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Responses of *Hyalella azteca* and *Ceriodaphnia dubia* to Reservoir Sediments Following Chelated Copper Herbicide Applications

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ABSTRACT

In response to nuisance growths of algae and vascular plants, such as dioecious hydrilla (*Hydrilla verticillata* L.f. Royle), copper formulations have been applied in lakes and reservoirs for a number of years. Concerns have arisen regarding the long-term consequences of copper applications and those concerns have appropriately focused on sediment residues. In this study, we evaluated the toxicity of sediments from treated (for a decade) and untreated areas in Lake Murray, South Carolina and estimated the capacity of those sediments to bind additional copper. Two sentinel aquatic invertebrates, *Hyalella azteca* Saussure and *Ceriodaphnia dubia* Richard, were used to measure residual toxicity of treated and untreated sediments from the field and after laboratory amendments. The copper concentrations in sediments were not significantly different for the treated and the untreated areas in Lake Murray and no toxicity was observed with either experimental organism or for sediments from either site. Based on the copper binding capacity of the collected Lake Murray sediments, the sediment copper concentration would have to increase eleven fold (from 16.3 to 180 mg Cu/kg) before adverse effects are observed if no new binding sites accrue. Measures of sediment copper concentrations coupled with toxicity testing using sensitive sentinel invertebrates provide evidence of a lack of bioavailable copper due to excess sediment binding capacity for copper at these sites.

Key words: Komeen®, Lake Murray, toxicity, *Hydrilla verticillata.*

INTRODUCTION

Copper has been widely used as an aquatic plant herbicide or algaecide to control growths of vascular and non-vascular plants in lakes and reservoirs throughout the world (Murray-Gulde et al. 2002). Komeen®[3], a chelated-copper herbicide containing 8% elemental copper from copper sulfate pentahydrate and copper ethylenediamine complex, can be applied to aquatic systems (i.e., aquaculture ponds, irrigation systems, and potable water reservoirs) to control invasive or problematic vegetation such as dioecious hydrilla. In North America, hydrilla is an invasive, submerged vascular plant inhabiting freshwater lentic systems, and often reaches nuisance levels if not controlled. Komeen has been used to manage hydrilla in Lake Murray, a 20,234-ha accreting reservoir located in Lexington County, South Carolina, U.S.A. In specific areas of Lake Murray, annual applications of Komeen at a concentration of 1 mg Cu/L have been used for ten years to control dioecious hydrilla. This situation presented an opportunity to address the issue of residual copper toxicity associated with sediments. Copper applied as a herbicide or an algaecide is introduced into the water column and ultimately moves into the sediments where it resides, based on its lithic biogeochemistry (Murray-Gulde et al. 2005a). It is critical to understand the long-term effects of chemical applications such as copper.

Concern has arisen regarding the environmental or ecological significance of copper accumulation in sediments due to repeated applications of copper-based herbicides or algaecides. Excess copper in sediments may result in adverse effects to aquatic macroinvertebrates or fish under specific water and sediment conditions. If the applied copper is bioavailable, then the concerns may be justified; however, if the applied copper speciates into non-bioavailable forms, the risk may be negligible. In 2000, MacDonald et al. derived a consensus-based sediment quality guideline for copper of 149 mg Cu/kg. Harmful effects to the environment will result, according to MacDonald et al., if this sediment copper concentration is exceeded. Also, the copper concentration in sediments, above which adverse effects are probable, was reported as 197 mg Cu/kg by the National Oceanic and Atmospheric Administration's screening quick reference tables or SQuiRTs (SQuiRTs 1999). However, these are generic guidelines and are likely very conservative or not applicable at specific sites. For example, since Lake Murray is an accreting system, it has increased binding sites for copper over time due to sedimentation of autochthonous and allochthonous materials (Murray-Gulde et al. 2005b). The copper concentration in sediments at a specific site (e.g., Lake Murray) as well as bioavailability and toxicity data can accurately evaluate a site for effects of residual sediment copper concentrations. Aquatic invertebrates commonly used in toxicity experiments and sensitive to copper exposures can help to

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discern whether the copper present in Lake Murray sediments is bioavailable.

Hyalella azteca Saussure and *Ceriodaphnia dubia* Richard were used to assess the bioavailability of copper associated with Lake Murray sediments. *H. azteca* is an aquatic macro-invertebrate found throughout North and South America in benthic environments (Hoffman et al. 1995). *H. azteca* is easily cultured in the laboratory and is sensitive to a wide range of toxicants including copper (Borgmann et al. 1989, ASTM 1995). *C. dubia* is a sensitive aquatic crustacean that is found within pelagic zones of lentic environments. This micro-crustacean is commonly utilized in aquatic toxicity tests because it is also easily cultured, has a short life cycle, and is especially sensitive to many toxicants including copper (Murray-Gulde et al. 2005a). *H. azteca* and *C. dubia* were exposed to copper-amended sediments from both the laboratory and the field.

The specific objectives of this research were: 1) to evaluate the toxicity of Lake Murray sediments, collected from an untreated area and from an area treated for ten years with Komeen, to *H. azteca* and *C. dubia*, 2) to measure the toxicity of sediments collected from microcosms (containing Lake Murray water, sediment, and hydrilla) treated each with 1 or 10 mg Cu/L as Komeen, and 3) to estimate the whole-sediment copper concentration necessary to elicit toxicity to *H. azteca* and *C. dubia* through amendments of copper to sediment samples. By amending field-collected sediments with a series of concentrations of copper in the laboratory and subsequently exposing sensitive organisms to these copper amended sediments, we could estimate the instantaneous binding capacity and the concentration at which copper becomes bioavailable. In this research, instantaneous binding capacity is the concentration of copper at the instant when all of the sediment binding sites with respect to copper are occupied and is estimated from the concentration at which copper in sediment becomes bioavailable based upon responses of sensitive organisms such as *H. azteca* and *C. dubia*.

MATERIALS AND METHODS

Culturing and Testing of *Hyalella azteca* and *Ceriodaphnia dubia*

H. azteca was cultured in 39-L aquaria and tested at a temperature of 25 ± 2°C under a 16-h light/8-h dark photoperiod (de March 1981, U.S. EPA 2000). Maple leaves (*Acer rubrum*) were used as a substrate and food source for the culture. Second instar *H. azteca* (10-13 d) were sorted from the cultures with sieves for testing purposes (Suedel and Rodgers 1993). Ten *H. azteca* were placed into individual 250 cm^3 borosilicate beakers containing a 1:4 sediment (50 cm^3) to water (150 cm^3) ratio and three 7-mm maple leaf discs as a food source (U.S. EPA 2000). *H. azteca* were exposed in 10-d static non-renewal toxicity experiments following U.S. EPA guidelines (2000).

C. dubia culturing followed U.S. EPA methods (1994) with identical temperature and light cycle regimes as described above for *H. azteca*. Following U.S. EPA guidelines (U.S. EPA 2000), less than 24-hour-old *C. dubia* were exposed to homogenized sediment samples in 7-day static non-renewal toxicity experiments. Individual *C. dubia* were exposed (10 replications/treatment) in 50 cm^3 plastic containers with a 1:4 sediment (10 cm^3) to water (30 cm^3) ratio. Each organism was fed 100 µL of algae (*Raphidocelis subcapitata* Korshikov) and YCT (yeast: trout chow: Tetramin®) daily (U.S. EPA 1994). The measured response of *H. azteca* and *C. dubia* from the sediment toxicity experiments was a change in survival in treatments versus controls.

Evaluation of Lake Murray Sediment

Sediment, water, and hydrilla were collected from two areas in Lake Murray: Jake's Landing Marina (treated for a decade with Komeen - N 34°01.671' and W 081°13.540') and Lake Murray Marina (not previously treated with Komeen - N 34°07.025' and W 081°15.209'). The samples were returned to Clemson University for analysis of physical and chemical characteristics of the sediment and water from both marinas. *H. azteca* and *C. dubia* were independently exposed in ten- and seven-day sediment toxicity experiments, respectively, with sediment collected from both Jake's Landing Marina and Lake Murray Marina. Four replications were used for each sediment sample (treatment). The control sediment (90.4% sand) was collected from a local reference site (N 34°38.188' and W 082°48.251') in Clemson, SC, U.S.A. Since no adverse effects have been observed to *H. azteca*, *C. dubia*, or other sensitive invertebrates in toxicity experiments, this sediment has been used for years as a control in the laboratory (Murray-Gulde et al. 2002). The overlying water for the toxicity experiments was collected from the respective locations at Lake Murray and was included to simulate field conditions. Copper concentrations in the sediment samples were measured by atomic absorption spectroscopy, following sediment digestion using an Ethos® Microwave Digester (U.S. EPA 1998). The detection limit for copper in sediment was 0.1 mg Cu/kg.

Lake Murray Sediment Evaluation: Post-treatment in Microcosms

For this research, ten experimental microcosms were constructed in a completely randomized design. Komeen was applied at 1 mg Cu/L or 10 mg Cu/L (10 times the maximum label concentration) to eight of the ten 568-liter Rubbermaid® microcosms (140 cm × 94 cm × 61 cm) containing water, sediment, and established hydrilla from Jake's Landing Marina and Lake Murray Marina. The two control microcosms, one from Jake's Landing Marina and one from Lake Murray Marina, did not receive additional Komeen amendments. Located in a Clemson University greenhouse, each microcosm was filled with sediment and water to depths of 15 cm and 43 cm, respectively. Dioecious hydrilla was established in each microcosm at field (summer) densities of 48-80 g/m^2 (wet weight) nine days prior to the Komeen applications. *H. azteca* and *C. dubia* were exposed in the laboratory in ten- and seven-day sediment toxicity experiments, respectively, to sediment samples collected from the eight sediment-amended microcosms (four treated microcosms that were replicated twice) plus sediment collected from the two un-amended (control) microcosms. Sediment samples collected from the replicated treatment microcosms were composited. *H. azteca* and *C. dubia* were independently exposed to the four composited sediment samples (four replications per composite) plus sediment collected from the two un-amended (control) microcosms (four replications per control microcosm). The treatment sediments (top 5 cm) were collected six months after the Komeen application. A one-way analysis of variance (ANOVA) with Dunnett's multiple-range test was performed (SAS 2004) to analyze data from the sediment toxicity experiments ($p \leq 0.05$).

Copper Amendments to Lake Murray Sediments

Based on lack of observed toxicity from the experiments outlined above, Lake Murray Marina sediment was amended with copper following methods of Suedel and Rodgers (1993). The nominal sediment copper concentrations for this experiment were: un-amended control, 125, 250, 500, 1000, and 2000 mg Cu/kg sediment. *H. azteca* and *C. dubia* were exposed in ten- and seven-day sediment toxicity experiments, respectively, to the copper-amended sediments (four replicates/sediment sample). For these experiments, a lowest observed effects concentration (LOEC) was calculated using an ANOVA followed by Dunnett's test. The LOEC was the sediment copper concentration necessary to elicit detectable adverse effects on *H. azteca* and *C. dubia* that was significantly different from the un-amended control. ANOVA and Dunnett's test calculations were performed using Toxstat 3.5 (Western, Inc. and Gulley 1996).

RESULTS AND DISCUSSION

Evaluation of Lake Murray Sediment

Sediment samples from both treated areas (Jake's Landing Marina) and untreated areas (Lake Murray Marina) in Lake Murray were analyzed for physical and chemical characteristics (Table 1) that may influence partitioning of copper and its subsequent bioavailability. The sediments at both sites were dominated by silt-size particulates. The organic matter at the untreated site was 50% greater than at the treated site (2.4% vs. 1.6%, respectively). The oxidation-reduction potential (Eh) in the untreated site sediments was more than an order of magnitude less (-166 mV) than the Eh potential in the treated site sediments (9.1 mV). The background sediment copper concentrations from Jake's Landing Marina and Lake Murray Marina were 18.0 and 16.3 mg Cu/kg, respectively. The control sediment was dominated by sand-size particulates with low percent organic matter (<1.0) and high Eh (60 mV). The copper concentration in the control sediment was 0.3 mg Cu/kg. The remaining physical and chemical parameters were similar for both Jake's Landing Marina and Lake Murray Marina.

Although the sediment copper concentrations were similar for both the treated (18.0 mg Cu/kg) and the untreated (16.3 mg Cu/kg) marinas, we anticipated that invertebrate toxicity would differ due to significant differences in percent organic matter and Eh between sediments from Jake's Landing Marina and Lake Murray Marina. This difference in toxicity was anticipated because copper has a propensity to bind to organic matter due to its lithic biogeochemical cycle and when sediment Eh significantly decreases by more than an order of magnitude (9.1 mV to -166 mV), copper has a tendency to exist as an insoluble complex such as copper sulfide. As a result, copper may become less bioavailable to *H. azteca* and *C. dubia*. The lack of difference between the copper concentrations in the sediments from the treated and untreated sites was unexpected and might be due to accretion as well as dispersion and dilution of the copper herbicide from the treatment sites. Even though some of the physical and chemical sediment parameters were different, significant invertebrate toxicity was not observed. *H. azteca* survival was greater than eighty-seven percent when exposed to Jake's Landing Marina and Lake Murray Marina sediments in ten-day experiments (Figure 1). No mortality of

TABLE 1. JAKE'S LANDING MARINA (TREATED FOR A DECADE WITH KOMEEN), LAKE MURRAY MARINA (NOT PREVIOUSLY TREATED WITH KOMEEN), AND CONTROL SEDIMENT AND WATER PHYSICAL/CHEMICAL CHARACTERISTICS.

Physical/chemical characteristics	Jake's Landing Marina	Lake Murray Marina	Control
Sediment			
% sand	14.5	9.4	90.4
% silt	85.5	90.6	9.5
% clay	<1.0	<1.0	<1.0
% OM	1.6	2.4	<1.0
Bulk density (% solids)	67.0	62.0	92.0
Total CEC (meq/100g soil)	5.6	4.5	1.3
*p*H	7.0	6.2	7.9
Eh (mV)	9.1	-166.0	60.0
Background Cu concentration (mg/kg)	18.0	16.3	0.3
Water			
*p*H	7.8-8.6	7.4-8.6	7.5-8.4
D.O. (mg/L)	6.6-8.7	6.4-8.5	7.9-8.5
Alkalinity (mg $CaCO_3$/L)	40-64	24-64	63-78
Hardness (mg $CaCO_3$/L)	20-84	32-60	67-80
Conductivity (µS/cm)	196-359	169-208	206-254
Temperature (°C)	22-23	22-23	22-23

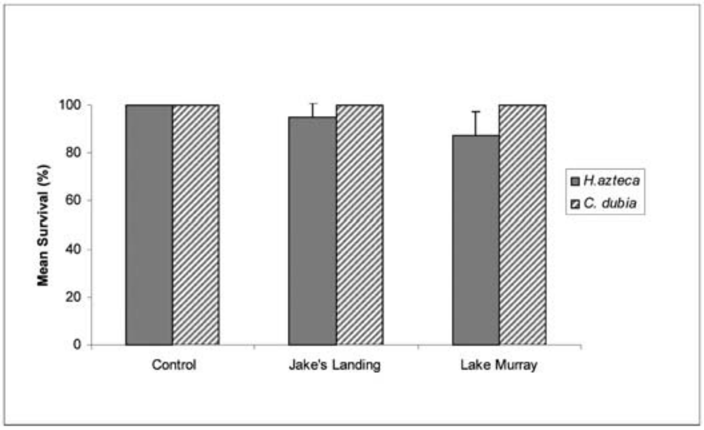

Figure 1. Responses of *H. azteca* and *C. dubia* (measured as survival) to Jake's Landing Marina (Jake's Landing) and Lake Murray Marina (Lake Murray) sediment exposures for 10- and 7-days, respectively. The "control" represents sediment collected from a local reference site in Clemson, SC, U.S.A. Error bars represent +1 standard deviation.

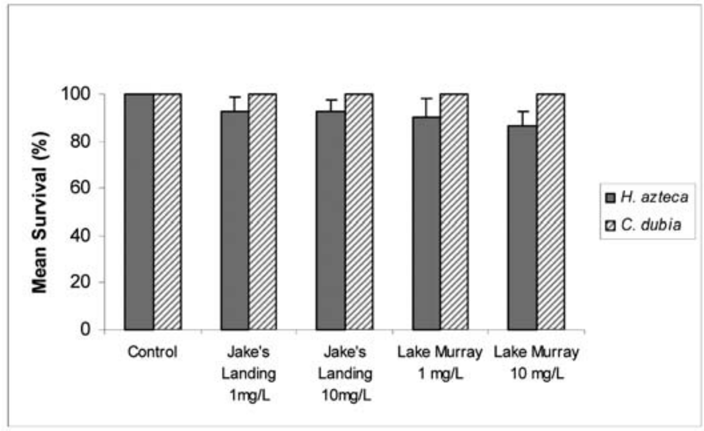

Figure 2. Responses of *H. azteca* and *C. dubia* (measured as survival) to Komeen®-amended Jake's Landing Marina (Jake's Landing) and Lake Murray Marina (Lake Murray) sediment exposures for 10- and 7-days, respectively. The "control" represents un-amended Lake Murray Marina sediment. Error bars represent +1 standard deviation.

C. dubia was observed when exposed to either Jake's Landing Marina or Lake Murray Marina sediments (Figure 1). There were no statistical differences in the responses of either *H. azteca* ($p = 0.512$) or *C. dubia* (no mortality) exposed to Jake's Landing Marina and Lake Murray Marina sediments when compared to the control sediment.

Lake Murray Sediment Evaluation: Post-treatment in Microcosms

Following copper amendments of 1 mg/L and 10 mg/L to eight of ten microcosms at Clemson University, *H. azteca* survival (Figure 2) in ten-day sediment experiments was not significantly different ($p = 0.298$) in any of the treatments when compared with the sediment controls collected from the two untreated microcosms. As in the previous experiment evaluating Lake Murray sediments, one hundred percent survival was observed for *C. dubia* in seven-day sediment toxicity experiments (Figure 2). Based on the volume of overlying water (374 L) and the mass of sediment (150 kg-dry weight) in each microcosm, a 1 mg Cu/L application was estimated to increase the copper concentration in the sediment by 2.5 mg Cu/kg. Therefore, the 1 mg Cu/L amendments increased the copper concentrations in Jake's Landing Marina sediment from 18.0 mg Cu/kg to 21.2 mg Cu/kg and in Lake Murray Marina sediment from 16.3 mg Cu/kg to 20.3 mg Cu/kg. For greater overlying water volumes or depths, for example, if the water depth increased from 43 cm (this study) to 2.5 m, the mass of applied copper would also increase and would be expected to result in a sediment copper concentration greater than 2.5 mg Cu/kg. Hence, site conditions, such as water depth and copper application rates, can lead to significant variability in copper mass loading and subsequent sediment accumulations. It is also important to note that microcosms containing sediment from Jake's Landing Marina, which had already been treated for a decade with Komeen, received an amendment equivalent to the amount that would be applied with an additional ten years of treatment (assuming 1 mg/L yearly treatment) in the two-10 mg Cu/L treated microcosms. As a result, the 10 mg Cu/L amendments did significantly increase the copper concentrations to 30.2 mg Cu/kg and to 29 mg Cu/kg for Jake's Landing Marina and Lake Murray Marina sediment, respectively. The copper concentrations in Jake's Landing Marina and Lake Murray Marina sediment following 1 mg/L and 10 mg/L applications further support the inference that the sediments are not toxic to *H. azteca* or *C. dubia*.

Copper Amendments to Lake Murray Sediments

H. azteca and *C. dubia* were exposed to five copper-amended samples of Lake Murray Marina sediment and an un-amended control. The measured copper concentrations in sediments were: un-amended Lake Murray Marina control (background concentration = 16.3), 132, 180, 440, 621, and 1460 mg Cu/kg sediment. The copper concentration in sediment necessary to elicit toxicity (LOEC) to *H. azteca* and *C. dubia* were both conservatively estimated at 180 mg Cu/kg (Figures 3 and 4) using Toxstat 3.5 (Western, Inc. and Gulley 1996). In Figure 3, the response of *H. azteca* in the 132 mg Cu/kg exposure was not statistically different from the response in the un-amended control. The instantaneous binding capacity of Lake Murray Marina sediments, the concentration of copper at the instant when all of the sediment binding sites with respect to copper are occupied, was estimated at 180 mg Cu/kg.

Since Jake's Landing Marina and Lake Murray Marina sediments were not toxic and an amount equivalent to an additional ten years of Komeen applications did not result in toxicity, it is unlikely that future Komeen treatments, applied at the legal limit of 1 mg Cu/L, in Lake Murray would increase the sediment copper concentrations from 16.3 to 180 mg Cu/kg (eleven fold). Thus, Komeen does not pose residual problems in treatment sites, such as Jake's Landing Marina. Furthermore, the additional sedimentation of autochthonous and allochthonous materials in Lake Murray, due to accretion, will decrease the overall copper concentra-

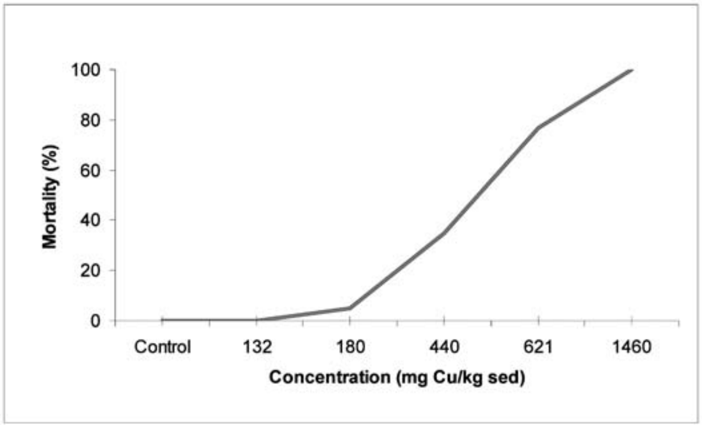

Figure 3. Response of *H. azteca* to ten-day Komeen®-amended sediment exposure. The "control" represents un-amended Lake Murray Marina sediment.

tion in the sediments and may also decrease risks (e.g., toxicity), due to copper, in Lake Murray sediments. Finally, using sensitive sentinel invertebrates (*H. azteca* and *C. dubia*) coupled with chemical analysis and experimental manipulations (sediment amendments), potential site-specific risks to aquatic biota following long-term chelated-copper herbicide applications can be more accurately assessed relative to solely using an arbitrary or generic chemical criterion based on total concentration of an element such as copper.

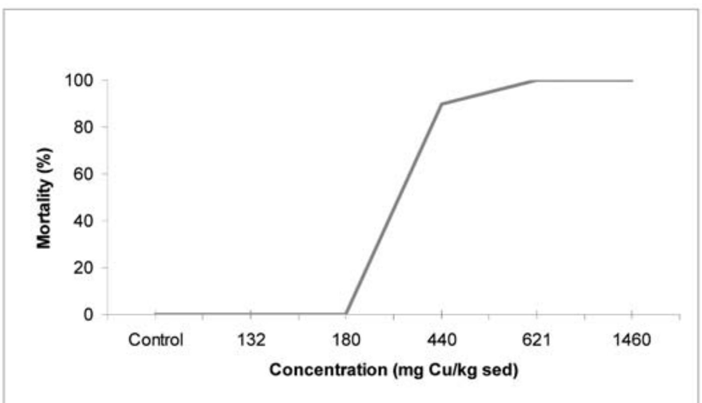

Figure 4. Response of *C. dubia* to seven-day Komeen®-amended sediment exposure. The "control" represents un-amended Lake Murray Marina sediment.

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