Toxicity of copper in an oxic stream sediment receiving aquaculture effluent

D.B. Huggett a,*, D. Schlenk a, B.R. Griffin b

a Department of Pharmacology, Environmental and Community Health Research, University of Mississippi, University, MS 38677, USA
b US Department of Agriculture, Stuttgart National Aquaculture Research Center, Stuttgart, AR 72160, USA

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Abstract

Sediments were collected from a stream (upstream, outfall and downstream) receiving copper laden catfish pond effluent to assess toxicity to non target biota. No significant reduction in Hyalella azteca survival or growth (10 d), or Typha latifolia germination and root and shoot growth (7 d) were observed after exposure to upstream and outfall sediments. A significant reduction in H. azteca survival was observed after exposure to the downstream sediment sample; however, no reduction in T. latifolia germination or seedling growth was detected. Bulk sediment copper concentrations in the upstream, outfall and downstream samples were 29, 31, and 25 mg Cu/kg dry weight, respectively. Interstitial water (IW) concentrations ranged from 0.053 to 0.14 mg Cu/l with 10 d IW toxicity units \( \geq 0.7 \). Outfall samples were amended with additional concentrations of copper sulfate so that bulk sediment measured concentrations in the amended samples were 172, 663, 1245, and 1515 mg Cu/kg dry weight. Survival was the most sensitive endpoint examined with respect to H. azteca with a no observed effects concentration (NOEC) and lowest observed effects concentration (LOEC) of 1245 and 1515 mg Cu/kg, respectively. NOEC and LOEC for T. latifolia root growth were 663 and 1245 mg Cu/kg, respectively. IW copper concentrations were \( \geq 0.86 \) mg Cu/l with H. azteca interstitial water toxicity unit (IWTU) concentrations \( \geq 1.2 \). Sequential extraction qualitatively revealed the carbonate and iron oxide fractions which accounted for a majority of the copper binding. In this instance, the copper which was applied to catfish ponds does not appear to be adversely impacting the receiving stream system. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Copper sulfate; Aquaculture; Sediment; Typha latifolia; Hyalella azteca

1. Introduction

The use of copper sulfate in the catfish aquaculture industry is historic and widespread. Used primarily to prevent infections (i.e., Ichthyophthirius multifilis) and algal blooms, copper sulfate (CuSO\(_4\) \cdot H\(_2\)O) may be applied to ponds several times a year at a suggested rate of 1 mg/l per 100 mg/l alkalinity (Tucker and Boyd, 1978; Schlenk et al., 1998). It has been observed in recreational lakes that copper sulfate has a short residence time in the water column after application (<24 h) (Button et al., 1977). Based on its water column residence time and its application rate, copper sulfate has the potential to concentrate in the sediment of aquaculture ponds.

A common practice in the aquaculture industry is the draining of catfish ponds. Often, the ponds are drained from the top down between yearly catfish harvests, with the water being discharged into a receiving stream. Few studies have investigated the fate and effects of copper sulfate in aquaculture facilities, especially the effects on...
non target biota in receiving streams. It is unknown to this point whether any of the applied copper is trans
ported into receiving streams during pond draining, thus potentially affecting aquatic organisms. Information re-
garding the fate and effects of copper in aquaculture facilities will soon be of importance, since catfish
aquaculture facilities may soon have to apply for and comply with National Pollutant Discharge Elimination System (NPDES) permits (USEPA, 1998).

In an effort to investigate the fate and effects of copper sulfate in aquaculture facilities (i.e., the receiv-
ing streams), eight catfish ponds were treated weekly with copper sulfate (CuSO₄ · 5H₂O) for 3 years. After the 3 yr treatment regime, sediments were collected from the stream, which received periodic discharge from the catfish ponds. The specific objectives of this study were to determine if: (1) copper sulfate in typical aquaculture ponds (0.4 ha) was transported to the receiving stream during pond draining; (2) copper sulfate in stream sediments was adversely affecting aquatic organisms by utilizing toxicity studies with the epi benthic invertebrate Hyalella azteca, the aquatic macrophyte Typha latifolia and various chemical pre-
dictors (interstitial water (IW) etc.); and, (3) a rela-
tionship exists between toxicity and important oxic sediment sorption sites (i.e., OC, FeOH etc.) for copper in stream sediments.

2. Materials and methods

2.1. Sample site description

Surface sediment samples (organic carbon = 1 3.4%,
PpH = 6.5 6.9, bulk density = 44%) were collected from the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS. During a 3 yr period, nine stocked channel catfish ponds (10 000 fish/ha) were treated with a total of 45 kg of dispersed copper (CuSO₄ · 5H₂O). Fol
lowing fish harvest, all ponds were drained. These ponds drained into a underground conduit, which then dis
charged into a receiving stream. Approximately 91.4 m separated the upstream and downstream samples with the outfall sample located in the middle of the two samples. A hand scoop was used to collect approximately 1 kg of each sample (duplicate samples were collected at each site), which were collected from the middle of the stream at each location. Stream samples were placed on ice in ziplock plastic bags and transported back to the University of Mississippi, where they were kept at 4°C until analysis. Prior to analysis, duplicate samples from each site were homogenized. Control sediments were collected at the University of Mississippi Field Station (UMFS). UMFS control sediments have been used successfully in several studies (Deaver and Rodgers, 1996; Huggett et al., 1999).

2.2. Test organisms

H. azteca used for testing were cultured at the Uni-
versity of Mississippi, Environmental Toxicology Re-
search Program (ETRP). Methods of culturing followed those prescribed by USEPA (1994). Two to three week old H. azteca’s were used for testing.

T. latifolia inflorescences were collected in April 1999
from UMFS. Inflorescences were placed in plastic bags
and transported to the University of Mississippi, ETRP,
where they were placed in a 20°C incubator until use.
For testing, viable seeds were separated from non viable seeds gravimetrically, by methods prescribed by Moore et al. (1999). Briefly, a part of the inflorescence was placed in a Hamilton beach commercial blender with approximately 500 ml water. Inflorescences were ho-
mogenized for 10 s, whereby the floating debris was
removed. Viable seeds, which were used for testing were
those which sank to the bottom of the blender (Moore et al., 1999).

2.3. Biological experimental design

H. azteca experiments consisted of 10 d static expo-
sures and followed methods according to Nebeker et al. (1984), while 7 d static T. latifolia exposures followed
methods prescribed by Muller et al. (2001). Briefly, a 1:4 wet sediment to water ratio was placed in glass borosi-
lcate beakers (250 ml beakers for H. azteca and 30 ml
beakers for T. latifolia). De ionized, reconstituted hard
water was utilized as overlying water in this study (APHA, 1992). After settling of sediment particles, 10 H. azteca or 10 T. latifolia seeds were added to each of their respective test vessels (three replicates per site plus a control). H. azteca tests were performed under constant aeration to insure that depleted oxygen would not be a confounding factor. H. azteca were fed one drop of YCT solution every other day. At the end of the exposure duration, sediments were sieved and H. azteca’s mor-
tality determined. H. azteca were then placed in 70% ethanol for growth determination at a later time. In
addition, T. latifolia germination was determined and the seedlings gently placed in a 70% ethanol solution for
root and shoot determination. Growth of H. azteca and T. latifolia after exposure were measured using a Vid
eometric 150 Image Analyzer (American Innovation) with Videometric software (version 2.1).

Sediment amendment (utilizing outfall sediment) with copper sulfate was accomplished on a dry weight basis with specific amounts of a 10 mg/ml copper sulfate (CuSO₄ · 5H₂O) stock solution added to enough sedi-
ment for all tests (300 g for each concentration). Upon addition of copper sulfate stock solution, sediments were homogenized by hand for 10 min with a spatula. Sedi-
ments were allowed to sit for 6 h whereby each sediment
concentration was split into three replicates and over lying water added (Huggett et al., 1999).

2.4. Chemical analysis

Total acid extractable copper sediment concentrations were determined by weighing 3–5 g of wet sediment in microwave digestion vessels. HCl (2 ml) and HNO₃ (5 ml) were added to each vessel prior to digestion. After microwave digestion, samples were filtered through polymembrane filters. Dissolved IW samples were collected at the end of each experiment by centrifugation of sediment at 2500 g for 15 min followed by vacuum filtration through a 0.45 µm polymembrane filter. All samples were acidified to a pH <2 with HNO₃ after collection. All copper concentrations were determined on a Varian Spectra Atomic Absorption Flame Spectrometer.

The sequential extraction procedure for the speciation of particulate copper followed methods prescribed by Yong and MacDonald (1998). Briefly, 0.75 g of dried sediment was weighed in a 40 ml centrifuge tube. For extraction of the exchangeable fraction, 8 ml of 1.0 M solution of MgCl₂ was added and allowed to react for 1 h. The carbonate bound fraction was extracted using the residue from the exchangeable fraction and 30 ml of 1 M NaOAc (pH 5). After a 5 h reaction, the amount of copper bound to Fe Mn oxides was determined by adding 30 ml of oxalic acid (0.1 M, pH 3) to the residue from the carbonate bound fractionation. Copper bound to organic matter was extracted by adding 8 ml of NaOCl (pH 9) to oxide bound residue for 30 min in a boiling water bath. The residual fraction is the sum of the above mentioned four fractions minus the total acid extractable copper concentration for that given sediment. Copper in specific sediment fractions were determined according to methods prescribed above.

2.5. Quality assurance

Aqueous reference toxicant tests with copper sulfate were conducted throughout the experimental period to insure that H. azteca sensitivity was remaining relatively constant (μ 48 h LC₅₀ was 83 ± 4 µg Cu/l). In addition, two 10 d LC₅₀ experiments were conducted (μ = 67 ± 6 µg Cu/l). Since T. latifolia seeds stay dormant until tested, no reference tests were conducted. Prior to analytical analyses, a three point calibration curve, which also included a reference blank, was established. Matrix spikes (85 115% recovery), initial and continuing calibration samples (ICV and CCV), and certifiable reference standards were utilized for quality assurance.

2.6. Statistical analyses

Effects on H. azteca survival and growth and T. latifolia germination and seedling growth were determined by statistically significant differences relative to controls (P ≤ 0.5). One way analysis of variance was performed with Dunnett’s multiple range test for significance compared to controls (Zar, 1996). No observed effects levels (NOEC) and lowest observed effects levels (LOEC) for H. azteca and T. latifolia in sediment spiking experiments were determined by significant differences relative to controls.

3. Results

Upstream and outfall samples had total bulk sediment copper concentrations of 29 and 31 mg Cu/kg dry weight, respectively (Table 1). These samples did not adversely affect H. azteca or T. latifolia (Tables 2 and 3). Generally, survival and growth of the sentinel organisms exposed to these samples was better in experimental sediments as compared to UMFS sediment. However, the downstream sediment sample (25 mg Cu/kg) did adversely affect H. azteca survival (67% survival). T. latifolia germination and seedling growth was not significantly different from controls.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bulk sediment copper (mg/kg)</th>
<th>IW</th>
<th>IWTU*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream</td>
<td>29</td>
<td>0.08</td>
<td>1.2</td>
</tr>
<tr>
<td>Downstream</td>
<td>25</td>
<td>0.14</td>
<td>2.1</td>
</tr>
<tr>
<td>Outfall</td>
<td>31</td>
<td>0.05</td>
<td>0.7</td>
</tr>
<tr>
<td>Outfall amended</td>
<td>172</td>
<td>0.08</td>
<td>1.2</td>
</tr>
<tr>
<td>Outfall amended</td>
<td>663</td>
<td>0.18</td>
<td>2.7</td>
</tr>
<tr>
<td>Outfall amended</td>
<td>1245</td>
<td>0.31</td>
<td>4.6</td>
</tr>
<tr>
<td>Outfall amended</td>
<td>1515</td>
<td>1.88</td>
<td>28.1</td>
</tr>
</tbody>
</table>

*IWTU = (IW)/H. azteca 10 d LC₅₀ (0.067 mg/l).

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Survival</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93</td>
<td>3.5 ± 0.02</td>
</tr>
<tr>
<td>Upstream</td>
<td>97</td>
<td>4.0 ± 0.05</td>
</tr>
<tr>
<td>Downstream</td>
<td>67*</td>
<td>4.1 ± 0.05</td>
</tr>
<tr>
<td>Outfall</td>
<td>93</td>
<td>3.7 ± 0.03</td>
</tr>
<tr>
<td>Amended 172 mg/kg</td>
<td>97</td>
<td>3.7 ± 0.02</td>
</tr>
<tr>
<td>Amended 663 mg/kg</td>
<td>93</td>
<td>3.6 ± 0.03</td>
</tr>
<tr>
<td>Amended 1245 mg/kg</td>
<td>97</td>
<td>3.5 ± 0.02</td>
</tr>
<tr>
<td>Amended 1515 mg/kg</td>
<td>40*</td>
<td>3.5 ± 0.02</td>
</tr>
</tbody>
</table>

*P < 0.5.
impacted by the downstream sediment sample. IW concentrations in these sediments were generally above the 10 d LC$_{50}$ for *H. azteca* (0.067 mg Cu/l).

Spiked sediment experiments (utilizing outfall sediment) revealed a NOEC and LOEC of 663 and 1245 mg Cu/kg, respectively, for *T. latifolia* root growth when compared to non spiked sediment. No effects were observed in regard to *Typha* germination and shoot growth; however, shoot growth did decrease with increasing concentrations. Survival was the most sensitive endpoint for *H. azteca*. NOEC and LOEC for *H. azteca* in this sediment were 663 and 1245 mg Cu/kg, respectively. Significant *H. azteca* mortality was observed in the 1515 mg Cu/kg spiked sediment, where the IW concentration was 1.88 mg/l with a corresponding in tertial water toxicity unit (IWTU) of 28.1. No effects were observed on *H. azteca* growth.

Sequential extraction of these sediments, both amended and unamended, revealed that the carbonate fraction accounted for the majority of the copper in these sediments (up to 70%) (Table 4). The oxide fraction did account for a significant amount (up to 40%), while the organic matter or exchangeable fractions accounted for very little (as low as 1%) of the total sediment associated copper.

### 4. Discussion

The copper concentration in the outfall sample was similar to that observed in the upstream and downstream samples. In addition, the concentrations observed at these sites were similar to those observed in aquaculture ponds where there is no documented use of copper (Xiang et al., 2001). This suggests that little to none of the copper applied to catfish ponds at this aquaculture facility was transported to the receiving stream. Xiang et al. (2001) reported that the sediments of the ponds utilized in this study contained 175 mg/kg, indicating accumulation of copper in the pond sediments. The downstream sediment sample did cause adverse effects on *H. azteca*. However, since total copper concentrations at the downstream site were similar to upstream and outfall copper concentrations (both have similar sediment characteristics), and no effects were observed in *T. latifolia* (Typha) is more sensitive to sediment associated copper than *H. azteca*, copper is probably not the causative agent eliciting the observed toxicity (Suedel et al., 1996; Muller et al., 2001).

Suedel et al. (1996) reported a *H. azteca* 10 d bulk sediment LC$_{50}$ of 262 mg Cu/kg in amended river sediments, while Huggett et al. (1999) reported a copper sulfate 10 d sediment NOEC and LOEC for *H. azteca* survival to be 2010 and >2010 mg Cu/kg, respectively in amended lake sediments. A 10 d NOEC for *H. azteca* survival was reported to be 23 mg Cu/kg (Deaver and Rodgers, 1996) in ponds treated with copper sulfate. Bennet and Cubbage (1992) reported *H. azteca* mortality to be <15% when exposed to freshwater sediments with copper concentrations 1100 mg Cu/kg. A 7 d NOEC for *T. latifolia* root growth was determined to be 19 mg Cu/kg in amended pond sediments (Muller et al.,

**Table 3**
*T. latifolia* germination (%) and root and shoot growth (mm ± S.D.) after exposure to field collected and amended samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Germination</th>
<th>Root growth</th>
<th>Shoot growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90</td>
<td>0.63 ± 0.10</td>
<td>1.16 ± 0.17</td>
</tr>
<tr>
<td>Upstream</td>
<td>87</td>
<td>0.83 ± 0.06</td>
<td>1.19 ± 0.07</td>
</tr>
<tr>
<td>Downstream</td>
<td>80</td>
<td>0.85 ± 0.03</td>
<td>1.12 ± 0.12</td>
</tr>
<tr>
<td>Outfall</td>
<td>93</td>
<td>0.82 ± 0.05</td>
<td>1.13 ± 0.02</td>
</tr>
<tr>
<td>Amended 172 mg/kg</td>
<td>90</td>
<td>0.74 ± 0.08</td>
<td>1.06 ± 0.03</td>
</tr>
<tr>
<td>Amended 663 mg/kg</td>
<td>100</td>
<td>0.57 ± 0.04</td>
<td>0.94 ± 0.15</td>
</tr>
<tr>
<td>Amended 1245 mg/kg</td>
<td>80</td>
<td>0.37 ± 0.05</td>
<td>0.88 ± 0.15</td>
</tr>
<tr>
<td>Amended 1515 mg/kg</td>
<td>97</td>
<td>0.35 ± 0.04</td>
<td>0.90 ± 0.10</td>
</tr>
</tbody>
</table>

* P < 0.5.

**Table 4**
Speciation of sediment associated copper (mg/kg dry weight) in field collected and amended samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Exchangeable</th>
<th>Carbonate</th>
<th>Oxide</th>
<th>Organic matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream</td>
<td>1.9</td>
<td>11.5</td>
<td>11.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Downstream</td>
<td>2.9</td>
<td>9.5</td>
<td>10.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Outfall</td>
<td>2.0</td>
<td>10.6</td>
<td>12.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Amended 172 mg/kg</td>
<td>6.9</td>
<td>79.0</td>
<td>43.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Amended 663 mg/kg</td>
<td>20.4</td>
<td>368.9</td>
<td>117.7</td>
<td>18.9</td>
</tr>
<tr>
<td>Amended 1245 mg/kg</td>
<td>22.4</td>
<td>462.6</td>
<td>145.4</td>
<td>10.8</td>
</tr>
<tr>
<td>Amended 1515 mg/kg</td>
<td>51.5</td>
<td>1065.5</td>
<td>255.1</td>
<td>9.8</td>
</tr>
</tbody>
</table>
2001). In this study, H. azteca NOEC and LOEC for survival were 1245 and 1515 mg Cu/kg, while Typha root growth was affected at 1245 mg Cu/kg. No effects were observed in terms of H. azteca growth, or T. latifolia germination and shoot growth in any of the sediments tested. Deaver (1996) observed no differences in H. azteca growth except in bulk sediment concentrations higher than LC\textsubscript{50} (45 mg/kg). Huggett et al. (1999) noted that there were no changes in H. azteca growth at bulk sediment concentrations up to 2010 mg Cu/kg. Longer exposure durations (i.e., 28 d) may have elucidated effects on H. azteca growth (Ingersoll et al., 1997). Root growth of T. latifolia seems to be the most sensitive indicator for this macrophyte at this given testing age. In a series of bioassays examining the effects of aqueous herbicides (paraquat and atrazine), aqueous and sediment associated copper sulfate, root growth was the most sensitive parameter investigated (Moore et al., 1999; Muller et al., 2001).

The difference in sediment effects concentrations in this study compared to others may be attributed to differences in sediment characteristics. Characteristics, such as or ganic carbon and particle size, significantly influence the bioavailability of copper in the sediment. For example, Deaver and Rodgers (1996) and Muller et al. (2001) used a sediment that had a high sand particle size (75%) and a low organic carbon (<1%) indicating little sorption of copper to the sediment. Sediments in this study had organic carbon levels between 1% and 3.4% indicating a potential to bind more copper. It has been suggested that normalization of total bulk sediment concentrations to organic carbon or iron oxides may provide a better prediction of toxicity (Chapman et al., 1998). However, the quality of organic carbon can vary, ultimately leading to variable predictions in metal partitioning and toxicity (Burgess et al., 1997).

Several chemical descriptors have been proposed as predictors of effects in metal contaminated sediments. Acid volatile sulfides (AVS) and IW have been proposed as the basis for which sediment quality is derived in anaerobic sediments, while oxides/hydroxides, organic carbon and IW have been proposed as regulators of ionic sediments (Ankley et al., 1996; Chapman et al., 1998). Catfish ponds represent a situation in which the pond sediment is oxic for most of the year (approximately nine to 10 months), but in the hot summer months the sediments turn anoxic. For substantial buildup of AVS, an anaerobic environment, organic carbon and sulfur sources are required. During fish production, each of these requirements for AVS production can be met; however, when cooler temperatures occur, the AVS may be oxidized thus causing the amount of AVS in the surface sediments to be drastically decreased. In addition, bioturbation due to the catfish and yearly pond draining could substantially reduce AVS concentrations. Utilizing the AVS model, AVS destruction could mean potential metal bioavailability (Ankley et al., 1996). Stream samples were collected in June, a time in which the sediments have the potential to turn anaerobic but redox monitoring indicated that the sediments were oxic. Therefore, aerobic sorption sites were qualitatively determined in stream samples. Consequently, this indicates that AVS concentrations are probably low and important oxic binding sites should be determined.

While sequential extraction techniques are limited (e.g., metal crossover between fractions, too short of extraction duration, etc.), a qualitative indication of bioavailability can be calculated (Biester and Scholz, 1997; Chapman et al., 1998). Generally, the organic carbon and iron oxide fractions are considered to be the most important sorption sites in aerobic sediments; however, it was observed that in these stream samples the carbonate fraction was an important short term binding site (Chapman et al., 1998). Xiang et al. (2001) indicated that oxide and organic matter fractions were important copper binding sites in catfish ponds. Therefore, in this particular aquaculture facility, carbonates may serve as short term binding sites, with a transition to the oxide and organic matter fractions for long term binding. Mahony et al. (1996) concluded that organic carbon may add an additional 10 100 μmol/g of copper binding. Alkalinity and hardness of the water in this part of Mississippi are both between 200 300 mg/l as CaCO\textsubscript{3} (Tucker et al., 1996), indicating the dominance of water quality parameters in metal fate and transport.

IW has been proposed as a major route of exposure for aquatic organisms to metals in sediment. By relating the dissolved metal concentration in the IW to the LC\textsubscript{50} of the organism, a prediction of toxicity may be made. Specifically, an IW/UTW is calculated, (IW Cu)/10 d LC\textsubscript{50}, which relates toxicity to IW concentration. An IW/UTW is >1 predicts a 50% invertebrate mortality (Ankley et al., 1996). Bennet and Cubbage (1992) reported an 82% survival of H. azteca after exposure to sediments with an IW concentration of 0.24 mg/l. In this study, the dissolved IW copper concentrations were generally above H. azteca LC\textsubscript{50} (0.067 mg Cu/l). Therefore, based on the IW model, a 50% H. azteca mortality should have occurred. Toxicity was not observed when the IW copper concentration was above the LC\textsubscript{50}, except at the highest amended sediment concentration. There may be several explanations for this observation. First, complexation of copper with ligands (HCO\textsubscript{3} and humic acids) in the IW may have reduced copper bioavailability (Chapman et al., 1998). This would not be surprising since the carbonate fraction accounted for a majority of the sediment associated copper in the sequential extraction experiment. Second, the main route of exposure for H. azteca in this study may have been through the diet (Chapman et al., 1998). While H. azteca may bury themselves in the sediment, often they are observed feeding on detrital particles on the sediment surface. So,
these organisms may not actually come into contact with the IW, but rather are exposed to the copper in their diet and possibly to the overlying water (Suedel et al., 1996; Chapman et al., 1998).

5. Conclusions

The copper concentrations in this Mississippi delta stream that received catfish pond effluent are similar to those observed in aquaculture ponds where no copper has been applied, indicating that copper enrichment was low. Toxicity associated with the downstream sediment cannot be attributed to copper because of the similarity of bulk sediment concentrations of copper and sediment characteristics between all three sites. In addition, spiked sediment tests revealed that the bulk sediment copper concentration would have to increase drastically (>663 mg Cu/kg) before effects would occur to the most sensitive species tested, *T. latifolia*. IW was not an accurate predictor of amphipod toxicity, possibly due to ligand complexation of copper. The role of carbonates as well as iron oxides need to be further investigated to assess to their role in limiting copper bioavailability in this system. The role of AVS during anaerobic situations should also be further examined to determine its usefulness in predicting bioavailability and toxicity of metals, like copper, in aquaculture ponds. Overall, the use of copper sulfate in these catfish ponds had little to no effect on the organisms investigated.

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Duane Huggett received his B.S. in Biology from Virginia Polytechnic and State University and his M.S. in Biological Sciences from the University of Mississippi. He is currently a research assistant in the Environmental and Community Health Research Program, School of Pharmacy, at the University of Mississippi.