A TROPICAL SEDIMENT TOXICITY TEST USING THE DIPTERAN CHIRONOMUS CRASSIFORCEPS TO TEST METAL BIOAVAILABILITY WITH SEDIMENT pH CHANGE IN TROPICAL ACID-SULFATE SEDIMENTS

MIKA R. PECK,*† DAVID A. KLESSA,‡ and DONALD J. BAIRD†
†Institute of Aquaculture, University of Stirling, Stirling, Scotland
‡Office of the Supervising Scientist, G.P.O. Box 461, Darwin, Northern Territory 0801, Australia

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Abstract—The wetlands of the Magela floodplain of northern Australia, which is the major sink for dissolved metals transported in the Magela Creek system, contain acid-sulfate sediments. The rewetting of oxidized acid-sulfate soil each wet season produces acidic pulses that have the potential to alter the bioavailability of sediment-associated metal contaminants. Acute toxicity tests (72-h mean lethal concentration [LC50]) using the tropical chironomid Chironomus crassiforceps Kieffer showed that copper toxicity decreased from 0.64 mg/L at pH 6 to 2.30 mg/L at pH 4. Uranium toxicity showed a similar trend (36 mg/L at pH 6 and 58 mg/L at pH 4). Sediment toxicity tests developed using C. crassiforceps also showed that both metals were less toxic at the lower sediment pH with pore-water copper toxicity having a lowest-observed-effect concentration of 4.73 mg/L at pH 4 compared to 1.72 mg/L at pH 6. However, a lower pH increased pore-water metal concentrations and overlying water concentrations in bioassays. Hydrogen ion competition on metal receptor sites in C. crassiforceps was proposed to explain the decrease in toxicity in response to increased H⁺ activity. This study highlights the need to consider site-specific physicochemical conditions before applying generic risk assessment methods.

Keywords—Sediment toxicity testing Chironomid Copper Uranium Acid-sulfate soil

INTRODUCTION

Although the risk assessment of sediments is currently being researched actively, sediment toxicity assessments are biased to environments and species from Europe and North America. Tropical species may differ markedly from these standard test organisms in their response to metals in sediments, the results from which may be further confounded by the choice of standardized laboratory conditions currently used to generate sediment toxicity data in Europe and North America [1].

In the highly seasonal environments of tropical wetlands, conditions can vary greatly from a suboxic–anoxic sediment system during the wet season to the prevalence of an oxic soil system over the dry season. In acid-sulfate sediments, this fluctuation from anaerobic to aerated conditions can result in the generation of acidity through the oxidation of sulfides. At present, no data exist with which to predict bioavailability of sediment-associated metal pollutants to tropical benthic species under the influence of such sediment-generated acidic pulse events.

The acid-sulfate sediments of the Magela Creek floodplain, within the Alligator Rivers region in the Northern Territory of Australia, are a result of the deposition of sulfidic marine alluvium overlayed by freshwater sediments. During the wet season, the flooded sediments exist under anaerobic conditions, and the reduction of sulfate and iron III oxides takes place. Pyrite is the stable endpoint of this process. Drainage, as a result of the lowering water table during the dry season, causes oxidation of pyrite to form sulfuric acid. On rewetting, an acidic pH pulse can be observed, as poor sediment buffering capacity is present [2]. Thus, the aim of the studies described here was to determine whether this change in pH in sediment affected the bioavailability and toxicity of sediment-associated metal contaminants. Benthic organisms, such as chironomids, have been shown to be major floodplain colonizers and provide food for breeding and migratory birds, which depend on tropical wetlands stopovers. Thus, any adverse impact on this important group of prey organisms from anthropogenically introduced metals is likely to have serious consequences for wetland ecosystem function. In addition, chironomids of the genus Chironomus are favored in sediment toxicity testing because of their easy culture, larval contact with the sediment, and the high probability of giving valid and sensitive test results [3]. Hence, a lethal and sublethal sediment toxicity test was developed after isolating and culturing Chironomus crassiforceps as a representative benthic chironomid species of the Magela Creek catchment and used to determine whether tropical acid-sulfate sediments and the resulting pH fluctuations within sediments resulted in changes in sediment-bound metals. Using copper-spiked sediment from the Magela Creek catchment, the most discriminatory sublethal test endpoint was determined from growth, emergence, development rate, and demographic endpoints.

The toxicity of copper and uranium to C. crassiforceps were then tested, as they were considered metals of potential ecotoxicological concern within the sediments of this region [4]. In this regard, metal speciation is critically important to assessing the bioavailability and toxicity of metals, including such forms as free hydrated ions, polymeric complexes with organic and inorganic ligands, and species bound to colloids and particulates. Factors such as pH, water hardness, available ligands, and other physicochemical parameters control the activities of metal species in solution and determine the nature
of metal reaction; whole sediment surfaces and receptor sites determine biological uptake. The toxicity of a metal in solution is generally considered to be proportional to the concentration of the free aqueous metal ion, that is, Cu$^{2+}$ [5]. Copper and uranium differ in their speciation changes with decreasing pH. For example, in a simple solution at pH 6, the free metal ion Cu$^{2+}$ predominates (>95%) with a small contribution from CuOH$^-$, and at pH 4 the free metal ion dominates (>99%). In contrast, the speciation of uranium shows a marked shift from polymeric uranyl species at pH 6 to the free uranyl ion UO$_2^+$ and UO$_2$OH$^-$, considered the toxic forms, at pH 4 [3].

In addition to altering the speciation of pore-water metals, a change in sediment pH will affect the concentration of pore-water metals. Hydrogen ions compete with metals for binding sites on sediment surfaces, and speciation changes in metals result in changes in the bonding affinities between the metals and the sediment surface [6]. Such processes are likely to affect the bioavailability of sediment-associated metals to benthic invertebrates.

To summarize, the objectives of the study were to develop a chronic sediment toxicity test using a chironomid native to northern Australia and assess the most sensitive endpoint to sediment-associated copper within a standard test design. The most sensitive test protocol was then used to determine whether any effect existed on metal bioavailability to the chironomid with changes in sediment pH.

**METHODS**

**Sediment toxicity test development**

Initially, a local chironomid species, *C. crassificorps*, was isolated from the field and cultured in the laboratory. During both culturing and testing, deionized water was used, as it reflects the water quality characteristics of the Magela floodplain during the wet season with conductivity values as low as 10 $\mu$s/cm and alkalinities of 1 mg/L [7]. Life history characteristics were determined at 27°C. The time from hatch to the end of the fourth larval instar lasted 8 d. Dry weights of fourth-instar larvae were 0.23 mg (standard deviation [SD] = 0.05 mg, $n$ = 10) for males and 0.42 mg (SD = 0.07 mg, $n$ = 8) for females. Following pupation for 24 h, emergence at a 2:1 male-to-female adult ratio was observed. Adults survived for 2 to 3 d, and laid egg masses that hatched in 2 to 3 d. Other life history characteristics are summarized in Table 1 [8].

**Acute toxicity experiments.** The 24-, 48-, and 72-h mean lethal concentration (LC50) of *C. crassificorps* to waterborne copper was determined at pH 4 and 6. Solutions containing nominal concentrations of 0.125, 0.250, 0.500, 1, 2, and 4 mg/L Cu were made up using copper sulfate, one series adjusted to pH 4 with sulfuric acid and the other to pH 6 with sodium hydroxide. The conductivity between copper-spiking treatment solutions was equalized to 32 $\mu$s/cm by titrating with 0.01 M sodium sulfate. Sand was collected from Magela Creek, put through a 500-μm sieve, ashed at 400°C to remove any organic material, and then rinsed in deionized water. Sand is required to allow tube building by the chironomid, so for each test the sand was titrated to pH 4 and 6 using sulfuric acid or sodium hydroxide, respectively. Three grams of sand were placed in each replicate petri dish. Each treatment consisted of four replicates with 60 ml of treatment water that was changed twice daily. Ten 4-d-old chironomids were added to each treatment replicate, and their survival was recorded every 24 h for 72 h. The chironomids were fed ground and sieved (<500 μm) fish feed (Aqua-lab aquarium products, Victoria, Australia) at a ration level of 2 mg dry feed/individual/d, a level that ensured control survival [8]. Water was collected from three replicates per treatment at the end of the experiment, acidified with 1% HNO$_3$, and then analyzed by atomic absorption spectroscopy.

The LC50 of *C. crassificorps* to uranium in water was determined at pH 4 and 6 by setting up replicates as described previously. Stock solutions with a nominal uranium concentration range of 0, 3, 5, 10, 25, 50, and 100 mg/L U were made up using uranyl sulfate and adjusted to the required pH as described previously. The conductivity between uranium-spiking treatment solutions was adjusted to 32 $\mu$s/cm as described previously.

**Sediment toxicity test**

**Sediment collection and characterization.** Sediment was collected from a small backflow billabong approximately 300 m south of gauging station 8210009 on Magela Creek. A representative subsample of sediment was air-dried at 40°C, then ground and sieved (<2 mm). A further subsample was ground and sieved (<0.015 mm) for total metal analyses. Methods and results are summarized in Table 2. Reference sediments were analyzed in all batches to ensure quality control.

**Sediment spiking.** Sediment was spiked by dissolving copper sulfate in 500 ml of water and then adding to 1 L of sediment in 5-L high-density polyethylene containers. Unless otherwise stated, sediment was spiked in the range of 0, 200, 400, 600, 800, and 1.000 mg Cu/kg sediment (dry wt). The mix was placed on a horizontal shaker for 12 h at 25°C, then left to settle for 6 h following standard methods [9]. Before pouring off, overlying water was sampled, filtered (<0.45 μm), acidified to 1% HNO$_3$, and then analyzed for copper to account for any losses.

**Sediment toxicity test methodology.** The design of the sediment toxicity test consisted of six treatments of eight replicates containing 10 larvae each. Aeration of individual replicates was necessary, as preliminary experiments showed that...
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without aeration, chironomids tended to spend extended periods near the surface in the overlying water in an effort to gain oxygen. The entire test system was designed to fit an incubator to ensure a constant temperature of 27°C and a 12:12-h light: dark tropical photocycle. Each replicate was set up in a 500-ml high-density polyethylene container designed to hold 100 ml of sediment and 175 ml of overlying water with an overflow covered by 250-μm plastic mesh. Overlying water was renewed twice a day using a static renewal system. All tests were initiated by the collection of at least four egg masses. Each egg mass was placed separately in 50-ml tubes containing deionized water. Approximately 48 h later, on hatching, larvae were transferred to separate plastic culture trays containing deionized water with sand to a depth of 2 to 3 mm, fed 100 mg food, and then aerated. Every 24 h, 200 mg of feed were added per tray.

For tests using growth as an endpoint, larvae were added to the test system 4 d after hatching. For other endpoints, larvae were added 2 d after hatching. In either case, at least 120 chironomids per egg mass from four egg masses were required for the test, with 10 larvae allocated randomly to each test chamber containing 100 ml of sediment using a completely randomized block design, with egg mass as the blocks. The replicates were then randomly allocated to the incubator, and the static water renewal and aeration system was attached; overlying water was renewed twice daily. A ration level of 2 mg/individual/d was provided [8].

**Chemical analysis of sediment.** At the end of the experiment, metal concentrations in overlying water and pore water were measured in three replicates per treatment. Pore-water copper concentration was estimated by decanting the overlying water, then transferring approximately 30 ml of sediment to a 50-ml centrifuge tube. As the use of a dense, inert hydrocarbon increases the recovery of pore water [10], 10 ml of 1,1,2-trichloro-trifluoroethane were added to the centrifuge tube in a fume cupboard, and the tube was sealed. Preliminary experiments verified the inert nature of the hydrocarbon, which did not complex copper from solution. The sediment was centrifuged at 2,500 rpm for 20 min, then 5 ml of overlying water were collected and filtered (<0.45 μm). The filtrate was diluted to 10 ml with deionized water, acidified to 1% HNO₃, and analyzed for copper by atomic absorption spectroscopy. Any chironomids collected in the sediment sample were added to chironomids recovered from the replicate.

**Test endpoints**

**Growth.** Sediment was spiked, and on termination water and sediment samples were taken for analysis, then overlying water was decanted from all replicates and ethanol added to a depth of 2 cm to all replicates to preserve chironomids. Chironomids were recovered from sediment by sorting on a 250-μm plastic mesh. Overlying water was decanted from all replicates and ethanol added to a depth of 2 cm to all replicates to preserve chironomids. Chironomids were recovered from sediment by sorting on a white tray, then each replicate was pooled, dried on a Teflon® tablet at 75°C, cooled in a desiccator, and weighed on a microbalance (±0.01 mg).

The average mass of chironomids per replicate was calculated by dividing pooled dry mass by the total number recovered. A two-way analysis of variance was carried out to determine the contribution of treatment and parental origin (egg mass) to the dry mass of chironomids at the end of the experiment. Egg mass was treated as a blocking factor, and the lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) were estimated for pore-water and total copper concentration. Homogeneity of variance was checked using plots of residuals versus fitted values. Regression analysis was carried out by linear regression of the arcsine transformation of percentage data, and the EC50 and 95% confidence intervals were estimated [11].

**Emergence, development, and demographic parameters.** The sediment toxicity test system was modified by sealing the top of each replicate with mesh, thus enabling any emerged adults to be collected from the replicate with an aspirator. Sediment was spiked and the test started with first-instar larvae by adding 10 individuals per replicate 2 d after hatching.

The number of males and females emerging daily was recorded and each female transferred to an individual oviposition chamber. The oviposition chambers were 100-ml plastic tubes with deionized water to a depth of 1 cm kept under the same standard test conditions as the toxicity test. At least one male was added to each female in the oviposition chambers. The chambers were checked daily for egg masses. Egg masses oviposited were placed under a binocular microscope, and the number of eggs was counted.

Emergence at each treatment concentration was calculated by dividing the number emerged by the number added at initiation of the test. The data were arcsine transformed, and a two-way analysis of variance was performed.

Mean development rate per vessel was calculated according to Equation 1

$$R = \frac{\sum_{i=1}^{n} f_{X_i} \cdot l_i}{n_e}$$

where $i$ = inspection interval, $m$ = maximum number of inspection intervals, $f_i$ = number of midges emerged in inspection interval $i$, $n_e$ = total number of midges emerged until end of experiment, and $x_i = 1/(day_i - l_i/2)$, where $day_i =$ inspection day (days since application) and $l_i = $ length of the inspection interval $l$ (days).

From the results at each Cu concentration, $\lambda$ was estimated from Equation 2

$$\lambda = \frac{e^{log\ survivorm} \cdot T}{day}$$

where $l = $ survivorm, $m = $ the number of daughters per female, and $T = $ generation time (days). Average $\lambda$ was estimated together with the standard deviation. To avoid making assumptions as to the distribution of $\lambda$, bootstrapping was used to estimate the mean and confidence intervals. Bootstrapping is a computer-intensive method that involves two steps. First, the unknown distribution of population values is estimated from the sample data, then the estimated population is repeatedly sampled to estimate the sampling distribution of the statistic [12]. An Excel® (Microsoft, Redmond, WA, USA) macro was programmed in Visual Basic to generate 1,000 bootstrap values. Mean $\lambda$ was estimated from the mean of bootstrapped values. Since population multiplication rate is not usually normally distributed [12], confidence limits were estimated by placing bootstrapped $\lambda$ values in ascending order. The lower 95% confidence limit was estimated as the mean of the 25th and 26th value and the upper 95% limit as the mean of the 975th and 976th value.

**Lethal and sublethal effect of pH on C. crassiforceps.** A 0.05-M solution of H₂SO₄ was prepared, and a series of stock solutions containing only deionized water were adjusted to pH 6.5, 6, 5.4, 5, 4, and 3.5. The conductivity of each solution was adjusted to 350 μS/cm by titration with a solution of sodium sulfate. Fifty grams of washed and dried sand were added to each replicate in the test chambers, and the overlying
water was added for each treatment from the stock solutions. The sediment toxicity test was initiated as described in the section Sediment Toxicity Test Methodology; however, in this case overlying water was renewed twice daily using the appropriate stock solutions. The pH was measured twice daily to ensure that it remained within 0.1 units of the set level. After 4 d, the test was terminated, and the chironomids were recovered, dried, and weighed.

Effect of sediment pH change on copper toxicity. One liter of sediment was added to 4.5 L of deionized water and the copper sulfate for each spike dissolved in 500 ml of deionized water. The copper solution was added to the sediment and stirred continuously for 5 min, then left for 24 h. A series of treatments were set up giving a nominal sediment concentration of 0, 400, 600, 1,200, 1,600, and 1,800 mg Cu/kg oven-dry sediment. The pH of the sediment/water mixture was adjusted to pH 6 by titrating with sodium hydroxide while stirring continuously. The conductivity of the mixture of all treatments was adjusted to 1,200 \( \mu \text{s/cm} \) with sodium sulfate. The sediment was left to equilibrate for 48 h and the pH readjusted if required. The overlying water was poured off (after samples for copper analysis were removed and filtered [\(< 0.45 \mu \text{m}\)], 100 ml were transferred to the test chambers, and the toxicity test initiated as described in the section Sediment Toxicity Test Methodology. On termination of the toxicity test, the pH and conductivity of the spiked sediment was recorded in three replicates [13], and pore-water and overlying water samples were taken for copper analysis as described in the section Chemical Analysis of Sediment. The nominal copper concentration in sediment following spiking was estimated after accounting for loss in water poured off after spiking. Data were analyzed by hypothesis testing and regression to compare NOEC, LOEC, and EC50 toxicity to the chironomids at the test pH value.

RESULTS

Acute tests

The acute toxicity of copper increased approximately three-fold from pH 4 to pH 6 (Table 3). As with copper, the toxicity of uranium was less at pH 4 than at pH 6. The acute toxicity of uranium (72-h LC50 = 36 mg/L; pH 6) was low compared to that of copper (72-h LC50 = 0.64 mg/L; pH 6). Even in molar terms, uranium is more than a factor of 10 less toxic than copper (72-h LC50 at pH 6 for uranium = 1.512 \( \times 10^{-4} \) M as opposed to 1.007 \( \times 10^{-5} \) M for copper).

Sublethal sediment copper toxicity test

Growth as an endpoint. Two-way analysis of variance was carried out on the mean dry weight of chironomids in each replicate, with parental source (egg mass) used as a blocking factor. Both parental source \( (p < 0.001) \) and treatment \( (p < 0.001) \) showed significant effects on body mass after 5 d.

Dunnett’s test was subsequently used to determine LOEC and NOEC values for dissolved Cu in sediment pore water and total copper in sediment (Table 4) showing that toxicity may be expected above a nominal sediment concentration of 400 mg/kg when the pore-water concentration rises above 0.3 mg/L under the conditions of this experiment. The EC50 values were estimated by regressing arcsine transformations of percentage reduction in mass, scaled to control for each blocked egg mass against pore-water copper concentration and nominal copper concentration. Both regressions resulted in a slope significantly different from zero (pore-water \( p < 0.001 \); nominal \( p < 0.001 \)), but response against nominal copper concentration gave a slightly better fit \( (r^2 = 0.64) \) than for pore water \( (r^2 = 0.57) \). A decrease in the pH of overlying deionized water (from the original pH of 5.82) in response to increasing copper concentration \( (p < 0.001, r^2 = 0.58) \) was also observed. Estimates of the power of the test showed that the probability of making a Type II error was <1% [11].

Emergence, development rate, and demographic endpoints. Clear effects of sediment copper concentration on mean number emerged and mean emergence time were observed. For total number emerging at each concentration, a LOEC of 800 mg/kg and a NOEC of 600 mg/kg was obtained. The characteristic emergence of males before females was observed with a time lag of approximately 1 d. Regression of development rate against sediment copper concentration showed development rate to be inversely proportional to copper concentration \( (p < 0.01) \) with an estimated EC50 of 941 mg/kg.

Survivorship, measured as survival to emergence, resulted in maximum survival of around only 50%. The addition of Cu to sediment increased the mean emergence time for females \( (p < 0.05) \). However, the mean number of eggs per treatment was not affected by Cu treatment \( (p = 0.28) \).

Table 3. Acute toxicity of copper and uranium to *Chironomus crassiforceps* with 95% confidence intervals in brackets from different times and pH (NA = no LC50 at this time)

<table>
<thead>
<tr>
<th>pH 6</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC50 copper (mg/L)</td>
<td>1.83 (1.69–1.96)</td>
<td>0.90 (0.84–0.95)</td>
<td>0.64 (0.59–0.69)</td>
</tr>
<tr>
<td>LC50 uranium (mg/L)</td>
<td>NA</td>
<td>NA</td>
<td>36 (34–39)</td>
</tr>
<tr>
<td>pH 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC50 copper (mg/L)</td>
<td>NA</td>
<td>3.47 (3.11–3.83)</td>
<td>2.30 (2.10–2.51)</td>
</tr>
<tr>
<td>LC50 uranium (mg/L)</td>
<td>NA</td>
<td>NA</td>
<td>58 (52–64)</td>
</tr>
</tbody>
</table>

* LC50 = mean lethal concentration.

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Table 4. Sublethal toxicity of copper to *Chironomus crassiforceps* using growth as the measured endpoint (95% confidence limits are shown for mean effective concentration [EC50])

<table>
<thead>
<tr>
<th>Pore-water copper concentration (mg/L)</th>
<th>Nominal sediment copper concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50</td>
<td>1.73</td>
</tr>
<tr>
<td>(1.37 &lt; EC50 &lt; 2.25)</td>
<td>(715 &lt; EC50 &lt; 981)</td>
</tr>
<tr>
<td>LOEC*</td>
<td>0.30</td>
</tr>
<tr>
<td>NOEC*</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* LOEC = lowest-observed-effect concentration.

* NOEC = no-observed-effect concentration.
the means and 95% confidence intervals from the bootstrapped
data (the larger confidence intervals) and 95% confidence in-
trvals from an assumed normal distribution (smaller con-

dence intervals). The data show that assumption of normality
is erroneous. The relative sensitivity of the test endpoints
are summarized in Table 5.

**Lethal and sublethal effect of pH on C. crassiforceps.**
Greater than 80% survival was observed under the pH values
tested, and no mortality attributable to pH was observed even
at pH 3.5. Figure 2 shows the mean weight per individual and
the 95% confidence intervals. A significant trend of continuous-
ously increasing growth performance with increasing pH was
observed (p = 0.03).

**Effect of sediment pH change.** Pore-water copper concentra-
tions are plotted against overlying water copper concentra-
tions in Figure 3. At pH 6, overlying water copper concentra-
tions are extremely low in comparison to sediment pore-water
copper concentrations. However, at pH 4, overlying water and
pore water contained similar concentrations of Cu showing rapid
mobilization of metal from sediment to overlying water. In terms
of toxicity, chironomids are expected to be exposed to copper
through pore water and overlying water, as they inhabit and
irigate their tubes, respectively. Analyzing the chemical data
alone, showing higher copper concentrations in both pore water
and overlying waters at the lower pH, might lead to the erro-
neous conclusion that toxicity is likely to increase with de-
creasing pH. However, two-way analysis of variance, which
tested treatment and parental source as factors in chironomid
dry mass at the end of the tests at pH 4 and 6, gave the LOEC
and NOEC values shown in Table 6, showing an increase in
toxicity with increased pH. Percentage decrease in dry mass
with treatment (scaled to control dry wt) was arcsine trans-
formed and then regressed against metal concentration. At pH
6, linear regression gave \( r^2 = 0.60 \) with slope significantly
different from zero (p < 0.001); at pH 4, a slope significantly
different from zero (p < 0.001) was also observed (\( r^2 = 0.358 \)).
Slopes were found to be significantly different from each other
(\( t = 4.9; df = 32; p < 0.01 \)). The EC50 values and 95%
confidence intervals were estimated [11] for pore-water copper
and nominal copper sediment concentrations and are shown in
Table 6. At pH 4, the EC50 value in terms of pore-water copper
was 9.58 mg/L (with 95% confidence interval = 7.10–14.38
mg/L) and increased at pH 6 to an EC50 of 6.08 mg/L (with
95% confidence interval = 5.00–7.40 mg/L).

**DISCUSSION**

The biogeographical range of C. crassiforceps (P.S. Cran-
ston, personal communication) means that this chironomid has
potential as a standard test organism with local relevance to
Southeast Asia, Indonesia, and Micronesia. The chironomid is
adapted to the wet-dry tropical conditions of the Northern
Territory of Australia, with a short life cycle ideal for aerial
colonization of newly formed water bodies created during the
wet seasons. This short life cycle at 27°C makes the sediment
toxicity test much faster (4 d) than current 10-d sediment tox-
icity tests using other species of chironomids at 21°C [14].
This faster life cycle under tropical conditions has implications
for interpreting sediment toxicity test data. For example, if egg
masses are collected daily to start the test, hatching could have
occurred any time over the previous 24 h. Hence, those larvae
that hatched just before collection would tend to be smaller
on termination of the test than those that hatched during the
previous day. Experimentation showed that egg masses under
these conditions could have a significant impact on size at test
end (p < 0.05) [8]. A difference in 23 h is a significant pro-
portion of the larval life cycle of 8 d at 27°C. To address this
problem, replicates should be blocked by egg mass.

A literature review of copper toxicity to chironomids was
undertaken to compare the toxicity response values obtained
to other studies of chironomid species. The 48-h LC50 value
for Polypedilum nubifer was 0.63 mg/L [15], for Chironomus
riparius 1.2 mg/L [16], for Chironomus decorus 0.7 mg/L
[17], and for Chironomus tentans 0.53 mg/L [18]. Chironomus

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**Table 5. Comparison of the sensitivity of various endpoints (mg Cu/ kg dry sediment)**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>NOEC(^a)</th>
<th>LOEC(^a)</th>
<th>EC50(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>200</td>
<td>400</td>
<td>832</td>
</tr>
<tr>
<td>Emergence</td>
<td>600</td>
<td>800</td>
<td>941</td>
</tr>
<tr>
<td>Development</td>
<td>600</td>
<td>800</td>
<td>941</td>
</tr>
</tbody>
</table>

\(^a\) NOEC = no-observed-effect concentration.

\(^b\) LOEC = lowest-observed-effect concentration.

\(^c\) EC50 = mean effective concentration.
Crassiforceps at pH 6 was used to allow comparisons with most other tests, which are normally carried out around neutrality, and gave an LC50 of 0.9 mg/L. The sensitivity of C. crassiforceps falls between that of C. riparius and C. tentans and is at a similar level to P. nubifer (also widely distributed throughout Southeast Asia and recorded from northern Australia) [15].

It is generally accepted that a valid test requires 80% survival or success of the control in chironomid larval growth tests [14]; however, for life cycle tests, control values are expected to be lower because of inherent mortality associated with pupation. The emergence of C. riparius has been observed at greater than 90% but as low as 15 to 43% for C. tentans (M. Watts, personal communication). It has been suggested (P. Sibley, University of Guelph, Ontario, Canada, personal communication) that the low emergence rate may be a result of cannibalism of pupae by larvae under low-feed conditions or high density, suggesting the need for further study if emergence or demographic endpoints are to be used. The most sensitive endpoint obtained using this experimental design was using growth as an endpoint, which is supported by other findings [16] that found the larval growth test to be more sensitive than emergence endpoints for C. decorus.

In summary, given the low emergence rates for C. crassiforceps, the use of demographic parameters as an endpoint under this experimental design became questionable because of the insensitivity in detecting differences between treatment and control. In the work described here, this arose as a result of experimental design, low emergence rates (50%), and chironomid sexual dimorphism. With relatively low numbers of individuals per replicate and sexual dimorphism in a 2:1 ratio in favor of males, as in C. riparius [19], a high probability existed that replicates exhibited zero female emergences, thus biasing λ in favor of zero. The inability to separate male from female until the fourth instar means that this effect is hard to control except by increasing the numbers of larvae per replicate. Nevertheless, demographic parameters are ecologically relevant and integrate sublethal and lethal effects [12], but the standard use of demographic parameters in toxicity testing requires further research to refine and reevaluate protocols for current ecotoxicological tests.

In terms of sensitivity, growth measured as dry weight after 4 d was more sensitive than population parameters. The increased sensitivity is not unexpected, if the individual parameters survivorship (l), the number of daughters per female (m), and generation time (t) are measured with minimum error since it is impossible for λ to be more sensitive. However, a combined, statistically undetectable difference in all the individual traits could be detected by λ [20].

Chironomus crassiforceps itself showed a high degree of tolerance to low pH with no lethal toxicity at pH 3.5. Other chironomids, such as C. riparius, have been shown to tolerate highly acidic conditions to pH 3, the lowest tolerance of invertebrates and fish in a review [21]. However, another study observed a reduction in survival of C. riparius larvae at pH 4 [22]. From studies under temperate conditions, it is generally thought that acidified streams show diminished species richness, increased abundance of tolerant species, and lower densities due to lower productivity as a result of increasing acidity [23]. Studies undertaken in southern tropical waters suggest that the response to acidity may be different. An experiment by Cranston et al. [24] within the Alligator Rivers region showed that the response to acid, heavy-metal-rich waters was a loss of some species typical to pristine conditions but overall an increase in species richness. The higher species richness at low pH is explained by the large tropical (Australian and Southeast Asian) pool of species that are tolerant of naturally occurring acidic aquatic habitats [24].

The acute toxicity data, showing increased toxicity of copper with a decrease in acidity, broadly agrees with reviews on fish, algae, fungi, crayfish, and benthos [21,25]. The reduced toxicity with decreasing pH is attributed to increased competition from protons on uptake sites on biological membranes [21,26] and elsewhere.

Using a chemical speciation approach, such as the free ion activity model [27], the toxicity of copper might be expected to increase slightly with decreasing pH, although little change is likely to be found in the concentration of Cu²⁺, thought to be the toxic form of copper, between pH 6 and 4. However, for uranium, marked speciation changes are expected to occur between pH 6 and 4. Markich et al. [28] showed an exponential increase in uranium toxicity when pH was reduced from 6 to 5 for the bivalve Venusium angasii. This toxicity was associated with a speciation change favoring the uranyl ion (UO₂⁺). This pattern was not observed for C. crassiforceps and highlights the observation that mechanisms of toxicity between species are variable [29]. The differences may be partially explained by the fact that, in sediments and soils,
sediment particles and sites on the biological membrane compete for the binding of metals present in the system. The effect of pH on the binding characteristics is difficult to predict unless the binding characteristics of both the biological surfaces and the sediments are known. Work by Plette et al. [6] shows that toxicity can increase or decrease as a result of the effect of pH on the surface-binding characteristics of the system. Hydrogen ion competition was proposed to explain the findings described here. Several mechanisms can be put forward to explain the way increasing the concentration of H$^+$ ions can reduce toxicity. One is that as pH decreases, the concentration of H$^+$ ions increases logarithmically, competing with metal ions for biological uptake sites on the membrane surface of the chironomid. This fits with a biological surface complexation model where uptake sites are occupied by either metal ions or protons before being taken up by the organism [5,26,30]. Changes in the surface potential of the cells of the chironomid may also contribute to increasing toxicity with increasing pH. With increasing pH, the electrostatic potential between the cell surface and the positively charged metal cations may increase, subsequently favoring metal sorption and uptake [30]. Another possibility is that hydrogen ions interact with the plasma membrane, altering transport [31]. The reasons for decreased toxicity with increasing hydrogen ion concentration may be a result of one or a combination of these mechanisms.

Within the sediment system studied, decreasing pH resulted in increased pore-water copper concentrations. This was attributed to hydrogen ion competition at the sediment–pore-water interface. The higher pore-water EC50 and LOEC values for C. crassiforceps at the lower pH of 4 compared to 6 were hypothesized to be due primarily to proton competition at the biological uptake sites of the chironomid [21,25,26]. Krantzberg and Stokes [5] found that chironomids accumulated metals to a greater degree with increasing pH. However, the effect may have arisen from increased surface adsorption as opposed to an intake mechanism. Although this study did not determine tissue copper concentrations, the ecologically relevant endpoints clearly demonstrated that sublethal chronic toxicity of copper to C. crassiforceps increased with increasing pore-water pH.

In terms of nominal sediment copper concentrations, hypothesis testing gave lower LOEC and NOEC values for copper at pH 6 than at pH 4, reflecting the trend for toxicity observed in pore-water data. Using regression analysis, the nominal sediment copper concentrations associated with pore-water EC50 values did not differ significantly over the two pH treatments. The effect of decreased pore-water copper toxicity at lower sediment pH contrasted with an increase in pore-water metal concentrations. The net effect on C. crassiforceps was that toxicity, in terms of nominal sediment copper loading (EC50 nominal), remained the same at both pH values. This highlights the importance of evaluating the impact of pH on both the speciation in sediments and the interactions with the biological surface/uptake sites.

Uranium was found to have no sublethal effect on C. crassiforceps at all levels expected in the environment (up to 5,000 mg U/kg dry sediment). This was attributed to a low affinity for protein-binding sites, as the UO$_2^{2-}$ ion exhibits ionic (hard acid) characteristics in soft/hard Lewis acid theory [32]. Hence, UO$_2^{2-}$ does not form strong bonds with soft bases that make up nucleophilic binding sites on proteins. Copper, on the other hand, is classified as a soft Lewis acid and forms complexes (often very stable chelate complexes) with thiol and amino groups of proteins, changing their conformation and either degrading gas exchange and/or altering fluid retention/exclusion [33].

Comparing the copper pore-water sublethal EC50 value (1.73 mg/L—an effect over 4 d) to the 48-h acute LC50 (0.9 mg/L at pH 6, 3.47 at pH 4), it can be seen that copper is less bioavailable in pore water than water. A number of reasons potentially exist for this. The correlation between pore-water copper concentration and a toxic effect on the chironomid is related to the degree of interaction of C. crassiforceps with pore water. The tubes that chironomids inhabit are irrigated with overlying water, thus potentially reducing their direct contact with pore-water copper; however, this is unlikely, as the concentration of copper in overlying water was similar to pore-water concentrations at pH 4. Another possibility is that copper forms extremely strong complexes with dissolved organic matter. The dissolved organic matter–copper complexes are generally considered nonbioavailable [34]. Dissolved organic matter concentration in Magela Creek water, as in deionized water, in which the LC50 tests were carried out, was negligible [7], but pore water, in equilibrium with the organic-rich sediment, would be expected to contain dissolved organic matter. It has been observed that 98% of copper is normally complexed with organic matter in soil solution [6]. This could potentially reduce toxicity in terms of total pore-water copper concentration.

CONCLUSIONS

The sediment toxicity test developed has potential for use as a generic toxicity test with ecological relevance from Southeast Asia through to northern Australia. The acute sensitivity of C. crassiforceps to copper fell within the range of sensitivities of chironomids used as standard test organisms in Europe and the United States. A major advantage of the test was that, using growth as an endpoint, the 4-d sublethal sediment toxicity test under tropical temperatures was more than twice as rapid as standard temperate sediment toxicity tests. Endpoints other than growth showed poor sensitivity under this experimental design. The implication is that the use of other endpoints requires changes in the test design to ensure an acceptable level of sensitivity, yet it is suggested that growth is both ecologically relevant [8] and sensitive for sediment toxicity testing.

This study highlights the need to consider pH in risk assessment of sediment in regions of acid-sulfate sediments. The interstitial water criteria toxic units are calculated by dividing the estimated pore-water concentrations by a chronic water quality guideline value that should first be adjusted for the measured pore-water hardness to account for hardness effects on toxicity [34]. This emphasizes the fact that adjustment for pore-water pH may also be necessary.

However, one of the problems in considering the effect of pH change on toxicity is that contradictory results have been obtained for different organisms [6]. For other organisms, the pattern seen here for the chironomid C. crassiforceps of decreasing toxicity of copper with increasing acidity was not observed. For example, heavy metal uptake by plant root systems is favored under low pH conditions [35]. As a tropical wetland ecosystem can exist as a soil system for a number of months of the year, it is also important to assess risk to the ecosystem in its phase both as sediment and as a soil [36]. Further work to understand the effect of pH on the competitive binding of metals between sediments and biological mem-
branes could help risk assessment models in environments affected by pH changes.

Another aspect of acid-sulfate sediments of concern is the potential impact on overlying water quality when generated acidity releases sediment-bound contaminants. The oxidation of acid-sulfate soil from the Magela floodplain showed that pH levels could reach as low as 1.5 and that soluble manganese, iron, and aluminum concentrations increase from the breakdown of clay minerals under such extremely acidic conditions [2]. The implication for risk assessment of acid-sulfate sediments is that even occluded metals may become bioavailable following the generation of acidity and the dissolution of mineral matrices. From the work contained here, metals were released from sediment to the overlying water column with decreasing pH. Water quality can be affected as a result of this phenomenon, and hence water quality assessment in regions of acid-sulfate sediments should consider sediment as a potential source of metal contamination.

Although recognized as a major influence on speciation and bioavailability of metals in freshwater systems, pH is currently not used to modify guideline values for water or sediment assessment, such as in the Draft Australian Water and Sediment Quality Guidelines [26]. The reason is due primarily to contradictory findings and poor-quality data [3]. However, rather than ignore the role of pH in influencing bioavailability, this study highlights the need to understand and include the effect of pH on metal toxicity, especially for sediment quality assessment in acid-sulfate soil regions.

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