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Effects of Copper-Contaminated Sediments on *Hyalella azteca, Daphnia magna,* and *Ceriodaphnia dubia:* Survival, Growth, and Enzyme Inhibition

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Abstract. The results of two newly developed sediment toxicity tests were compared to the standard, 48-h acute *Daphnia magna* and *Ceriodaphnia dubia* tests. The 14-day *Hyalella azteca* growth inhibition test is a definitive test of chronic exposure to toxic sediments. The rapid, fluorescent *D. magna* test, which is based on *in vivo* inhibition of enzymatic processes, has been suggested as a rapid screening tool. *H. azteca* mortality was the least sensitive bioassay endpoint, while *H. azteca* growth, *D. magna* fluorescence, *C. dubia* mortality, and *D. magna* mortality were equally sensitive to the effects of sediments spiked with $CuSO_4$. In sediments collected in copper-contaminated lakes, the standard, acute 48-h *D. magna* bioassay was the least sensitive test, while the *D. magna* fluorescence test and *H. azteca* growth reduction test were the most sensitive bioassays.

Sediment toxicity tests are an important part of the procedures used to assess the ecological effects of contaminated sediments. An approach that uses a battery of tests has been prescribed as an appropriate method for assessing sediment toxicity (Giesy and Hoke 1989). This research examines two new toxicity test methods for possible inclusion in a battery of tests: the 14-day *Hyalella azteca* growth reduction bioassay, and the rapid, fluorescent *Daphnia magna* toxicity test.

Current batteries of sediment toxicity tests do not include many benthic species. The freshwater amphipod *H. azteca* is an epibenthic species suitable for sediment toxicity testing (Nebeker *et al.* 1984; Borgmann and Munawar 1989) because it is: 1) sensitive to contamination (Schubauer-Berigan *et al.* 1993; West *et al.* 1993); 2) easy to rear in laboratory cultures (Borgmann *et al.* 1989); 3) it typically survives (>80%) in control sediments during experiments (Nebeker *et al.* 1984); and 4) its entire life cycle is aquatic, with individuals reaching maturity in approximately 30 days at 23°C (Geisler 1944).

Most bioassays require at least 24 h to complete, and several tests require 10 or even 28 days. Thus, sediment toxicity assessment of a water body may require weeks to months. The rapid, fluorescent *D. magna* toxicity test may allow at least preliminary sediment toxicity assessments to be completed within a few hours to a few days. The basis of the fluorescent *D. magna* test is the ingestion and enzymatic hydrolysis of 4-methylumbelliferyl-b-D-galactoside (MUF-G) to yield the dye 4-methylumbelliferone (MUF), which is fluorescent in alkaline solutions (Elnabarawy *et al.* 1988; Janssen and Persoone 1993). This enzymatic reaction produces individuals that fluoresce under 385 nm ultraviolet light. The endpoint of this test is quantal (fluorescent: not fluorescent) within 60 min, and has been calibrated to 48 h LC_{50} values for several compounds to *D. magna* (Janssen and Persoone 1993).

The objectives of this research were to: 1) compare the sensitivities of the *H. azteca* toxicity test and the fluorescent *D. magna* toxicity test to the standard, 48 h *D. magna* and *C. dubia* toxicity tests; and 2) to compare the toxicity of laboratory-spiked soil to the toxicity of sediments collected from the field. Copper was chosen as the toxicant for these experiments because: 1) the literature has information on copper toxicity for many species, which allows comparison of these results to other research; and 2) Michigan's copper mining district provides copper-contaminated sediments that can be used for field-laboratory comparisons.

Materials and Methods

Sediment Spiking

Soil was obtained from a clean site near Florissant, Missouri, in April, 1989 and stored in a barn at Michigan State University, East Lansing,

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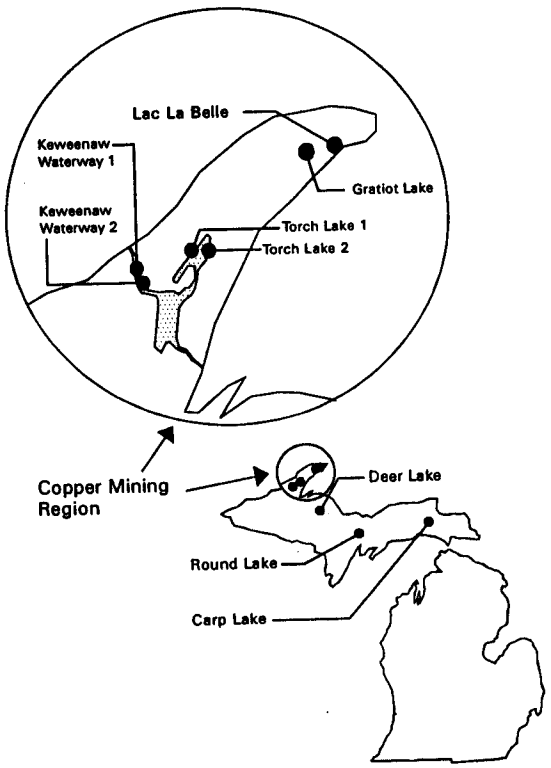

Fig.1. Map of Michigan showing the locations of sediments representing a range of copper contamination. Samples ofthese sediments were tested for toxicity to *Daphnia magna, Ceriodaphnia dubia,* and *Hyalella azteca*

Michigan. This soil was chosen for spiking studies because it was well-characterized, readily available, and easily stored. The soil was 8% sand, 26% clay and 66% silt (USDA classification = silt-loam) and contained 39% H_2O (by weight) and 1% total organic carbon. The mean PAH concentration was ≤15 ng/g dry soil; total PCB = 30 ng/g dry soil. Metal concentrations in the dry soil were 0.24 mg Cd/g, 18.0 mg Cr/g, 14.6 mg Pb/g, 0.07 mg Hg/g, 18.4 mg Ni/g, 44.4 mg Zn/g, 8.35 mg As/g, and 10.4 mg Cu/g (Ingersoll and Nelson, US Fish & Wildlife Service, Columbia, MO, personal communication). Spiking solutions were made with reagent grade $CuSO_4 \cdot 5H_2O$ (JT Baker) in analytical-grade water. The nominal concentrations of the spiking solutions were 0, 45, 67, 100, 150, and 225 mg Cu/L. Three kilograms of homogenized soil were combined with 12 L of each spiking solution in a 20 L polyethylene bucket (pre-rinsed with 10% v/v HCl), and mixed with a stainless steel spoon. When a visibly homogeneous mixture was achieved, an additional 3 kg of soil were added and re-mixed to a uniform consistency. The spiked soils were stored at 25°C for four months, and stirred every third week to facilitate equilibrium. The partitioning of Cu between soil, overlying water, and pore water are discussed elsewhere (Leweke 1993).

Field Sediment Collection

Sediments from nine locations in Michigan (Figure 1) were selected for the field testing component of this study. These locations encompass a range of copper concentrations from relatively uncontaminated locations for reference samples, to water bodies with sediments comprised primarily of stamp mill tailings. Gratiot Lake has the smallest concentration of copper (52 mg/kg dry sediment) in the surficial (0-10 cm depth) sediments (Evans *et al.* 1991b). Carp (Big Trout) and Round Lakes have slightly higher surficial sediment copper concentrations of approximately 123 and 142 mg/kg, respectively (Evans *et al.* 1991b). Deer Lake sediment has 203 mg/kg copper (Evans *et al.* 1991b), but is significantly enriched with up to 16 mg/kg mercury (Evans *et al.* 1991a). Lac La Belle had a brief history of copper mining, milling, and smelting, and contains 390 mg/kg copper in the surficial sediments (Evans *et al.* 1991b). The Keweenaw Waterway had numerous copper stamp mills and smelters along its shores. We chose two locations along the waterway: Keweenaw Waterway #1 was located near smelter tailings on the shore, while Keweenaw Waterway #2 was located away from visible tailings. Torch Lake received an estimated 200 million tons of stamp sand tailings that filled approximately 20% of the lake's original volume (Markham 1985). We also chose two locations within Torch Lake: Torch Lake #1 was located near a closed smelter and stamp mill, where the sediments overlay tailings, and Torch Lake #2 was in the open basin of the lake, at least two kilometers from visible tailings.

Sediment samples were collected with a petite ponar dredge between August 15 and 19, 1992. Samples were stored in acid rinsed (10% v/v HCl) 12 L polyethylene buckets with minimum head space. Sediment buckets were stored in coolers during sampling, then refrigerated at 4°C for two weeks prior to toxicity testing and chemical analyses.

Pore Water Extraction

Pore water for chemical analyses and toxicity testing was separated from the sediments by centrifugation (Giesy *et al.* 1988). To prepare the spiked sediments, the overlying water (spiking solution) was decanted and the sediment was stirred with a stainless steel spoon to a homogeneous consistency. Field-collected sediments were also homogenized. Aliquats of sediment were centrifuged in acid-rinsed, 250 mL polycarbonate bottles at 6730 g for 60 min (at 4°C). The supernatant pore water was decanted into acid-washed 500 mL polyethylene bottles and stored for less than 7 d in the dark at 4°C until used for bioassays or chemical analyses.

Metal Analysis

Pore water samples were filtered through 0.45 mm Acrodisc® PVDF (Gelman) membrane filters, preserved with 1% (v/v) HNO_3, and analyzed with a Hitachi® model 180-80 atomic absorption spectrophotometer (AAS) with polarized Zeeman background correction. Copper concentrations greater than 100 mg/L were determined by flame AAS; all other samples were quantified by graphite furnace AAS. Quality assurance/quality control (QA/QC) procedures included frequent calibration to standards, duplicate analyses, and recovery of EPA quality control standard ICAP19 (EMSL, Cincinnati, OH) and NIST standard reference material 1643C (Gaithersburg, MD). Standard recoveries ranged from 80-100%, and relative percent differences for duplicate analyses of reference materials were <10%. Detection limits were 12.7 mg/L for flame AAS and 0.6 mg/L for graphite furnace AAS.

Laboratory Cultures

Cultures of 120 adult *H. azteca* were reared in glass quart jars containing six 5 cm × 10 cm pieces of presoaked cotton gauze (3 mm mesh cheesecloth). Cultures were aerated and fed 20 mg of Tetra Min® (TetraWerke, Melle, Germany) flake food daily. Cultures were sieved every 7 d to remove the young for bioassays and to start new cultures. During the sieving process, 25-30% of the water was replaced, and gauze was added to keep a relatively constant volume. Amphipod

The mechanisms controlling the bioavailability of copper in freshwater sediments are still unclear. There is evidence that (cold acid extractable, or "acid-volatile) metal sulfides bind divalent metals and make them less bioavailable. If the molar ratio of cold acid extractable metal:sulfide (SEM/AVS ratio) is less than 1.0, then no "free" metal is expected to be bioavailable (Di Toro *et al.* 1990). Copper bioavailability also appears to be influenced by dissolved organic carbon (Allen *et al.* 1993). In this study, the SEM/AVS ratios for copper ranged from 0.4 to 26,000 in these samples and did not accurately predict toxicity. This result has been reported by other researchers (Ankley *et al.* 1993) who found a better correlation between copper concentrations in filtered pore water and toxicity. Filtered pore water copper concentrations that were an order of magnitude greater than the spiked sediment LC_{50} values (also for copper in filtered pore water) were not consistently acutely toxic to any of the species tested. Differences in pH can explain variability in metal toxicity (Schubauer-Berigan *et al.* 1993), where a 10-fold change in $[H^+]$ (one pH unit) produced a 10-fold change in water-only LC_{50} values. In this study, pH differences were not great enough to explain the observed differences in toxicity. Observations made during these experiments and others (Fu *et al.* 1992; Allen *et al.* 1993) suggest that humic materials $\leq$0.45 mm in size were contributing to copper bioavailability in these samples.

Conclusions

1) Both the *D. magna* test based on enzyme inhibition and the *H. azteca* test based on growth were useful for assessing sediment toxicity and more sensitive than the standard 48 h *D. magna* and *C. dubia* tests. 2) Spiking studies did not necessarily predict toxicity in field-collected sediments, even when the same substance, copper, was the causative agent. 3) The greater sensitivity and rapidity of the *D. magna* fluorescence test makes it a potentially useful screening tool for identifying nontoxic sediments in a tiered testing approach. Because this test has demonstrated a tendency to overestimate the toxicity of field-collected samples, sediments that are identified as toxic by this technique should be verified by a battery of sediment toxicity tests. 4) Amphipod growth is a more sensitive bioassay endpoint than lethality. Bioassays that use growth reduction of *H. azteca* as a measurement of toxicity should be further developed.

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cultures were reared at 25°C (± 1°C) under a 16 h light: 8 h dark regimen. Light was supplied by two 20-watt cool white fluorescent bulbs (General Electric, Cleveland, OH) 40 cm above the cultures with an intensity of 23 $\mu E\ m^{-2}\ sec^{-1}$. The culture water was a mixture of 30% well water and 70% Barnstead E-pure® (Dubuque, IA) with a hardness of 94 mg $CaCO_3$/L, an alkalinity of 104 mg $CaCO_3$/L, and a pH of 8.2.

Cultures of *D. magna* and *C. dubia* were grown in brood boards containing one adult female per chamber. The chambers were 30 mL polystyrene (Beverage Ware®, St. Paul, MN) food service cups. The brood boards were covered with 5 mm thick clear plastic sheets to retard water evaporation. The cultures were fed 100 mL yeast-cerophyll-trout chow (YCT) mixture (USEPA 1985), and 100 mL *Selanastrum capricornutum* algae daily. The cladocerans were grown in culture water comprised of 90% Barnstead E-pure® water and 10% bottled Perrier® (Vergeze, France) water with a hardness of 39 mg $CaCO_3$/L, an alkalinity of 45 mg $CaCO_3$/L, and a pH of 8.0. Cultures were reared at 25°C (± 1°C) under a 16 h light: 8 h dark regimen. Light was supplied by two 20-watt cool white fluorescent bulbs 20 cm above the cultures for an intensity of 32 $\mu E\ m^{-2}\ sec^{-1}$. The neonates were removed every 24 h to produce individuals of known age for toxicity testing. New brood boards were initiated weekly (with neonates) and maintained for 21 and 14 d, respectively, for *D. magna* and *C. dubia*.

Toxicity Tests

Hyalella azteca bioassays used 3 to 10-d old juveniles and lasted 14 d. Juvenile amphipods were collected from cultures with sieves and held in a separate jar for 72 h to allow them to recover from handling stress. The amphipods molt during this period, and control survival increases from 80% to ≥ 90% (Kubitz 1994). Five replicate groups of 5 animals were added to 30 mL polyethylene (SOLO®, Chicago, IL) cups that contained 5 g sediment and 20 mL culture water. Water lost from evaporation was replaced, and 50 mL of a 20 mg/mL suspension of Tetra Min® was added daily to each cup. Survival and dry weight were measured at test termination. Test animals were dried at 60°C for 24 h prior to weighing. Amphipod survival in the reference (Gratiot Lake) sediment was greater than 90%.

Enzyme inhibition (fluorescence) tests were performed with 24- to 48-h-old *D. magna* neonates that had been starved for 24 h (Leweke 1993). Three replicate groups of 6 animals were added to 30 mL polyethylene cups containing 10 mL of pore water. After a one-hour exposure, 100 mL of MUF-G suspension ($1.42\ E^{-2}$ Mol) was added to each cup. The treatments were incubated for 15 min, and the number of animals that were fluorescent under UV light (385 nm) was recorded for each cup. Fluorescence for all test control treatments was greater than 90%.

Standard, 48-h acute, *D. magna* and *C. dubia* tests were performed with ≤24 h old neonates (USEPA 1985). Four replicate groups of 5 animals were added to 30 mL polystyrene cups containing 25 mL of pore water for *D. magna*, and 20 mL pore water for *C. dubia*. All pore water samples were allowed at least 3 h to equilibrate to incubator temperature before use in a test. Air bubbles that formed in the cups due to oversaturation of gasses in the pore water were removed with a Pasteur pipette. Test animals were not fed during exposure. Survival was measured at test termination; control survival was greater than 90%.

Water quality parameters were measured in the pore water samples and overlying water of the bulk sediment bioassay, including alkalinity, hardness, electrical conductivity, pH, dissolved oxygen, temperature, and ammonia. Hardness was measured by the EDTA titrimetric method (with colorimetric endpoint) and total alkalinity by titration with 0.02 N H_2SO_4 (APHA 1985). Dissolved oxygen, pH, and ammonia were measured with ion-selective probes. Acid-volatile sulfide/simultaneously extracted metal (AVS/SEM)(copper only) molar ratios were measured in sediment samples using the methods described by Allen and others (1991). The colorimetric method was used to determine AVS concentrations.

Median lethal concentrations for spiked and field-collected sediments were calculated using the probit method; where the data was not suitable for probit analysis, the trimmed Spearman-Karber method (Hamilton *et al.* 1977) was used. Significant differences among field-collected sediments were calculated using analysis of variance (ANOVA) on rank-transformed data, followed by Dunnett's one-tailed test for *C. dubia*, D. magna, and *H. azteca* survival, and *D. magna* enzyme inhibition. Because amphipods from each sample were pooled for weight measurements, statistical tests could not be performed. Recent experiments have confirmed that *H. azteca* growth reductions ≥50% (of reference, or control weight) with this experimental design are statistically significant (Kubitz 1994). Using this information, sediment samples that reduced *H. azteca* dry weight ≥50% relative to the reference sediment (Gratiot Lake) were considered toxic (Table 1).

Results

In the spiking studies, *D. magna* and *C. dubia* were equally sensitive to the added copper, based on their respective LC_{50} values of 22 and 24 mg Cu/L filtered pore water. *H. azteca* was slightly less sensitive with an LC_{50} of 43 mg/L. The *D. magna* enzyme inhibition test was more sensitive than any of the lethality-based bioassays, with an EC_{50} of 13 mg/L. A valid EC_{50} value could not be calculated for the *H. azteca* growth bioassay because the weight measurements were not replicated (df = 0), but regression analysis (% weight v log [Cu]; $R^2 = 0.94$) predicts that the median effect level for amphipod growth would be 19 mg Cu/L filtered pore water. Both sublethal tests were more sensitive than the either of the lethality-based bioassays.

The median effects values from the spiked sediment experiments were also within the range reported in the literature from similar studies (Table 2). Schubauer-Berigan and others (1993) found that changing the test solution pH by one unit can alter the toxicity of metals to cladocerans by an order of magnitude. Within this context, the responses listed in Table 2 are reasonable.

In the experiments using field-collected sediments, there was no observed difference in lethality between *D. magna*, *C. dubia* and *H. azteca*. Furthermore, lethality was not a sensitive endpoint for any species; survival greater than 90% was often recorded in sediments with filtered pore water copper concentrations greater than the LC_{50} values measured in the spiking studies (Table 1). The sublethal endpoints were more sensitive than lethality; both the *D. magna* fluorescence test and the *H. azteca* growth test routinely produced toxic responses when exposed to samples with greater copper concentrations than their spiked sediment EC_{50} values (Table 1). The sublethal tests were also in agreement as to which sediments were toxic, with the exception of Round Lake, which was "toxic" in the *D. magna* fluorescence and *C. dubia* lethality tests, but "nontoxic" in the *H. azteca* growth test.

The rapidity (1 h) and small sample requirements (60 mL) of the *D. magna* fluorescence test provided an opportunity to perform toxicity tests on dilution series of each lake's sediment pore water. This technique allowed independent EC_{50} values to be calculated for each sample, which show good precision (Table 3). The EC_{50} values from the field-collected samples were typically less (Table 3) than the spiked sediment EC_{50}

Table 3. Comparison of median effects concentrations from field-collected sediments. All concentrations are mg Cu/L filtered pore water. Independent EC_{50} values for *D. magna* enzyme inhibition were calculated from dilution series of pore water from each sample location. Numbers in parentheses are 95% confidence intervals

Location	Pore water pH	*Daphnia magna* EC_{50}
Spiked soil (sediment) pore waters	7.1–7.4	13.1 (9–16)
Gratiot Lake pore water dilution series	6.9	>100% [Cu]
Round Lake pore water dilution series	7.1	2.6 (2.1–3.1)
Carp (Big Trout) Lake pore water dilution series	7.4	2.2 (1.7–2.8)
Keweenaw Waterway #2 pore water dilution series	7.1	3.0 (1.6–3.9)
Keweenaw Waterway #1 pore water dilution series	7.5	3.5 (1.8–4.7)
Torch Lake #2 pore water dilution series	7.4	6.0 (5.1–7.0)
Torch Lake #1 pore water dilution series	7.5	15.2 (10.8–18.7)
Deer Lake pore water dilution series	7.2	1.1 (0.8–1.3)[a]
Lac La Belle pore water dilution series	7.0	64.7 (46.0–152.0)[b]

[a]Presence of mercury in this sediment is a likely source of confounding toxicity
[b]Pore water for this sample was stained by humic acids, which appear to have bound Cu, making it unavailable

copper was bound to "dissolved" (≤0.45 mm) humic materials and was unavailable for biological uptake. The *D. magna* fluorescence test and the *H. azteca* growth test identified most field-collected sediments with filtered pore water copper concentrations below the spiked sediment EC_{50} values as nontoxic (Table 1). The exceptions were Deer Lake, which is contaminated with up to 16 mg/kg mercury (Evans *et al.* 1991a), and Round Lake, which was toxic in the 1 h *D. magna* fluorescence and 48 h *C. dubia* lethality tests.

Discussion

The *D. magna* fluorescence, *H. azteca* survival, and *H. azteca* growth tests all show promise as definitive measures of sediment toxicity. Survival of *H. azteca* in bulk sediment exposures is equivalent to survival of *D. magna* and *C. dubia* in pore water, demonstrating that these three species are equally sensitive. These results support the use of upper water column species, especially cladocerans, for sediment toxicity assessment, provided that a relevant sediment extract such as pore water is used for the exposures. Similarly, the agreement between pore water (*D. magna* and *C. dubia*) and bulk sediment (*H. azteca*) toxicity tests provides empirical evidence that pore water is the route of exposure to copper in sediments. The *H. azteca* growth test is more sensitive than the survival test with the same species, and should be developed further so it may be used to calculate EC_{50} values. The *D. magna* fluorescence (enzyme inhibition) test correlates well with the more definitive *H. azteca* growth test that integrates sediment toxicity over a longer time. The *D. magna* fluorescence test is especially attractive for rapid toxicity assessment, but has exhibited a potential to overestimate sediment toxicity and should not be used as the sole test for measuring sediment toxicity. At this time, the rapid, fluorescent *D. magna* test is only useful as a screening test to identify nontoxic samples. Samples that are toxic in the 1 h *D. magna* fluorescence test should be examined further with a battery of more definitive tests.

We observed a tendency for the 1 h *D. magna* fluorescence test to be most sensitive in field-collected sediment pore waters, less sensitive in spiked sediment pore waters, and least sensitive in water-only exposures. This tendency is not explained by expected differences in copper bioavailability between these matrices. In theory, there are more partitioning matrices and competing ions in sediment pore waters (humic acids, Ca, Fe, Mn, etc.) than in culture water. These factors predict that copper would be most available in pure culture water, less available in spiked inorganic soil, and even less available in organic field-collected sediments. The results of the *D. magna* lethality test, both *H. azteca* tests, and the *C. dubia* test support this theoretical basis for bioavailability. These bioassays indicate that copper was less available in field-collected pore water than in spiked sediment pore water or water-only exposures. In sediment samples from Torch Lake and Lac La Belle, the filtered pore water copper concentrations were greater than the LC_{50} values for *C. dubia*, *D. magna*, and *H. azteca*, yet lethality for these species was not significant, indicating that copper was not available. Copper bioavailability does not, therefore, explain the greater sensitivity of the *D. magna* fluorescence test in pore waters.

The increased sensitivity (lack of fluorescence) observed in pore waters may be caused by interference from substances in the pore water. This interference may be produced in two ways: the uptake of the MUF-G substrate may be decreased, or the MUF-G may be interacting with elements of the pore water matrix and become less available. The MUF-G uptake may be decreased by the presence of other particles in the pore water. Since *D. magna* does not discriminate between particles on a chemosensory basis (DeMott 1986), this species is probably not avoiding MUF-G. The presence of other particles may simply dilute the effective concentration of MUF-G. Alternately, *D. magna* may decrease their feeding activity in pore water relative to clean water. The MUF-G may also interact chemically with substances in the pore water matrix. Possible interactions include: ionization of MUF-G, altering it's ability to be hydrolyzed; absorbance of MUF-G to elements in the pore water, causing it to aggregate or dissolve, and avoid being filtered; and chemical reactions between MUF-G and pore water substances. Microbial uptake and hydrolysis of MUF-G, in competition with the *D. magna*, is unlikely; such activity would have produced fluorescent water, which was not observed. The *D. magna* fluorescence test was also more sensitive to compounds in effluents than in exposures to pure compounds (Janssen *et al.* 1993). Clearly, more basic research is needed to understand the chemical, behavioral, and biochemical factors influencing the uptake and enzymatic hydrolysis of MUF-G by *D. magna*.

Table 1. Summary of results for field-collected sediments. Response values are percentages of reference response. The samples below the dashed line have copper concentrations above the median effect levels measured in the spiked soil (sediment) experiment and are expected to be toxic

Location	Pore water Copper (mg Cu/L)	*D. magna* % of reference Fluor.	*D. magna* % of reference Survival	*C. dubia* % of ref. Survival	*H. azteca* % of reference Survival	*H. azteca* % of reference Growth
Deer Lake	3.3	20.3*	100	100	52.2*	64.1
Gratiot Lake[a]	3.8	100	100	100	100	100
Round Lake	4.6	20.3*	95.0	85.0*	104.3	134.6
Carp (Big Trout) Lake	5.0	96.1	95.0	95.0	100	107.7
Keweenaw Waterway #2	45.6	0*	100	95.0	100	50.6
Lac La Belle	55.7	106.1	100	95.0	108.7	89.7
Keweenaw Waterway #1	186.8	0*	0*	n.t.[b]	39.1*	19.2*
Torch Lake #2	228.7	15.2*	100	35.0*	104.6	28.8*
Torch Lake #1	426.0	0*	95.0	n.t.[b]	91.3	24.4*

*Indicates significantly different from reference location sediment ($p = 0.05$) using analysis of variance, followed by Dunnett's one-tailed test. These sediments are considered toxic

[a]Reference sediment: response arbitrarily set = 100 for comparative purposes

[b]n.t. indicates sample was not tested

Table 2. Comparison of results from these studies‡ to similar published experiments. All copper concentrations, unless otherwise noted, are for mg Cu/L filtered pore water. Numbers in parentheses are 95% confidence intervals

	D. magna 1 h EC_{50}	*D. magna* 48 h LC_{50}	*C. dubia* 48 h LC_{50}	*H. azteca* 10 or 14 d LC_{50}
‡Spiked soil	13 (9–16)	22 (19–25)	24 (21–28)	43 (32–61)
Spiked sediment	—	30 (34–46)[a]	—	39 (18–49)[a]
Water only	230[b]	140[b]	—	31 (28–35)[c]
Water only	36[d]	—	28 (19–41)[e]	24 (4.9–120)[e]
Water only	—	—	200 (150–270)[f]	87 (65–120)[f]
‡Field sediment	n.c.[g]	n.c.[g]	26 (20–35)	n.c.[g]
Field sediment	—	—	27[h]	28 (21–38)[i]

[a]Cairns *et al.* 1984. 10 d bulk sediment exposure; Soap Creek Pond (OR) sediment spiked with $CuCl_2 \cdot 2H_2O$, soluble copper concentration in overlying water; overlying water pH = 6.8

[b]Janssen and Persoone 1993. 1 h, water-only exposure to $CuSO_4 \cdot 5H_2O$

[c]Ankley *et al.* 1993. Water-only exposures to $CuSO_4$

[d]Leweke 1993. 1 h, water-only exposure to $CuSO_4 \cdot 5H_2O$, mean EC_{50} of 5 tests

[e]Schubauer-Berigan *et al.* 1993. Water-only exposures to $Cu(NO_3) \cdot 3H_2O$ pH = 7.0–7.5

[f]Schubauer-Berigan *et al.* 1993. Water-only exposures to $Cu(NO_3) \cdot 3H_2O$ pH = 8.0–8.5

[g]n.c. = not calculated; the lack of response at high concentrations, as seen in Table 3, made data unsuitable for median level calculations

[h]Schubauer-Berigan *et al.* 1993. Keweenaw Waterway (MI) sediment pore water exposure; pH = 7–7.5

[i]Ankley *et al.* 1993. 10 d, Keweenaw Waterway (MI) bulk sediment exposure

values. This difference is not explained by pH (Table 3). This increased sensitivity and unexpected toxicity of Round Lake sediment pore water suggests that factors other than copper were present in the field sediments that affected the outcome of the *D. magna* fluorescence test.

The information from the spiked soil experiments was only partly effective for predicting toxicity in field-collected sediments. When the lethality test endpoints were used, only sediment from the Keweenaw Waterway location #1 was toxic, even though the copper concentrations in filtered pore water from both Torch Lake sediments contained 5–20 times the LC_{50} values for any of the exposed species (Table 1). The field-collected sediments did not emulate the characteristic dose-response relationship for *D. magna* or *H. azteca*, and LC_{50} values could not be calculated for these two species. The *C. dubia* test produced acceptable data for median effects level calculations, and the field sediment LC_{50} for this species (26 mg/L) is comparable to both the spiked sediment LC_{50} (24 mg/L) and the results of other researchers (Table 2). The sublethal endpoints more successfully predicted toxicity in sediments from the field, based on results from the spiking studies. Nearly all field-collected sediment samples with filtered pore water concentrations of copper greater than the median lethal levels in the spiked sediments were identified as toxic by both the *D. magna* fluorescence test and the *H. azteca* growth test. The exception was sediment from Lac La Belle, which had a filtered pore water copper concentration of 55.7 mg/L, four times the 1 h *D. magna* EC_{50}. The pore water from Lac La Belle was yellow, even after filtering, indicating the presence of humic substances. Humic acids can bind metals (Fu *et al.* 1992). A plausible explanation for the lack of toxicity in sediment and pore water from the Lac La Belle sample is that the