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Effects of Copper Sulfate on *Typha latifolia* Seed Germination and Early Seedling Growth in Aqueous and Sediment Exposures

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Abstract. The vascular macrophyte *Typha latifolia* Linnaeus (common cattail) may be a sentinel for evaluating potential phytotoxicity to rooted aquatic macrophytes in aquatic systems. To further evaluate the potential utility of this species, *T. latifolia* seed germination, shoot growth, and root elongation were measured in 7-day aqueous exposures using mean measured aqueous copper concentrations of 10.0, 23, 41, 62, 174, and 402 μg Cu/L, which were $\geq$ 62% of nominal concentrations. Seed germination and seedling shoot growth were not significantly affected by any of these copper concentrations as compared to controls. Mean measured no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) for root elongation were 18.6 μg Cu/L and 35.0 μg Cu/L, respectively. Seven-day sediment tests were conducted by amending uncontaminated sediments with copper sulfate to mean measured concentrations of 7.9, 17.1, 21.0, 51.2, 89.5, and 173.5 mg Cu/kg, which were $\geq$ 84% of nominal concentrations. Seed germination was not significantly different from controls. Mean measured NOEC and LOEC values for seedling shoot growth were 89.5 mg Cu/kg and 173.5 mg Cu/kg, respectively, and mean measured NOEC and LOEC values for root growth were 14.0 mg Cu/kg and 19.7 mg Cu/kg, respectively. These results demonstrate that *T. latifolia* early seedling growth can be utilized for assessing aqueous and sediment toxicity of copper.

The purpose of this series of experiments was to develop a phytotoxicity testing protocol for aqueous and sediment tests using seeds from an aquatic macrophyte, *Typha latifolia* Linnaeus (common cattail). Previously, species of the genus *Lemna* have been used for phytotoxicity testing as surrogates for aquatic macrophytes (Wang 1992). However, these floating aquatic plants have vestigial vascular tissues and would not indicate adverse effects if a test material was active by altering transport in xylem and phloem (Sculthorpe 1985). Similarly, the algal species *Selanastrum capricornutum* Printz has been widely utilized in phytotoxicity testing but may not adequately represent rooted aquatic macrophytes. Although several terrestrial vascular plants have been utilized in phytotoxicity testing for soils, many of these species will not survive saturated soils or inundation (Sculthorpe 1985). Historic emphasis on aquatic animals has led to the general assumption that members of the plant kingdom are less sensitive and results of testing with animals would be protective of aquatic plants (Lewis 1995). To initially evaluate *T. latifolia* as a candidate aquatic macrophyte for aqueous and sediment testing, a series of experiments were conducted to determine the response of this plant to copper.

Aspects of the life history and growth habits of *T. latifolia* make this plant a viable candidate for aquatic phytotoxicity testing (Yeo 1964; Grace and Harrison 1986). Previous studies have been directed toward responses of mature cattails to phytotoxic exposures (Powell *et al.* 1996). However, seed germination and early seedling growth may be the most sensitive stages in development of these plants (Wang and Williams 1988; Boutin *et al.* 1995). Under most conditions *T. latifolia* is a prodigious producer of seeds that can be used to evaluate effects of individual chemicals as well as mixtures, such as effluents, on seed germination. Influences of these chemicals or mixtures on growth and development of the early seedling can be determined as well. This aquatic macrophyte, which grows as an emergent plant in the adult stage, is amenable to both aqueous and sediment testing as a seed or young seedling.

In this series of experiments, *T. latifolia* seeds were exposed for 7 days to aqueous copper sulfate and copper sulfate–amended sediments. Copper sulfate, a standard reference toxicant (Suedel *et al.* 1996), was chosen for this study because of relatively abundant published aquatic toxicity information. Aqueous and sediment exposure concentrations were selected based on current aquatic invertebrate data to make comparisons between the relative sensitivities of *T. latifolia* and invertebrates. To assess phytotoxic responses to copper exposures, seed germination and seedling shoot and root growth were

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measured. The responses of *T. latifolia* in this series of experiments were contrasted with results from other experiments with plants as well as animals.

Materials and Methods

Typha latifolia

Mature *T. latifolia* inflorescences were collected in March 1996 at a rural wetland site in Lafayette County, MS. Flowers were placed in plastic bags, transported to the laboratory, and incubated at 20 ± 1°C until testing. Seeds were separated from bristle hairs by placing them in an Osterizer® blender filled with Milli-Q™ water and blended for 30 s. Seeds that sank to the bottom were considered viable and used for testing (McNaughton 1968).

Aqueous Concentrations of Copper Sulfate

Stock solutions for both aqueous and sediment tests were prepared by dissolving reagent grade cupric sulfate ($CuSO_4 \cdot 5H_2O$) (Fisher Scientific, Pittsburgh, PA) in Milli-Q water. Experimental concentrations (12.5, 25, 50, 100, 200, and 400 μg Cu/L) were prepared by serial dilution of stock solution with spring water, which had been filtered using MFS 0.45 μm polymembrane filters to remove particulates (Rodgers *et al.* 1997). Previous analysis of dilution spring water showed no detectable levels of metals, including copper (Rodgers *et al.* 1997). Hardness and alkalinity of filtered spring water were adjusted with $NaHCO_3$ and $CaCl_2$ (Fisher Scientific) to total hardness of 80 mg/L as $CaCO_3$ and alkalinity of 60 mg/L as $CaCO_3$. Filtered spring water (control) and aqueous copper solutions (40 ml) were placed in 50-ml beakers with three replicates at each concentration. Samples were acidified with redistilled nitric acid (GFS Chemicals, Lot #L507260) to pH 1–2 to determine copper concentrations. Experimental copper concentrations were analyzed using a Buck™ model 200-A flame atomic absorption spectrophotometer according to Standard Methods (APHA 1992). Samples with nominal concentrations ≤ 50 μg Cu/L were evaporated to concentrate the solution to ensure measured concentrations were above analytical detection.

Sediment Concentrations of Copper Sulfate

Sediments characterized by Deaver and Rodgers (1996) were utilized from the same wetland that *T. latifolia* inflorescences were collected. These sediments have been successfully characterized and utilized in previous experiments (Deaver and Rodgers 1996). Sediments were amended with copper sulfate to nominal concentrations of 6.25, 12.5, 25, 50, 100, and 200 mg Cu/kg. Approximately 15 g of control or amended sediments and 35 ml of filtered, adjusted spring water were placed into 50-ml beakers with three replicates at each concentration. Average sediment quality values were as follows: pH 6.0–6.7, redox +57 mV, organic matter 1.68%, organic carbon 0.26%, bulk density 72.95%, sand 75.9%, silt 21.4%, and clay 2.7%.

To measure copper concentrations, sediment samples (> 3.5 g) from each concentration, in duplicate, were placed in aluminum weighboats and dried at 70°C in a drying oven for 24 h. Dried samples were homogenized using a mortar and pestle, and approximately 2 g from each sample were placed into 50-ml beakers. Sediment weight for each sample was recorded. The samples were acidified to pH 1–2 with 10 ml redistilled nitric acid and digested for 5 h at 200°C to extract sediment-associated copper (Plumb 1981). After cooling to room temperature, samples were vacuum filtered (0.45 μm). Acid filtrate was collected and poured into a graduated cylinder, brought to known volume with Milli-Q water, and poured into labeled amber bottles. Samples were analyzed on a Buck model 200-A flame atomic absorption spectrophotometer as prescribed in APHA (1992).

Exposure Procedures

Aqueous and sediment experiments were initiated by adding 15 viable *T. latifolia* seeds to each replicate 50-ml beaker (three replicates/concentration) under 1,500–3,000 Lux fluorescent lighting with a 16 h light/8 h dark photoperiod. Previous experiments have determined that *T. latifolia* requires a 16-h light photoperiod to germinate (Bonnewell *et al.* 1983; Rivard and Woordard 1989). Experiments were conducted at the recommended temperature of 24 ± 1°C (McNaughton 1968). After a 7-day test period, the number of seeds at each concentration that did not germinate were counted, and seedlings preserved in 70% ethanol. Root and shoot lengths (mm) of 10 seedlings from each replicate were measured using a Videometric 150 Image Analyzer (American Innovision) with Videometric software (version 2.1).

Statistical Analysis

Mean root and shoot lengths of seedlings and percent germination of seeds at each measured concentration for each experimental trial ($n = 3$) were calculated. Differences in *T. latifolia* seed germination and early seedling growth were determined by statistically significant differences relative to controls ($p \leq 0.05$). One-way analysis of variance (ANOVA) was performed with Dunnett's multiple range test for significance relative to controls ($p \leq 0.05$) (Zar 1984). If assumptions for a parametric ANOVA were not met, ANOVA on ranks with Kruskal-Wallis was performed. The no-observed-effects-concentration (NOEC) and lowest-observed-effects-concentration (LOEC) for *T. latifolia* root and shoot growth were determined by statistically significant differences relative to the controls. NOEC and LOEC values for each measured endpoint in aqueous and sediment exposures were determined by averaging the NOEC and LOEC values from the three experimental trials according to the mean measured copper concentration.

Results

Aqueous Exposures

In three aqueous experimental trials, 7-day aqueous exposures of copper sulfate to *T. latifolia* seeds significantly affected seedling root elongation. Mean measured NOEC and LOEC for root elongation were 18.6 μg Cu/L and 35.0 μg Cu/L, respectively. There were no observed significant effects on seed germination and seedling shoot growth as compared to controls. Although significant effects on *T. latifolia* seed germination were not observed, a trend toward seed germination inhibition was observed as aqueous copper concentrations increased (Table 1). Mean measured aqueous copper concentrations in this exposure series were 10, 23, 42, 62, 174, and 402 μg Cu/L (≥ 62% of nominal concentrations) and were statistically different from each other ($p \leq 0.05$).

Table 1. Seven-day root and shoot elongation (± SD) and germination (%) of *Typha latifolia* seeds exposed to aqueous copper sulfate (based on three independent experiments; n = 3)

Nominal Concentration (μg Cu/L)	Mean Measured Concentration (μg Cu/L)	Mean Root Length (mm) (± SD)	Mean Shoot Length (mm) (± SD)	Seed Germination (#germ/text#)	Percent Germination %
0.00	0.00	14.3 (0.6)	9.7 (0.4)	126/135	93
12.5	10.0	13.2 (0.6)	9.4 (0.4)	117/135	87
25.0	23.0	12.1 (0.7)	9.6 (0.3)	120/135	89
50.0	41.0	6.8 (0.4)[a]	10.0 (0.4)	114/135	84
100.0	62.0	1.8 (0.03)[b]	10.1 (0.4)	112/135	83
200.0	174.0	0.55 (0.02)[b]	10.2 (0.2)	104/135	77
400.0	402.0	0.18 (0.03)[b]	9.7 (0.3)	107/135	79

[a] Denotes significant differences ($p < 0.05$) between treatment and control values
[b] Denotes significant differences ($p < 0.01$) between treatment and control values

Sediment Exposures

In three amended sediment trials, 7-days exposures of copper sulfate–amended sediment to *T. latifolia* seeds were observed to significantly affect seedling root elongation and shoot growth when compared with controls (Table 3). Mean measured NOEC and LOEC values for root elongation were 14.0 mg Cu/kg and 19.7 mg Cu/kg, respectively. Mean NOEC and LOEC values for seedling shoot growth were 89.5 mg Cu/kg and 173.5 mg Cu/kg, respectively. Percent seed germination was not significantly different from controls. Mean measured sediment copper concentrations were 7.9, 17.1, 21.0, 51.2, 89.5, and 173.5 mg Cu/kg (≥ 84% of nominal concentrations) and were statistically different from each other ($p \leq 0.05$).

Discussion

Due to characteristics of the water used (neutral pH and moderate hardness and alkalinity) (Table 2), the expected dominant copper species in aqueous exposures was the divalent form (Cu^{2+}). Sediment characteristics that affect copper toxicity include pH, organic carbon and other organic ligands, sulfides, and other inorganic binding sites (Flemming and Trevors 1989). Copper was bioavailable to *T. latifolia* in sediment exposures due to limited binding sites associated with low amounts of organic matter and clay. An oxidizing environment was indicated by a positive oxidation-reduction potential in all amended sediment exposures; therefore, acid volatile sulfides were not significant for regulating copper bioavailability.

Overall, *T. latifolia* root elongation was the most sensitive parameter evaluated in this study. Previous studies have proposed that the chief mechanism of copper toxicity to vascular aquatic vegetation is through copper penetration of cell membranes, binding to receptors in chloroplasts, and blocking photosynthetic electron transport (Stauber and Florence 1987). However, in this study there were no indications of chlorosis or etiolation occurring in *T. latifolia* seedling shoots in either aqueous or sediment exposures. It is hypothesized that copper uptake occurred primarily through the roots, inhibiting cell division and cell elongation in the apical and subapical region, respectively, thus eliciting the observed responses.

To compare the relative sensitivity of *T. latifolia* to copper in aqueous and sediment exposures, the results from this series of experiments were contrasted with results from similar plant and animal studies. *Selenastrum capricornutum* and *Chlorella vulgaris* had reported nominal EC50 values (growth inhibition) of 400.0 μg Cu/L and 200.0 μg Cu/L, respectively, in aqueous cupric chloride ($CuCl_2$) exposures. For nominal aqueous exposures to copper sulfate, the reported 7-day EC50 for *Lemna minor* oxygen production was 600.0μg Cu/L (Table 4). Brown and Rattigan (1979) reported a 28-day IC50 (plant damage) of 3,100 μg Cu/L, for *Elodea canadensis* exposed to copper sulfate (nominal copper concentration). Radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), and perennial ryegrass (*Lolium perenne*), terrestrial plants commonly utilized for toxicity testing, had nominal 7-day NOEC values of 260.0 μg Cu/L for root elongation when exposed to aqueous copper sulfate (Table 4). Compared to *S. capricornutum*, *C. vulgaris*, *E. canadensis*, *L. minor*, *R. sativus*, *L. sativa*, and *L. perenne*, *T. latifolia* was generally more sensitive to 7-day aqueous exposures of copper sulfate. Therefore, copper toxicity information on these species would not be adequately protective of *T. latifolia* and potentially other emergent macrophytes.

Sensitivity of *T. latifolia* to copper also differs as compared with aquatic animal species. Responses to copper sulfate–amended sediment include a 7-day LC50 for *Chironomus tentans* of 1,600 mg Cu/kg and a 7-day LC50 of 319 mg Cu/kg for *Hyalella azteca* (Table 5). *T. latifolia* had a 7-day LOEC for root elongation of 19.7 mg Cu/kg and a 7-day LOEC of 173.5 mg Cu/kg for shoot elongation, adverse responses at concentrations well below the 7-day LC50s for *C. tentans* and *H. azteca*. Other animals routinely utilized in toxicity evaluations include *Pimephales promelas* (fathead minnow) and *Ceriodaphnia dubia*. Suedel *et al.* (1996) reported 7-day LC50 values of 1.16 μg Cu/L for *C. dubia* and 8.2 μg Cu/L for *P. promelas* in measured aqueous copper exposures (Table 5). Bishop and Perry (1981) reported a nominal 48-h LC50 of 20.0 μg Cu/L for *Daphnia magna* and aqueous copper sulfate. *T. latifolia* had a 7-day LOEC for root elongation of 35.0 μg Cu/L. Although *C. dubia*, *P. promelas*, and *D. magna* were more sensitive to aqueous copper exposures than *T. latifolia*, such a trend should not be expected for other potential toxicants that could enter aquatic systems. For example, *T. latifolia*

Table 2. Water characteristics of modified spring water[a] in 7-day aqueous and sediment growth experiments with *Typha latifolia* seeds

Parameter	Aqueous Exposures	Sediment Exposures
Temperature (°C)	22.5	22.5
pH	6.6–7.9	6.0–6.2
Dissolved oxygen (mg/L)	8.0–8.1	7.9–8.0
Alkalinity (mg/L as $CaCO_3$)	63–68	66–68
Hardness (mg/L as $CaCO_3$)	74–98	60–68
Conductivity (μmhos/cm)	432–442	435–439

[a] Spring water was filtered (0.45 μm) and adjusted (alkalinity: 60–80 mg/L $CaCO_3$; hardness: 60–80 mg/L as $CaCO_3$)

Table 3. Seven-day root and shoot elongation (± SD) and germination (%) of *Typha latifolia* seeds exposed to copper-amended sediment (based on three independent experiments; $n = 3$)

Nominal Concentration (mg Cu/kg)	Mean Measured Concentration (mg Cu/kg)	Mean Root Length (mm) (± SD)	Mean Shoot Length (mm) (± SD)	Seed Germination (#germ/test#)	Percent Germination (%)
0.00	0.00	14.4 (0.3)	12.9 (0.3)	128/135	95
6.25	7.9	13.5 (0.3)	14.0 (0.4)	135/135	100
12.5	17.1	13.1 (0.4)	13.6 (0.4)	124/135	92
25.0	21.0	10.8 (0.3)	12.8 (0.4)	126/135	93
50.0	51.2	9.4 (0.5)	14.1 (0.6)	130/135	96
100.0	89.5	4.9 (0.4)[a]	2.9 (0.3)[a]	129/135	95
200.0	173.5	0.0 (0)[b]	2.6 (0.1)[b]	110/135	81

[a] Denotes significant differences ($p < 0.05$) between treatment and control values

[b] Denotes significant differences ($p < 0.01$) between treatment and control values

Table 4. Relative sensitivities of aquatic and terrestrial plants to aqueous and sediment copper sulfate exposures

Plant	Exposure Type	Parameter	Concentration	Reference
Selenastrum capricornutum	Aqueous	96-h EC50	400 μg Cu/L	Blaylock *et al.* (1985)
Chlorella vulgaris	Aqueous	96-h EC50	200 μg Cu/L	Blaylock *et al.* (1985)
Lemna minor	Aqueous	7-day EC50	600 μg Cu/L	Bishop and Perry (1981)
Elodea canadensis	Aqueous	28-day IC50	3,100 μg Cu/L	Brown and Rattigan (1979)
Panicum miliaceum (seeds)	Aqueous	5-day NOEC	700 μg Cu/L	Wang (1987)
Raphanus sativus (seeds)	Aqueous	7-day NOEC	260 μg Cu/L	Gorsuch *et al.* (1990)
Lactuca sativa (seeds)	Aqueous	7-day NOEC	< 260 μg Cu/L	Gorsuch *et al.* (1990)
Lolium perenne (seeds)	Aqueous	7-day NOEC	260 μg Cu/L	Gorsuch *et al.* (1990)
Typha latifolia (seeds)	Aqueous	7-day LOEC	35.0 μg Cu/L	This study

was found to be more sensitive to selected herbicides than aquatic invertebrates (Moore *et al.* 1999).

T. latifolia was selected for this series of experiments as an aquatic vascular macrophyte because of its pandemic distribution throughout North America and its occupation of diverse habitats (*i.e.*, agricultural and roadside ditches, brackish marshes, and freshwater marshes). Obtaining and maintaining a collection of *T. latifolia* seeds for testing is relatively easy because one cattail inflorescence contains thousands of viable seeds, and little space is required for their storage. *T. latifolia* seeds can be stored in plastic bags (without prior storage preparation) in an incubator at constant temperature (20 ± 1°C) for over a year, giving researchers the ability to conduct experiments at any time. Preparation of *T. latifolia* seeds for testing by blending in a commercial blender for 30 s is a simple and inexpensive technique, resulting in hundreds of viable seeds for evaluation. Seeds of other aquatic macrophytes exhibit periods of natural dormancy and require bleach treatments, heating, acid baths, or scarification to facilitate germination (Plyler and Proseus 1996; Ponzio 1998). Because viable *T. latifolia* seeds can be readily separated from nonviable seeds, germination rates are relatively high (> 85%). Therefore, any differences in seed germination observed in a test are likely due to the material being tested and not a result of seed

Table 5. Relative sensitivities of aquatic testing organisms (animals) to aqueous and sediment copper sulfate exposures

Animal	Exposure Type	Parameter	Concentration	Reference
Ceriodaphnia dubia	Sediment	7-day NOEC	18.1 mg Cu/kg	Suedel *et al.* (1996)
	Aqueous	7-day NOEC	3.7 μg Cu/L	Suedel *et al.* (1996)
Daphnia magna	Sediment	7-day LC50	38.7 mg Cu/kg	Suedel *et al.* (1996)
	Aqueous	2-day LC50	20.0 μg Cu/L	Bishop and Perry (1981)
Chironomus tentans	Sediment	7-day LC50	1,600 mg Cu/kg	Suedel *et al.* (1996)
	Aqueous	7-day LC50	657 μg Cu/L	Suedel *et al.* (1996)
Hyalella azteca	Sediment	7-day LC50	319 mg Cu/kg	Suedel *et al.* (1996)
	Aqueous	10-day NOEC	82.0 μg Cu/L	Deaver and Rodgers (1996)
Pimephales promelas	Sediment	10-day NOEC	136.9 mg Cu/kg	Suedel *et al.* (1996)
	Aqueous	10-day NOEC	8.6 μg Cu/L	Suedel *et al.* (1996)
Typha latifolia	Sediment	7-day LOEC	19.7 mg Cu/kg	This study
	Aqueous	7-day LOEC	35.0 μg Cu/L	This study

viability. Phytotoxicity experiments with *T. latifolia* seeds utilize little space because large testing vessels are not required, and seeds germinate within 4 days, allowing for a standard 7-day or 10-day experiment to be concluded with observable effects in that duration. This laboratory has also used this testing protocol for evaluating the effects of atrazine and paraquat to *T. latifolia* seed germination and early seedling growth in 7-day aqueous exposures (Moore *et al.* 1999).

Conclusion

This series of experiments focused on developing a phytotoxicity testing method with potential for assessing the effects of chemical stressors on aquatic macrophytes using *T. latifolia*. *T. latifolia* was chosen for this study as an aquatic vascular macrophyte because of its widespread distribution throughout North America. *T. latifolia* seeds are easy to collect and require minimal storage maintenance; the endpoints of germination and root/shoot growth are readily measurable. Emphasis on testing with algal species, *Lemna* sp., and animal species has resulted in the assumption that these species are more sensitive or representative of aquatic macrophytes. However, when *T. latifolia* responses from this study were contrasted with results from other plant and animal experiments, *T. latifolia* was more sensitive to copper in both aqueous and sediment exposures. Therefore, the sensitivity of *T. latifolia* to sediment and aqueous copper exposures would not be adequately represented by these other species. If aquatic macrophytes are to be represented in toxicity evaluations, then a sentinel species, such as *T. latifolia*, should be included in testing protocols. Aquatic animals, nonvascular aquatic plants, and terrestrial plants possess entirely different growth habits, physiologies, and sensitivities than rooted aquatic macrophytes; therefore, it is not adequate for their responses to various exposures to be considered inclusive and protective for aquatic macrophytes. As demonstrated by this study, *T. latifolia* is a potential sentinel for utilization in aqueous and sediment phytotoxicity tests.

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