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Acute Toxicity of Copper Sulfate and Chelated Copper to Channel Catfish *Ictalurus punctatus*

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Abstract

Channel catfish fingerlings *Ictalurus punctatus* were exposed to copper sulfate or a commercial chelated copper product in a series of static toxicity tests conducted using waters with a wide range of total alkalinity and hardness values. Estimates of mean 96 h LC50 values were 0.05, 0.73, 0.95, and 0.98 mg/L as Cu for copper sulfate and 0.06, 1.51, 1.97, and 1.74 mg/L as Cu for the chelated copper product in waters having total alkalinities of 16, 76, 127, and 240 mg/L $CaCO_3$ respectively. On a copper basis, the chelated product was significantly ($P < 0.05$) less toxic to fish than copper sulfate in all waters except that of the lowest total alkalinity. Highly significant ($P < 0.01$) linear relationships were found between LC50 values for copper from copper sulfate and pH, log [total alkalinity], and log [total hardness], of test waters. These results cast doubt on the validity of the formula commonly used to calculate practical copper sulfate pond treatment rates, which is based upon a simple linear relationship between application rate and total alkalinity.

Copper sulfate ($CuSO_4 \cdot 5H_2O$) is registered by the United States Environmental Protection Agency as an algicide in waters used to raise fish for human consumption. It is commonly applied for this purpose in ponds used to culture channel catfish *Ictalurus punctatus*. The compound is also used as a parasiticide for control of fish ectoparasites such as *Ichthyophthirius, Trichodina, Ichthyobodo* (*Costia*), and *Ambiphrya* (*Scyphidia*). The effectiveness of copper sulfate as an algicide and parasiticide is diminished as the total alkalinity and total hardness of waters increase. To account for the reduced effectiveness, current practice is to increase the rate of copper sulfate application in direct proportion to the total alkalinity of the water (MacMillan 1985).

The toxicity of copper sulfate to fish also decreases as pH, total alkalinity, and total hardness increase, and as copper binds to inorganic or organic substrates. Acute toxicity tests for copper have been conducted on at least 27 fish species (Environmental Protection Agency 1980), but only chronic toxicity tests on eggs and fry (Sauter et al. 1976) and embryo-larval bioassays (Birge and Black 1977) have been reported for channel catfish. Also, the relationship between suggested pond application rates and toxicity to fish over the range of pH, total alkalinity, and total hardness values encountered in channel catfish culture ponds is not known.

Chelation of copper by organic compounds such as ethanolamines or ethanolamine complexes protects copper from precipitation and complexation. Commercial products containing chelated copper are claimed to be more effective than copper sulfate as an algicide in hard, alkaline water. However, little is known regarding the toxicity of chelated copper compounds relative to copper sulfate or factors affecting toxicity of chelated copper compounds to fish.

The present study determined toxicity of copper sulfate and a commercial chelated copper product to channel catfish in waters of various alkalinities and hardnesses. Such information will help formulate safer and more effective application rates for copper compounds in channel catfish ponds.

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TABLE 1. *Fish sizes, water quality, and copper LC50 values for tests of the toxicity of copper from copper sulfate or a chelated copper product to channel catfish in four waters. All values, except LC50 values, are mean ± SD. Estimates of LC50 values are given for both replicate tests. Values in parentheses are 95% confidence intervals for the estimated LC50 value. NC = confidence interval could not be calculated using the probit procedure.*

Variable	Water 1	Water 2	Water 3	Water 4
Fish wet weight (g)	3.9 ± 0.5	3.0 ± 0.5	2.3 ± 0.4	3.2 ± 0.8
Fish total length (cm)	8.6 ± 0.3	7.9 ± 0.5	6.9 ± 0.5	7.8 ± 0.6
pH	7.3 ± 0.1	8.2 ± 0.1	8.4 ± 0.1	8.7 ± 0.1
Total alkalinity (mg/L $CaCO_3$)	16 ± 1	76 ± 2	127 ± 8	239 ± 10
Total hardness (mg/L $CaCO_3$)	16 ± 1	83 ± 2	161 ± 12	287 ± 12
96-h LC50 (mg Cu/L)				
Copper sulfate 1	0.054 (0.036–0.081)	0.762 (0.600–0.953)	0.768 (NC)	1.041 (NC)
Copper sulfate 2	0.055 (0.035–0.085)	0.700 (0.559–0.866)	1.139 (NC)	0.925 (0.041–1.151)
Chelated copper 1	0.065 (0.043–0.103)	1.657 (1.214–2.389)	1.503 (1.129–1.955)	1.878 (1.447–2.408)
Chelated copper 2	0.051 (0.031–0.081)	1.362 (0.888–1.993)	2.436 (1.752–4.282)	1.603 (1.320–1.903)

Materials and Methods

Channel catfish fingerlings, *Ictalurus punctatus* were obtained from the Delta Research and Extension Center, Stoneville, Mississippi and held for several weeks at 17.0 ± 1.0 C. Fish were fed a commercial, 32% protein, crumbled feed every other day to maintain body weight. Fish were not fed for 48 h prior to or during toxicity tests.

Toxicity tests were conducted in 120 L glass aquaria equipped with airstones. Water temperatures were maintained at 17.0 ± 0.5 C except during trial 3 where they varied from 18.0 to 21.5 C due to air conditioning difficulties in the laboratory. Aquaria were filled with 80 L of a mix of filtered pond water diluted with distilled water to give four waters with a range of pH, total alkalinity, and total hardness values (Table 1). Ten fish were present in each aquarium.

Static test conditions (APHA 1989) were chosen to imitate conditions during use of copper products in fish culture ponds; i.e., the chemical is applied to the pond in a single dose and concentrations of dissolved copper decline after application (Boyd 1990). The study was conducted as a 4 × 2 factorial arrangement of four waters and two treatments (copper sulfate and chelated copper) in a completely random design with two replications. For each replicate test, fish were exposed to a control (no copper added) and five concentrations of copper. Copper was added from stock solutions prepared from copper sulfate pentahydrate (25.4% Cu) or Copper Control® (8.5% Cu from mixed copper-triethanolamine-diethanolamine complexes, available from Argent Chemical Laboratories, Redmond, Washington). The concentrations used in each test were chosen to give from 0% to 100% mortality of the test animals within 96 h based on the results of preliminary experiments. The following water quality variables were monitored daily: total ammonia (phenate method, APHA 1989), nitrite (sulfanilamide method, APHA 1989), pH (Orion Research EA 940 Meter), temperature (mercury thermometer), un-ionized ammonia (calculation of Emerson et al. 1975), and dissolved oxygen (YSI Model 54 ARC). Total hardness (titration with EDTA, APHA 1989) and total alkalinity (titration with standard acid, APHA 1989) were measured at the beginning of each test in all aquaria.

Aquaria were checked every 8 h and dead fish were removed immediately. Median lethal concentration (LC50) and associated

95% confidence intervals for each replicate toxicity test were calculated by probit analysis (Finney 1971). Differences among mean LC50 values were assessed by two-way analysis of variance. If significant differences were shown to exist, means were separated using the least significant difference test. The nature of the relationships between LC50 values and water quality variables was determined using linear regression. Adequacy of the linear relationships was assessed with the lack-of-fit test (Woolson 1987). Analyses were conducted with the Statistical Analysis System (SAS Institute, Inc. 1985), Statements of significance refer to $P \leq 0.05$.

Results and Discussion

Differences in fish size among experiments (Table 1) were not considered great enough to affect toxicity. Monitoring of water quality parameters indicated values were below concentrations that have been determined to cause stress: nitrite and un-ionized ammonia were present only in trace amounts and dissolved oxygen concentrations were greater than 90% of saturation throughout all tests. Convulsions, lethargy, and loss of equilibrium were noticed in many fish prior to death in copper-treated waters. No fish died in the control tanks. Estimates of 96 h LC50 values and associated 95% confidence intervals for replicate tests of the two copper products in each of the four waters are given in Table 1.

The toxicity of copper from copper sulfate decreased as total alkalinity, total hardness, and pH increased. This trend was expected based on current understanding of the chemistry of copper in water. The form of copper most toxic to fish, and presumably also to algae and protozoans, is thought to be the cupric ion, Cu^{2+}. The stock solution of copper sulfate consisted of cupric and sulfate ions. When added to water in the aquaria, cupric ions react to form precipitates of tenorite, CuO, and various dissolved complexes. Environmental pH and total alkalinity are major modulators of copper toxicity because they affect the total concentration and speciation of dissolved copper in solution (Boyd 1990). As pH increases over the range of 7 to 9, total dissolved copper concentration and cupric ion concentration decrease; as total alkalinity increases, a larger proportion of the total copper in solution is present as various carbonate complexes. Hardness (specifically calcium hardness) also affects the toxicity of copper to fish, presumably by modulating the fish's biological response to the metal. Calcium ions may compete with cupric ions for cation binding or adsorption sites at the gill surface, resulting in decreased copper uptake by fish (Cusimano et al. 1986).

Expressed on a copper basis, the chelated copper product was significantly less toxic to fish than copper sulfate, except in the test water with the lowest pH, total alkalinity, and total hardness. Although less toxic to fish, chelated copper compounds are claimed to be more effective algicides than copper sulfate in waters of moderate to high pH and total alkalinity. The organic complexing agent prevents rapid precipitation of copper from solution. Presumably, this makes available a low, but algicidal concentration of cupric ion over a longer period as copper dissociates from the chelated complex. Although chelation supposedly prevents rapid loss of copper from the water, toxicity of chelated compounds to channel catfish also varied with total alkalinity, pH, and total hardness. As Cu^{2+} dissociated from the organic chelating agent, it apparently was precipitated or complexed to a greater extent as total alkalinity and pH increased. Displacement of copper from the chelator by calcium or magnesium, and subsequent precipitation or inorganic complexation of the displaced copper may also have contributed to the decreased concentration of the toxic cupric ion species.

Results of the present study cast some doubt on the basis for the formula commonly used to calculate copper sulfate treatment rates as a function of total alkalinity. Practical treatment rates (as mg/L $CuSO_4 \cdot$

$5H_2O$) are calculated by dividing the total alkalinity expressed as mg/L $CaCO_3$ by 100 (MacMillan 1985; Tucker and Robinson 1990). The basis for this formula is unclear and it is not supported by experimental data or theory. Implicit in the formula is a linear relationship between total alkalinity and copper sulfate toxicity. Analysis of data from the present study showed a significant lack of fit for the linear relationship (Fig. 1) between total alkalinity and LC50 values for copper from copper sulfate, but there was no significant lack of fit for the linear relationship between log [total alkalinity] and LC50 values.

The test waters in this study were prepared to simulate waters in northwest Mississippi channel catfish culture ponds where ponds are supplied with well water from a dolomitic limestone-gravel aquifer. The aquifer yields water of moderately high total alkalinity and hardness, and the two are approximately equal on an equivalents/L basis. Total alkalinity and hardness are conservative variables, and significant changes in magnitude occur only as a result of dilution (as by rainfall) or concentration (as by evaporation). In these pond waters, and in the test waters used in this study, total hardness varies directly with total alkalinity. Accordingly, it was not possible to differentiate between the effects of the individual variables on copper toxicity. Also, in the test system used in the present study, the dissolved carbon dioxide concentration, [$CO_{2(aq)}$], can be assumed constant because the bioassay tanks were vigorously aerated and [$CO_{2(aq)}$] was at near equilibrium with atmospheric carbon dioxide. At pH values below about 8.5 to 9, pH is proportional log [HCO_3^-] at constant [$CO_{2(aq)}$]. So, in the test waters of the present study, pH also varied with total alkalinity. Thus, highly significant linear relationships also existed between LC50 values and log [total hardness] and between LC50 values and pH (Fig. 2).

If cupric ion is assumed to be the toxic dissolved copper species, then hydrogen ion concentration should be the major modulator of copper toxicity because it controls the solubility of tenorite, CuO, the stable solid phase of copper above pH 7 (Stumm and Morgan 1970):

$$CuO + 2H^+ = Cu^{2+} + H_2O; \quad K = 10^{7.65}$$

Based upon this equation, cupric ion concentrations decline by 100-fold for every unit increase in pH. This implies that copper toxicity should also decline by 100-fold per unit pH increase. In this study, copper toxicity from copper sulfate decreased roughly 20-fold over the pH range of 7.3 to 8.7. In other words, copper sulfate was more toxic at higher pH values than expected based solely upon the relationship between pH and tenorite solubility. The presence of

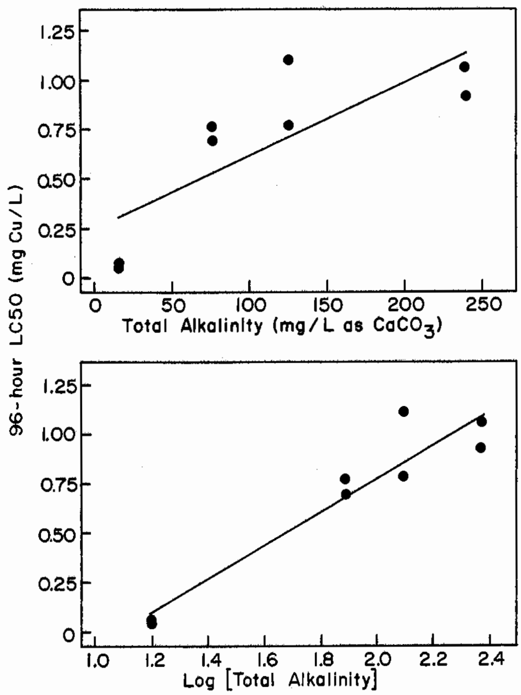

FIGURE 1. *Relationships between total alkalinity and the 96 h LC50 for copper sulfate, as copper, to channel catfish fingerlings (top) and between log [total alkalinity] and the 96 h LC50 values (bottom). Regression equations describing the relationships are: (top) $y = 0.2523\ (\pm 0.1650) + 0.0037\ (\pm 0.0012)\ x$, $r^2 = 0.63$; (bottom) $y = -0.9092\ (\pm 0.2328) + 0.8401\ (\pm 0.1199)\ x$, $r^2 = 0.89$. Values in parentheses are standard errors for parameter estimates.*

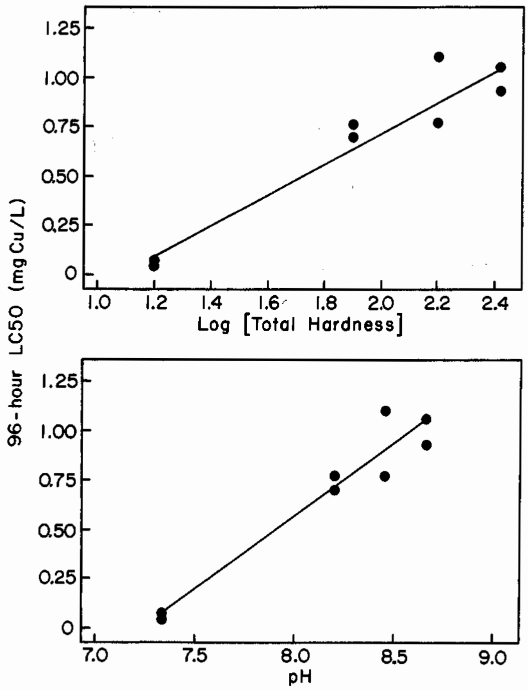

FIGURE 2. *Relationships between log [total hardness] and the 96 h LC50 for copper sulfate, as copper, to channel catfish fingerlings (top) and between pH and the 96 h LC50 values (bottom). Regression equations describing the relationships are: (top) $y = -0.8421 \pm (0.2139) + 0.7820(\pm 0.1068)x$, $r^2 = 0.90$; (bottom) $y -5.3388$ (± 0.7533) $+$ 0.7381 (± 0.0922) x, $r^2 = 0.91$. Values in parentheses are standard errors for the parameter estimates.*

postulated toxic hydroxide species such as $Cu(OH)^+$ and $Cu(OH)_2{}^o$ (Chakoumakos et al. 1979), which increasingly contribute to the total dissolved copper pool as pH increases, may explain the lack of decrease in toxicity of the magnitude expected as the pH of test waters increased.

The relationships among pH, total alkalinity, and copper toxicity will be more complex in natural waters than in the simple system used in this study because $[CO_{2(aq)}]$ is not constant in natural waters. Relative changes in community respiration and gross photosynthesis cause $[CO_{2(aq)}]$ to cycle diurnally, in turn causing pH to vary independent of changes in total alkalinity. Also, the normal relationship among the variables may be distorted in some waters: waters of low hardness and high alkalinity, and vice versa, are not uncommon. Because the relative importance of the three variables as modulators of copper toxicity is unknown (Chakoumakos et al. 1979; Cusimano et al. 1986; Lauren and McDonald 1986), the use of a single variable (such as total alkalinity) as a universal predictor of copper toxicity may carry some risk.

Deriving appropriate copper treatment rates for use in pond aquaculture will be further complicated by interactions with other variables within the pond ecosystem. Elder and Horne (1978) list five fates of copper in the aquatic envirorunent: 1) movement of dissolved copper to living cells; 2) complexation of dissolved copper by organic and inorganic ligands; 3) precipitation of dissolved copper and formation of suspended particulate forms; 4) loss of copper to sediments; and 5) solubilization of copper precipitates in the sediments. In the present study, sediment and organic matter were excluded from the bioassays. Sediment and dissolved organic matter may, however, be major factors influencing copper toxicity to fish in ponds. For example, dissolved organic material is associated with 5–28% of the total soluble copper in sea water (Williams 1969). Further studies need to be conducted under a variety of field conditions to assess the importance of sediment and dissolved organic matter as modulators of copper toxicity.

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