®-Plus. However, the 4 -day and 7-day LOEC typical laboratory exposures were 51.2 (±1.27) µg Cu / L and 29 (±1.76) µg Cu / L as Cutrine®-Plus. A 7-d NOEC for reproduction of 20 (± 2.19) µg Cu / L, as Cutrine®-Plus for the typical laboratory exposure experiment was estimated, and a potency slope (% mortality / µg Cu / L) of 0.491 (0.212 – 0.769) was calculated (Figures 4.2 and 4.4).

Pimephales promelas and Ceriodaphnia dubia toxicity tests using Cutrine®- Plus (declining laboratory exposures)

The 4-day and 7-day LC_{50} values for *P. promelas* using declining laboratory exposures were 973 (804 – 1176) and 690 (574 - 831) µg Cu / L, respectively, as copper Cutrine®-Plus (Table 4.3). The 4-day and 7-day NOECs for the declining laboratory exposures for *P. promelas* were estimated to be 793 (±4.95) and 392 (± 3.54) µg Cu / L for Cutrine®-Plus, respectively (Table 4.5). The 4-day and 7-day LOECs for the declining laboratory exposures for *P. promelas* were estimated to be 1029 (±5.66) and 598 (±4.95) µg Cu / L for

Cutrine®-Plus, respectively (Table 4.5), and a potency slope (% mortality / µg Cu / L) of 0.049 (0.046 - 0.052) was calculated (Figures 4.1 and 4.3).

The 4-day and 7-day LC_{50} values for *C. dubia* in declining laboratory exposure experiments were 70 (55-90) and 48 (33-68) µg Cu / L, respectively, as Cutrine®-Plus (Table 4). The 4-day and 7-day NOEC for typical laboratory exposure experiments for *C. dubia* was estimated to be 41 (± 2.47) µg Cu / L as Cutrine®-Plus. The 4-day and 7-day LOEC in declining laboratory exposures was estimated to be 72 (±2.47) µg Cu / L as Cutrine®-Plus. A 7-d NOEC of 51 (±1.27) µg Cu / L, as Cutrine®-Plus, and an LOEC of 72 (± 2.42) µg Cu / L for the declining laboratory exposure experiment were estimated for *C. dubia* with reproduction as the endpoint, and a potency slope (% mortality / µg Cu / L) of 0.349 (0.561 - 0.138) was calculated (Figures 4.2 and 4.4).

Comparison of responses of P. promelas and C. dubia to typical l aboratory exposures and declining laboratory exposures of copper sulfate pentahydrate and Cutrine®-Plus

In general, declining exposures are less toxic than typical exposures with the same concentration of an element or compound (Handy 1994). In these experiments, declining exposures were less toxic than typical laboratory exposures when responses of *P. promelas* and *C. dubia* using copper sulfate pentahydrate and Cutrine®-Plus were measured. Using *P. promelas* and *C. dubia*, the 4d LC50s for declining exposures were 22 and 27 % higher for copper sulfate pentahydrate, respectively, and the 7 d LC50s for declining exposures were 33 and 46 % higher, respectively, than the typical laboratory exposures when using copper sulfate pentahydrate. Using *P. promelas* and *C. dubia*, the 4d LC50s for

declining exposures were 33 and 30 % higher for Cutrine®-Plus, respectively, and the 7 d LC50s for declining exposures were 42 and 50 % higher, respectively, than the typical laboratory exposures when using Cutrine®-Plus. Data from these experiments indicated that typical laboratory experiments tend to overestimate risks relative to declining exposures. Thus risks in the field estimated from typical laboratory exposures may greatly overestimate actual risks.

Based upon these data, the responses of the sentinel non-target species to the copper-containing algaecides differ significantly. The invertebrate, *C. dubia*, was generally an order of magnitude more sensitive than the vertebrate, *P. promelas* (e.g. *C. dubia* 4d LOEC was 28 (±2.80) ug Cu / L, as copper sulfate pentahydrate and P. promelas 4 d LOEC was 169 (±4.24) µg Cu / L, as copper sulfate pentahydrate; Tables 4.6 and 4.7). The non-chelated algaecide, copper sulfate pentahydrate, was more toxic to these non-target species than the chelated algaecide Cutrine®-Plus. Further, the half-lives or residence times for the algaecides in the water columns post-application differ for copper containing algaecides (Mastin and Rodgers 2000; Murray-Gulde et al. 2002). Reported half lives for copper containing algaecides in treated waters range from < 24 h (Tedrow 2007) to 20 days (Effler et al. 1980; Skeaff et al. 2002). For algaecides with relatively short half-lives after application, declining exposures should be a more realistic prediction of risks to non-target species in the field. In actual field situations, the exposures are bounded and risks to non-target species can be estimated. Applications of algaecides in the field are bounded by the maximum amount allowed by the registration label. Risks to non-target species in the field

will depend on the exposure and the relative sensitivities of the non-target species. There is likely a margin of safety for fish and little or no margin of safety for sensitive invertebrates based upon these data and published results from other studies (Typical *P. promelas* LOECs were 355 (±2.83) and 793 (±4.95) µg Cu / L, as copper sulfate pentahydrate and Cutirne®-Plus; Declining *P. promelas* LOECs were 355 (±2.83) and 882 (±7.78) µg Cu / L, as copper sulfate pentahydrate and Cutirne®-Plus, respectively; Table 4.7). However in actual field situations entire water bodies are not typically treated and invertebrates usually recover quickly (Heckman 2005). Therefore, typical laboratory exposures may overestimate risks of declining exposures. Since concentrations of copper-containing algaecides rapidly decline after treatment, use of laboratory data with typical exposures to estimate risks in field is likely very conservative given the propensity of copper to interact with a variety of ligands and decrease bioavailability.

Table 4.6. Responses of non-target species to copper (Cutrine®-Plus and Copper Sulfate). Estimated LC_{50} values are based on acid-extractable concentrations unless otherwise indicated.

Organism	Algaecide	Test Duration	Copper Concentration (µg acid-extractable Cu/L)	Citation
C. dubia	$CuSO_4*5H_2O$	96-h LC_{50}	41	This Study (Typical)
		96-h LC_{50}	56	This Study (Declining)
		96-h EC_{50}	34	Murray-Gulde 2002[a]
		48-h EC_{50}	2.72 (soluble)	Suedel et al. 1996[b]
		96-h EC_{50}	304.8	Erickson et al. 1996[c]
	Cutrine®-Plus	96-h LC_{50}	54	This Study (Typical)
		96-h EC_{50}	70	This Study (Declining)
		96-h LC_{50}	124	Murray-Gulde 2002[a]
P. promelas	$CuSO_4*5H_2O$	96-h LC_{50}	519	This Study (Typical)
		96-h LC_{50}	634	This Study (Declining)
		96-h EC_{50}	656	Murray-Gulde 2002[a]
		48-h LC_{50}	20.2 (soluble)	Suedel et al. 1996[b]
		96-h LC_{50}	12.5 (soluble)	Suedel et al. 1996[b]
		7-d LC_{50}	8.2 (soluble)	Suedel et al. 1996[b]
	Cutrine®-Plus	96-h LC_{50}	731	This Study (Typical)
		96-h LC_{50}	973	This Study (Declining)
		96-h EC_{50}	863	Murray-Gulde 2002[a]
		48-h EC_{50}	255.4	Mastin and Rodgers 2000[d]

[a]Water chemistry for Murray-Gulde 2002 – alkalinity (88-92); hardness (72-80); conductivity (208-255); pH (7.2-8.0)
[b]Water chemistry for Suedel et al. 1996 – alkalinity (9-21); hardness (6-10); conductivity (20-50); pH (6.9-8.0)
[c]Water chemistry for Erickson et al. 1996 –alkalinity (42.5); hardness (45); pH (6.8-7.2)
[d]Water chemistry for Mastin and Rodgers 2000 – alkalinity (55-96); hardness (48-96); conductivity (270-450); pH (6.4-8.0)

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CHAPTER 5

SUMMARY

Problematic growths of algae have become more apparent as the human population increases. As people move to locations adjacent to water resources, their awareness of problematic growths of algae becomes more acute. Some of the problems that algae blooms have caused include: 1) altered aesthetics and decline in adjacent property values (WHO 2003; Figueiredo et al. 2004); 2) interference with recreational activities such as fishing, boating, and swimming (WHO 2003; Figueiredo et al. 2004); 3) adverse effects on drinking water including production of taste and odor compounds (e.g. geosmin and 2-methylisoborneal) (Mastin and Rodgers 2000); 4) the off flavor compounds in fish and other vertebrates as well as injury and toxicity (e.g. Avian Vacuolar Myelinopathy; Wilde et al. 2005); and 5) production of toxins directly impacting invertebrates, fish and mammals including humans (Behm 2003; WHO 2003). Therefore, as water resources are used for more purposes, as well as more intensively, control of algal growths that cause adverse impacts on water resource usages is often required.

Algaecides are often efficient and effective tactics for water resource managers to respond to algal growths that prohibit the use of lakes, reservoirs and streams. We need information regarding the responses of specific algae in site waters to algaecide exposures in order to efficiently and effectively deal with these problems. In the absence of this information, ineffective algaecides or

excessive treatments may be used. Development of an efficient algaecide assay using site specific samples could contribute to better decisions regarding algaecide application and increase margins of safety for non-target species. Since these laboratory assays or experiments predict responses of algae in site waters to algaecide exposures, predictions need to be verified in the field. Confirmation of laboratory responses with results from field application of algaecides will increase confidence in the laboratory assay. Additional research on the responses of non-target species such as sensitive fish and invertebrates to algaecide exposures is also needed to permit better decisions by water resource managers when evaluating potential risks associated with site specific algaecide applications. Laboratory toxicity data for non-target species are typically developed using laboratory waters and relatively constant or non - varying exposures. In field applications of algaecides, the exposures rapidly decline with time. Fundamentally, the responses of non-target species to these exposures should differ significantly. If water resources managers use the laboratory data to directly predict responses of non-target species in the field to algaecide exposures, the risks of an application may be greatly overestimated. Therefore, research is needed to clarify this situation.

Chapter Two, "An Efficient Planktonic Algal Bioassay using Site Water and Copper-containing Algaecides", focused on developing a more efficient laboratory assay for responses of field-collected algae to algaecide exposures. Representative samples of algae in site water were shipped to the laboratory and responses of the algae to algaecide exposures were measured. Obviously, algal

species vary in their responses to an algaecide (Murray-Gulde et al. 2002), and algaecides vary in their potency for a given algal species (Heatley et al. 2002). Importantly, site water characteristics may also alter the bioavailability of an algaecide resulting in a strong influence on the responses of an algal species to an exposure. Current procedures for evaluating responses of algal species in site waters to algaecide exposures involve collection and shipping of considerable volumes of water (8-16 L). If the volume of water could be reduced, and the same results could be obtained, then considerable savings in terms of resources would be achieved. The current procedures require approximately 10 to 14 days to predict responses of the algae at a field site to an algaecide exposure. If the time required to obtain predictive results regarding algal responses to algaecide exposures could be reduced to a few days, then field applications could be implemented in a timely fashion. This research was prompted by the need for a laboratory algaecide screening approach producing the same valuable information as the current method (Murray-Gulde et al. 2002), but requiring a lesser volume of site water and shorter test duration. Therefore, intervention in the field could occur sooner. The objectives of these experiments were: 1) to determine if a lesser volume (100, 50, or 25 ml) and a shorter duration of exposure (72, 48h, or 24 h) can produce the same results (e.g. not significantly different) as a larger volume (200 ml) and longer duration exposure (96 h) using copper-containing algaecides in laboratory toxicity tests with site water containing problematic algae, and 2) to determine if algal responses (chlorophyll *a*, and cell density) to exposures of algaecides change with decreases in volume and time. Two site

waters were evaluated in this experiment. Data supported using an exposure volume of no less than 100 ml with an exposure duration of at least 72 hours for a planktonic algal assay to predict field responses.

Chapter Three, "Responses of *Lyngbya* to Algaecide Exposures in the Laboratory and the Field" involved comparison of laboratory and field exposures and responses. Application of laboratory results to field situations has been an area of interest particularly in the case of problematic algal species. Laboratory tests have been developed to predict responses of algae to nutrient exposures (USEPA 1985). These laboratory experiments were verified in field studies (Auer et al. 1986). Similarly, we need to confirm through *in situ* studies the accuracy of predictions from laboratory studies of responses of algal species to algaecides. The fundamental principle underlying this question is: can similar responses be expected if laboratory exposures are essentially duplicated in the field?

Laboratory screening approaches are efficient and effective for identifying efficacious approaches for controlling benthic algae (Quimby 1981; Kay et al. 1983; Kay et al. 1984; Mastin et al. 2002; Murray-Gulde et al. 2002; Tedrow 2007; Chapter two of this dissertation). Laboratory experiments to determine efficacious approaches for treatment of *Lyngbya* decrease time, effort and expense relative to field-scale treatments seeking a viable approach for managing benthic algae. Confirmation is needed of results from the laboratory that are applied directly to field situations. With the forgoing in mind, the specific objectives of this research were: 1) to contrast responses of *Lyngbya* to exposures of algaecides and adjuvants in a laboratory study to determine efficient and efficacious

treatment options, 2) to measure field responses of *Lyngbya* to exposures of algaecides and an adjuvant, and 3) to contrast responses of *Lyngbya* in laboratory exposures of algaecide and adjuvant with responses to field exposures. Experiments were conducted exposing *Lyngbya* in the laboratory and in field sites in two Alabama reservoirs (Lay Lake and Lake Jordan). A unique combination of algaecides was required to control the growth of this benthic species in the laboratory. This treatment involved PAK™-27 (sodium carbonate peroxyhydrate) followed 24 hrs later with an application of copper, as chelated copper (Algimycin®), and Cide-Kick II®. These treatments were applied to four field sites in two reservoirs. *Lyngbya* biomass and chlorophyll *a* decreased significantly following applications of this combination treatment. Field responses of *Lyngbya* were similar to those observed in the laboratory, however, lesser exposures in the field yielded similar responses. These results infer that there is a cumulative effect over longer durations of time when using multiple field treatments.

Chapter Four, "Contrasting Responses of *Pimephales promela*s and *Ceriodaphnia dubia* to laboratory and simulated field exposures of Cutrine®-Plus and copper-sulfate pentahydrate", involved evaluation of non-target species' responses to simulated field exposures of algaecides. Water resource managers evaluate responses of both target and non-target species to potential treatment chemicals (i.e. algaecides). Typically, data from laboratory studies of responses of sensitive invertebrates and fish (USEPA 2002; USEPA 1985) are contrasted with the treatment concentrations of algaecides required to control problematic

algae. The result of this comparison is often called the "margin of safety" for the non-target species, where the lowest observed effects concentration for the non-target species is divided by the concentration of the algaecide that is required to control the algae. If the ratio is greater than one there is a margin of safety associated with the algaecide use. Such a simple calculation and evaluation can greatly overestimate the risk to non-target species associated with an algaecide application if the laboratory results do not accurately predict the responses of the non-target species in the field situation. Field observations indicate that the laboratory results developed from toxicity testing that utilizes continuous, essentially non-varying exposures, over-predict the toxicity observed in the field due to a declining (i.e. pulse) exposure of an algaecide application. Data are needed to determine whether or not the laboratory exposures are predictive of field responses of non-target species. More accurate predictions of the responses of non-target species to algaecide exposures will permit water resource managers to make more defensible decisions regarding mitigation strategies for problematic algae.

The primary purpose of this research was to contrast responses of a sentinel aquatic invertebrate and vertebrate to exposures of copper-containing algaecides. The exposures in this study encompass typical exposures during laboratory testing situations (e.g. "typical exposures") and declining exposures simulating field conditions after an application. Specific objectives of this research are to contrast: 1) responses of *Pimephales promelas* in typical exposures of copper sulfate pentahydrate versus declining exposures; 2) responses of *P.*

promelas in typical exposures of Cutrine®-Plus versus declining exposures; 3) responses of *Ceriodaphnia dubia* in typical exposures of copper sulfate versus declining exposures; 4) responses of *C. dubia* in typical exposures of Cutrine®-Plus versus declining exposures, and to compare 5) responses of these two species to copper sulfate pentahydrate exposures and Cutrine®-Plus exposures to determine the relative risk of these algaecides. *C. dubia* was more sensitive than *P. promelas* in these experiments. The declining exposures were less toxic than the typical laboratory exposures. There is likely a margin of safety for fish and little or no margin of safety for sensitive invertebrates based upon these data and published results from other studies. However, in actual field situations, entire water bodies are not typically treated and invertebrates usually recover quickly (Heckman et al. 2005).

In order for water resource managers to effectively and efficiently react to algal growths that are prohibiting use of a lake, reservoir or stream, information must be obtained on the response of the specific algae in site waters to algaecide exposures. In the absence of this information, ineffective algaecide or excessive treatments may be implemented. Research on development of an efficient algaecide assay using site specific samples can contribute to better decisions regarding algaecide applications and increase margins of safety for non-target species. Since these laboratory assays or experiments provide predictions of responses of algae in site waters to algaecide exposures, these predictions need to be verified in the field. Confirmation of laboratory responses with results from field application of algaecides will increase confidence in the laboratory assay.

Additional research measuring responses of non-target species such as sensitive fish and invertebrates to algaecide exposures will permit better decisions by water resource managers when evaluating the risks associated with a site specific application of algaecide. Laboratory toxicity data for non-target species are typically developed using laboratory waters and relatively constant or non-varying exposures. In field applications of algaecides, the exposures rapidly decline with time. Fundamentally, the responses of non-target species to these exposures should differ significantly. If water resources managers use the laboratory data to directly predict responses of non-target species in the field to algaecide exposures, the risks of an application may be greatly overestimated. In order to more efficiently and effectively manage crucial water resources that have been impaired by algal blooms, water resource managers need reliable data. Importantly, they need accurate predictions of responses of the target algal species to algaecide exposures. They also need accurate characterizations of potential risks to non-target species following algaecide applications. This research has proposed a strategy to obtain laboratory information that can improve risk prediction in the field.

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