

Effectiveness of Coated, Controlled-Release Copper Sulfate  
as an Algicide for Phytoplankton Control in Ponds

by

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## ABSTRACT

Copper sulfate and diuron are the two common treatments for controlling blue-green algae, the cause of off-flavor in aquaculture ponds, especially catfish ponds. Copper sulfate has been used most widely because of its lower cost. However, a number of copper sulfate applications may be required at low dose application to prevent excessive algal bloom and off-flavor of the culture product. Agrium Advanced Technologies produced coated algicide products and the products were investigated. The effectiveness of each product was compared with regular algicide treatments: copper sulfate and diuron.

The effectiveness of a coated copper sulfate algicide designed for controlled release of copper was compared with that of regular copper sulfate, the algicide normally used in aquaculture ponds. The coated product released copper for about 10 weeks. Initially, concentrations of copper in ponds treated with the coated product were similar or greater than those in ponds receiving weekly applications of regular copper sulfate. After 3 weeks, ponds receiving regular copper sulfate had higher concentrations of copper than were observed in ponds treated with the coated copper product. Phytoplankton abundance was no greater in the ponds to which the coated product was applied than in ponds treated weekly with regular copper sulfate. The coated product was applied in ponds treated weekly with regular copper sulfate. The coated copper product appears to be a potentially effective method for controlling phytoplankton in aquaculture ponds, and it would be easier to apply than regular copper sulfate.

A coated copper product was further investigated in catfish ponds. Copper concentrations were greater in the regular copper sulfate treatment, but the coated copper sulfate treatment gave equal or possibly better phytoplankton control. Copper additions did not negatively impact hybrid catfish survival, production, or feed conversion in either the regular copper sulfate treatment or the coated copper sulfate treatment as compared to the control. Moreover, there was no difference in copper sulfate treatment as compared to the control or the two copper treatments. Flavor scores for fish did not differ among control and treatments.

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## Table of Contents

Abstract.....	ii
Acknowledgments.....	iii
List of Tables .....	vi
List of Illustrations.....	vi
Introduction .....	1
Literature Review .....	3
Materials and Methods .....	22
Result and Discussion .....	34
Literature Cited .....	77

## List of Tables

Table 1 Fish flavor intensity score .....	32
Table 2 Fish taste descriptive .....	33
Table 3 Means and standard deviations in control pond and ponds treated with different formulations of copper sulfate and diuron by two application methods.....	37
Table 4 Results of tests of grand mean of water quality variables for significance (P = 0.05) by the Duncan's Multiple Range Test.....	43
Table 5 Grand means and standard errors of the mean for water quality variables in control and copper treated, hybrid catfish ponds .....	61
Table 6 Means and standard errors for hybrid catfish production in control and copper-treated ponds.....	62
Table 7 Means, standard errors, and ranges for copper concentrations in hybrid catfish fillets from control and copper-treated ponds.....	63
Table 8 Results of flavors evaluation of fillets from catfish in control and copper-treated ponds.....	64

## List of Figures

Figure 1 Mean water temperature on different dates in ponds treated once with coated copper sulfate at low and high rate and weekly with regular copper sulfate.....	44
Figure 2 Mean specific conductance on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	45
Figure 3 Mean pH on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	46
Figure 4 Mean copper concentration on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	47
Figure 5 Mean secchi disk visibility on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	48
Figure 6 Mean turbidity on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	49
Figure 7 Mean chlorophyll <i>a</i> concentration on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	50
Figure 8 Means for total phytoplanktonic algal abundance and abundance of blue-green algae on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate.....	51
Figure 9 Means for dissolved oxygen concentration chlorophyll <i>a</i> concentration on different dates in ponds treated once with coated copper sulfate at low and high rates and weekly with regular copper sulfate .....	52
Figure 10 Means and standard errors for water temperature in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	65

Figure 11 Means and standard errors for conductivity in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	66
Figure 12 Means and standard errors for pH in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	67
Figure 13 Means and standard errors for total alkalinity concentration in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	68
Figure 14 Means and standard errors for dissolved oxygen concentration in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	69
Figure 15 Means and standard errors for copper concentration in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	70
Figure 16 Means and standard errors for secchi disk visibility in control ponds and in ponds treated with regular copper sulfate and coated sulfate .....	71
Figure 17 Means and standard errors for turbidity in control ponds and in ponds treated with regular copper sulfate and coated sulfate .....	72
Figure 18 Means and standard errors for chlorophyll <i>a</i> concentration in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	73
Figure 19 Means and standard errors for total number of individual planktonic algae in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	74
Figure 20 Means and standard errors for total number of individual blue green algae in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	75
Figure 21 Means and standard errors for percentage of blue green algae in control ponds and in ponds treated with regular copper sulfate and coated sulfate.....	76



## INTRODUCTION

Fertilizers coated with special materials that control the release rate of nutrients are called slow-release or controlled-release fertilizers. Coated fertilizers are used for several purposes – especially for supplying nutrients to ornamental plants and lawns. Studies done at Auburn University (Kastner and Boyd 1996; Rushton and Boyd 1995) revealed that slow-release fertilizers were effective as sportfish pond fertilizers. But, for reasons mentioned below, slow-release fertilizers have not been offered as pond fertilizers.

Odorous compounds produced by blue-green algae in ponds can be absorbed by fish to impart a bad taste or off-flavor to their flesh. Copper sulfate is frequently applied to ponds to kill blue-green algae responsible for off-flavor in ponds for commercial production of channel catfish (Tucker 2000). Copper from copper sulfate has a relatively brief residence time in water (McNevin and Boyd 2004), and frequent re-treatment usually is necessary to combat off-flavor in catfish. Diuron is used less frequently to control off-flavor-producing algae in catfish ponds (Tucker et al. 2003), but the application schedule recommended by the United States Environmental Protection Agency (USEPA) for this purpose requires small weekly applications of 0.01 mg/L active ingredient for up to a total of 9 weeks. Controlled-release copper or diuron products would seem to have potential merit as algicides for controlling blue-green algae responsible for off-flavor in commercial catfish ponds.

The events leading up to the study of coated algicides in fish ponds is interesting. In 1991, representatives of the Grace-Sierra Horticultural Products Company visited the

Department of Fisheries and Allied Aquacultures at Auburn University and offered to support a small research effort to investigate the possible use of controlled-release fertilizers in ponds. Findings of this research led Grace-Sierra to obtain patents for controlled-release fertilizers for use in sportfish and aquaculture ponds, and they initiated a program to further test and introduce these products into the US and international markets. Unfortunately, Scotts Miracle-Gro Company completed the purchase of Grace-Sierra Horticultural Products Company on 17 December 1993 (<http://www.thefreelibrary.com>), and the controlled-release fertilizer project for ponds was discontinued. In December 2009, a group from Agrium Advanced Technologies, a specialty fertilizer producer with corporate headquarters in Loveland, Colorado and a product innovation center and production facility in Sylacauga, Alabama, visited Auburn University to discuss aquaculture product needs. Among the group was a former Grace-Sierra employee who had been involved in the project to develop a controlled-release fertilizer for ponds. The idea of coating copper sulfate and diuron for use as an algicide was discussed, and this resulted in Agrium providing funding to Auburn University to investigate two algicides, coated copper sulfate and coated diuron, for possible use in catfish farming to control off-flavor.

## LITERATURE REVIEW

The enrichment of surface waters with plant nutrients is known as eutrophication, and it can lead to an increase in production and biomass of phytoplankton causing what is known as an algal bloom (Boyd, 2000). Through their photosynthetic processes, algae utilize carbon dioxide, converting it into organic matter and oxygen. They are an essential part of the lake and reservoir ecosystem and constitute the base of the aquatic food chain. Generally, water quality problems develop when excessive algal growth occurs, and sometimes the algae float to the water surface causing a scum. These blooms may be harmful to water quality, toxic to some species, and they can affect human activities in water bodies.

High levels of nutrients in water bodies are usually the result of anthropogenic inputs, and nutrient enrichment not only affects algal abundance, it affects phytoplankton species composition. The species favored by nutrient enrichment often is undesirable, and some of these species are problematic worldwide. Algal blooms can occur in any aquatic system such as rivers, lakes, estuarines, pond impoundments, and even coastal waters. They are spread worldwide by water, wind, transport of algae in ship ballast water, etc. (Johnk et al., 2008; Sunda et al., 2006).

Exceptionally dense accumulations of algae can affect both ecosystem and public health through algal toxin production, rapid oxygen consumption from the decaying biomass, and blockage of light penetration. The characteristic of algal communities

widely vary from undetectable to very high densities. A bloom is defined as enough algae to discolor the water. Toxicity varies with algae species; some algae are toxic only at very high densities, while others can be toxic at very low densities. “Red tide” and “brown tide” are terms used to describe algal blooms that discolor the water.(Shumway, 1990)

The production of algal toxins associated with a proliferation of algae is commonly called a harmful algal bloom (HAB). HABs have become a common threat, and their numbers are increasing around the world, especially along the U.S. coastline. (Rodgers, 2008). The National Oceanic and Atmospheric Administration (NOAA) has taken this issue seriously and produced a visual system called “Harmful Algal Blooms Observing System” to show the HABs event’s sites classification, and intensity on spatial maps (<http://service.ncddc.noaa.gov/website/AGSViewers/HABSOS/maps.htm>). A variety of algal toxins can be produced in marine, brackish and fresh waters, as well as on wet soils. These toxins are mostly organic molecules (Falconer, 1993; Johnk et al., 2008). Sunda et al. (2006) mentioned the high potential of HABs occurrence in the future; toxin-producing algae may become more prevalent, especially in eutropic freshwater systems. There is no doubt that HABs are occurring in more locations than ever before and new sightings are reported regularly. This trend is due to increasing eutrophication throughout the world and there are several classic examples relating HABs frequency to anthropogenic activities (Smayda, 1990).

For certain toxin-producing species, significant impacts occur at population densities of only several hundred cells per liter. For example, *Dinophysis* need only to be present at 100s of cells per liter to induce diarrhetic symptoms in humans, because it

toxin is concentrated by shellfish and then ingested by human consumers. *Pfiesteria piscicida* and *P. shumwayiae* are associated with fish lesions, skin and eye irritation, and short-term neurocognitive disorders (Gratta et al., 2001), and the algae need only reach levels of 250 zoospores per liter to be of concern. Toxin-producing species can be found in other groups besides the dinoflagellates, including raphidophytes, diatoms, cyanobacterial, and several other groups.

Certain species can exert their effects through the synthesis of compounds that alter cellular processes of other organisms from plankton to humans. The most severe effects of HABs include mortality of fish, birds, and mammals (including humans), respiratory or digestive tract problems, memory loss, seizures, lesions and skin irritation. These blooms also lead to losses of coastal resources such as submerged aquatic vegetation and benthic epi- and in-fauna. The primary groupings of HAB toxins according to syndrome include ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP), paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), azaspiracid shellfish poisoning (AZP), amnesic shellfish poisoning (ASP), and cyanobacteria toxin poisoning (CTP). Represented in the diverse group of harmful chemicals synthesized by algae are neurotoxins, carcinogens, and a number of other compounds. These compounds can negatively affect humans and other organisms that consume filter-feeding bivalves or plankton feeders that have concentrated algal toxins (Kevin et al., 2003).

### Consideration of HABs impact on public health, and living resources

The increasing interest in HABs include not only public health concerns, but impaired water use for municipal supply because of muddy-earthly flavor or some other unpleasant taste and odors caused by certain algal species. Algal blooms have adverse effects on living resources of many coastal systems, cause economic losses because of reduced tourism, recreation, or seafood sales. High costs are associated with maintaining public advisory services and monitoring for shellfish toxins, water quality, and plankton composition.

A 5-year study (1987-1992) by Hoagland et al. (2002) estimated that approximately US \$49 million were lost annually to HAB related impacts in the United States. Many areas ideal for establishing productive and profitable wild shellfisheries remain closed year-round due to persistent toxicity of the resource from repeated toxin exposure and an inability to depurate accumulated toxins from the contaminated shellfish. For example, the Georges Bank surf clam fishery has been closed since 1989 due to continuing PSP toxicity, and the United States roe-on scallop industry in this area has consequently not been developed. They also reported that the Alaskan shellfishery with an estimated value of US \$50 million annually cannot be realized because of HABs.

Many extreme cases of HABs effects on public health have been reported in many countries. Severe problems in drinking water supplies related to releasing of MIB by *Phormidium tenue* (Negoro et al., 1998) and also a similar situation associated with *Oscillatoria curviceps* in the USA. Beyruth (2000) reported many HABs related on

public health events in water supply resources in Brazil. Fifty-four people died in Caruarú following exposure to *Microcystis aeruginos* toxins (Jochimsen et al., 1998), and over 140 people, mostly children, required medical treatment at the hospital in a Palm Island case, reportedly caused by *Cylindrospermopsis raciborskii* toxicity (Bourke et al., 1983). Many recent reports have stimulated interest in gaining a better understanding of the dynamics of HABs, especially from cyanobacteria (CHABs) in order to improve management approaches to the control of the growth of potentially toxic algae.

#### HABs impact on the aquaculture industry

HABs can cause serious economic losses in aquaculture if they kill cultured organisms or cause consumers concern about food safety. Landsburg (2002) and Hudnell (2008) estimated that the effect of HAB outbreaks on the U.S. economy is more than \$40 million per year or \$ 1 billion per decade. Numbers of cultured species affected by HABs vary widely from mollusks, crustaceans, to finfish.

Even severe blooms of non-toxic algae can bring disaster for cultured animals when oxygen depletion occurs in the shallow waters of many aquaculture systems from decaying algae residue. Algal toxins are a problem in aquaculture when they are produced in sufficient quantities and with sufficient potency to kill cultured organisms, decrease feeding and growth rates, cause food safety issues, or adversely affect the quality of the product (Shumway, 1990). John (2008) concluded the problems caused by algal toxins in the freshwater aquaculture systems of both shellfish and finfish are

capable of lowering product quality by imparting “off-flavor”, resulting in indirect toxicity through changes in water quality, and causing direct toxicity to cultured species to result in less production.

Lack of proper management may cause excessive algal blooms in aquaculture systems. The most common causes are over feeding and insufficient use of mechanical aeration. The most common toxin-producing algae are blue-green algae, golden algae and euglenoids. Blue-green algae can rapidly overtake an aquaculture pond and contribute to unstable environmental conditions. Off-flavor in both shellfish and finfish adversely affect the fishing and aquaculture industries through consumer dissatisfaction and economic losses caused by reduced supply of fish and inability to sell market-size fish (Sindelar et al., 1987).

### Off-flavor

Off-flavor can alter the taste of products and affect consumer acceptability and market development. Comparisons of wild-caught seafood and aquaculture products indicate that both are good sources of protein and are valuable foods. If aquaculture products with disagreeable flavors are marketed, first-buyers may assume that the objectionable flavor is inherent in the product or in aquaculture products in general. After the disagreeable initial encounter, the consumer may avoid future purchases of foods produced in aquaculture in favor of poultry, pork, beef or seafood from capture fisheries (Tucker, 2000). Objectionable flavor causes fish farmers to hold market-size fish for a



longer period of time until the off-flavor has disappeared and fish are acceptable to the processor and consumer. This delay increases production costs as well as the chance of losing a harvest through oxygen depletion or disease.

Hanson (2006) reported that U.S. catfish producers suffer economic losses from off-flavor as high as U.S. \$47 million annually. The cost of off-flavor harvest delays were estimated to be \$8.85/ha/day in 2001. The range of the additional costs of production associated with decays can be \$0.01-0.25/kg of catfish production. The typical U.S. catfish production cost is approximately \$1.43/kg and the additional cost by off-flavor could take from 3 to 17 % of total production cost.

Off-flavor literally refers to usual or unsatisfactory tastes or odors. Obviously detecting disagreeable tastes and odors of any food may make people refuse the product even though it is otherwise of good quality. Moreover, the absence of usual taste and odors does not guarantee food safety because toxins and microbial pathogens can be present in foods with no discernible off-flavor. Thus, the presence of off-flavors does not necessarily mean that foods are unsafe. As an important example, the most common environmentally-derived off-flavors in aquaculture products are caused by chemicals of low, practically non-existent toxicity to the animal under culture and to human consumers of the product (Dionigi et al., 1993; Nakajima et al., 1996).

Off-flavor generally is viewed as a water quality-related phenomenon. These odorous compounds formed in the environment can be ingested or absorbed by fish and impart off-flavor to fish flesh. Lovell (1979) suggested that the problem has been

intensified because farmers have greatly increased the rates of feed application to fish in ponds. Lovell (1979) mentioned that off-flavor is not a permanent thing; it disappears soon after its source in the pond is gone. This may take only a week or as long as several months may be required for off-flavor compounds to be depurated. Off-flavor creates considerable inconvenience in maintaining pond harvest schedules. However, commercial aquaculture systems occasionally cause the animal under culture to acquire undesirable flavors because of the inability to control the environment through management (Tucker and Martin, 1991; Haard, 1992). Off-flavor acquired during growout, unlike most other production problems encountered in aquaculture, do not involve food safety issues or affect the growth or health of the animal under culture.

Off-flavor is caused by odorous, waterborne chemicals that are absorbed from the water and deposited in edible tissues. These chemicals are usually produced by naturally-occurring pond microorganisms or enter the water through anthropogenic pollution. Also, off-flavor may result from lipid oxidation or bacterial spillage associated with improper post-harvest handling (Tucker 2000).

Types of off-flavor in aquaculture products are described by many familiar flavors or odors that may be described as smelling like sewage, rotten, moldy, kale, cardboard, and gasoline (Vander Ploeg 1991). However, the most common undesirable flavors in aquatic systems that causes a huge concern throughout the aquaculture industry are earthy-muddy flavors that occur when cultured organisms are cultured in environments containing geosmin and 2-methylisoborneol.

### Geosmin and 2-methylisoborneol

Geosmin is a bicyclic tertiary alcohol and identified as trans-1, 10-dimethyl-trans-9-decalol with earthy-muddy odor character in dilute solution. The compound is an extraordinarily potent flavor-impairing chemical in water and fish, although humans differ greatly in their sensory sensitivity to the compound (Tucker, 2000). Sensory panels who have been trained to taste geosmin can detect concentrations in water of about 0.02 µg/L (Buttery et al., 1976; Persson, 1980). The sensory threshold concentration or the lowest concentration that can be tasted or smelled in fish ranges from 6 to 10 µg/kg (Yurkowski and Tabachek, 1974). In channel catfish, Grimm et al. (2004) reported a threshold of geosmin range 0.25-0.5 µg/kg.

The compound 2-Methylisoborneol or MIB is also a bicyclic tertiary alcohol and classified as 1,2,7,7-tetramethyl-exo-bicyclo(2.2.1.)heptan-2-ol. It has a musty-medicinal odor in dilute aqueous solution, and it has a camphorous odor when concentrated (Tucker, 2000). Persson (1979) reported that the mean threshold odor concentration of MIB is about 0.04 µg/L in water, and the taste panels could detect the concentration of MIB at about 0.1 µg/kg in mild tasting fish: MIB can be tasted in pike and rainbow trout at 0.6 µg/kg. Also, the MIB threshold sensory in channel catfish is 0.1-0.2 µg/kg. The blue-green algae most important in releasing MIB is *Oscillatoria chalybea* (Van der Ploet et al. 1992; Vander Ploeg et al. 1995). Currently, this species has been re-named as *Oscillatoria perornata*.

Geosmin and MIB both are produced by certain microorganisms that are abundant and accumulate in aquaculture systems, especially cyanobacteria and actinomycetes (Zaitlin and Watson, 2006; Smith et al., 2008; Scharader and Summerfelt, 2010). The origin of geosmin has been reported by many studies. Van der Ploeg (1992) reported that blue-green algae of the genus *Anabaena sp.* was associated with high levels of geosmin in ponds at Auburn, Alabama, and *Anabaena spiroides* blooms in Mississippi ponds were associated with geosmin levels from 0.05 to 6.25 µg/L. Tucker (2000) concluded that geosmin production was associated with species of *Anabaena*, *Aphanizomenon*, and *Nostoc* in the family Nostocaceae; species of *Oscillatoria*, *Lynbya*, *Phormidium*, *Symploca*, and *Schizothrix* in the family Oscillatoria; and *Fischerella* in the family Stigonemataceae. Species of *Lynbya*, *Oscillatoria*, and *Phormidium* are also genera-producing MIB.

Besides blue-green algae and actinomycetes, Armstrong et al. (1986) reported fish with off-flavor associated with green algae; *Scenedesmus*, *Dictyosphaerium*, *Coelastrum*, *Sphaerocystis*, *Pediastrum*, and *Closterium*. Also, there is an observation of the release of MIB from aerobic biofilter material in intensive tilapia culture systems (Guttman and Van Rijn, 2008) that apparently was produced by *Streptomyces* bacteria. Van der Ploeg et al. (1992) reported that most likely geosmin appears in soft water with total alkalinity and hardness below 50 mg/L while MIB is more common in harder water.

The environment affects odorous compounds. For example, MIB in warm water is rapidly absorbed by fish, but it is also lost rather easily from the fish once the fish is no

longer exposed to MIB in the water. On the other hand, when fish acquire MIB in cold water it may take longer to purge (Van der Ploeg, 2001).

Geosmin and MIB can be lost from water by volatilization and by biodegradation by microorganisms in water. A study by Lelana (1987) suggests that volatilization is an important loss of the compounds from aquaculture systems over the long term. However, it can be lost much faster through biodegradation; the same study reported that 98% of the geosmin in pond water samples was metabolized by naturally occurring microorganisms within 72 hr. MIB appears to be more resistant to biodegradation than geosmin.

#### Algal control: algicides

To reduce excessive phytoplankton blooms, various chemical, mechanical, and biological treatments have been used with various degrees of effectiveness (Boyd 1990). Chemical control methods have been most effective and they are easy to apply. Various algicides have been used in the aquaculture industry: copper sulfate, simazine (2-chloro-4, 6-bis (ethylamino)-s-triazine), Solricin 135 (potassium ricinoleate), and diuron.

Although these compounds have been used successfully to control algal in aquaculture, they can have negative effects. Tucker (2002) summarized the negative effects as follows: 1) broad spectrum toxicity towards phytoplankton resulting in the death of entire phytoplankton community; 2) potential toxicity toward non-target

organisms; 3) negative public perception of the use of synthetic compound in agriculture; 4) environmental safety issues.

The USEPA (2002) approves chemical use in ponds. Only copper-based products are registered for controlling algae in aquaculture. However, Hanson (2001) reported that diuron was granted an exception by USEPA in April 1999 for use in aquaculture ponds to control algae that produce off-flavor in channel catfish.

The first registration for a copper-containing pesticide for agricultural use was issued in 1956. However, copper sulfate was issued an exception in March 1986 for aquatic application for algaecide applications, herbicide applications, and molluscicide and macro-invertebrate applications (USEPA, 2008). Currently, 16 products containing copper have active food use registrations subject to tolerance reassessment and reregistration review. Although various synthetic algicides are available for use in aquaculture, only diuron and copper sulfate or copper based products can be legally applied in aquaculture systems.

### Diuron

Diuron is classified as 3-(3,4-dichlorophenyl)-1,1-dimethylurea that is a substituted urea herbicide used for selective pre-emergence or early post-emergence control of seedling grasses and broad-leaved weeds in certain croplands. At low concentration, it has algicide characteristics making it useful for off-flavor management (Tucker and Leard 1999). These characteristics include a wide margin of safety between algicidal concentrations and concentrations toxic to fish and humans, freedom from

complex interactions with other water chemistry variables, and lack of long-term persistence in the pond environment because the chemical is decomposed by natural microbiological activity (Hanson, 2001).

### Copper

The metal copper is a trace element essential as a micronutrient for plants at low concentration because it is a reactant in biochemical functions of photosynthetic organisms (Barón et al., 1995). At high concentration, it can be toxic by interrupting electron transport through photosystem II, and it can modify or inhibit fundamental enzymatic activities (Pinto et al., 2003; Bruda et al., 2003). The most important mechanism of phytotoxicity is binding of copper with sulfhydryl groups of proteins, which lead to inhibition of critical enzymes.

Palmer and Maloney (1955) and Fitzgerald (1959) were concerned about algistatic properties of copper sulfate. Cultures of algae treated with certain chemicals appear to die at first, but then recover after further incubation. This has been frequently observed in toxicity tests with copper sulfate; as the incubation time increases, cultures that appeared to be dead recover and grown over time, in much the same density as untreated control cultures. Recovery of certain treated algal cultures is due to the fact that cells of the algae are not dead but their growth is temporarily inhibited (Fitzgerald and Faust (1963). Tucker (2000) noted that some of the noxious species of cyanobacteria appear to be relatively sensitive to copper. Boyd (2005) discussed that it is still difficult

to take advantage of differential toxicity of copper under field conditions and that copper tolerance also varies among species.

USEPA (2008) listed copper as a pesticide and copper compounds are extensively used in various agricultural settings. Tens of millions of pounds of copper are applied annually, predominately in crops protection. Most importantly, the ecological risk assessments indicate that copper is relatively safe for use as a broad-spectrum fungicide on many foods and ornamental crops, and for direct use in water applications as an algicide, aquatic herbicide, bactericide, and molluscicide. Copper compounds also are registered for antimicrobial application.

Although there are several forms of copper-containing active ingredients under review, the active component of toxicological interest is cupric ion. The USEPA based their assumption on the fact that there are no residential or occupational risks resulting from exposure to copper products. Several current copper products for agriculture do not specify typical application rates, minimum retreatment intervals or frequency of treatments. However, screening-level, ecological assessment indicated that copper can pose acute risks to various organisms, with the greatest risk to aquatic organisms resulting from direct water applications and runoff from fields adjacent to water bodies (USEPA, 2006).



### Copper sulfate application

Copper sulfate quickly dissolves in water and the cupric ion reacts with a wide variety of inorganic, organic and biological components. Copper bioavailability is related to its speciation, which is linked to various parameters including pH, redox potential, alkalinity, organic and inorganic ligand concentration. Boyd (2005) demonstrated the fate of cupric ion in aquaculture ponds by assuming that tenorite is the mineral controlling the solubility of copper. The equilibrium cupric ion concentration at pH 7 is ten times higher at pH 8. In aquaculture ponds, cupric ions react with carbonate ions to form a soluble cupric carbonate ion pair and to form soluble complexes with naturally organic acids in water. This suggests that after applying copper in ponds, it may be present more in the complexed form, also called the chelated form, than in cupric ions. Cupric ions precipitate rapidly as cupric oxide, and they can be adsorbed on colloidal clay and organic matter in bottom soil through cation exchange processes.

Copper toxicity is related to cupric ion activity rather than total copper concentration. To avoid a higher cupric concentration than needed to control algae that might kill fish or culture organisms, the maximum recommendation of copper for fish is about 0.02 mg/L in acidic, low-alkalinity water and 0.2 mg/L in water with high alkalinity and pH (Boyd, 2005). Water quality analyses, especially total alkalinity, are required before applying copper sulfate. The most common dosage is:

$$\text{Copper sulfate (mg/liter)} = \text{total alkalinity (mg/liter as CaCO}_3) \div 100$$

Masada and Boyd (1993) discussed the advantage of chelated copper algicide application over copper sulfate. Chelated copper algicide provides a higher concentration of copper in water, and the loss of copper from the water to the pond bottom is slightly slower. The problem is that chelated copper is several times more expensive than copper sulfate.

#### Copper accumulation in fish and environment

After copper sulfate application, copper quickly disappears from the pond water and copper concentration falls to the pretreatment level within 72 hr (McNevin et al. (2004). Silapajarn and Boyd (2006) reported that copper accumulation in ponds tended to increase with high organic matter concentration and pH. Copper has low solubility at pH 6 and above. Boyd (2005) suggested that there was no detectable contamination of fillets of fish by copper treatment in ponds. According to Stokes (1979), fish muscle normally contains low concentrations of copper, even at high levels of copper concentration in water. Muscle does not often reflect increases in copper in the external environment. Stangg and Shuttle worth (1982) found that copper was first accumulated in the gill tissue. Avenant-Oldewage and Marx (2000) also found that gills accumulated copper first, but the fate of copper ingested through the mouth or uptake through the gills will ultimately be deposition in the liver.

### Controlled-release technology

Time release technology may be described as a method to control the rate of release of an active ingredient of a fertilizer, medicine, or pesticide over time. There are many terms that can be used to describe such products: slow-release (SR); sustained-release (SR); sustained-action (SA); extended-release (XR); timed-release (TR); modified release (MR); continuous-release (CR); delayed-release (DR); controlled-release (CR) technology. This technology has been used in various fields such as surgery, pharmacology, and agriculture. (Teasley and Onochie, 2009)

The advantage of this method in fertilization of agricultural crops is that traditional fertilization presents the concern of environmental losses such as volatilization, denitrification, leaching, and runoff. Use of slow-release nitrogen can minimize leaching and other environmental losses. It also can reduce the cost of fertilization.

Time-release technology for fertilizers can be categorized into two groups, coated and uncoated. An example of uncoated, slow-release fertilizer is urea-formaldehyde reaction products that decompose in soil by chemical processes, biological processes, or a combination of both. Isobutylidene diurea (IBDU) relies solely on soil chemical processes to breakdown the product to the inorganic salts magnesium, ammonium, and phosphate.

Coated, slow-release fertilizers are usually coated by one or the other of two major coating compounds. Sulfur-coated urea releases nitrogen through oxidation of its

sulfur coating. The other kind of coating is a polymer coat. For example, urea is coated with special polymer coating – special to each manufacturer. Polymer coating allows water to move in and dissolve urea, and afterward nitrogen diffuses out through the porous polymer membrane. Polymer-coated urea (PCU) is beneficial in reducing split applications in sandy soils for potatoes and reduction in nitrogen leaching loss on sandy soils.

Well-known coated fertilizers for agricultural systems are ESN<sup>®</sup> (Environmental Smart Nitrogen, Agrium, Calgary, AB), Polyo<sup>®</sup> (Agrium, Calgary, AB), and Nutricote<sup>®</sup> (Chisso-Ashahi Fertilizer Co., Ltd., Tokyo, Japan).

Time-release technology is increasingly employed in other fields. Teasley and Onochie (2009) applied it for chemical oxidation or chemox for environmental remediation. It is an emerging method for treating subsurface water and soil. It can be used in a contaminated zone where the oxidants rapidly react to oxidize and breakdown subsurface contaminants to less toxic by-products.

Collins et al. (1973) first applied the controlled release concept to herbicides. They created a theoretical paper entitled “Controlled release of herbicides-theory”. Its purpose was to development guidelines for experimentation. This paper contained a mathematical model to compute the release rate of herbicides from coated granules. The controlled release of the herbicide from spherical granules can be described by the following expression:

$$\frac{dv}{dt} = 4\pi r^3 \frac{dr}{dt}$$

d = derivative, v = volume, r = radius, t= time

## MATERIALS AND METHODS

The work to evaluate coated algicides was conducted in a series of three studies over three summers. The ponds and their general management and the methods that applied to all studies will be presented before the individual studies are described.

### Experimental ponds and water management

Earthen ponds used in the study are located at the E. W. Shell (EWS) Fisheries Center of Auburn University located about 5 km north of Auburn, Alabama on Alabama Highway 147. Ponds of the E-Series ( $400 \text{ m}^2 \times 1 \text{ m}$  average depth) and H-Series ( $200 \text{ m}^2 \times 0.75 \text{ m}$  average depth) were used. These ponds were constructed on soil of the Piedmont Plateau that are acidic, fine-loamy, kaolinitic, thermic Typic Kanhapludults (McNutt, 1981). The water source is a reservoir filled by runoff from a wooded watershed and supplied by gravity flow through pipes to the research ponds. This water has total alkalinity and total hardness concentrations of 8 to 12 mg/L as  $\text{CaCO}_3$ , nutrients and organic matter concentrations are low (Boyd, 1990). Water was added to ponds to maintain surface level 10-15 cm below the top of the overflow pipe to prevent outflow following rains and to keep water deep enough to discourage aquatic weed growth.

Total alkalinity concentration in pond waters was measured two weeks before the beginning of each study. Ponds with concentration below 25 mg/L were treated in mid-March with agricultural limestone at 2,000 kg/ha (Boyd, 1990).

### Pond fertilization

All ponds received inputs of nitrogen and phosphorus fertilization at rate equal to 6 kg N/ha and 3 kg P<sub>2</sub>O<sub>5</sub>/ha per application as recommended by Wudtisin and Boyd (2005) and Boyd et al. (2000). Potassium fertilization of ponds on the EWS Fisheries Center is not necessary (Viriyatum and Boyd, 2009).

Fertilizers were weighed and transferred to 20 L of pond water in a plastic bucket. The water was stirred to dissolve the fertilizer. The resulting slurry was splashed over pond surfaces. Fertilizers were applied three times at 2-week intervals at beginning of studies, and afterwards at 3-week intervals.

### Algicides and their application

Agrium Advanced Technologies, Sylacauga, Alabama, supplied both coated copper sulfate and diuron materials for the studies. The coated copper sulfate contained 20% copper. Coated algicide materials were applied only one-time at the beginning of each experiment. Regular copper sulfate contains 25% copper and was purchased from a local feed-and-seed store. The treatment rate for regular copper sulfate was calculated as a dose that supplied a concentration equal to 0.01X the total alkalinity (Boyd and Tucker, 1998). Copper sulfate crystals were weighed to provide the necessary amount and dissolved in 20 L of pond water. The solution was splashed over pond surfaces, and aerators were operated for 15 min to completely mix copper sulfate with the water.

The diuron application was based on the USEPA rule that allows the use of diuron at 4.25 active ingredient/ha per week for 10 weeks. Regular diuron was available for the EWS Fisheries Center.

### Water quality analyses

Water samples were collected with a 90-cm water column sampler (Boyd and Tucker, 1992). Samples were taken between 06:00 and 08:00 am. They were transported to the laboratory where analyses were initiated at once. Total alkalinity was estimated by acidimetry; total hardness was determined by titration with EDTA; turbidity was determined by nephelometry and expressed in nephelometer turbidity units (NTU) (Cleceri et al., 1998). In addition, water samples were dipped from pond surfaces on several occasions and water temperature, pH and conductance measured with portable meters. Secchi disk visibility was also determined on each sampling date.

Chlorophyll a concentration was determined by spectroscopy of acetone-methanol (5:1) extracts of phytoplankton removed from samples by membrane filtration. (Marker, 1972) Primary productivity was determined by light-dark bottle. Three-BOD bottles were placed about 1-foot in water column.

Water samples for copper analysis were preserved immediately with 1:1 nitric acid solution and brought to the laboratory. All glassware in this analysis was carefully washed with 1:1 nitric acid solution and rinsed with deionised water. Samples were filtered by Whatman. No. 42 and adjusted the pH of the preserved sample to between 2 to 6 with 5.0 N sodium hydroxide. Samples were measured by using the Hach Porphyrin Method (Hach Chemical Company, Loveland, Colorado) to develop a copper-induced color. The color was evaluated with a standard spectrophotometer rather than a Hach kit spectrophotometer.



### Filamentous algae

The abundance of filamentous algae and other aquatic weeds was assessed at monthly intervals. Each pond was visually divided down the middle of its long dimension and each of the two halves was visually divided into three single sections. The approximate area in each of the six sections that was covered by underwater or floating aquatic weeds was estimated and the coverage of the sections with weeds was sketched on an outline map of each pond. The percentages for the sections were averaged to obtain the percentage of filamentous algae for the entire pond.

### Phytoplankton sampling and classification

Water samples were collected and transferred into 100-ml glass bottles containing 1-ml of Lugol's solution as a preservative. The samples were observed periodically and more Lugol's solution was added when the solution color began to fade.

Samples were concentrated by homogenization and sedimentation (Mazziotti and Vadrucci, 2007). Samples were gently shaken and allowed to stand overnight. After the dead phytoplankton settled to the bottom of container, solution was removed carefully with a siphon to prevent re-suspension of settled algae. The original volume and final volume of sample solution were recorded precisely to compute the dilution factor. The concentrated samples were transferred in 50-ml brown vial and stored in the dark (American Public Health Association, 1999). Phytoplankton were counted and identified to genus at a total magnification of 150X or 600X in a Sedgewick-Rafter counting cell under a microscope fitted with a Whipple grid (American Public Health Association, 1989; Bernard, 1971; Gertrud and Annadotter, 2006).

### Data analysis

Data were analyzed using the general linear model (GLM) after checking for normality with Shapiro-Wilk test and homogeneity. When the null hypothesis of GLM was not satisfied, data on a particular variable were transformed. Following by Duncan's ranges test to check for the differences between groups, when the interaction was significant ( $P < 0.05$ ), they were evaluated using ANOVA across treatment levels at each sampling date compare to entire experimental period. These procedures were performed by SAS (version 9.1). Graphical data were plotted by using Sigma Plot (version 10.0)

### Proof of concept study – 2009 study

This small study was conducted in 2009. It consisted of a control and four treatments – regular diuron, coated diuron, regular copper sulfate, and coated copper sulfate – applied to unreplicated ponds with no fish. The coated products were made and supplied by Agrium.

This study consisted of two trials conducted in the same manner. In one trial the coated products were placed in the bottoms of plastic pans (30 × 15 cm), covered with nylon mesh material (1-mm), and put on pond bottom s where the water depth was about 60 cm. The mesh covers were checked weekly and cleaned as necessary. A single plastic pan was used per pond. In the other trial, the coated products were broadcast over the pond surfaces.

Ponds were fertilized beginning on 1 June, but fish were not placed in ponds and no aeration was applied. The regular copper sulfate was applied at 129 g/pond per application for 10 weeks. Regular diuron was applied at 4.25 g active ingredients/pond

per application for 10 weeks. The coated copper sulfate and diuron were applied in single applications of 742 g and 490 g, respectively, of the coated products. According to the company, the amounts in coated products were equal in total active ingredient to the total amount of regular copper sulfate and diuron applied.

Surface water samples were analyzed for Secchi disk visibility, turbidity, and chlorophyll *a* concentration, primary productivity was measured, and phytoplankton abundance assessed as described earlier.

#### Coated copper sulfate rates – 2010 study

This study was conducted in 15, E-Series (treatments) and five H-Series ponds (controls) on the SFC. Three treatments were evaluated, regular copper sulfate, low rate of coated copper sulfate (750 g/pond), and high rate of coated copper sulfate (1,500 g/pond). Treatments and control ponds were randomly selected by the ballot method – a slip of paper with a pond number was drawn from one jar and a slip with a treatment for this pond was drawn from a second jar. Each treatment and control were assigned to 5 replicated ponds.

The ponds were fertilized beginning on 1 June 2010, but fish were not stocked and aerator was not applied. The quantity of coated product for each pond was divided into three equal aliquots, and each aliquot was placed in a nylon bag 1-mm mesh size that would retain the smallest granules of the product. The bags were suspended at a depth of 30 cm beneath the water surface in each pond on 10 June. Copper sulfate application began on the same day at 157 g/pond for a total of 14 applications. Treatment rates in terms of total copper applied were: control, 0 g/pond; low-coated copper sulfate, 150

g/pond (0.375 g/m<sup>2</sup>); high-coated copper sulfate, 300 g/pond (0.750 g/m<sup>2</sup>); regular copper sulfate, 550 g/pond (1.38 g/m<sup>2</sup>).

Pond waters were analyzed for pH, water temperature, Secchi disk visibility, specific conductance, turbidity, chlorophyll *a*, dissolved oxygen, and soluble copper. Phytoplankton was identified to genus and enumerated. Methods for analyses were described above.

#### Evaluation of coated copper sulfate in catfish ponds – 2011 study

The high-rate coated sulfate (1,500 g/pond) was compared with regular copper sulfate for phytoplankton control in catfish ponds. Fifteen, E-Series ponds were used in this study. Five ponds each were assigned randomly to three treatments: control, regular copper sulfate, and coated copper sulfate by drawing lots. Each pond was stocked with 600 fingerling hybrid catfish of 20-cm average length on 12 April 2011. Four grass carp *Ctenopharynodon idella* (88 g/fish) were placed in each pond for weed control on May 2011.

Pond management followed standard commercial practices. A commercial, pellet catfish feed containing 32% crude protein was applied to ponds 6 days per week at an estimated 3% of body weight adjusted weekly for weight gain (Cole, 1984). No attempt was made to remove uneaten feed from ponds. A 0.33-hp aerator was placed in each pond and operated nightly to supplement natural sources of dissolved oxygen.

Coated copper sulfate treatment consisted of a single application of 1,500 g/pond of the product (300 g copper). Coated copper sulfate for each pond was divided into three, 500 g aliquots, and each aliquot was confined in a mesh bag (1.0-mm opening).

Nylon lines were tied to bags and the lines were attached to bamboo stakes to suspend the bags at a depth of 30 cm. Ponds assigned to the regular copper sulfate treatment were treated with crystalline copper sulfate at one one-hundreth of the total alkalinity at weekly intervals starting on 1 June 2011 and continuing until 14 September 2011 for 16 applications.

Water samples were analyzed for total alkalinity, total hardness, turbidity, pH, water temperature, specific conductance, chlorophyll *a*, dissolved oxygen, and soluble copper. The environmental monitoring also included filamentous algae and phytoplankton assessments and primary productivity determination. Methods have already been described.

Ponds were drained and fish were harvested on 18 and 19 October 2011. At harvest, the number of fish present was counted and the total weight of fish determined. Samples of three fish from each pond were filleted, and the fillets were frozen immediately until analyzed for copper concentration and use in sensory tests for flavor evaluation

#### Fish fillet copper analysis

Fish were filleted, and fillets were skinned and trimmed using ceramic knife. Fillets were lyophilized to constant weight with a Labconco Lyph Lock Model 6 freezer drier. Dried fillets were ground with the IKA Economical Analytical Mill (Cole-Parmer, Vernon Hills, IL, USA). Aliquots of each sample were digested in a mixture of concentrated nitric and perchloric acids (7:3) distilled water. Digested samples were

diluted to 50 ml and analyzed for copper by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Li 2012)

### Sensory tests

The fish samples were taken from each pond and filleted as described above. The samples were defrosted and packed in microwaveable plastic container separately for each pond. They were wrapped in paper and cooked in a microwave oven in a separate room from where the testing was done. Each fish was divided into five small pieces, placed in 150-ml plastic cup, and covered with saran wrap immediately. Each piece was coded randomly according to pond number and collected fish.

The panel was assigned to test randomly. The five-member taste panel evaluated all three fish samples of each pond for both flavor intensity and flavor description. At the beginning and between samples, panel members rinsed their mouth with freshwater and bit a piece of cracker. Taste test methodology described by van der Ploeg, (1991) was used. It evaluates both flavor intensity and description of taste based on common references.

The flavor intensity scales from 0 to 3 or 4 as described in Table 1. The threshold concentration is the lowest level at which an odorous compound can be perceived. Threshold concentrations vary among individuals because people are not equally sensitive to odors. Therefore, a threshold concentration of a consumer population is defined as the lowest level that 50 percent of the people can detect. Typically, people who judge flavor quality are expected to be sensitive enough to detect the compounds of interest at a concentration lower than the threshold level for the consumer population.

The threshold levels for geosmin and methylisoborneol have been established and can be used to select judges for fish flavor analysis. Fish without off-flavors receive the score 0 because undesirable flavors are perceived to be absent. Off-flavor fish were scored as follows: slight (1); distinct (2); strong (3); or extreme (4).

The same piece of fish was used to define the type of flavor. Most flavor descriptors are standardized by and referenced to names of commonly known materials and odor characteristics (Table 2). The two common off-flavors have been named the odor descriptors following by the causative agents: “earth/geosmin” and “musty/methylisoborneol (MIB).

**Table 1. Fish flavor intensity score: Source: van der Ploeg, 1991**

<b>Verbal Description</b>	<b>Intensity Scale</b>
No off-flavors	0
Threshold	T
Very slight	0.5
Slight	1
Slight to distinct	1.5
Distinct	2
Distinct to strong	2.5
Strong	3



**Table 2. Taste descriptive modified from flavor wheel: Source: Van der Ploeg, 1991**

Acceptable	Objectionable				
	Vegetable	Decay	Blue-Green Algae	Chemical	Fishy
Corn	Celery	Egg-Sulfury	Woody	Metallic	Crawfish
Nut-like	Mushroom	Sewage	Pine	Pesticide	Fish Oil
Buttery/Fat	Greens/Grassy	Decaying Vegetation	Musty/MIB	Diesel	Stale
Chicken	Onion	Rotten	Earthy/Geosmin		Rancid
		Moldy			Cardboard

## RESULTS AND DISCUSSION

### Proof of concept study-2009

The proof of concept study conducted in 2009 was not truly replicated. But, in a sense, it was duplicated in order to determine which of two ways of exposing the coated product was most feasible.

Water quality variables exhibited much variation among ponds and over time, making it difficult to meaningfully assess differences among treatments and between individual treatments and control. For this reason, data for all dates in each pond were averaged for comparisons among treatments and control (Table 3).

Secchi disk visibility is an estimate of the depth of visibility (turbidity) in water. In aquaculture ponds, turbidity often results primarily from plankton (both phytoplankton and zooplankton). However, suspended soil particles and humic substances also create turbidity and lessen depth of visibility. In trial 1, Secchi disk visibility was lower and turbidity averaged higher (this does not mean statistically higher because the lack of replication prevented use of statistic tests) in the control pond than in the treated ponds (Table 3). In trial 2, differences between control and treatments were not so clear, but the pond treated with coated copper sulfate had rather low Secchi dish visibility and high turbidity.

In this study, we were concerned with the effect of algicides on algal abundance, and most particularly, with the abundance of blue-green. Chlorophyll *a* concentration is a better variable for assessing algal abundance than Secchi disk visibility or turbidity,

because chlorophyll *a* concentration is not influenced by factors other than the amount of phytoplankton in the water.

In trial 1 where algicides were placed in pans, all products caused a large reduction in both chlorophyll *a* and blue-green algal abundance. The coated products performed as well as the regular products. Trial 2 in which algicides were broadcast gave similar results with the exception that the chlorophyll *a* concentration averaged very high in the pond receiving the coated copper sulfate. This was the result of a dense phytoplankton bloom for several weeks in that pond; the reason that this pond had a dense phytoplankton bloom while the others did not cannot be explained. This pond should be considered an outlier as far as data evaluation is concerned.

The results of these two, un-replicated trials show that both copper sulfate and diuron treatment can lead to a reduction in both chlorophyll *a* concentration and abundance of blue-green algae. With the exception of the high chlorophyll *a* concentration in the coated copper sulfate treatment of trial 2, the coated products performed as well as the regular products. This is, however, advantage that the coated products would not have to be applied but once during the growing season. The coated product also would allow better control over copper and diuron concentration in the water.

The pans were placed about 1-foot below water surface where water movement was less than in surface water, and particles settled on top of the ponds necessitating frequent cleaning. Nevertheless, the pan application seemed to be superior for coated copper sulfate than did the broadcast applications (Table 3). Thus, for future studies, it was decided to place the coated copper products in mesh bags (same material used to

cover pan) and suspend the bags in the surface water where circulation is better than at the pond bottom.

Agrium Advanced Technologies decided to discontinue work with diuron. The reason was not explained, but possibility it was because this compound has for several years been given approval by USEPA in catfish ponds on a year to year basis, and the company may have feared that its approval might be discontinued in the future. Copper sulfate, on the other hand, has permanent USEPA approval for use in food fish. Thus, in 2010 and 2011, only copper sulfate was investigated.

#### Coated copper sulfate rates - 2010 study

This study was an evaluation of regular copper sulfate and two concentrations of coated copper sulfate as algicides. The ponds were fertilized to provide high nutrient concentrations necessary for dense algal blooms, but fish were not stocked in ponds, and aeration were not applied.

Water temperature was similar among ponds of controls and treatments on all sampling dates (Figure 1), but when grand means were compared, control ponds had average temperatures of 0.90 to 1.03°C less than treatment ponds (Table 1). These differences were likely related to control ponds being shallower than treatment ponds and thereby losing proportionally more heat at night.

Specific conductance (Figure 2, Table 4) was slightly less in control ponds than in treatment ponds. No explanation for this difference is provided, but amounts of copper sulfate applied to treatment ponds were not enough to measurably influence specific conductance.

Table 3. Means and standard deviations in control pond and ponds treated with different formulations of copper sulfate and diuron by two application methods.

Treatment	Secchi disk (cm)	Turbidity (NTU)	Chlorophyll <i>a</i> (µg/L)	Blue-green algae (cellsx10 <sup>3</sup> /ml)
Trial 1 (pan application)				
Control	44 ± 9	59 ± 93	171 ± 275	54 ± 54
Coated diuron	63 ± 8	10 ± 7	27 ± 28	18 ± 10
Regular diuron	77 ± 9	5 ± 3	19 ± 19	19 ± 8
Coated copper sulfate	57 ± 14	20 ± 12	22 ± 14	19 ± 4
Regular copper sulfate	58 ± 15	15 ± 13	17 ± 18	18 ± 6
Trial 2 (broadcast application)				
Control	73 ± 10	9 ± 8	41 ± 56	10 ± 7
Coated diuron	75 ± 8	8 ± 10	23 ± 21	8 ± 8
Regular diuron	74 ± 10	6 ± 3	24 ± 29	9 ± 7
Coated copper sulfate	50 ± 22	32 ± 24	209 ± 235	51 ± 53
Regular copper sulfate	66 ± 15	8 ± 5	28 ± 17	15 ± 8

The pH did not vary greatly among control and treated ponds on any sampling dates (Figure 3), but grand means for control ponds was higher than grand means of copper treatment ponds (Table 4). Ponds of coated copper algicide treatments did not differ in mean pH, but they had higher mean pHs than ponds treated with regular copper sulfate (Table 4).

Copper concentration was much higher in copper sulfate-treated ponds than in control ponds throughout the study (Figure 4). Coated copper sulfate-treated ponds had higher copper concentrations than control ponds until 5 August in the low treatment and until 8 September in the high treatment. Grand means of copper were higher in copper-treated ponds (Table 4). However, regular copper sulfate treatment resulted in greater copper concentrations than occurred in ponds treated with coated copper sulfate. Of course, considerably more copper was applied to ponds of the regular copper sulfate treatment than to the other copper-treated ponds. Ponds of the high coated copper sulfate treatment were higher in copper concentration than ponds of the low coated copper sulfate treatment.

Phytoplankton abundance was estimated by four techniques: Secchi disk visibility, turbidity, chlorophyll *a* concentration, and total algal abundance. Secchi disk visibility was higher in control ponds than in treated ponds throughout the study –treated ponds had clearer water (and contained less phytoplankton ) than control ponds (Figure 5). The ponds of low coated copper sulfate treatment often had greater Secchi disk visibility than ponds of either the high coated copper sulfate treatment or the regular copper sulfate treatment. There was no trend of differences in Secchi disk visibility

between ponds of the high coated copper sulfate treatment and ponds of the regular copper sulfate treatment. Grand means for Secchi disk visibility differed among control and treatment ponds as follows: control < regular copper sulfate < high coated copper sulfate treatment < low coated copper sulfate treatment (Table 4).

Turbidity was higher on all dates in copper-treated ponds than in control ponds, but turbidity levels fluctuated among treated ponds from date to date (Figure 6). The grand mean for turbidity in control ponds was higher than grand means of treated ponds, but grand means for turbidity did not differ among ponds of different copper treatments (Table 4).

Chlorophyll *a* concentrations followed a trend similar to turbidity (figure 7). Grand means of chlorophyll *a* for control ponds were greater than those of copper-treated ponds, but grand means for copper-treated ponds did not differ (Table 4).

Total algal abundance revealed similar trends and differences among control ponds and treated ponds as observed for chlorophyll *a* concentration (Figure 8, Table 4). Blue-green algal abundance did not differ between control ponds and ponds of the low coated copper sulfate treatment (Figure 8, Table 4), but ponds of the other two copper treatments had less blue-green algae. There was no difference in blue-green algal abundance between the high coated copper sulfate treatment and the regular copper sulfate treatment. There also was no apparent difference in the types of blue-green algae in the treatment and control ponds. Although several genera of blue-green algae were observed in the ponds, by far the most common and abundant genus was *Anabeana*. Species of this genus has been associated with geosmin-related off-flavor in fish at the SFC (van der Ploeg et al., 1992).

Dissolved oxygen concentration was always above 2 mg/L in ponds despite the decision to not aerate, and mean dissolved oxygen concentrations usually were above 5 mg/L (Figure 9). Grand means for dissolved oxygen concentration were higher in control ponds than in copper treated ponds. However, there were no differences in dissolved oxygen concentration among the three copper treatments (Table 4).

The copper concentration deserves further discussion, because maintaining a high enough concentration of copper to control algae is the crux of the study. Mean copper concentration in control ponds varied from 4.6 to 11.1  $\mu\text{g/L}$  during the study. Moreover, on individual sampling dates, there was variation in copper concentration among the five, replicate control ponds, e.g., on 7 July, the copper concentration varied from 2.8 to 6.3  $\mu\text{g/L}$ . On the same date, similar variation also was observed in treatment ponds; copper concentration ranged from 12.9 to 21.1  $\mu\text{g/L}$  in the high-rate, coated-product treatment. Variation possibly resulted from several sources to include copper contamination of sampling bottles, analytical error, and changes in conditions in ponds that affected copper concentration. However, variation in copper concentrations was no greater than the amount of variation typically encountered in measurements of other water quality variables in ponds (Boyd et al., 1979; Boyd et al., 1994).

Control ponds and ponds treated with regular copper sulfate had great differences in copper concentration on all dates – differences ranged from 6.6 to 20.0  $\mu\text{g/L}$ . The weekly treatment rate of copper sulfate was equal to a copper concentration of 98  $\mu\text{g/L}$ , but the total input of copper to a pond of the regular copper sulfate treatment was equivalent to 1,372  $\mu\text{g/L}$ . However, at the end of the study, copper concentration in ponds of the regular copper sulfate treatment averaged only 16.94  $\mu\text{g/L}$ . The rapid and



large disappearance of copper from water was not unexpected, because earlier studies showed that copper applied to ponds is either adsorbed by phytoplankton, precipitated from water as copper oxide, deposited in a sediment as organically-bound copper in dead phytoplankton, or adsorbed directly by sediment (McNevin and Boyd, 2004; Han et al., 2001).

Ponds treated with regular copper sulfate had copper inputs more than three-fold greater than those of ponds treated with the low rate of coated product, and almost twice those of ponds treated with the high rate of coated product. Mean copper concentrations were understandably lower on most dates in ponds treated with the coated product. However, on the first date that copper concentration was measured (13 days after copper treatments were initiated), the high rate of coated product caused a much greater copper concentration than observed in ponds treated with regular copper sulfate. Moreover, ponds receiving a low rate of coated product had copper concentration equal to those measured in ponds of the regular copper sulfate treatment. On June 30, copper concentration was approximately equal in ponds receiving a high rate of coated product and those treated with regular copper sulfate. On this date, ponds treated with the low rate of coated product had less copper than ponds treated with regular copper sulfate. After 30 June, ponds with regular copper sulfate treatment had higher copper concentration than found in ponds to which the coated product was applied. After 5 August, ponds treated with the low rate of coated product had copper concentration approximately equal to those of control ponds. There was clear elevation of copper concentration in ponds receiving the high rate of coated product until 1 September.

On 20 August, bags containing copper product were removed from one pond receiving the high rate of coated-product. The mesh of bags was not clogged with debris that would have interfered with copper release to the water. But, when particles of coated product were observed and handled, it was obvious that nearly all of them were empty shells-copper sulfate had completely dissolved. This suggests that coating thickness for copper sulfate was sufficient to control copper releases for about 10 weeks.

These findings indicate the coating should be thicker to prevent the high, initial rate of copper release, and to allow copper to be released over a longer period. In channel catfish culture, excessive phytoplankton growth and related problems are most common from May through September-a period 5 months (Boyd and Tucker, 1998). This indicates that the product should be designed to release copper at a relatively continuous rate for 5 to 6 months.

Excellent phytoplankton control was achieved using the coated product. Based on grand means for the four indicators of phytoplankton abundance (Table 1), both rates of coated product were statistically indistinguishable from regular copper sulfate treatment. Moreover, phytoplankton did not rapidly regrow in ponds treated with the coated copper product for at least 1 month after copper sulfate had dissolved from the granules. By mid October, phytoplankton blooms had become re-established in ponds that had been treated with coated copper product at both rates.

Table 4. Results of tests of grand mean of water quality variables for significance (P = 0.05) by the Duncan's Multiple Range Test.

Means indicated by the same letter are not different.

Treatment	Secchi disk (cm)	DO (mg/L)	Temperature (°C)	pH	Conductance (µmhos/cm)	Turbidity (NTU)	Cu (µg/L)	Blue- green (cell/mL)	Total algae (cell/mL)	Chlorophyll <i>a</i> (µg/L)
Control	20 a	6.9	29.01	9.02 a	102	100	6.6 a	130,586	213,433	118
High coated CuSO <sub>4</sub>	67 b	5.97	29.92 a	8.84 b	113 a	21 a	13.2 c	45,198 a	78,783 a	46.0 a
Low coated CuSO <sub>4</sub>	73 c	5.68	29.92 a	8.78 b	113 a	23 a	8.4 b	74,078 a	86,934 a	56.8 a
Regular CuSO <sub>4</sub>	57 d	5.55	30.04 a	8.60 c	113 a	15 a	18.8 d	61,877 a	79,351 a	42.4 a

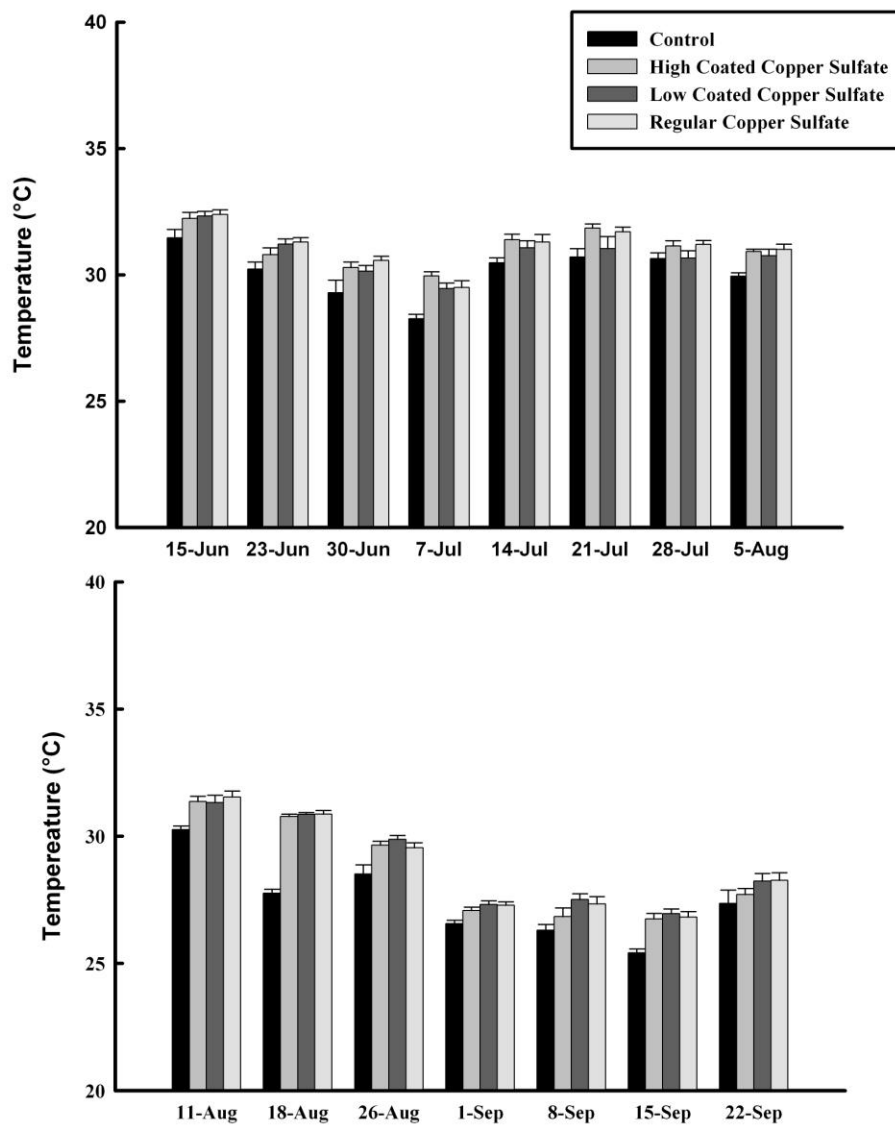


Figure 1. Mean water temperature on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

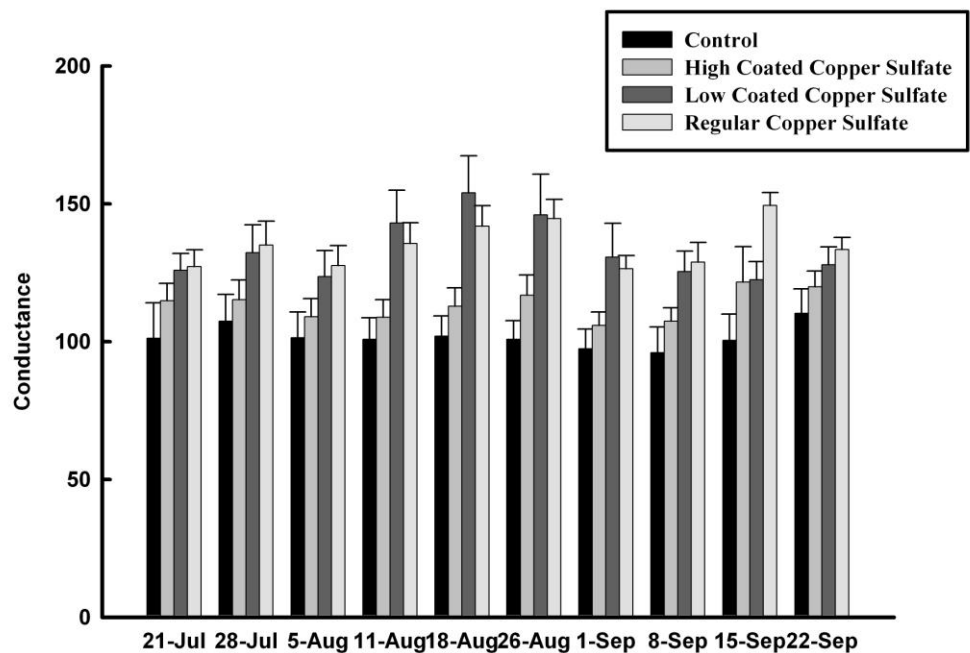


Figure 2. Mean specific conductance on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

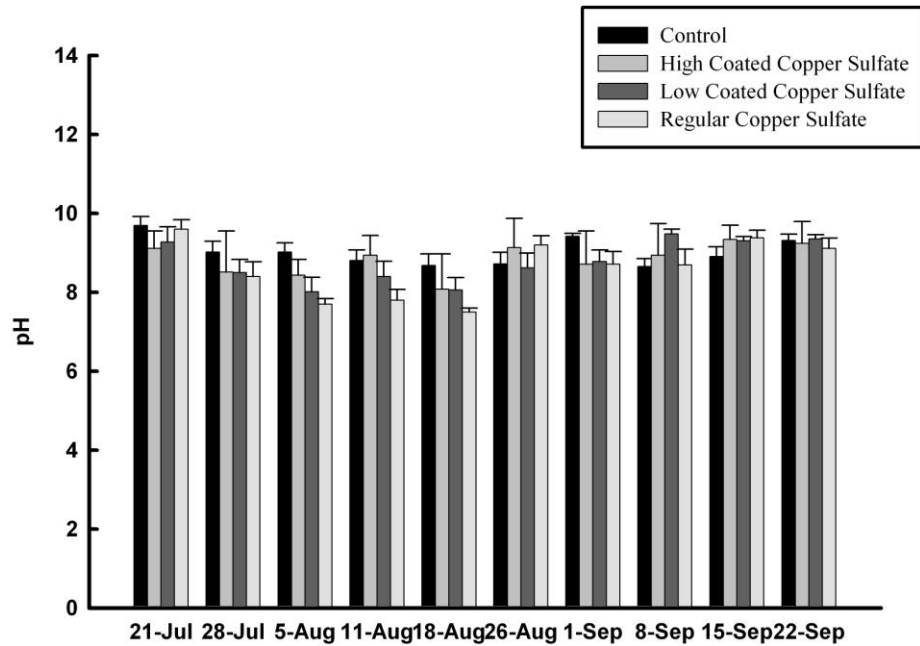


Figure 3. Mean pH on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

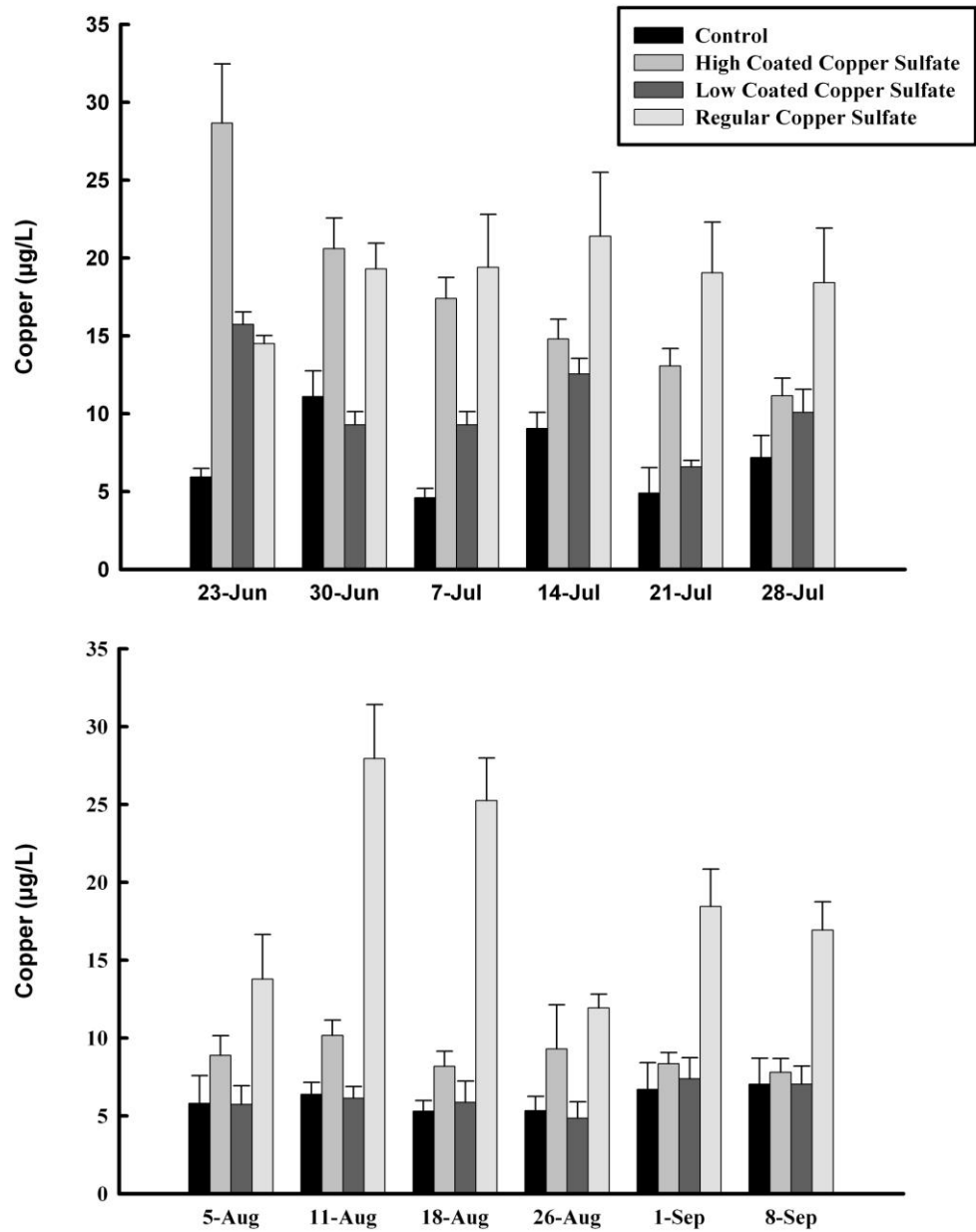


Figure 4. Mean copper concentration on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

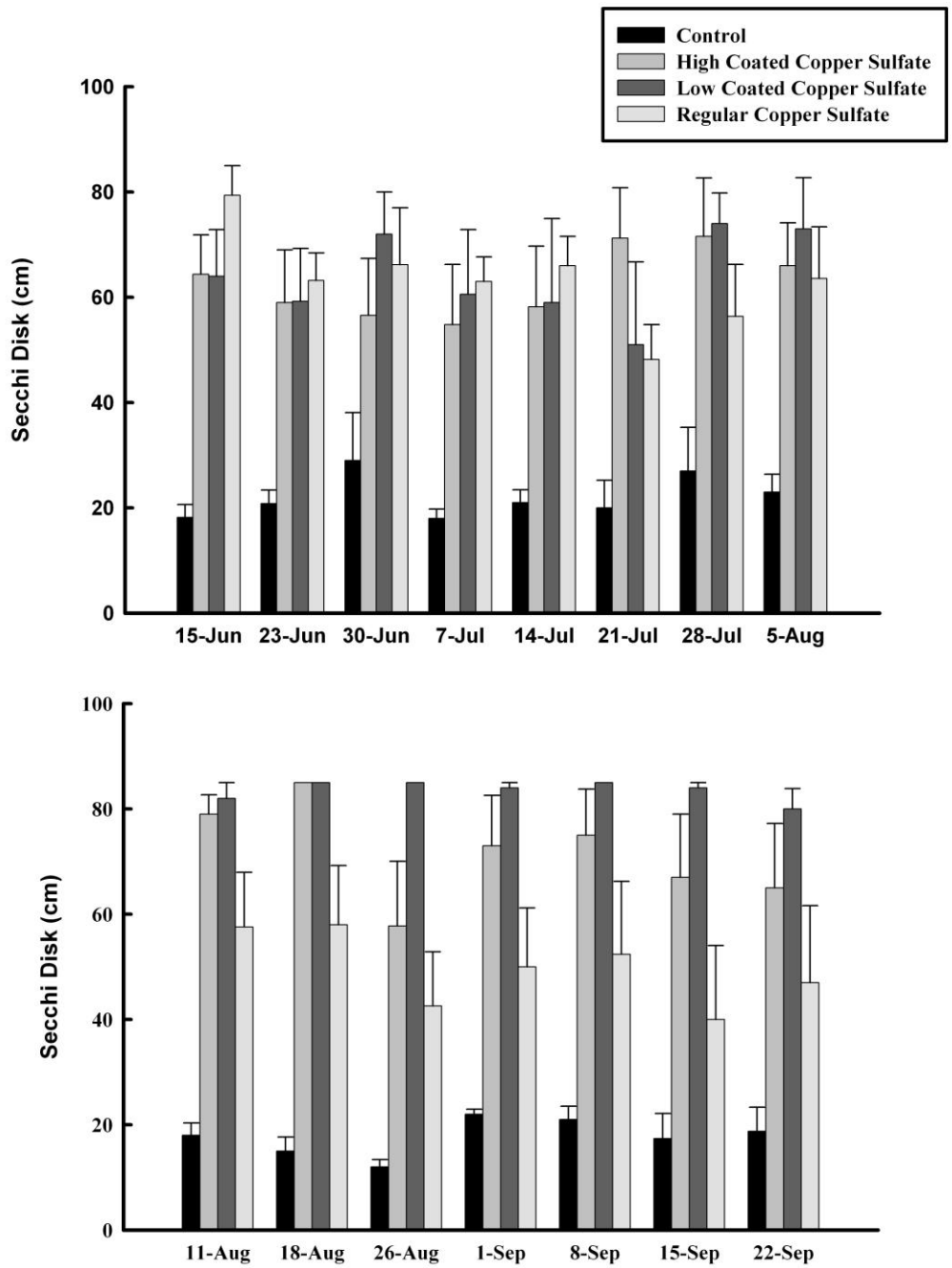


Figure 5. Mean secchi disk visibility on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.



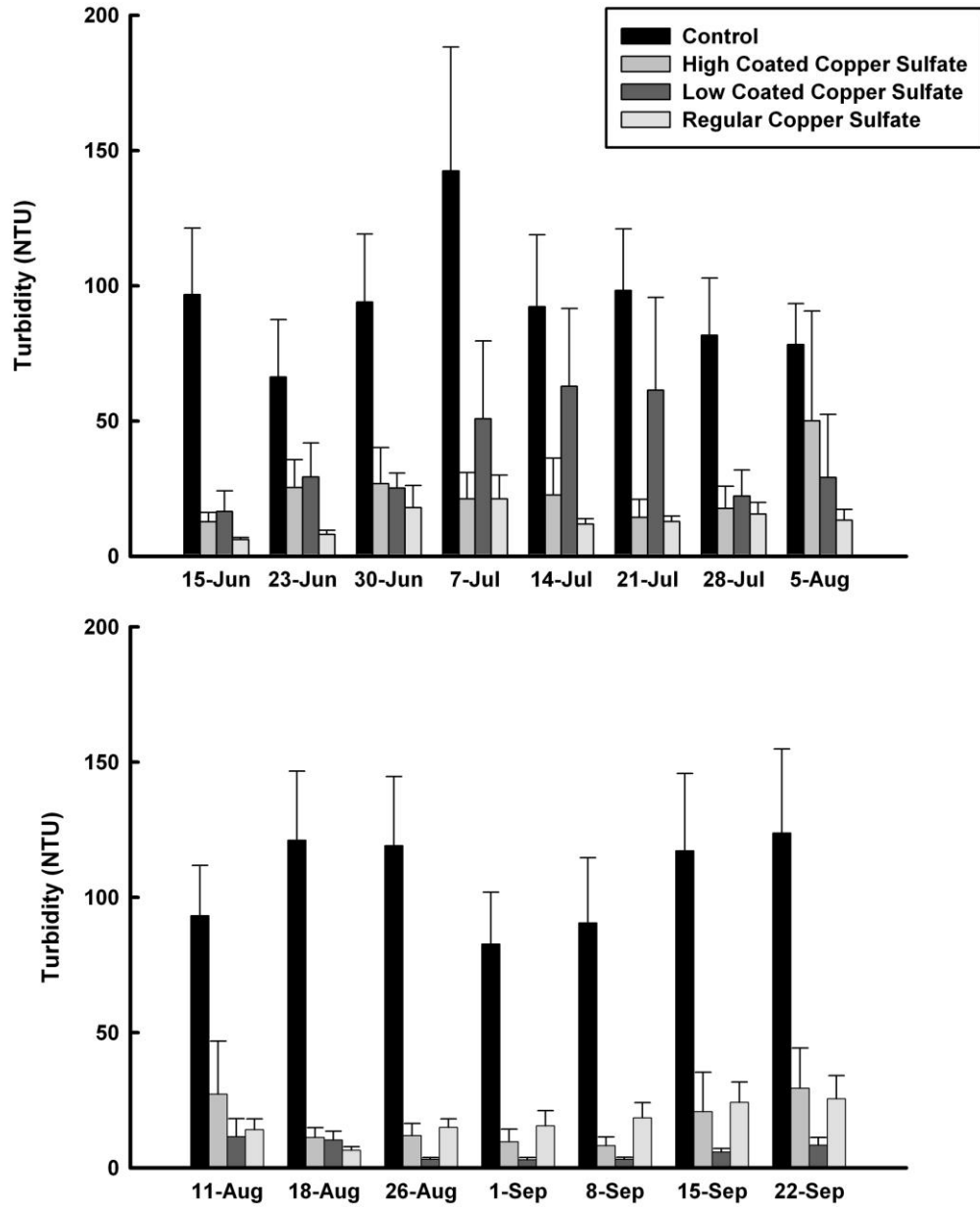


Figure 6. Mean turbidity on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

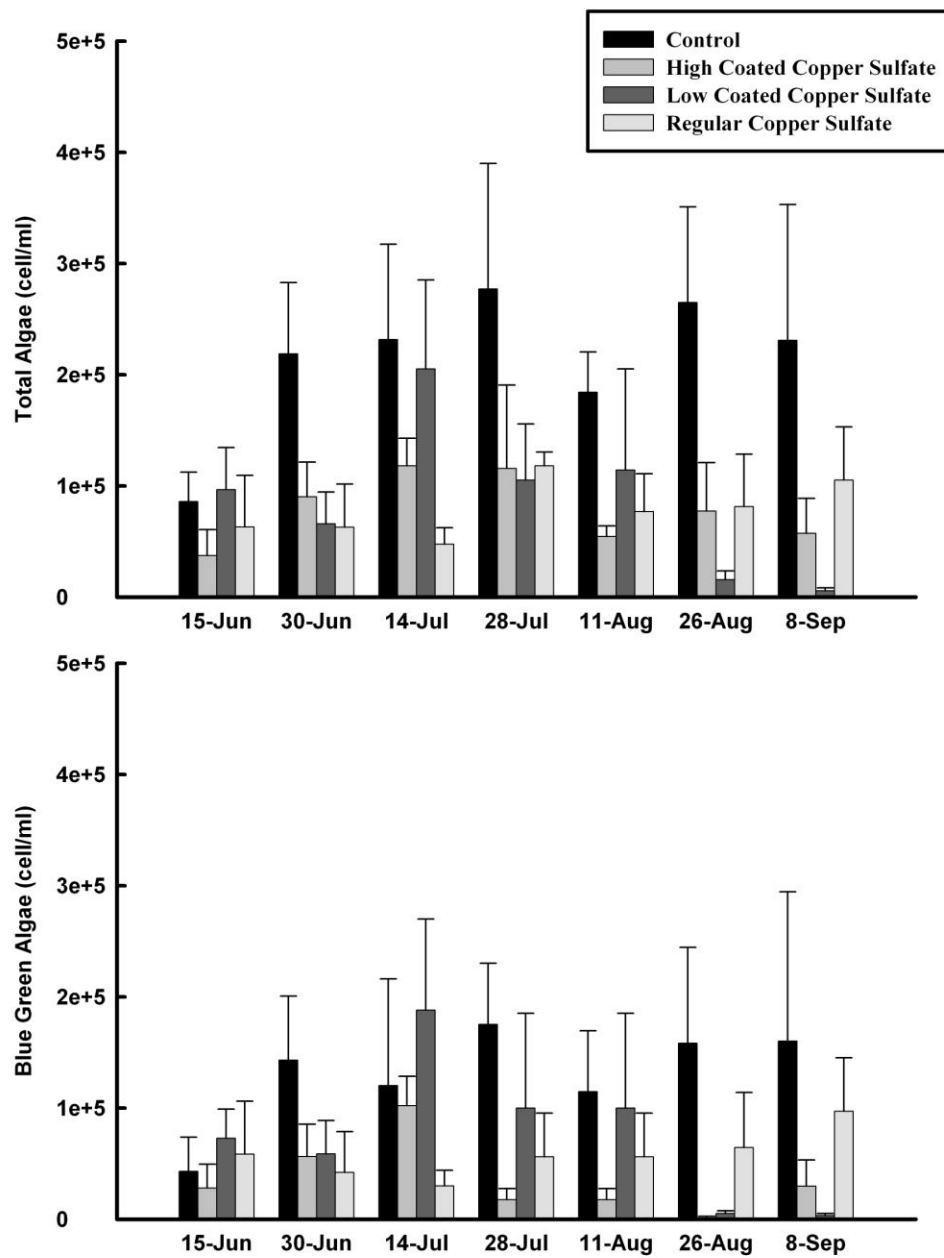


Figure 8. Means for total phytoplanktonic algal abundance and abundance of blue-green algae on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

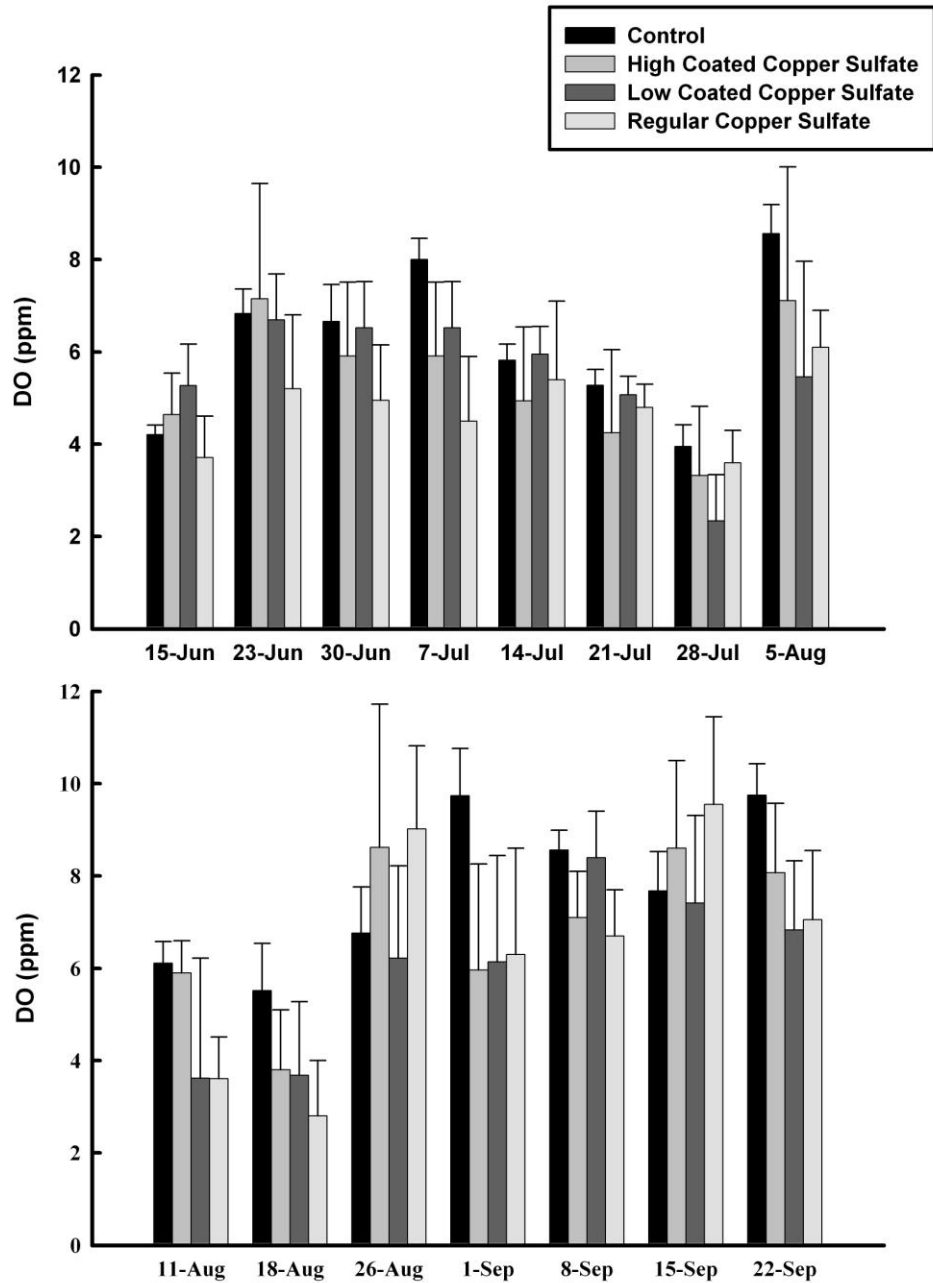


Figure 9. Means for dissolved oxygen concentration on different dates in ponds treated once with coated copper sulfate (low = 150 g Cu/pond; high = 300 g Cu/pond) and weekly with regular copper sulfate (39.3 g Cu/pond/week). Whiskers indicate standard errors of the mean.

### Evaluation of coated copper sulfate in catfish ponds – 2011 study

The 2011 experiment was a comparison of regular copper sulfate and coated copper sulfate in catfish ponds managed according to procedures used in commercial catfish ponds. Controls and treatments were replicated five times.

Water temperatures measured in early morning (Figure 10) were above 25°C between 1 June and 8 September, and dropped to about 20°C on 15 September. Although there were occasional differences in temperature among treatments, e.g., ponds receiving coated copper sulfate had lower water temperature than other ponds on 21 July, mean temperature in control and treatments for the grow-out period was quite similar. Grand means and standard deviations for water temperature did not differ among control or treatments (Table 5).

Conductivity varied during the study, but followed similar patterns of change over time (Figure 11). Weekly means for conductivity in control and treatment ponds ranged from 101 to 168  $\mu\text{mhos/cm}$ , and individual values ranged from 73 to 221  $\mu\text{mhos/cm}$  during the study. Variation resulted from factors affecting concentrations of major ions that contribute most of the conductivity. The main factors probably were: difference among ponds in initial bottom soil quality; variation among ponds in response to liming; rainfall; concentration of ions over time by evaporation. The grand mean for conductivity was slightly lower in the coated copper sulfate treatment than in the control and regular copper sulfate treatment (Table 5). However, the difference in conductivity between ponds of the coated sulfate treatment and the other ponds was considered too small to influence phytoplankton growth or copper solubility.

The pH was measured in samples collected in the morning and ranged from 7.0 to 9.1 in individual ponds. Typically, afternoon pH would be somewhat greater (Boyd and Tucker, 1998). Treatment means for pH on individual dates ranged from 7.24 to 8.56, but no differences were observed among treatments for individual sampling date (Figure 12). Likewise, grand means for treatment did not differ in pH (Table 5).

Because total alkalinity concentration in ponds of the regular copper sulfate treatment was used to estimate the copper dose, this variable was determined weekly. Alkalinity tended to increase until mid July, and then decline slightly during remaining weeks of the study (Figure 13). The decrease in alkalinity after mid July probably was caused by an increased rate of nitrification, and acidic reaction, in response to higher feeding rates and greater ammonia input to pond water. Weekly, mean concentrations for alkalinity were seldom below 50 mg/L or above 70 mg/L—an acceptable range for catfish culture (Boyd and Tucker, 1998). The grand mean for total alkalinity was lower in the coated copper sulfate treatment than in control or regular copper sulfate treatment (Table 5). The difference was noted from the beginning of the study and was unrelated to the use of the coated product.

Mechanical aerators were operated from midnight to 0600 h in ponds to avoid low dissolved oxygen concentration. On a few occasions, dissolved oxygen concentration in individual ponds fell between 2 and 3 mg/L, but treatment means never fell below 3.5 mg/L (Figure 14). Dissolved oxygen stress to fish probably was not a problem in any of the ponds. The grand mean for dissolved oxygen concentration was slightly lower in ponds of the coated copper sulfate treatment than in control ponds or in ponds of the regular copper sulfate treatment (Table 5). This difference was too small to affect fish.

Copper concentration was quite low in the control pond: individual measurements ranged from 0.0 to 12.1 µg/L; weekly means varied from 0.27 to 4.01 µg/L (Figure 15); the grand mean was  $1.74 \pm 0.19$  µg/L (Table 5). Ponds of the regular copper sulfate treatment had higher copper concentrations than ponds of the control and coated product treatment on nearly all dates (Figure 15). Grand means for copper differed among control and treatments and between the two treatments (Table 5).

The amount of copper applied to ponds in the regular copper sulfate treatment averaged 987 g as compared to 300 g in ponds receiving the coated copper sulfate treatment. Thus, it is not surprising that the regular copper sulfate treatment had the greatest copper concentration in the water.

Although the copper concentration was not elevated in ponds of the coated copper sulfate treatment after 29 July, it is likely that product was still releasing copper, but at a rate so slow that its uptake by phytoplankton prevented an increase above the ambient concentration. This could explain why the coated product continued to control phytoplankton after it no longer provided greater dissolved copper concentrations than those found in control ponds.

### Phytoplankton

Two control ponds developed infestations of underwater aquatic weeds, *Najas* sp. and *Chara* sp., over their entire bottoms, and phytoplankton blooms did not develop these two ponds in spite of large nutrient inputs in feed-underwater weeds outcompeted phytoplankton for nutrients. Underwater weed infestations did not develop in other control ponds or treatment ponds. However, a comparison of phytoplankton abundance

between weed-infested ponds and copper treatment ponds could be misleading, and the two weed-infested ponds were deleted from the phytoplankton abundance assessment.

Phytoplankton abundance was evaluated by three, indirect methods: Secchi disk visibility; turbidity; chlorophyll *a*. Treatments and controls had Secchi disk visibilities less than 40 cm on nearly all dates suggesting an abundance of plankton. However, the coated copper sulfate treatment had clearer water – higher Secchi disk visibility – than the control or regular copper sulfate treatment on most sampling dates (Figure 16). The regular copper sulfate treatment had higher Secchi disk visibility than the control only on a few dates (Figure 16). Grand means for Secchi disk visibility did not differ between control ponds and ponds treated with regular copper sulfate, but the coated copper sulfate treatment had a higher grand mean than either. (Table 5)

Turbidity was similar among control and treatments until August when turbidity tended to escalate in the control and regular copper sulfate treatment (Figure 17). Grand means for turbidity were greater in the control than in treatments, but the regular copper sulfate treatment and the coated copper sulfate treatment did not differ with respect to turbidity (Table 5).

Chlorophyll *a* concentration followed a trend similar to that observed for turbidity (Figure 18). It is interesting to note, however, that the coated copper sulfate treatment often had less chlorophyll *a* than either the control or regular copper sulfate treatment. Grand means for chlorophyll *a* differed between control and treatments but not between treatments (Table 5).

A total of 55 genera of planktonic algae were observed in sample from the ponds. However, 13 genera were observed dominate (over 10% of total phytoplankton

community) individual ponds on single sampling dates. Only four genera of blue-green algae were among the dominant taxa. Moreover, only two of the dominant blue-green algal genera, *Oscillatoria* and *Microcystis*, have previously been associated with off-flavor in catfish from pond at E. W. Shell Fisheries Research Center. *Anabaena*, the genera most often associated with off-flavor at this station in previous studies, was not dominant in any of the experimental ponds during the 2011 growing season.

As usual, variation in algal abundance was great-among ponds in the same treatment, among ponds in different treatments, and in the same pond over time – leading to a general lack of differences among treatment means for total phytoplankton abundance, total blue-green algae, and percentage blue-green (Table 5). The changes over time in the three, direct measurements of algal abundance are depicted in Figures 19, 20, and 21. Data on direct phytoplankton enumeration did not provide as good of an assessment of phytoplankton abundance in ponds as was obtained with the indirect measurements of phytoplankton density. This is not surprising, because observation on the inadequacy of direct algal enumeration for assessing the status of phytoplankton blooms in ponds have been made many times (Boyd and Tucker, 1998). The main problem is the wide variation in the sizes of individuals of different genera and of the great variation among ponds and dates with respect to genera present in samples.

Despite application of less copper, the coated copper sulfate treatment resulted in as good or possibly better algal control than did the regular copper sulfate treatment. The regular copper sulfate treatment was applied weekly, and water samples for copper analyses were taken 2 days later each week. Earlier work showed that copper concentration in ponds increases by 100 to 200  $\mu\text{g/L}$  immediately after application of



regular copper sulfate, but within 48 to 72 hr. copper concentration will fall to ambient level (McNevin and Boyd, 2004). Conversely, coated copper sulfate releases copper continuously, and it is plausible that a sustained, low, but above ambient concentration of copper, persisted in ponds treated with the coated product. This would explain the good results obtained with the coated product in spite of rather low concentration of copper in ponds where it was used.

### Fish production

Fish survival averaged near 90% in control and treatments (Table 6), but there was a fish kill in one of the regular copper sulfate-treated ponds on 26 July. A total of 152 dead fish were recovered. The cause of this fish kill was not established with certainty, but dissolved oxygen concentration was not low and no disease was noted in the fish. The mortality occurred soon after copper sulfate treatment and possibly could have been caused by high copper concentration soon after the weekly treatment. Control ponds and ponds treated with coated copper sulfate had almost identical average production- 6,688 kg/ha, and 6,662 kg/ha respectively (Table 6). Irrespective of the fish kill, average production of 6,080 kg/ha in the regular copper sulfate treatment was not significantly less ( $P>0.05$ ) than production achieved in the control and coated product treatment. The feed conversion ratio (FCR) of about 1.6 in control and both treatments (Table 6) suggests that feed was used quite efficiently – commercial catfish producers usually report FCR values of 2.0 to 2.5. In summary, the coated copper product had no adverse effects of fish survival, fish production, or efficiency of feed use, Application of copper in catfish ponds obviously raise the question of contamination of fish flesh with

this metal. Copper sulfate has long been used in catfish ponds, and there are no reports of copper contamination of fish flesh. In the present study, analyses of fillets revealed low, highly-variable concentrations of copper (Table 7), but mean copper concentrations for fish from controls and treatments did not differ ( $P > 0.05$ ). To put the observed copper concentrations in fillets in perspective, concentrations in fillets were less than the US Environmental Protection Agency drinking water standard of 1.3 ppm of copper. That does not seem to be reason for food safety concerns over copper treatment in catfish ponds.

#### Off-flavor

Off-flavor scores are summarized in Table 8. There was variation from pond to pond in scores, but fish from all ponds has relatively low scores and would have been acceptable to the processing plant. There was no difference in average score among the control and treatments.

It is interesting to note that the flavor description given by the taste panel members for the slight off-flavor in the fish as a stale, fishy taste. Flavors characteristically caused by blue-green algae commonly associated with off-flavor in catfish at the E.W. Shell Fisheries Research Center were not abundant in 2011 – this phenomenon cannot be explained from information available. Moreover, fish from ponds on the Center seldom have off-flavor problems as severe as those often found in fish from ponds on the commercial farms in west-central Alabama and in Mississippi and Arkansas. This difference also has not been explained adequately, because the abundance of blue-green algae in ponds at the Center is usually as great as in commercial ponds.

Ponds at the Center have much softer water than commercial ponds in west-central Alabama and in Mississippi and Arkansas, but there have been no studies to determine if a relationship exists between the degree of water hardness and off-flavor.

The main point is that despite the similarity in flavors scores among the control and treatment ponds of this study, one should not infer that copper treatment is not beneficial. All ponds do not exhibit the off-flavor phenomenon, and the magnitude of the problem tends to vary from year to year and from location to location. There is sample evidence that copper sulfate can control phytoplankton responsible for off-flavor in commercial catfish ponds, and the present study suggest that coated copper sulfate could be as effective or even more effective than regular copper sulfate in controlling phytoplankton.

In practice, ponds are treated with copper sulfate if fish are off-flavor near the desired harvest date. This treatment may have to be repeated two or more times before fish are of acceptable flavor. Trials should be conducted in commercial ponds using coated copper sulfate instead of regular copper sulfate. Results of the present study show that the coated product does not cause toxicity or lower production, and it does not impair food safety. Thus, it would be possible to find catfish producers willing to participate in off-flavor trials of a coated copper product.

Table 5. Grand means and standard errors of the mean for water quality variables in control and copper treated, catfish ponds.<sup>1,2</sup>

Variable <sup>3</sup>	Control	Copper treatment	
		Regular	Coated
pH (standard units)	7.76 ± 0.0055 a	7.67 ± 0.041a	7.67 ± 0.046a
Total alkalinity (mg/L as CaCO <sub>3</sub> )	61.5 ± 1.87 a	61.7 ± 1.29 a	55.9 ± 1.38 b
Conductivity (µmhos/cm)	152 ± 3.9 a	150 ± 2.7 a	145 ± 3.1 b
Water temperature (°C)	27 ± 0.35 a	27.3 ± 0.27 a	27.3 ± 0.26 a
Dissolved copper (µg/L)	1.9 ± 0.26	14.3 ± 0.95	4.1 ± 0.34
Secchi disk (cm)	18.1 ± 0.98	20.5 ± 0.8	29.2 ± 2.58
Turbidity (NTU)	76.5 ± 9.48	51.9 ± 0.27	61.1 ± 5.73
Chlorophyll <i>a</i> (µg/L)	172 ± 3.9 a	150 ± 2.7 b	145 ± 3.1 c
Total phytoplankton (individuals/ml)	21.2 ± 4,647a	15,74 ± 3,600a 3	15,770 ± 3,600a
Total blue-green algae (individuals/ml)	14.6 ± 4,149a	7,507 ± 3,214a	11,965 ± 3,213a
Percentage blue-green algae	59.7 ± 5.1 a	34.2 ± 3.9 b	59.1 ± 3.9 a
Dissolved oxygen (mg/L)	6.1 ± 3.9 a	5.9 ± 0.10 a	5.1 ± 0.20a

<sup>1</sup> Weekly measurements between 1 June and 15 September.

<sup>2</sup> There were five ponds for control and treatments, but data from two weed-infested, control ponds were omitted for Secchi disk visibility, turbidity, chlorophyll *a*, total plankton, total blue-green algae, and percentage blue-green algae.

<sup>3</sup> Means indicated by the same letter do not differ ( $P > 0.05$ ) in horizontal comparisons as determined by Duncan's test

Table 6. Means and standard errors for catfish production in control and copper-treated ponds.

Treatment <sup>1,2</sup>	Survival (%)	Net production (kg/ha)	FCR
Control	90.6 ± 1.63 a	6,688 ± 112.9 a	1.63 ± 0.028 a
Regular copper sulfate	87.2 ± 4.96 a	6,080 ± 585.8 a	1.67 ± 0.039 a
Coated copper sulfate	87.4 ± 4.20 a	6,662 ± 53.9 a	1.63 ± 0.014 a

<sup>1</sup>Five replications per treatment.

<sup>2</sup>Means indicated by the same letter do not differ ( $P>0.05$ ) in horizontal comparisons as determined by Duncan's test.

Table 7. Means, standard errors, and ranges for copper concentrations in catfish filets from control and copper-treated ponds.

Treatment <sup>1,2</sup>	Mean±SE	Min.	Max
Control	0.54 ± 0.080 a	0.35	0.78
Regular copper sulfate	0.62 ± 0.036 a	0.50	0.68
Coated copper sulfate	0.70 ± 0.056 a	0.52	0.82

<sup>1</sup>Five replications per treatment.

<sup>2</sup>Means indicated by the same letter do not differ ( $P>0.05$ ) in horizontal comparisons as determined by Duncan's test.

Table 8. Results of flavors evaluation of fillets from catfish in control and copper-treated ponds

Treatment	Pond	Score	Mean <sup>1</sup> ±SE
Control	E-38	0.625	0.725±0.092 a
	E-40	1.000	
	E-44	0.875	
	E-47	0.625	
	E-58	0.500	
Regular copper sulfate	E-37	0.625	0.700±0.050 a
	E-39	0.625	
	E-41	0.625	
	E-42	0.875	
	E-59	0.750	
Coated copper sulfate	E-43	0.625	0.675±0.151 a
	E-45	0.625	
	E-46	0.375	
	E-60	0.50	
	E-61	1.25	

<sup>1</sup>Means indicated by the same letter do not differ ( $P>0.05$ ) in horizontal comparisons as determined by Duncan's test.

### Temperature (Celcius)

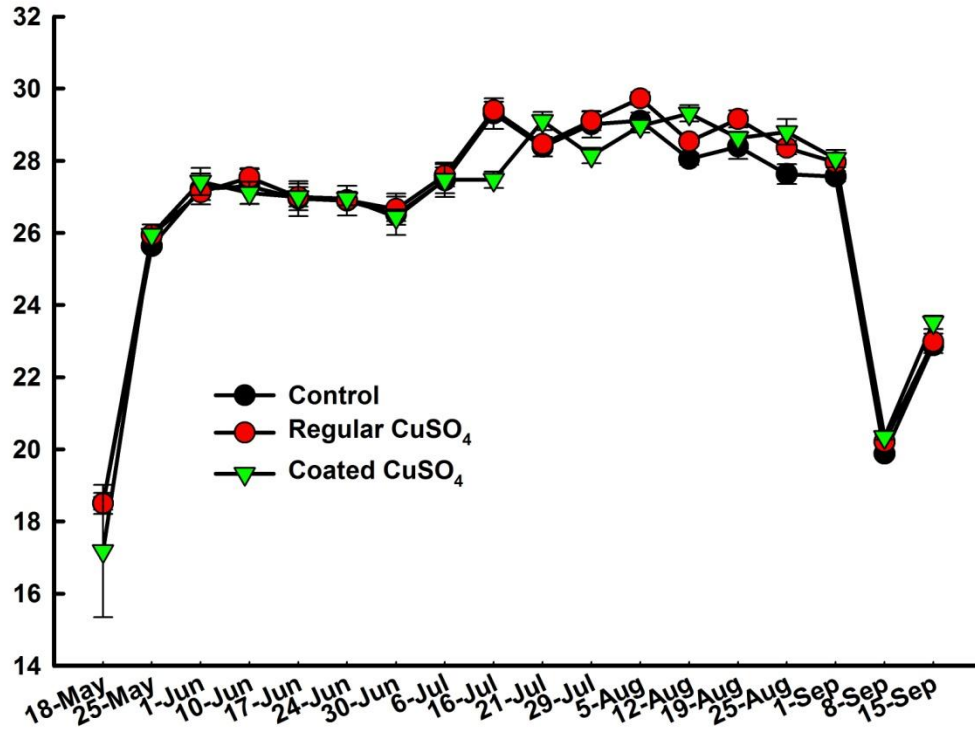


Figure 10. Means and standard errors for water temperature in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate



Conductivity ( $\mu\text{mhos/cm}$ )

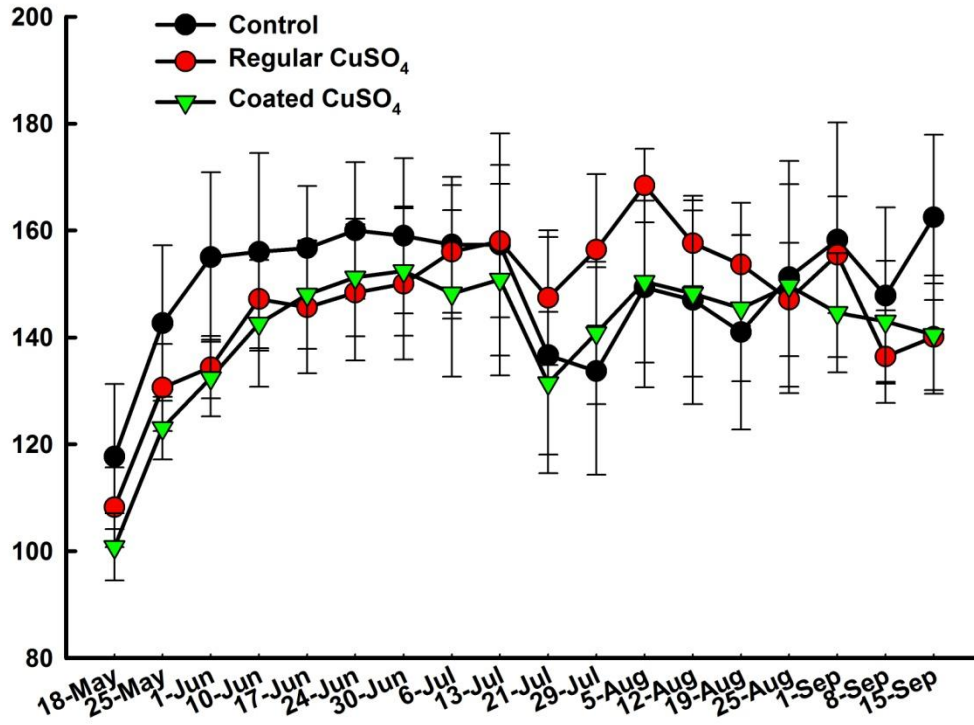


Figure 11. Means and standard errors for conductivity in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

# pH

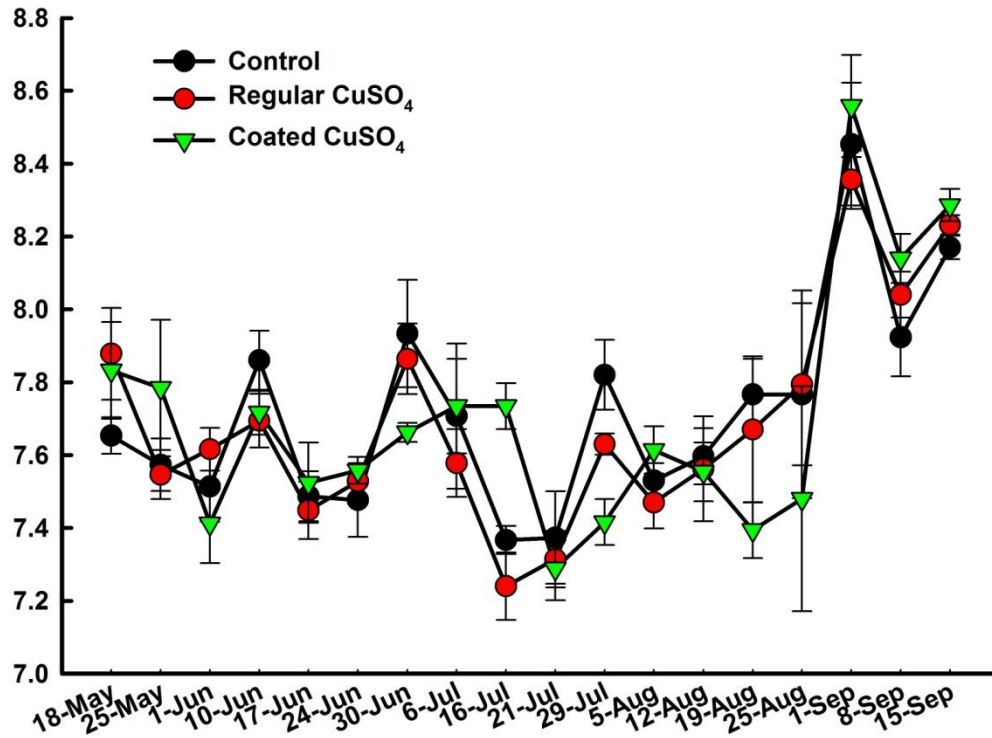


Figure 12. Means and standard errors for pH in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Total Alkalinity (mg/L)

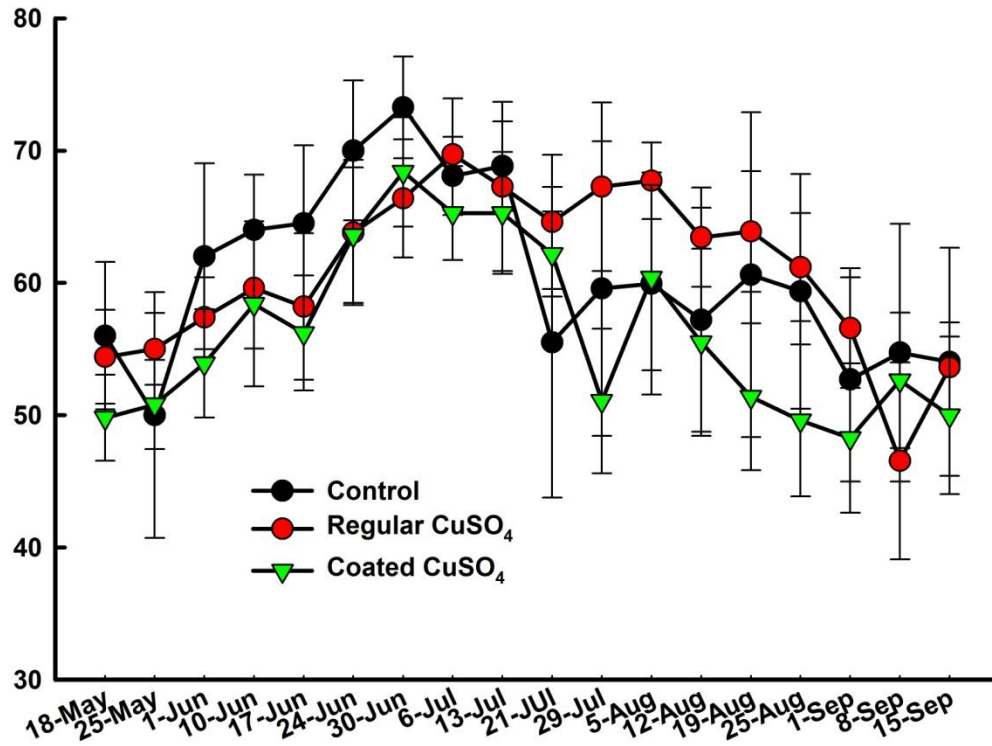


Figure 13. Means and standard errors for total alkalinity concentration in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Dissolved Oxygen (mg/L)

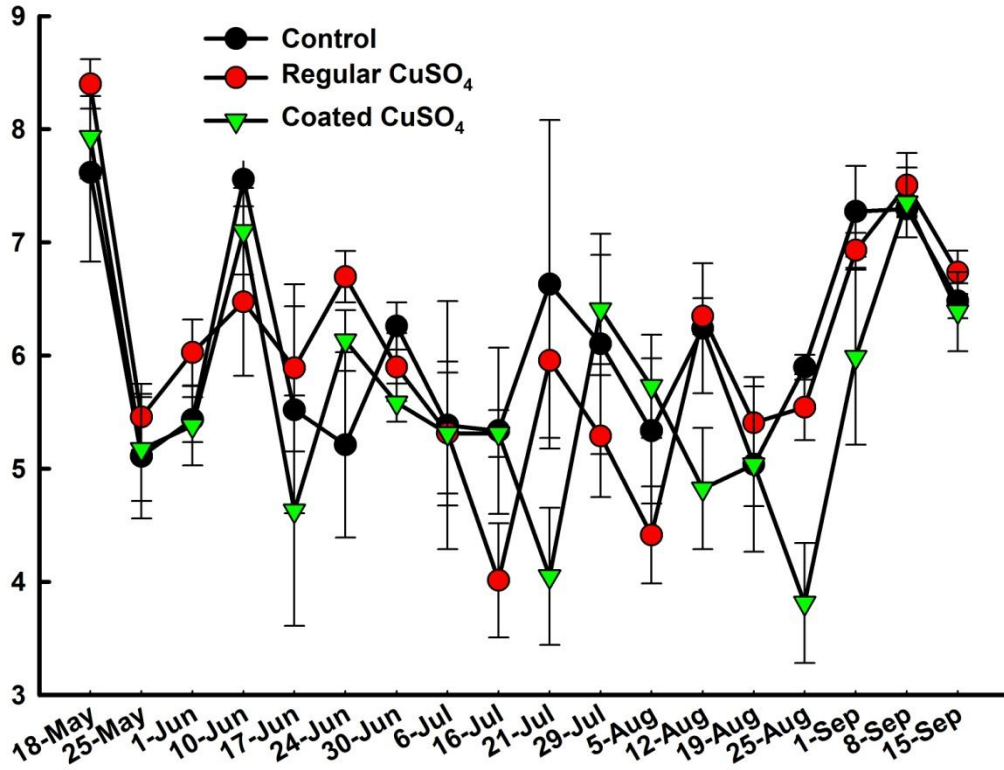


Figure 14. Means and standard errors for dissolved oxygen concentration in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Copper (ug/L)

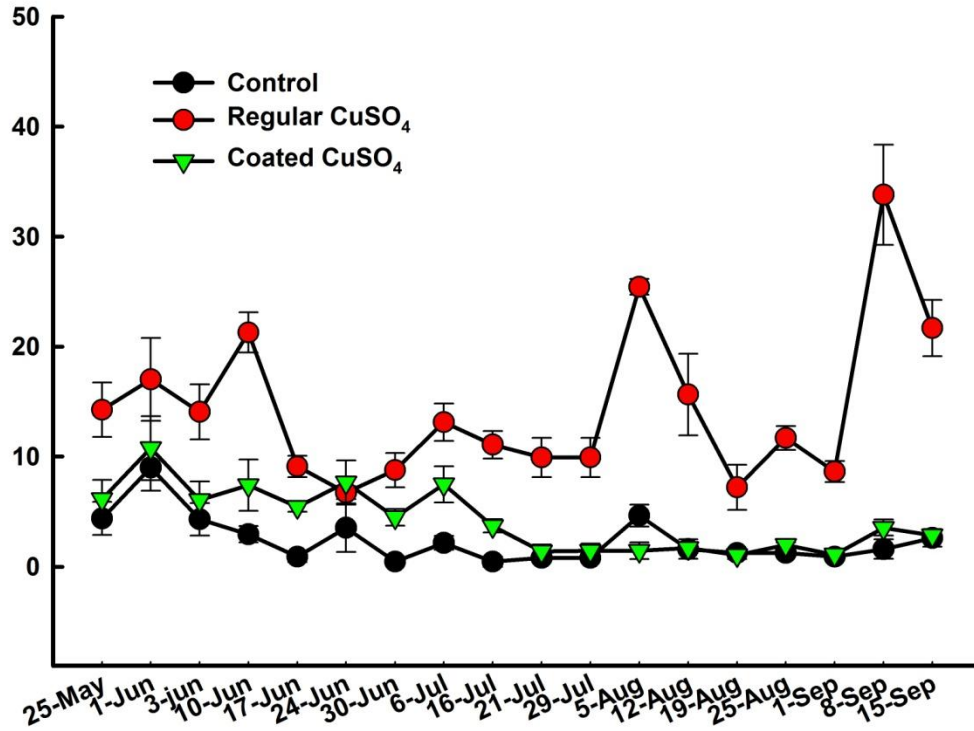


Figure 15. Means and standard errors for copper concentration in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Secchi Disk (cm)

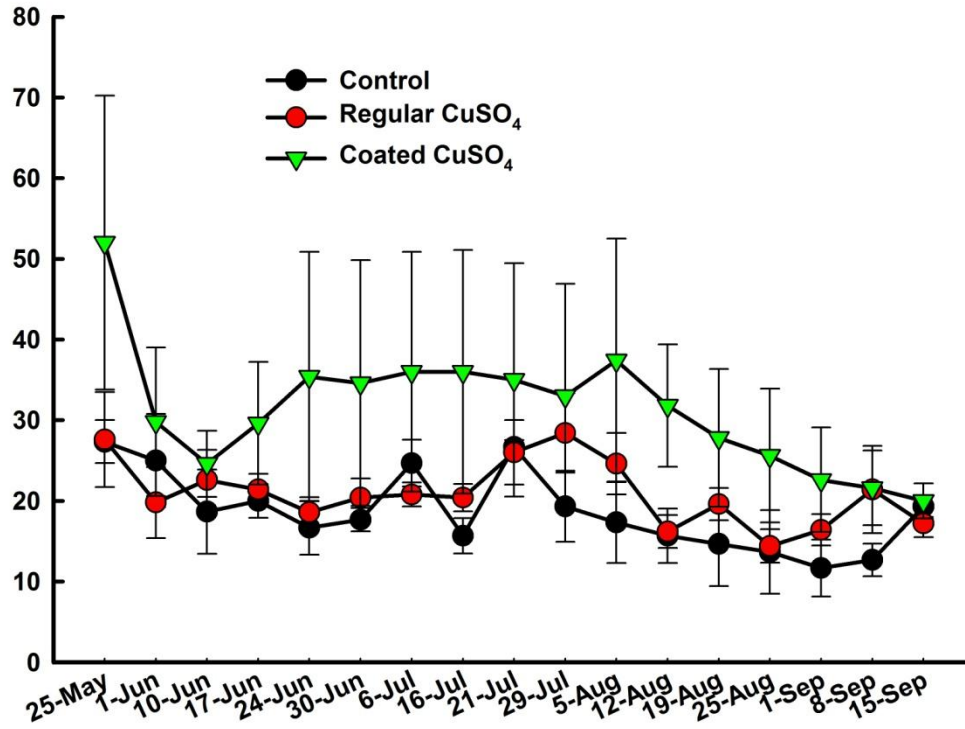


Figure 16. Means and standard errors for secchi disk visibility in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Turbidity (NTU)

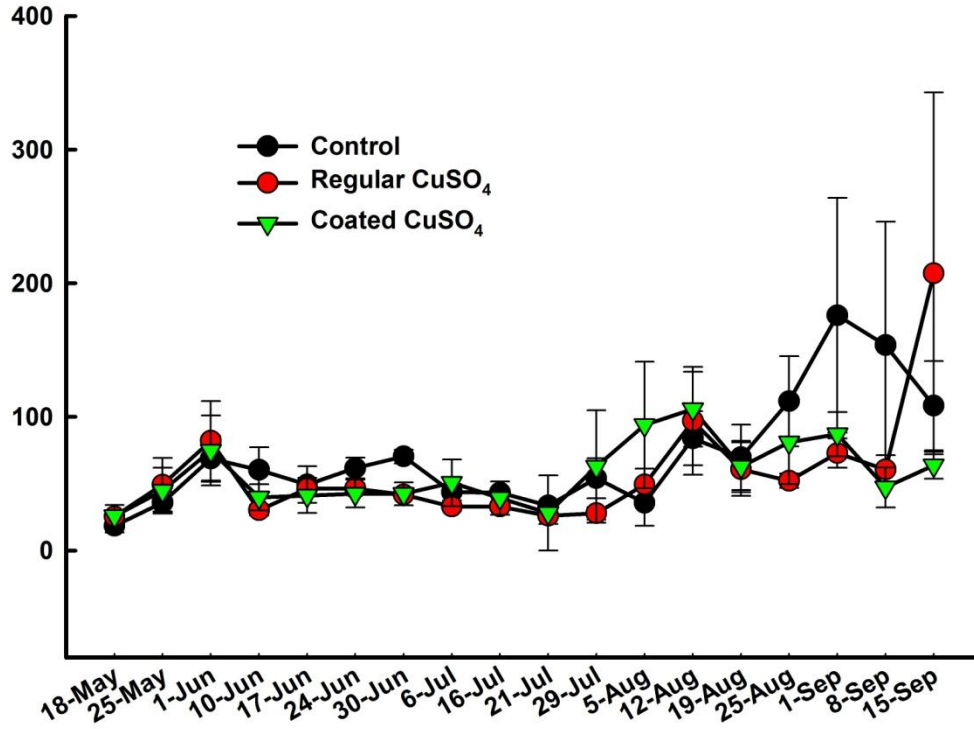


Figure 17 Means and standard errors for turbidity in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Chlorophyll A (ug/L)

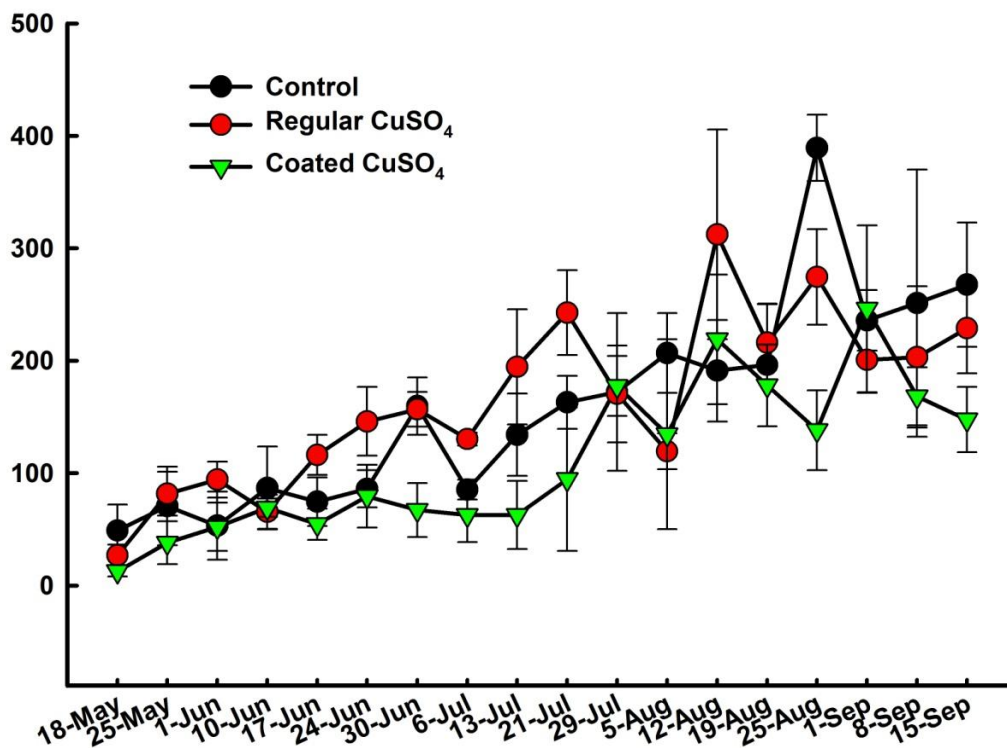


Figure 18. Means and standard errors for chlorophyll *a* concentration in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate



### Total Algae (cell/ml)

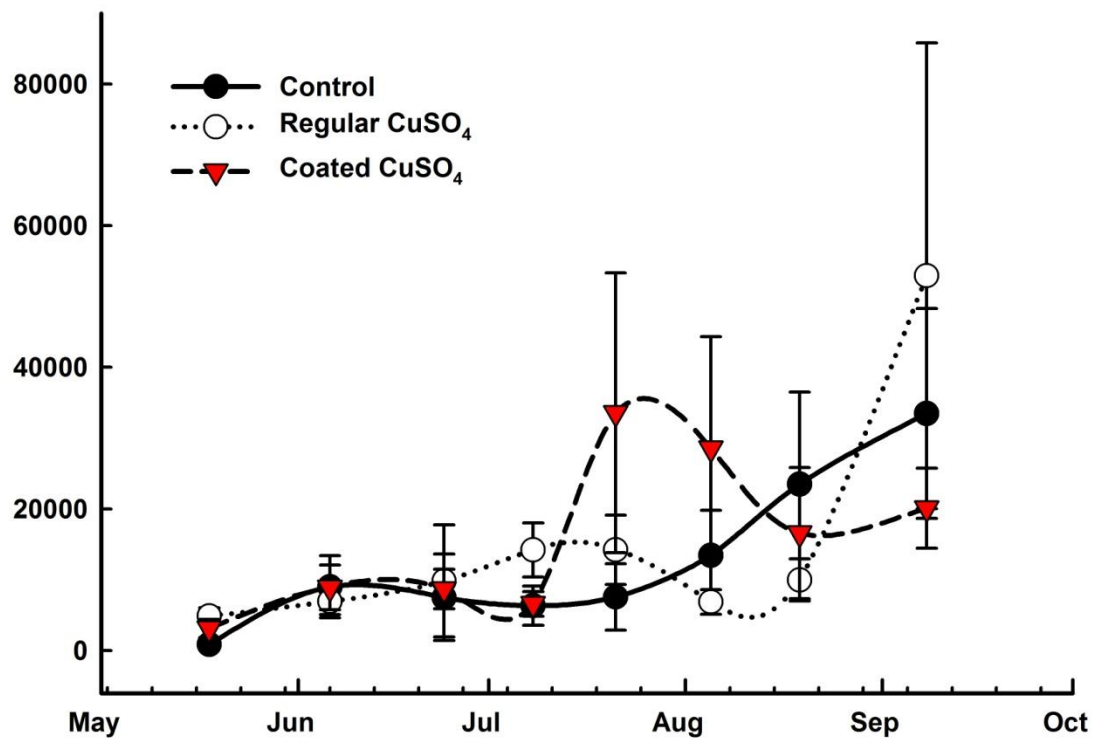


Figure 19. Means and standard errors for total number of individual planktonic algae in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

### Blue Green Algae (cell/ml)

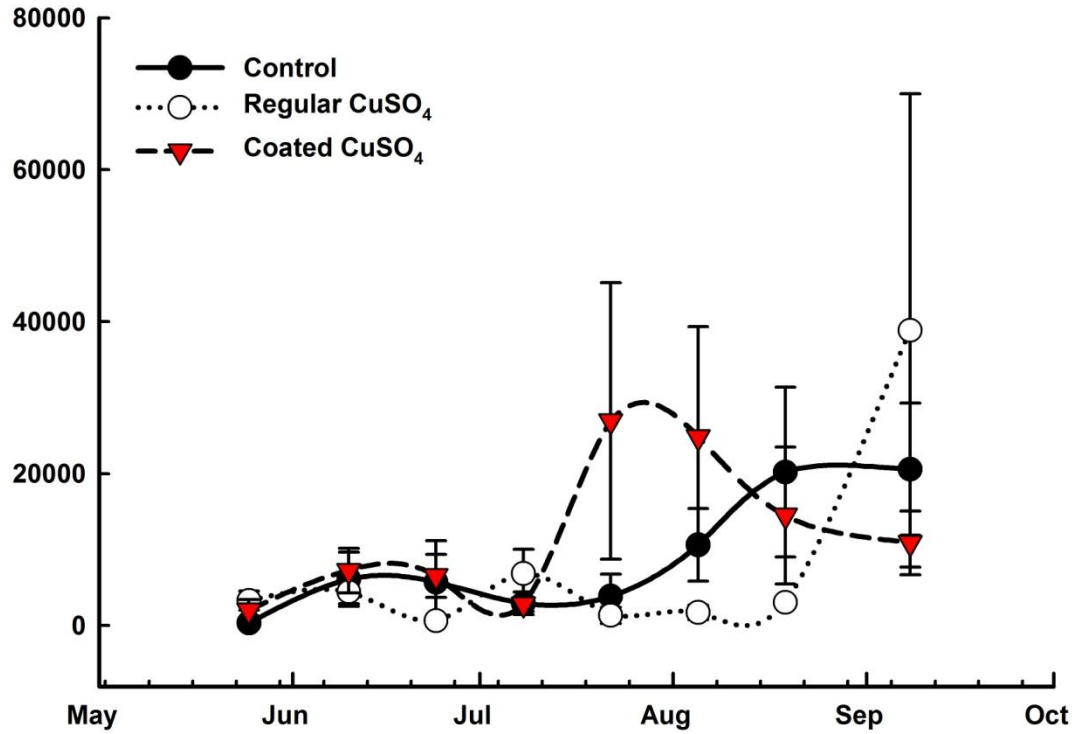


Figure 20. Means and standard errors for total number of individual blue green algae in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

## Percentage of Blue Green Algae

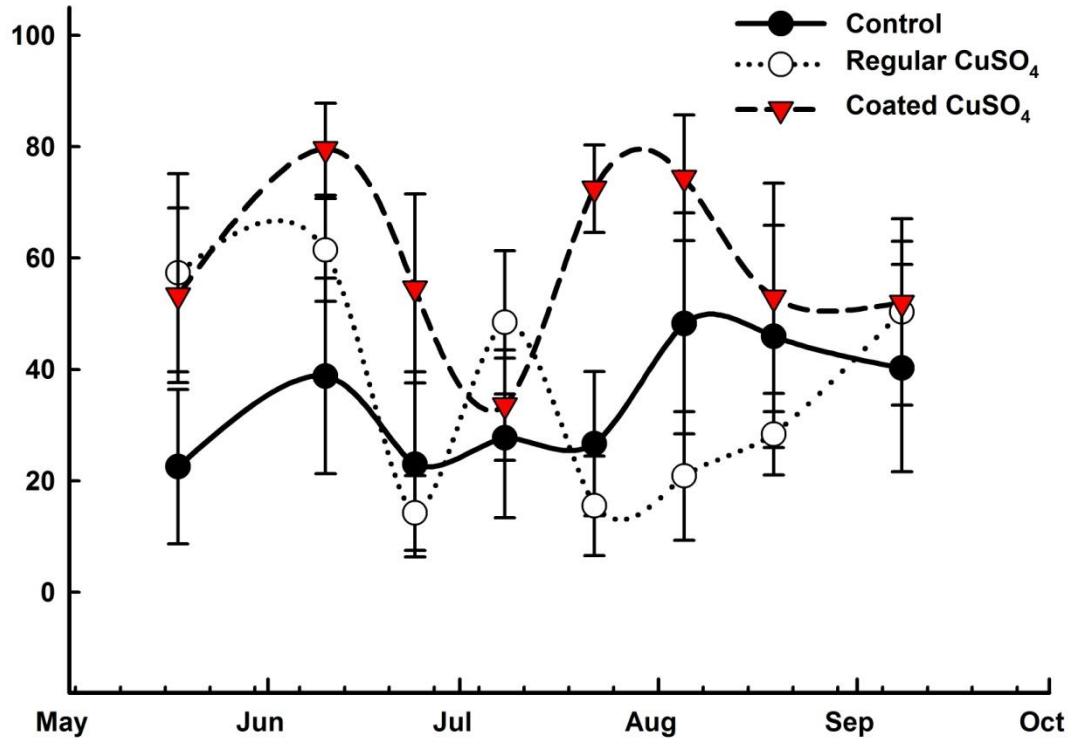


Figure 21. Means and standard errors for percentage of blue green algae in control ponds and in ponds treated with regular copper sulfate and coated copper sulfate

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