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Toxicity of Alkalinity to *Hyalella azteca*

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Toxicity testing and chemical analyses of pore water have been suggested for inclusion in the process of sediment quality assessment (USEPA 1994a) and for use in sediment toxicity identification evaluation procedures (Ankley and Schubauer-Berigan 1995). Incorporation of pore water into sediment evaluations has been prompted by the understanding that bioavailability of sediment contaminants is primarily through exposure to the pore water (Burton 1991). However, interpretation of porewater toxicity is highly dependent upon the porewater chemistry. Inherent chemical characteristics and rapid shifts in chemical equilibria associated with the oxidation of freshly-extracted pore water confound the relationship between porewater chemistry, contaminant availability and toxicity (Lasier 1995). For example, high concentrations of alkalinity, which are typical of sediment pore water from many regions, can be toxic to test animals (Cowgill and Milazzo 1991; Hoke et al. 1992). In research with cladocerans, Hoke et al. (1992) attributed the toxicity of elevated levels of carbonate in solution to the inhibition of chloride uptake.

Total alkalinity is the sum of all bases in solution titratable with a strong acid, including carbonate, bicarbonate, ammonia, phosphate, silicate and some organic bases (Stumm and Morgan 1981). Porewater alkalinity concentrations up to 1600 mg/L as $CaCO_3$ have been found in estuarine sediments (Winger and Lasier 1993, 1995) and we have measured porewater alkalinities from sediments of arid regions (Utah) up to 4500 mg/L as $CaCO_3$ (unpublished data). Porewater concentrations of these bases can be extremely high due to a combination of ionic strength, dissolved organic carbon, sulfate reduction, carbon dioxide and ammonium production and dissolution/precipitation of mineral solid-phases (Presley and Trefry 1980). However in carbonate-bearing waters, carbonate and bicarbonate contribute all but minor amounts of the acid-neutralizing capacity measured as alkalinity (Stumm and Morgan 1981).

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Hyalella azteca is a freshwater amphipod suggested for use in sediment and porewater evaluations (USEPA 1994a; Ankley and Schubauer-Berigan 1995). The toxicity of alkalinity to *H. azteca* is unknown. This study focused on the toxicity of carbonate alkalinity, which at the pH range tested was comprised of primarily bicarbonate. The objective of this study was to assess the toxicity of alkalinity to *H. azteca* due to its potential significance in the interpretation of sediment and porewater toxicity. Tests were also conducted to compare the tolerance of different aged animals to alkalinity.

MATERIALS AND METHODS

Toxicity of alkalinity was tested by exposing *H. azteca* to different concentrations of sodium bicarbonate ($NaHCO_3$) added to moderately-hard reconstituted water. A toxicity test of sodium chloride (NaCl) was conducted alongside the initial alkalinity test to establish a toxic level of sodium and to verify that toxicity was due to bicarbonate and not to the sodium component of the salt. Initial tests were conducted with 14-d old *H. azteca;* however, updated guidelines on sediment and porewater testing procedures recommend the use of 7-14 d old animals (USEPA 1994b). Subsequently, four tests were preformed with 7-d old animals, and then two additional tests were conducted to provide a side-by-side comparison of the two ages.

Hyalella azteca were cultured in our laboratory and separated from mass cultures using U.S. Standard sieves. Amphipods which passed through a 710µm sieve but were held on a 500µm sieve averaged 11-d old, and those that passed through a 425µm sieve but were held on a 355µm sieve averaged 4-d old (Winger et al. 1993). After sieving, animals were held in 10 L of aerated reconstituted water for three days with food (Tetrafin®) added immediately and the day before testing. At the beginning of the first side-by-side age comparison, twenty animals of each age were preserved and measured for total length. Lengths were measured by projecting images of the animals onto a large screen with a microscope-slide projector. Length was measured on the projected image using a piece of thick-walled plastic tubing that had been calibrated with a stage micrometer. The resolution afforded by this method was 0.01 mm.

Hyalella azteca were exposed for 96 h to serial dilutions of $NaHCO_3$. The initial test included six dilutions of $NaHCO_3$ (6,000 mg $NaHCO_3$/L serially diluted by 0.5x) that were prepared using moderately-hard reconstituted water (USEPA 1994b). Subsequent tests were reduced to four serial dilutions (0.5x) of $NaHCO_3$ starting at 3000 mg $NaHCO_3$/L. Reconstituted water that had been aged for 3 d served as diluent and control treatment in all tests. Sodium bicarbonate and NaCl solutions were prepared 1 d before testing. Sodium chloride toxicity was evaluated during the initial test to establish the toxicity of sodium. Six serial dilutions (0.5x) of a 10,000 mg NaCl/L solution were tested. Sodium chloride additions do not significantly alter alkalinity, therefore all NaCl solutions had alkalinities around 70 mg/L (alkalinity of the reconstituted water).

In all tests, five replicate 30-mL test chambers were prepared for each test solution by adding 20 mL of test solution, 10 *H. azteca,* and a 2 cm x 2 cm square of Nytex netting. Chambers were randomized, covered with plastic wrap and stored in an environmental chamber at 23 °C with 16 h light:8 h dark photoperiod. Animals in the initial test were fed 0.25 mL of Tetrafin® solution (4 g/L solids) at test initiation and at 48 h. Feeding during the testing procedure was discontinued after the initial test.

Hyalella azteca survival was determined at the end of 96 h and the median lethal concentration (LC_{50}) calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977). LC_{50}s were calculated for alkalinity (as mg/L $CaCO_3$), $NaHCO_3$ (mg/L), and NaCl (mg/L). Measured concentrations of alkalinity at test initiation were used for alkalinity LC_{50} calculations, whereas nominal concentrations of $NaHCO_3$ and NaCl were used for their respective LC_{50} calculations. The criterion for a successful test was control survival ≥ 90%.

Alkalinity, reported as mg/L $CaCO_3$, was measured with a LaMotte Alkalinity Test Kit that provides a colorimetric titration with 0.05% H_2SO_4. The sensitivity of this method was ± 4 mg/L. In addition to alkalinity, pH (Orion pH electrode), conductivity (Fisher conductivity meter), and hardness by EDTA titration method (APHA 1992), were measured at test initiation and after 96 h of storage under test conditions but without test organisms.

Chemical speciation and calculations of ion concentrations and activities were performed with the geochemical assessment model MINTEQA2/PRODEFA2 (Allison et al. 1990). Nominal concentrations were used for these calculations.

RESULTS AND DISCUSSION

Measurements of *H. azteca* length at test initiation indicated the mean length of the younger animals to be 1.87 mm (SD 0.20) and the mean length of the older animals to be 2.75 mm (SD 0.27). These data correspond to the lengths of 7-day old and 14-day old animals, respectively (Winger et al. 1993).

Alkalinity was toxic to *H. azteca* at concentrations sometimes encountered in sediment pore water (Fig. 1). There was also a significant difference between the tolerances to alkalinity of the two different ages of animals. The mean 96-h LC_{50} for 14-d old animals was 1212 (SD 64) mg/L $CaCO_3$ whereas the mean for the 7-d old animals was 662 (SD 18) mg/L $CaCO_3$. In the initial test which was conducted with 14-d old animals, the alkalinity LC_{50} was 1272 mg/L $CaCO_3$ which corresponded to 1979 mg/L $NaHCO_3$. The following four tests conducted with the 7-d old animals resulted in alkalinity LC_{50} values ranging from 629 to 675 mg/L $CaCO_3$. Side-by-side comparisons in the final two tests verified a significant difference in tolerance to alkalinity between the two ages of *H. azteca.* In these two tests, the younger animals had a mean LC_{50} of 674 mg/L $CaCO_3$ and the 14-d old animals had a mean LC_{50} of 1182 mg/L $CaCO_3$. The difference

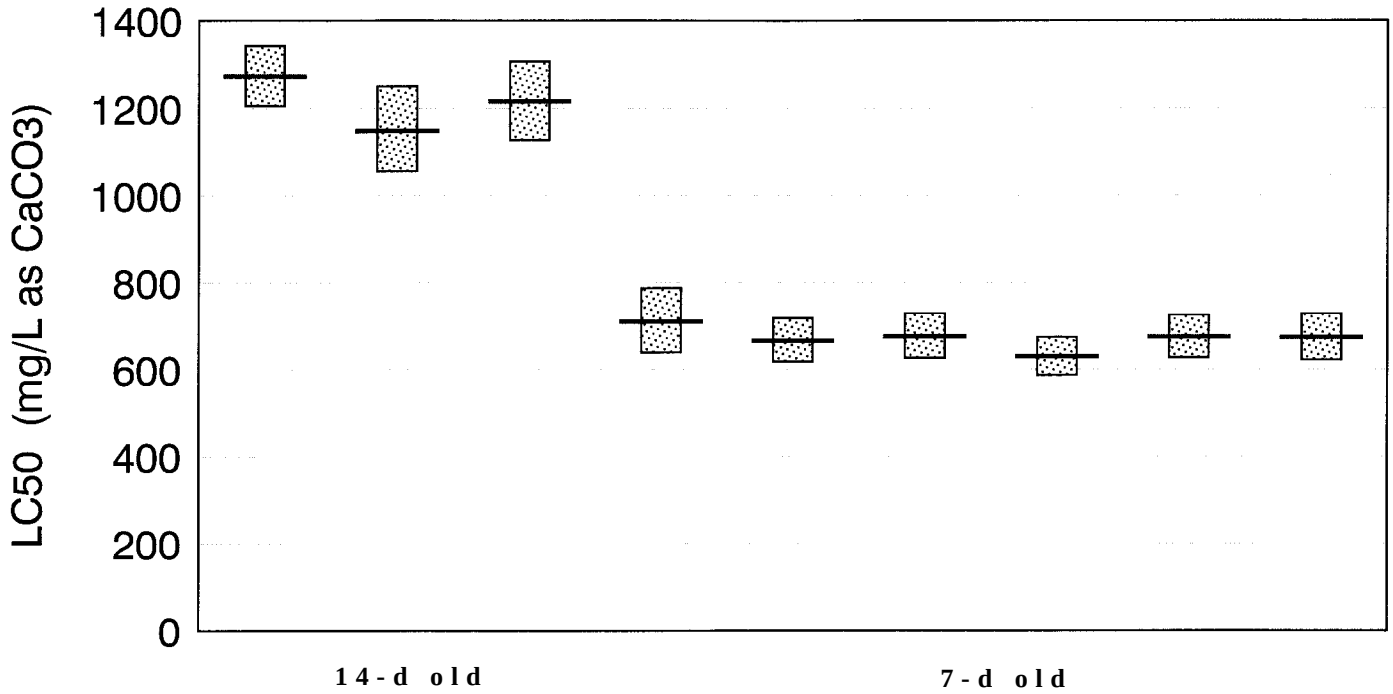

Figure 1. Alkalinity LC50 values with 95% CI for 14- and 7-d old *Hyalella azteca* in $NaHCO_3$ toxicity tests.

between the first test with 14-d old animals (LC_{50} of 1272) and the following two tests with them (LC_{50}s of 1055 and 1128) may reflect the modifying influence of food additions. For example, we have observed in our cultures that Tetrafin^{R} increases acidity. Control survival for all tests averaged 98% with a minimum survival of 96%.

The toxicity of these solutions was due to elevated bicarbonate concentrations, not the simultaneously elevated sodium concentrations. The 96-h LC_{50} for NaCl was 6507 mg/L. The concentration of sodium present in the theoretical alkalinity LC_{50} solution (1979 mg $NaHCO_3$/L) was about 570 mg/L (value includes LC_{50} sodium concentration plus sodium in dilution water), whereas the sodium concentration in the NaCl LC_{50} solution was around 2600 mg/L.

Toxicity of bicarbonate alkalinity to *H. azteca* was similar to that reported for two species of cladocerans. Cowgill and Milazzo (1991) and Hoke et al. (1992) determined that carbonate/bicarbonate 48-h LC_{50}, values for cladocerans ranged between 500 and 1000 mg/L as $CaCO_3$. The bicarbonate toxicity was attributed to an inhibition of chloride uptake (Hoke et al. 1992). If that was the mode of toxicity observed in this study, inhibition of chloride uptake was due to the high concentration of bicarbonate, not a reduction in chloride activity. Speciation by MINTEQA2 of the highest $NaHCO_3$ LC_{50} solution (1979 mg/L) revealed that chloride activity was not affected at these concentrations. Inhibition may have occurred either by direct competition with bicarbonate ions at membrane surfaces or by reducing the concentration gradient and retarding the exchange of bicarbonate for chloride.

Alkalinity toxicity varied with organism age (Fig. 1). The older animals could tolerate almost twice the alkalinity as the younger animals. Collyard et al. (1994) found that sensitivity to five toxicants differed between ages of *H. azteca*. In several cases the youngest animals (0-2 d old) were more tolerant than animals 2-10 d old, but with all the contaminants that they examined except diazinon the oldest animals were the least sensitive. For copper and zinc, they reported significant differences in sensitivity between 7- and 14-d old age classes. Based on these findings (Collyard et al. 1994) and results from this study, we recommend that the preferred ages of *H. azteca* to be used in porewater toxicity testing be narrowed to 6- to 8-d old.

Water chemistries in the test solutions were consistent across the seven test periods, but chemistries changed over the course of the test which produced dissimilar levels of pH and hardness among treatments (Table 1). There was an increase in pH accompanied by a significant decrease in hardness and a slight decrease in alkalinity in the treatment solutions. Solution pH levels increased from 8.3 (pH of dilution water) to a high of 9.3. Alkalinity and hardness declined due to the precipitation of calcite ($CaCO_3$). This precipitation was measurable after 1 d (at test initiation) and seemed to reach equilibrium within 5 d. MINTEQA2 predicted a complete loss of Ca due to this process, but Ca was still

Table 1. Average water chemistry (with SD, n=7) of $NaHCO_3$ solutions at the start and end of 96-h tests.

Solution (mg $NaHCO_3$/L)	Alkalinity (mg/L as $CaCO_3$)		pH		Hardness (mg/L as $CaCO_3$)		Conductivity (µS)	
	Start	End	Start	End	Start	End	Start	End
375	294 (22)	270 (9)	8.5 (0.1)	8.8 (0.1)	97 (9)	47 (5)	740 (18)	709 (28)
750	532 (26)	498 (14)	8.5 (0.1)	9.1 (0.1)	85 (20)	39 (2)	1099 (20)	1077 (37)
1500	1004 (15)	979 (22)	8.6 (0.2)	9.3 (0.1)	74 (27)	36 (0)	1801 (23)	1824 (35)
3000	1940 (43)	1883 (47)	8.5 (0.1)	9.2 (0.1)	62 (21)	36 (0)	3142 (36)	3177 (37)
control	74 (4)	74 (4)	8.3 (0.0)	8.3 (0.1)	103 (3)	102 (2)	375 (11)	373 (9)

in solution after 5 d. Hardness levels declined in all $NaHCO_3$ solutions, but fell consistently to 36 mg/L in the 1500 and 3000 mg $NaHCO_3$/L solutions and to 30 mg/L in the 6000 mg $NaHCO_3$/L solution used in the initial test (data not shown). The reconstituted water contained slightly over 100 mg/L hardness which was comprised of about 30 mg/L Ca (equivalent to 75 mg/L as $CaCO_3$ hardness) and 6 mg/L Mg (equivalent to 25 mg/L as $CaCO_3$ hardness). After accounting for the hardness contributed by Mg at the end of the test, about 11 mg/L of hardness was provided by Ca, which corresponds to a Ca concentration of roughly 5 mg/L in solution.

These changes in basic chemistries due to precipitation of solids from formulated test solutions (and pore waters) are often ignored thermodynamic phenomena that can have significant effects on observed toxicities. As demonstrated here, toxicant-ameliorating ions such as calcium can precipitate out of solution reducing protection against divalent contaminants. Precipitation or coprecipitation can reduce nominal toxicant concentrations that result in solutions of lesser toxicity and underestimates of toxicity. Caution must be exercised when using formulated test solutions or pore waters to ensure chemical conditions remain constant throughout the duration of the test or that these changes be documented.

Porewater chemistry and toxicity are important aspects of a complete assessment of sediment contamination, and are the basis for evolving procedures for sediment toxicity identification evaluations. Interpretation of toxicity should consider the test organism's tolerances to alkalinity as well as elevated concentrations of other ions. Natural characteristics of some sediment pore waters (i.e. concentrations and ratios of the major cations and anions) may overshadow anthropogenic sources of toxicity. As demonstrated in this study, alkalinity at concentrations occasionally found in sediment pore waters may be toxic. Therefore, tolerances of test species to alkalinity should be known prior to use in porewater toxicity tests, and alkalinity should be routinely measured when the toxicity of pore water is being evaluated.

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