TOXICITY ASSESSMENT OF SEDIMENT ASSOCIATED COPPER SULFATE IN A MISSISSIPPI DELTA CATFISH AQUACULTURE FACILITY

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INTRODUCTION

The use of copper sulfate in the catfish aquaculture industry is historic and widespread. Used primarily to prevent infections (i.e. *Ichthyophtirius multifiliis*) and algal blooms, copper sulfate may be applied to ponds several times a year at a suggested rate of 1 mg/L per 100 mg/L alkalinity (Schlenk et al. 1998, and Tucker and Boyd 1978). 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 practice in the aquaculture industry is the draining of catfish ponds. Often times, 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 transported into receiving streams during pond draining, thus potentially effecting aquatic organisms. Information regarding 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, sediments were collected from catfish ponds and a stream which received discharge from the catfish ponds, which were treated with copper sulfate for a extended period of time. The specific objective of this study was to determine if copper sulfate added to catfish ponds was adversely effecting aquatic organisms in the ponds themselves or the stream receiving aquaculture discharge. Effects were quantified utilizing toxicity studies with the epibenthic invertebrate *Hyalella azteca*, the aquatic macrophyte *Typha latifolia* and various chemical predictors (interstitial water). If no toxicity was observed, then sediments were to be spiked with additional amounts of copper sulfate to determine effects thresholds.

MATERIALS AND METHODS

<u>Sample Site Description</u>

Sediment samples were collected from the Mississippi State University Delta Research and Extension Center aquaculture facility in Stoneville, MS. During a three year period, eight catfish ponds at this facility were treated with a total of 45 kg of dispersed copper. These ponds drained into a underground conduit, which then discharged into a receiving stream. Eight treated and three control pond samples were collected and subsequently dried. Approximately 100 yards separated the up-stream and downstream samples, with the outfall sample located in the middle of the two samples. A hand scoop was used to collect all samples, which were placed in ziplock plastic bags. Stream samples (organic carbon = 1 - 3.4%, pH = 6.5-6.9, bulk density = 44%) were placed on ice and transported back to the University of Mississippi, where they were kept at 4°C until analysis. Control sediments for the stream exposure were collected at the University of Mississippi Biological Field Station (UMFS). UMFS control sediments have been used successfully in several studies (Huggett et al. 1999, Deaver and Rodgers 1996).

Test Organisms

H.azteca used for testing were cultured at the University of Mississippi, Environmental Toxicology Research Program (ETRP). Methods of culturing followed those prescribed by USEPA (1994). Two-three week old *H. azteca* were used for testing.

T.latifolia inflorescences were collected in April 1999, from the University of Mississippi Field Station (UMFS). I iflorecscences were placed in plastic bags and transported to the University of Mississippi, ETRP, where they were placed in a 20 °C incubator until used. For testing, viable seeds were separated from non-viable seeds gravimetrically, by methods prescribed by Moore et al. (1999). Briefly, part of the inflorescence was placed in a Hamilton Beach commercial Blender with approximately 500 ml water. Inflorescences were homogenized for 10 seconds, 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).

Experimental Design

H. azteca experiments consisted of 10d static exposures and followed methods according to Nebeker et al. (1984), while 7d static *T.latifolia* exposures followed methods prescribed by Muller et al (1999) and Huggett (1998). Briefly, a 1:4 wet sediment to water ratio was placed in glass borosilicate beakers. 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, *H.azteca* were sieved,with mortality determined and subsequently placed in 70% ethanol for growth determination. 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 Videometric 150 Image Analyzer (American Innovision) 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 10mg/ml copper sulfate stock solution added to enough sediment for all tests. Upon addition of copper sulfate stock solution, sediments were homogenized by hand for 10 minutes with a spatula. Sediments were allowed to sit for 6 h whereby each sediment concentration was split into three replicates and overlying water added (Huggett et al. 1999).

Chemical Analysis

Total acid extractable copper sediment concentrations were determined by weighing 3-5 g of wet sediment into microwave digestion vessels. HCl (2 ml) and HNO_3 (5ml) were added to each vessel prior to digestion. After microwave digestion, samples were filtered through polymembrane filters. Dissolved interstitial water samples were collected at the end of each experiment by centrifugation of sediment at 2,500 *g* for 15 minutes followed by vacuum filtration through a 0.45 um polymembrane filter. All samples were acidified to a pH < 2 with HNO_3 after collection. All copper concentrations were determined on a Varian Spectra Atomic Absorption Flame Spectrometer.

Quality Assurance

Reference toxicant tests with copper sulfate were conducted throughout the experimental period to ensure that *H. azteca* sensitivity was remaining relatively constant (average 48 h LC_{50} was 83 ± 4 ug 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.

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 ($p \leq 0.5$). One way analysis of variance was performed with Dunnett's multiple range test for significance compared to controls (Zar 1985). No observed effects levels (NOEC) and lowest observed effects levels (LOEC) for *H.*

azteca and *T.latifolia* in sediment spiking experiments was determined by significant differences relative to controls.

RESULTS

Up-stream 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 effect *H. azteca* or *T. latifolia* (Table 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 effect *H. azteca* survival (67 % survival). *T.latifolia* germination and seedling growth was not impacted by the down stream sediment sample. No toxicity was observed in *H.azteca* or *T.latifolia* after exposure to treated pond sediment, even though pond sediment concentrations were as high as 175 mg Cu/kg (Table 4 and 5) (Xiang et al. 2000). Large variation in *T. latifolia* shoot growth was observed.

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 regards 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 was 663 and 1245 mg Cu/kg, respectively. No effects were observed on *H.azteca* growth. Interstitial water concentrations in these sediments were generally above the 10 d LC_{50} for *H.azteca* (0.067 mg Cu/L). Significant *H. azteca* mortality was observed in the 1515 mg Cu/kg spiked sediment where the interstitial water concentration was 1.88 mg/l, with a corresponding IWTU of 28.1.

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. 2000). This suggests that little to none of the copper applied to catfish ponds at this aquaculture facility was transported to the receiving stream. 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 copper than *H. azteca*), copper is probably not the causitive agent eliciting the observed toxicity (et al. 1999, Suedel et al. 1996).

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 sulfate10 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. A 7 d NOEC for *T.latifolia* root growth, was determined to be 19 mg Cu/kg in amended pond sediments. In this study, *H. azteca* NOEC and LOEC for survival were 1245 and 1515 mg Cu/kg, while *Typha* root growth was effected at 1245 mg Cu/kg. No effects were observed in terms of *H.azteca* growth, or *T.latifolia* germination and root growth in any of the sediments tested. Deaver (1996) observed no differences in *H. azteca* growth except at bulk sediment concentrations higher than LC_{50} (45 mg/kg). Huggett et al. (1999) note 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, and industrially contaminated sediments, root growth was the most sensitive parameter investigated (Moore et al. 1999, Muller et al. 2000, Huggett 1998).

The difference in sediment effects concentrations in this study compared to others maybe attributed to differences in sediment characteristics. Characteristics, such as organic carbon and particle size, significantly influence the bioavailability of copper in the sediment. For example, Deaver and Rodgers (1996) and Muller et al (1999) used a sediment that had a high sand particle size (75 %) and a low organic carbon (<1%), indicating that 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).

Results in this study are comparable to established apparent effect threshold-low and apparent effect threshold-high (AET-L and AET-H) values for copper. AET-L and AET-H values for copper are 390 and 1300 mg Cu/kg dry weight, respectively (USEPA 1997). Minimal effects are predicted at the AET-L, while adverse effects would be observed at the AET-H. In this study, the *H. azteca* LC_{50} was 1477 with a confidence interval of ± 80 mg Cu/kg. The lower limit would therefore be 1397 mg Cu/Kg, which is similar to the AET-H. In addition, results of the *Typha* assay (i.e. LOEC of 1245 mg Cu/kg) fall in between the AET-L and the AET-H, representing a range in which there are possible adverse effects.

Interstitial water has been proposed as a major route of exposure for aquatic organisms to metals in sediment. By relating the dissolved metal concentration in the interstitial water to the LC_{50} of the organism, a prediction of toxicity may be made. Specifically, a interstitial water toxicity unit (IWTU)is calculated, [interstitial water Cu]/ 10 d LC_{50}, which relates toxicity to interstitial water concentration. When a IWTU is > 1, toxicity is predicted to occur; whereas, when a IWTU is < 1, toxicity is predicted not to occur (Ankley et al. 1996). In this study, the dissolved interstitial water copper concentrations were generally above *H. azteca* LC_{50}. Therefore, based on the interstitial water model, toxicity should have occurred. Toxicity was not observed when the interstitial water copper concentration was above the LC_{50}, except at the highest amended sediment concentration. There may be several explanations for this observation. First, complexation of copper with ligands (HCO_3- and humic acids) in the interstitial water may have reduced copper bioavailability (Chapman et al. 1998). 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 times they are observed feeding on detrital particles on the sediment surface. So these organisms may not actually come into contact with the interstitial water, but rather are exposed to the copper in their diet and possibly in the overlying water (Chapman et al. 1998, Suedel et al. 1996).

CONCLUSIONS

While long term application of copper sulphate sis increase copper concentrations in the pond sediments, no toxicity was observed. Caution must be applied when interpreting the pond toxicity results, because the samples were dried prior to testing. Drying of the sediments may have altered the bioavailability of the copper in those sediments. Copper concentrations in this Mississippi Delta stream sediment 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*. Interstitial water was not a accurate predictor of amphipod toxicity, possibly due to ligand complexation of copper.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Craig Tucker (Mississippi State University) and Mr. David Block (University of Mississippi) for assistance with sediment sampling, Mr. James Allgood for analytical assistance (University of Mississippi) and Mr. Cory McCulloch for assistance with data collection.

REFERENCES

Ankley, G.T., Di Toro, D.M., Hansen, D.J., Berry, W.J. 1996. Assessing ecological risk of metals in sediments. Environmental Toxicology and Chemistry. 15:2056-2066.

Button, S.B., Hostetler, H.P., Mair, D.M. 1977. Copper dispersal in a water supply reservoir. Water Research. 11:539-544.

Chapman, P.M., Wang, F., Janssen, C., Persoone, G., Allen, H.E. 1998. Ecotoxicology of metals in aquatic sediments: binding, release, bioavailability, risk assessment and remediation. Canadian Journal of Fisheries and Aquatic Sciences. 55:2221-2243.

Deaver, E., Rodgers, J.H. 1996. Measuring bioavailable copper using anodic stripping voltammetry. Environmental Toxicology and Chemistry. 15:1925-1930.

Deaver, E. 1995. Investigations of copper speciation and bioavailability in wetland sediments. PhD Dissertation, University of Mississippi.

Huggett, D.B., Gillespie, W.B., Rodgers, J.H. 1999. Copper bioavailability in Steilacoom Lake sediments. Archives of Environmental Contamination and Toxicology. 36:120-123.

Huggett, D.B. 1998. Assessing risks associated with exposures of aquatic organisms to contaminated sediments. Masters Thesis, University of Mississippi.

Ingersoll, C.G., Brunson, E.L., Dwyer, F.J., Hardesty, D.K., Kemble, N.E. 1997. Use of sublethal endpoints in sediment toxicity tests with the amphipod *Hyalella azteca*. Environmental Toxicology and Chemistry. 17:1508-1523.

Moore, M.T., Huggett, D.B., Huddleston, G.M., Rodgers, J.H., Cooper, C.M. 1999. Herbicide effects on Typha latifolia germination and root and shoot development. Chemosphere. 38:3637-3647.

Muller, S.L., Huggett, D.B., Rodgers, J.H. 2000. Effects of aqueous and sediment associated copper sulfate on Typha latifolia germination and early seedling growth. Archives of Environmental Contamination and Toxicology. (In Review).

Nebeker A.V., Cairns, M.A., Gakstatter, J.H., Malueg, K.W., Schuytema, G.S., Krawczyk, D.F. 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. Environmental Toxicology and Chemistry. 3:617-630.

Schlenk, D., Gollon, J.L., Griffin, B.R. 1998. Efficacy of copper sulfate for the treatment of *Ichthyophthiriasis* in channel catfish. Journal of Aquatic Animal Health. 10:390-396.

Suedel, B.C., Deaver, E., Rodgers, J.H. 1996. Experimental factors that may effect toxicity of aqueous and sediment bound copper to freshwater organisms. Archives of Environmental Contamination and Toxicology. 30:40-46.

Tucker, C.S., Boyd, C.E. 1978. Consequences of periodic applications of copper sulfate and simazine for phytoplankton control in catfish ponds. Transactions of the American Fisheries Society. 107:316-320.

Tucker, C.S., Kingsbury, S.K., Pote, J.W., Wax, C.L. 1996. Effects of water management practices on discharge of nutrients and organic matter from channel catfish ponds. Aquaculture. 147:57-69..

USEPA1998.www.epa.gov/fedrgstr/EPA-WATER/1998/September/Day-04/w23891.htm

USEPA. 1997. The incidence and severity of sediment contamination in surface waters of the United States. EPA.823-R-97-006

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA. 600/R-94/024

Xiang, H.F., Hargreaves, J.A., Kingery, W.L., Huggett, D.B., Schlenk, D.K. 2000. Accumulation, distribution and toxicity of copper sulfate in soils of catfish ponds receiving periodic copper sulfate applications. Journal of Environmental Quality. (In review).

Zar, J.H. 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ.

Sample	Bulk Sediment Copper	Interstitial Water	IWTU*
upstream	29	0.08	1.2
downstream	25	0.14	2.1
outfall	31	0.05	0.7
outfall amended 1	172	0.08	1.2
outfall amended 2	663	0.18	2.7
outfall amended 3	1245	0.31	4.6
outfall amended 4	1515	1.88	28.1

*IWTU = [interstitial water]/ *H.azteca* 10d LC_{50} (0.067 mg /l)

Table 1. Bulk sediment copper concentrations (mg/kg), interstitial water concentrations (mg/l) and interstitial water toxicity units (IWTUs) associated with the stream collected and amended sediments.

Sample	Survival	Growth
control	93	3.5 ± 0.02
upstream	97	4.0 ± 0.05
downstream	67*	4.1 ± 0.05
outfall	93	3.7 ± 0.03
amended-172 mg/kg	97	3.7 ± 0.02
amended-663 mg/kg	93	3.6 ± 0.03
amended-1245 mg/kg	97	3.5 ± 0.02
amended-1515 mg/kg	40*	3.5 ± 0.02

* $p < 0.5$

Table 2. Survival (%) and growth (mm ± standard deviation) of *H.azteca* exposed to stream and amended sediments.

Sample	Germination	Root Growth	Shoot Growth
control	90	0.63 ± 0.10	1.16 ± 0.17
upstream	87	0.83 ± 0.06	1.19 ± 0.07
downstream	80	0.85 ± 0.03	1.12 ± 0.12
outfall	93	0.82 ± 0.05	1.13 ± 0.02
amended-172 mg/kg	90	0.74 ± 0.08	1.06 ± 0.03
amended-663 mg/kg	100	0.57 ± 0.04	0.94 ± 0.15
amended-1245 mg/kg	80	0.37 ± 0.05*	0.88 ± 0.15
amended-1515 mg/kg	97	0.35 ± 0.04*	0.90 ± 0.10

*p< 0.5

Table 3. *T.latifolia* germination (%) and root and shoot growth (mm ± standard deviation) after exposure to stream collected and amended samples.

Sample	Survival	Growth
Control 1	83	3.9 ± 0.7
Control 2	83	3.6 ± 0.1
Control 3	87	3.9 ± 0.3
1	90	3.7 ± 0.4
2	90	4.3 ± 0.7
3	83	3.9 ± 0.1
4	93	3.5 ± 0.1
5	97	3.9 ± 0.5
6	87	3.3 ± 0.2
7	83	3.8 ± 0.8
8	80	3.9 ± 0.3

Table 4. Survival (%) and growth (mm ± standard deviation) of *H.azteca* exposed to pond sediment samples.

Site	Germination	Root Growth	Shoot Growth
Control 1	87	6.0 ± 0.5	9.9 ± 1.5
Control 2	83	7.8 ± 0.3	9.8 ± 0.6
Control 3	93	7.7 ± 0.9	10.9 ± 1.4
1	87	8.6 ± 0.9	11.6 ± 0.6
2	93	7.4 ± 0.5	10.7 ± 0.5
3	90	6.1 ± 0.2	10.6 ± 0.8
4	100	7.2 ± 0.7	10.6 ± 0.2
5	97	6.4 ± 0.2	10.4 ± 0.3
6	100	6.9 ± 0.8	10.8 ± 0.3
7	93	7.0 ± 0.3	9.9 ± 0.4
8	90	6.4 ± 1.1	10.2 ± 0.4

Table 5. *T.latifolia* germination (%) and root and shoot growth (mm ± standard deviation) after exposure to pond sediment samples.