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Experimental Factors That May Affect Toxicity of Aqueous and Sediment-Bound Copper to Freshwater Organisms

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Abstract. Because of recent concerns regarding the ability of acute (48–96 h) sediment toxicity tests to accurately assess the potency of sediment-bound contaminants, effects of exposure duration, test organism selection, and test endpoint on the observed toxicity of aqueous phase copper and a copper-contaminated freshwater sediment were evaluated. Toxicity of sediment-bound copper was assessed by monitoring survival and reproduction of *Ceriodaphnia dubia* Richard, survival of *Daphnia magna* Straus and *Hyalella azteca* Saussure, and survival and growth of *Chironomus tentans* Fabricius and *Pimephales promelas* Rafinesque. Organisms were exposed in static systems for 48 h, 96 h, 7 d, 10 d, and 14 d, which enabled measurement of acute and chronic toxicity. Relative sensitivities of test organisms exposed to copper in water and copper-contaminated sediment varied with test duration and test endpoint. In general, *C. dubia* was the most sensitive organism tested, followed in decreasing sensitivity by *D. magna*, *P. promelas*, *H. azteca*, and *C. tentans*. A temporal mortality threshold was demonstrated by *C. dubia* and *D. magna* when exposed to copper, with little mortality occurring after 96 h of exposure. Effects of test duration on copper toxicity were most pronounced for *H. azteca* and *C. tentans*, with mortality and growth effects becoming increasingly sensitive with increasing test duration. Formulated sediment served as a suitable control and reference sediment in this study, and matched a variety of field-collected test sediment characteristics. In tests utilizing copper-contaminated sediments, observed responses (mortality, growth, reproduction) corresponded with overlying water concentration of copper rather than concentration of copper in bulk sediment or pore water.

Recent concerns regarding sediments as sources and sinks for contaminants in aquatic systems has focused attention on our ability to predict potential adverse effects of sediment-bound materials such as copper (Ankley *et al.* 1993). Copper is an essential element for organisms and is also considered a priority pollutant by U.S. EPA (U.S. EPA 1980). Because of the widespread use of copper as an algicide, aquatic herbicide, fungicide, and bactericide, as well as discharges from smelting, refining, and other copper-producing industries, copper may accumulate sufficiently in sediments to produce adverse effects on aquatic organisms.

Copper occurs in particulate, colloidal, dissolved, organic, and inorganic chemical forms (Leckie and Davis 1979). Organic ligands and pH are important factors affecting copper speciation, as well as transport and deposition, in aquatic environments (Lewis 1992). Divalent copper (Cu^{+2}) is the predominant oxidation state in soluble aqueous complexes. Copper is generally more toxic at lower pH values, which is usually attributed to a greater concentration of free copper ion (Campbell and Stokes 1985). The chemical form of copper is critical to its behavior in biological processes, its bioavailability, and its toxicity to aquatic organisms from sediments. Sediment characteristics that affect copper toxicity include organic carbon (Malueg *et al.* 1986), pH (Leckie and Davis 1979), and organic ligands (Lewis 1992). Acid volatile sulfides (AVS) have been hypothesized as a sediment characteristic regulating copper bioavailability (Di Toro *et al.* 1992); however, some studies have suggested that this situation is more complex, with other partitioning phases operative (Ankley *et al.* 1993).

The ability of laboratory experiments to detect adverse effects of sediment-sorbed materials on aquatic and benthic organisms depends on several factors, including exposure duration, species selection, and test endpoints. Previous studies conducted with contaminated field-collected sediments (Burton *et al.* 1989), contaminated dredged sediments (LeBlanc and Suprenant 1985), and sediments amended with fluoranthene (Suedel and Rodgers 1993) found that sufficient test duration was crucial in order to accurately assess contaminated sediments. Sensitive test endpoints are also a necessary component of sediment toxicity tests in order to verify the toxicity of sediments (Burton 1991) since there is a wide range of sensitivity of test endpoints to aqueous and sediment-bound contaminants (Ingersoll and Nelson 1990; Winner 1988). As such, selection of appropriate test endpoints is also essential for accurately assessing sediment toxicity.

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To further the understanding of factors that affect the toxicity of aqueous and sediment-bound copper to common freshwater sediment toxicity testing organisms in laboratory tests, a series of experiments were conducted. Test species *Chironomus tentans* Fabricius, *Hyalella azteca* Saussure, *Daphnia magna* Straus, *Ceriodaphnia dubia* Richard, and *Pimephales promelas* Rafinesque were used to 1) determine the relative sensitivities of these organisms to copper in water-only exposures and a field-collected copper-contaminated sediment; 2) determine the duration of exposure required to elicit mortality or sublethal biological effects (e.g., reduced growth or reproduction) by a copper-contaminated sediment; and 3) determine if sublethal test endpoints are more sensitive than survival of test organisms used in this study. Test organism mortality in water-only copper tests was compared with concentrations of copper in overlying water, pore water, and sediment in sediment tests.

Materials and Methods

Test Organism Culture Procedures

All test organisms were cultured at the University of Mississippi Biology Department laboratory. *H. azteca* culturing procedures followed the methods of de March (1981). Amphipods used for testing were removed from cultures and gently washed through a 1.0 mm mesh sieve. Organisms that passed through the 1.0 mm sieve but were retained by a 0.6 mm sieve (approximately 2–3 weeks old) were collected and used for testing. *C. tentans* culture methods followed those of Townsend *et al.* (1981). Midges used for testing were second instar larvae (10 d old). *D. magna* and *C. dubia* culturing procedures followed the methods of Peltier and Weber (1985). Hardness and alkalinity of University of Mississippi Biological Field Station (UMBFS) water were adjusted with (0.1 g/L) $NaHCO_3$ and $CaCl_2$ to a total hardness of 80 mg/L as $CaCO_3$ and alkalinity of 60 mg/L as $CaCO_3$. *P. promelas* culturing procedures followed the methods of Peltier and Weber (1985). Fatheads used in testing were 2–4 d old.

Experimental Design

All experiments were conducted in incubators at 20 ± 1°C under a 16 h light/8 h dark photoperiod. Experiments were started by adding eight *H. azteca*, six *C. tentans*, eight *D. magna*, or eight *P. promelas* to each of four replicate beakers. Experiments with *C. dubia* were started by adding one neonate to each of 10 replicate beakers per treatment. Water-only tests were conducted in 250 ml borosilicate glass beakers with 200 ml of UMBFS water, except for *C. dubia* tests, which were conducted in 50 ml beakers with 40 ml of UMBFS water. UMBFS water was used as a control, except for *D. magna* tests, where adjusted UMBFS pond water (see culture procedures) was used. Glass beads (150–212 μm, Sigma Chemical Co., St. Louis, MO) were used as a substrate in *C. tentans* tests to allow for tube building and to reduce stress (Suedel and Rodgers 1993).

Organisms were exposed to sediments in 250 ml glass beakers, with each beaker containing 40 ml of sediment and 160 ml UMBFS pond water, except for *C. dubia* tests, which were conducted in 50 ml beakers with 8 ml of sediment and 32 ml UMBFS pond water (1:4 sediment to water ratio). Formulated sediment (100%) was used as a control sediment in all experiments (Suedel *et al.* 1995; this volume). Sediment/water mixtures were allowed an overnight contact period before adding organisms. In all tests, feeding regimes for each organism were as follows: *D. magna* and *C. dubia*—0.5 ml and 0.05 ml, respectively, of *S. capricornutum* algae daily; *H. azteca*—0.5 g wet wt. of leached and ground maple leaves at test initiation; *C. tentans*—0.1 ml cerophyll suspension at test initiation and every other day thereafter; *P. promelas*—10–20 newly hatched *Artemia nauplii* per fish daily. Dissolved oxygen concentrations did not drop below 40% of saturation in any test; therefore, aeration was not required. Water quality parameters measured are presented in Table 1.

Table 1. Water characteristics measured in water-only and sediment experiments

Parameter	Water-only experiments	Sediment experiments
Temperature (°C)	19.7–24.1	19.5–22.3
pH	6.9–8.0	6.7–7.8
D.O. (mg/L)	6.2–8.5	5.2–7.7
Alkalinity[a] (mg/L as $CaCO_3$)	9–21	34–56
Hardness[a] (mg/L as $CaCO_3$)	6–10	8–120
Conductivity[a] (uS/cm)	20–50	50–180

[a] Data from *D. magna* tests were not included because adjusted UMBFS pond water was used, resulting in higher values for alkalinity, hardness, and conductivity (water-only tests: alkalinity = 68–70, hardness = 72–80, conductivity = 280–305; sediment tests: alkalinity = 67–71, hardness = 136–148, conductivity = 320–360)

Sediments

Copper-contaminated sediment was collected from a creek site downstream from a copper smelting operation. Sediment was collected by a grab sampler, transported on ice to the UMBFS laboratory, and stored at 4°C until use. Total copper concentration in this sediment was 18,259 ± 1,884 mg/kg (mean ± S.D.). Barium and lead concentrations were below analytical detection limit (<5 mg/kg). Characteristics of this sediment were as follows: solids, 63%; organic matter, 5.5%; cation exchange capacity, 3.91 meq/100 g; redox potential, +4 mv; pH 6.6; sand, 20%; silt, 80%; and clay, 0%. Formulated sediment was used as a control and dilution sediment in all tests. Additional information regarding formulated sediment preparation and use in this study is given in Suedel *et al.* (1995; this volume).

Analytical Procedures

Stock solutions used for water-only tests were prepared by dissolving reagent grade cupric sulfate ($CuSO_4 \cdot 5H_2O$) in Milli-Q (MQ) water. Samples for water-only copper analyses were obtained from sacrificial beakers (one replicate per concentration) at the start of each test. Water samples were filtered through a 0.45 μm filter and acidified with redistilled nitric acid (Aldrich Chemical Co., Milwaukee, WI) to a pH of 1–2 before analysis. In sediment tests, after the overlying water was sampled and removed, approximately 4.0 g subsample of wet sediment from each sacrificial beaker (one replicate per copper concentration) was placed (via precleaned stainless steel spatula) in an aluminum weighing boat, dried at 80°C for 24 h, acid digested for 5 h at 100°C, filtered (0.45 μm), and diluted to 25 ml with MQ water before analysis. Pore water copper samples were extracted from the remaining sediment (approximately 50 g wet wt.) by centrifugation at 12,000 × g for 10 minutes. After centrifuging, pore water was removed by pipet, filtered through a 0.45 μm filter, and acidified to pH 1–2 before analysis.

Table 2. LC_{50} values for test species exposed to copper in water-only exposures (μg/L) vs contaminated sediment tests in overlying water (μg/L), pore water (μg/L), and sediment (mg/kg)

			Sediment tests		
Species	Duration	Water-only tests	Overlying water	Pore water	Sediment
C. dubia	48 h	2.72	6.23	84.0	129.3
	96 h	1.46	9.06	121.7	35.8
	7 d	1.16	6.56	117.9	32.0
	10 d	4.18	6.56	117.9	32.0
	14 d	4.18	6.56	117.9	32.0
D. magna	48 h	11.3	7.25	170.0	169.7
	96 h	10.1	7.96	36.8	40.3
	7 d	10.6	7.68	35.9	38.7
	10 d	9.5	7.62	35.7	38.4
	14 d	9.5	7.43	35.2	37.4
P. promelas	48 h	20.2	17.7	68.9	428
	96 h	12.5	12.8	54.1	265
	7 d	8.2	10.1	44.7	182.9
	10 d	6.9	9.2	42.2	162.4
	14 d	8.8	2.3	35.9	286
H. azteca	48 h	72.2	59.0	754	424
	96 h	65.6	47.2	674	351
	7 d	52.6	42.4	638	319
	10 d	67.2	35.2	584	262
	14 d	44.1	30.5	545	247
C. tentans	48 h	529	323	5820	4522
	96 h	630	57.1	135.0	1905
	7 d	657	49.4	106.9	1600
	10 d	1502	36.3	61.5	1026
	14 d	>1175	ND[a]	ND	ND

[a] No data

Copper samples of <30 μg/L were concentrated by boiling, while copper samples of >30 μg/L were not concentrated prior to analysis. Copper concentrations in all water and sediment samples were determined using a Buck model 200-A flame atomic absorption spectrophotometer. Mean (±S.D.) copper recovery in both water-only and sediment tests was 96.5 ± 8.15%.

Statistical Analyses

Lethal concentrations (LC_{50}s) and 95% confidence intervals (C.I.) for all tests were calculated using the moving average method of Stephan (1977). Tests for normality and homogeneity of variance of data were performed using Shapiro-Wilk's test and Bartlett's test, respectively (Gulley *et al.* 1989). Analysis of variance (ANOVA) and Dunnett's multiple range test were used to detect differences between control and treatment survival means (Gulley *et al.* 1989). Bonferroni's t-Test was used to detect differences between control and treatment means of growth and reproductive endpoints (Gulley *et al.* 1989). Regression analysis (*SAS* 1989) was used to determine the magnitude of the relationships between sediment copper concentrations and pore water and overlying water copper. The 5% alpha level was used in all statistical tests.

Results and Discussion

Water-Only Experiments

Survival of organisms in controls in all water-only experiments ranged from 80 to 100%. LC_{50} and NOEC values were based on copper concentrations measured at the start of each experiment. Copper concentrations measured at experiment termination were, on average, 83 ± 16.8% of initial copper concentrations in all tests. As shown in Table 1, low hardness values (6-10 mg/L as $CaCO_3$) may have resulted in a "worst case" copper exposure, as increasing water hardness and associated carbonate alkalinity are thought to reduce the acute toxicity of copper (USEPA 1980; Gauss *et al.* 1985).

In water-only experiments, *C. dubia* was the most sensitive organism tested, followed in decreasing sensitivity by *P. promelas, D. magna, H. azteca,* and *C. tentans* (Table 2). LC_{50} values for *C. dubia* exposed to copper in water-only experiments decreased through 7 d of exposure, but then slightly increased in the longer duration tests of 10 d and 14 d. A mortality threshold within 7 d of exposure was observed for *C. dubia* exposed to aqueous copper. *D. magna* responded to aqueous copper predominantly within the first 96 h of exposure, resulting in a mortality threshold at 96 h. No appreciable increase in *D. magna* mortality was observed after 96 h of aqueous copper exposure. *P. promelas* survival decreased through 10 d of exposure but increased slightly at 14 d. The majority of *P. promelas* mortality was manifested after 7 to 10 days of exposure to aqueous copper. LC_{50} values for *H. azteca* exposed to aqueous copper were 5 to 8 times higher than *P. promelas* and *D. magna* LC_{50} values. The 10 d LC_{50} value obtained in this study (67.2 μg/L) was similar to the 10 d LC_{50} value reported for *H. azteca* by Cairns *et al.* (1984) of 59 μg/L. *H. azteca* did not achieve a mortality threshold through 14 d of exposure. *C. tentans* LC_{50} values were at least 10 times greater than the LC_{50} values for other species tested. *C. tentans* survival in water-only copper exposures increased (rather than decreased) through time, illustrating the ability of this organism to sequester copper and reduce copper toxicity.

Sediment Experiments

Control survival in all sediment experiments ranged from 85 to 100%, indicating that formulated sediment provided a suitable substrate for all test species. LC_{50} and NOEC values were based on copper concentrations at the start of each experiment which were, on average, 107 ± 22% of measured copper concentrations at test termination. The effects of the test sediment on water quality of the UMBFS control water were apparent, resulting in increased alkalinity, hardness, and conductivity of the overlying water compared to water-only experiments (Table 1), with anticipated concomitant effects on copper speciation.

Ceriodaphnia dubia

In general, *C. dubia* was the most sensitive organism examined, and responded to copper-contaminated sediment predominantly within the first 96 h of exposure. LC_{50} values based on both overlying water and pore water increased slightly in the 96 h test compared to the 48 h test, but otherwise remained constant through 14 d of exposure (Table 2). Based on sediment concentrations, LC_{50} values decreased ten times from the 48 h test (129 mg/kg) to the 96 h test (36 mg/kg), but remained relatively constant thereafter. *C. dubia* reproduction, expressed as the total number of offspring per female, was less sensitive than survival in the 7 d and 10 d tests, but was more sensitive than survival in the 14 d test. Similar to the water-only tests, sediment test data showed that a 96 h exposure duration was sufficient to elicit mortality in this species when exposed to copper.

Daphnia magna

Test duration had little effect on survival of *D. magna* exposed to copper-contaminated sediment (Table 2). LC_{50} values for overlying water were similar in all tests. LC_{50} values based on pore water and sediment copper concentrations decreased four times from the 48 h test to the 96 h test. However, pore water and sediment LC_{50} values did not decrease at 7 d, 10 d, or 14 d of exposure. As in the water-only tests, *D. magna* achieved a mortality threshold at 96 h of exposure. The 48 h LC_{50} values for overlying water (7.25 μg/L) and sediment (170 mg/kg) determined in this study were four times lower than 48 h LC_{50} values of 30 μg/L in overlying water and 681 mg/kg in sediment reported for *D. magna* exposed to a copper-amended sediment (Cairns *et al.* 1984).

Pimephales promelas

P. promelas response to copper-contaminated sediment showed a general decrease in survival with increasing exposure duration (Table 2). LC_{50} values based on overlying water and sediment concentration decreased through 10 d of exposure, but increased at 14 d. LC_{50} values based on pore water concentrations decreased through time, from 69 μg/L at 48 h to 36 μg/L at 14 d. *P. promelas* growth (expressed as dry weight) was equally sensitive as survival in the 10 d test, but in tests of 14 d duration, NOEC values for growth were two times greater than for survival.

Hyalella azteca

H. azteca response to copper-contaminated sediment resulted in decreasing survival with increasing test duration through 14 d of exposure (Table 2). No threshold for mortality was achieved for *H. azteca* exposed to copper-contaminated sediment through 14 d of exposure. Test durations of at least 14 d may be required to accurately assess the toxicity of sediment-sorbed copper to *H. azteca*. The 10 d LC_{50} values reported in this study of 35 μg/L in overlying water for *H. azteca* were similar to 10 d LC_{50} values reported by Cairns *et al.* (1984) of 39 μg/L with copper-amended sediments. LC_{50} values based on sediment copper concentrations, however, were considerably different, ranging from 262 mg/kg in this study to 1,078 mg/kg by Cairns *et al.* (1984). The variability in LC_{50} values between studies based on sediment concentrations reflects the influence of sediment characteristics on copper bioavailability.

Chironomus tentans

C. tentans survival decreased through time when exposed to copper-contaminated sediment (Table 2). *C. tentans* growth, expressed as dry weight, was considerably more sensitive than survival in the 10 d test (Table 3). In this study, no temporal threshold for mortality or growth was achieved for *C. tentans* exposed to copper-contaminated sediment for up to 14 d of exposure. *C. tentans* survival in sediment tests decreased through time, whereas survival in water-only tests increased through time. Test durations of at least 10 d may be required to accurately assess the toxicity of sediment-sorbed copper to *C. tentans*. Conducting laboratory evaluations with *C. tentans* with an insufficient test duration (*i.e.*, ≤10 d) or insensitive endpoint (*i.e.*, survival) may lead to erroneously concluding that a sediment is not toxic.

The 10 d LC_{50} values reported here of 36 μg/L and 1,026 mg/kg (overlying water and sediment, respectively) for *C. tentans* exposed to a copper-contaminated sediment were similar to 10 d LC_{50} values of 38 μg/L and 857 mg/kg reported for *C. tentans* exposed to a copper-amended sediment (Cairns *et al.* 1984). Cairns *et al.* (1984) concluded that the route of exposure was via the overlying water, but based this conclusion on water-only data for *H. azteca* and not *C. tentans* data. If exposure duration and test endpoint are properly selected (*i.e.*, 10 d duration or greater), *C. tentans* sensitivity to this copper-contaminated sediment approaches that of the other organisms examined.

Sediment Experiments—Relative Sensitivities, Test Duration, and Endpoints

Relative sensitivities of the organisms examined in this study when exposed to a copper-contaminated sediment were depen-

Table 3. Comparison of no observed effects concentrations (NOECs) for lethal and sublethal endpoints of test organisms exposed to copper-contaminated sediment

			No observed effects concentration (NOEC)		
Organism	Test duration	Endpoint	Water (µg Cu/L)	Pore water (µg Cu/L)	Sediment (mg Cu/kg dry wt)
C. dubia	7 d	Survival	3.7	79.9	18.1
		Reproduction	14.1	163	52.5
	10 d	Survival	3.7	79.9	18.1
		Reproduction	9.6	132	45.9
	14 d	Survival	3.7	79.9	18.1
		Reproduction	3.2	48.9	11.9
P. promelas	10 d	Survival	8.6	42.8	136.9
		Growth (dry wt)	8.6	42.8	136.9
	14 d	Survival	15.1	20.2	129.3
		Growth (dry wt)	>32	>52	>461
C. tentans	10 d	Survival	22.9	36.1	>>216
		Growth (dry wt)	<21.6	<16.3	<216

dent upon test duration and test endpoint (Table 3). Based on copper concentrations measured in sediment, *H. azteca* and *C. tentans* are more sensitive than *P. promelas,* with significant effects on survival and growth observed for *H. azteca* at 193 mg/kg (14 d), for *C. tentans* at 216 mg/kg (10 d), and for *P. promelas* at 259 mg/kg (14 d). As in the water-only experiments, *C. dubia* was again the most sensitive organism based on sediment concentrations, with adverse impacts detected at concentrations as low as 18.1 mg/kg (Table 3).

Organism physiology plays an important role in organisms' sensitivities to copper in sediment. Chironomid larvae regulate accumulation of copper, nickel, and zinc in their tissues when exposed to these metals in sediments (Krantzberg and Stokes 1989). Metal-binding proteins (*e.g.*, metallothioneins) act as sinks or sequester metals such as zinc, copper, cadmium, and mercury in organism tissues (Petering and Fowler 1986; Fowler 1987; Olsson and Haux 1986). Increased exposure to copper resulted in increased mortality and reduced growth, with no lower threshold observed, indicating that a minimum exposure of 7 d to copper is required to manifest an adverse response in *C. tentans.*

The effect of test duration and endpoint on test results were species-dependent (Table 3). In general, the cladocerans, *C. dubia* and *D. magna,* responded to the copper-contaminated sediment within 96 h of exposure, demonstrating a mortality threshold at 96 h of exposure. *C. dubia* reproduction was more sensitive than survival only after 14 d of exposure, indicating that at least 14 d of exposure may be required to adversely affect reproduction. *P. promelas* growth in 10 and 14 d tests was equal to or less sensitive than survival, even though tests were started with 2-4 d old fry. *H. azteca* and *C. tentans* survival both decreased through time, with no mortality threshold being achieved through exposure for either organism. Laboratory sediment tests of at least 10-14 d duration were required to accurately assess the potency of a copper-contaminated sediment to *H. azteca* and *C. tentans.* In this study, *C. tentans* growth as dry weight was more sensitive than survival.

A regression analysis was performed to evaluate the relationship between copper concentrations in sediment and copper concentrations in pore water and overlying water in sediment tests (Figure 1). Both pore water and overlying water copper concentrations were highly dependent on the concentration of copper in sediment (r^2 = 0.94 and 0.97, respectively). For a given sediment copper concentration, pore water concentrations of copper varied up to 30 times, whereas overlying water concentrations varied less than three times. In these experiments, the relationship between copper in sediment and overlying water was more predictable than the relationship between copper in sediment and pore water. Even though highly significant r^2 values were obtained for the relationships between copper concentrations measured in sediments and those measured in either overlying water or pore water, the factor of three to 30 difference in variability for a given sediment concentration is sufficient to encompass from 100% survival to 100% mortality for the organisms examined in this study. Thus, measuring copper concentrations in sediment or pore water would only provide presumptive evidence of sediment toxicity.

To gain some insight into the source of copper toxicity, LC_{50} values from water-only tests for each organism were compared to concentrations of copper in overlying water, pore water, and sediment in sediment tests (Table 2). For *C. dubia, D. magna, P. promelas,* and *H. azteca,* toxicity observed in copper-contaminated sediment tests was presumed to be associated with copper in the overlying water, since copper concentrations in overlying water in sediment tests were similar to copper concentrations eliciting the same responses in water-only tests. For *C. tentans,* mortality corresponded with copper concentrations in sediments rather than overlying water or pore water (Table 2). These results reflect the physiological differences as well as differing routes of exposure between test organisms examined in this study.

Sediment Experiments—Risk Characterization

Laboratory toxicity tests are frequently used to determine the presence of toxicity (screening tests) and degree of toxicity (definitive tests) of sediments. *D. magna* and *C. dubia* 96 h tests were suitable as screening level tests in this study due to the immediate response of these organisms to copper exposure in sediment and water, but did not provide information regarding the degree of toxicity (Table 3). *H. azteca* and *C. tentans* were not as sensitive to copper during 48 and 96 h tests. *H. azteca* survival and *C. tentans* growth in 10 and 14 d tests were

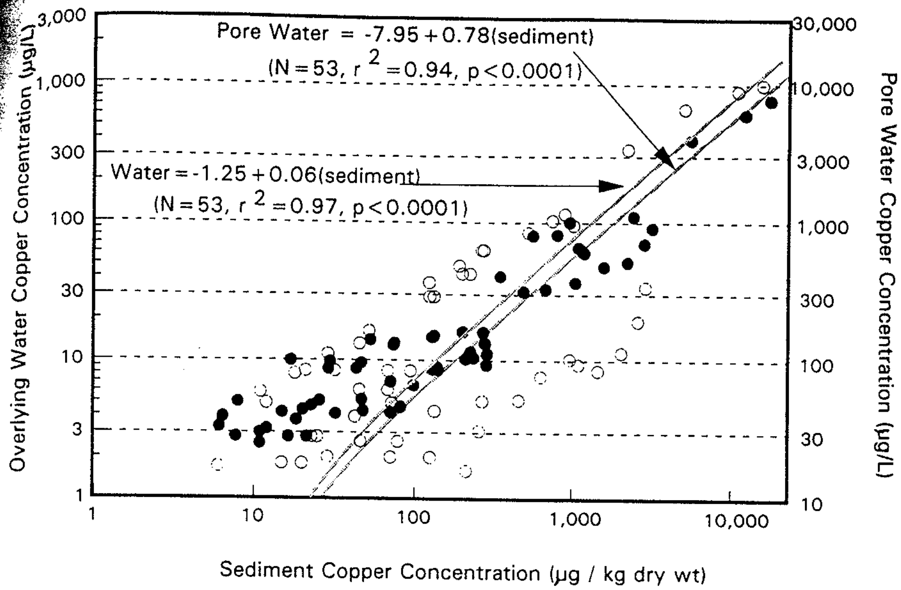

Fig. 1. Relationship between concentrations of copper in sediment and concentrations of copper in pore water (open circles) and overlying water (closed circles) in sediment tests

suitable for determining the degree of copper toxicity in water and sediment tests. The 10 d *C. tentans* growth test and the 14 d *C. dubia* reproduction test were particularly useful for assessing sediment potency because they provided a more continuous response than mortality, which provided only a quantal, or all-or-nothing response. The exposure-response relationship developed from these two tests could be used to estimate to what degree a sediment may be above or below the threshold of toxicity.

This study provided evidence that these test species were useful for assessing copper toxicity in sediment. The species examined occupy different niches in aquatic systems. For example, daphnids are generally considered water column organisms, but *D. magna* ⩾48 h old actively graze on sediment surfaces. *C. dubia* also has been observed in this study to feed on sediments by rapidly striking the sediment surface to suspend sediment surface particles. *H. azteca* is an epibenthic detritivore, which feeds on organic material on the sediment surface and on epiphytes of rooted aquatic vegetation. *C. tentans*, however, is a benthic infaunal organism, which during its larval stage, constructs a case from organic material in sediment. By using several organisms that represent a variety of niches, the likelihood of making an erroneous conclusion regarding sediment toxicity can be reduced. When this is not possible, one should use caution in extrapolation of laboratory results to field situations.

Summary

Effects of experimental conditions including exposure duration, test organism selection, and test endpoint on the observed toxicity of aqueous phase copper and a copper- contaminated freshwater sediment were evaluated. Relative sensitivities of test organisms exposed to copper in water and copper-contaminated sediment varied with test duration and test endpoint. In general, *C. dubia* was the most sensitive organism tested, followed in decreasing sensitivity by *D. magna*, *P. promelas*, *H. azteca*, and *C. tentans*. Effects of test duration on copper toxicity were most pronounced for *H. azteca* and *C. tentans*, with mortality and growth effects becoming increasingly sensitive with increasing test duration. Formulated sediment served as a suitable control and reference sediment in this study, matching characteristics (except redox potential) of the test sediment.

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References

Ankley GT, Mattson VR, Leonard EN, West CW, Bennett JL (1993) Predicting the acute toxicity of copper in freshwater sediments: Evaluation of the role of acid volatile sulfide. Environ Toxicol Chem 12:315–320

Burton GA Jr, Stemmer BL, Winks KL, Ross PE, Burnett LC (1989) A multitrophic level evaluation of sediment toxicity in Waukegan and Indiana Harbors. Environ Toxicol Chem 8:1057–1066

Burton GA (1991) Assessing the toxicity of freshwater sediments. Environ Toxicol Chem 10:1585–1627

Cairns MA, Nebeker AV, Gakstatter JH, Griffis WL (1984) Toxicity of copper-spiked sediments to freshwater invertebrates. Environ Toxicol Chem 3:435–445

Campbell PGC, Stokes PM (1985) Acidification and toxicity of metals to aquatic biota. Can J Fish Aquat Sci 42:2034–2049

de March BGH (1981) *Hyalella azteca* (Saussure). In: Lawrence SG (ed) Manual for the Culture of Selected Freshwater Invertebrates. Can Spec Publ Fish Aquat Sci 54:61–77

Di Toro DM, Mahoney JD, Hansen DJ, Scott KJ, Carlson AR, Ankley GT (1992) Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. Environ Sci Technol 26:96–101

Fowler BA (1987) Intracellular compartmentation of metals in aquatic organisms: Roles in mechanisms of cell injury. Environ Health Persp 71:121–128

Gauss JD, Woods PE, Winner RW, Skillings JH (1985) Acute toxicity of copper to three life stages of *Chironomus tentans* as affected by water hardness-alkalinity. Environ Pollut Series A 37:149–157

Gulley DD, Boelter AM, Bergman HL (1989) Toxstat, Release 3.0. University of Wyoming, Laramie, WY

Ingersoll CG, Dwyer FJ, May TW (1990) Toxicity of inorganic and organic selenium to *Daphnia magna* (Cladocera) and *Chironomus riparius* (Diptera). Environ Toxicol Chem 9:1171–1181

Ingersoll CG, Nelson MK (1990) Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera). In: Landis WG, van der Schalie WH (eds) Aquatic Toxicology and Risk Assessment: Thirteenth Volume, ASTM STP 1096. American Society for Testing and Materials, Philadelphia, PA, pp 93–109

Krantzberg G, Stokes PM (1989) Metal regulation, tolerance, and body burdens in the larvae of the genus *Chironomus*. Can J Fish Aquat Sci 42:389–398

LeBlanc GA, Suprenant DC (1985) A method of assessing the toxicity of contaminated freshwater sediments. In: Cardwell RD, Purdy R, Bahner RC (eds) Aquatic Toxicology and Hazard Assessment: Seventh Symposium, ASTM STP 854. American Society for Testing and Materials, Philadelphia, PA, pp 269–283

Leckie JO, Davis JA III (1979) Aqueous environmental chemistry of copper. In: Nriagu JO (ed) Copper in the Environment, Part I: Ecological Cycling. Wiley-Interscience, NY, pp 89–121

Lewis AG (1992) The biological importance of copper: A literature review. Contractor report for the International Copper Association, New York, NY

Malueg KW, Schuytema GS, Krawczyk DF (1986) Effects of sample storage on a copper-spiked freshwater sediment. Environ Toxicol Chem 5:245–253

Olsson P, Haux C (1986) Increased hepatic metallothionein content correlates to cadmium accumulation in environmentally exposed perch (*Perca fluviatilis*). Aquat Toxicol 9:231–242

Peltier WH, Weber CI (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. USEPA Rept. No. EPA/600/4-85/013. EMSL, Cincinnati, OH

Petering DH, Fowler BA (1986) Discussion summary: Roles of metallothionein and related proteins in metal metabolism and toxicity: Problems and perspectives. Environ Health Persp 65:217–224

SAS Institute (1989) SAS User's Guide: Statistics, Version 6 edition. Cary, NC

Stephan CE (1977) Methods for calculating an LC_{50}. In: Mayer FL, Hamelink JL (eds) Aquatic Toxicology and Hazard Evaluation, STP 634. American Society for Testing and Materials, Philadelphia, PA, pp 65–84

Suedel BC, Rodgers JH Jr (1993) Bioavailability of fluoranthene in freshwater sediment toxicity tests. Environ Toxicol Chem 12:155–165

Suedel BC, Deaver E, Rodgers JH Jr (1995) Formulated sediment as a reference and dilution sediment in definitive toxicity tests. Arch Environ Contam Toxicol 30:40–46

Townsend BE, Lawrence SG, Flannagan JF (1981) *Chironomus tentans* Fabricius. In: Lawrence SG (ed) Manual for the culture of selected freshwater invertebrates. Can Spec Publ Fish Aquat Sci 54:109–126

U.S. Environmental Protection Agency (1980) Ambient water quality criteria for copper, EPA 440/5-80-036. Criteria and Standards Division, Washington, DC

Wentsel R, McIntosh A, Atchison G (1978) Evidence of resistance to metals in larvae of the midge *Chironomus tentans* in a metal-contaminated lake. Bull Environ Contam Toxicol 20:451–455

Winner RW (1988) Evaluation of the relative sensitivities of 7-d *Daphnia magna* and *Ceriodaphnia dubia* toxicity tests for cadmium and sodium pentachlorophenate. Environ Toxicol Chem 7:153–159