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Effects of Calcium, Magnesium, and Sodium on Alleviating Cadmium Toxicity to *Hyalella azteca*

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Toxicity of trace metal ions to aquatic organisms, arising through either anthropogenic inputs or acidification of surface waters, continues to be both a regulatory and environmental problem. It is generally accepted that the free metal ion is the major toxic species (Florence et a1.,1992) and that inorganic or organic complexation renders the metal ion non-bioavailable (Meador, 1991, Galvez and Wood, 1997). However, water chemistry parameters such as alkalinity, hardness, dissolved organic carbon and pH influence metal ion toxicity either directly by lowering free metal ion concentration or indirectly through synergistic or antagonistic effects. Alkalinity and salinity can affect the speciation of metal ions by increasing ion-pair formation, thus decreasing free metal ion concentration. For example, Cu was found to be less toxic to rainbow trout in waters of high alkalinity (Miller and Mackay, 1980), due to formation of $CuCO_3$ ion pair, and corresponding reduction in free Cu^{2+} concentration. The influence of salinity on the toxicity of cadmium to various organisms has been demonstrated in a number of studies (Bervoets et al., 1995, Hall et al., 1995, Lin and Dunson, 1993, Blust et al., 1992). In all these studies the apparent toxicity of cadmium was lowered as salinity was increased due to increased formation of $CdCl^+$ and $CdCl_2$ aqueous complexes that are non-toxic or of much lower toxicity than the free Cd^{2+} ion. Changes in pH exert both a biological and chemical effect on metal ion toxicity (Campbell and Stokes, 1985). Low pH favors greater metal ion solubility, and, in the absence of complexing ions, reduced speciation of the metal ion, which tends to increase toxicity compared to higher pH. However, low pH also enhances competition between H^+ and metal ion for cell surface binding sites, which tends to decrease metal ion toxicity.

The effect of water hardness in ameliorating the toxicity of heavy metals to aquatic organisms has been established for a number of trace metals e.g. Zn (Bradley and Sprague, 1985), Cu (Winner and Gauss, 1985) and Cd (Stephenson and Mackie, 1989a). Increased water hardness decreases metal ion toxicity. Based primarily on studies of fish, the ameliorative effect of increased water hardness has been ascribed to competition between Ca and Mg ions with trace metal ions for gill surface binding sites. Charge balance dictates that an increase in water hardness is accompanied by increased anion concentrations. Changes in metal ion toxicity may arise through increased ion pair formation, in addition to Ca and Mg competition. By determining

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toxicity as a function of free metal ion concentration, a number of studies (e.g. Bradley and Sprague, 1985) have demonstrated that the effect of water hardness on ameliorating metal ion toxicity is independent of the concomitant increase in anionic concentration of the solution. Linear relationships between trace metal LC50s and water hardness have been developed using $\log_{10}$(LC50) as the dependent variable and $\log_{10}$(hardness) as the independent variable (Sprague, 1987). However, toxicity studies for a number of trace metals indicate that the major protective effect of water hardness arises through the Ca concentration of the water (Davies et al. 1993; Markich and Jeffree, 1994). Davies et al. (1993) found little effect of increased water hardness (50,200 and 400 mg L^{-1} as $CaCO_3$) on the toxicity of Cd to rainbow trout, when $MgSO_4$ was used to adjust water hardness.

This paper addresses the effects of Ca, Mg, Na and Cd speciation on the toxicity of Cd to *H. azteca*. This amphipod is commonly used in effluent and freshwater sediment toxicity testing and effluent water testing and is very sensitive to Cd. Cadmium concentrations bioaccumulated in *H. azteca* collected from 69 Ontario lakes were found to be a function of water hardness, total Cd and DOC concentrations (Stephenson and Mackie, 1988). In a laboratory study, Stephenson and Mackie (1989b) further showed that the Cd bioaccumulated by *H. azteca* increased significantly as the Ca concentration of the test solutions decreased. In this study acute 96-h LC50s for Cd were established in laboratory synthesized waters where the major cation was either Ca, Mg, or Na. LC50s were correlated with major solution cation concentrations and the resulting regression equations were used to predict toxicity in additional tests.

MATERIALS AND METHODS

The individual effects of increasing Ca, Mg, and Na concentrations on the acute toxicity of Cd to *H. azteca* were investigated in three 96-h static toxicity tests carried out using a 4 x 5 factorial design. Either Ca, Mg, or Na was added, as the Cl salt, to a base water (6.8 mg L^{-1} $NaHCO_3$, 4 mg L^{-1} KCl, 6 mg L^{-1} $MgSO_4$, and 7.3 mg L^{-1} $CaC1_2 \cdot H_2O$) to create four solutions of increasing concentration for each cation. To each of these solutions five increasing concentrations of Cd were added (including one of 0 Cd as a control). Cd additions were made from dilutions of 1000 mg L^{-1} Cd AA standards (Fisher Scientific, Fairlawn, NJ.). Based on these dilutions there were 20 solutions for each of the Ca, Mg, and Na tests. Five 20 ml replicates of each solution were placed in 30 ml polyethylene cups with ten 7-l O-d old *H. azteca* added to each cup. The cups were randomly distributed on a holding board and placed in an environmental chamber at 23°C with a 16 h light:8 h dark photo-period for 96 h. Animals were not fed for the duration of the test. Pilot studies revealed that *H. azteca* survive well for 96-h without feeding (>90% survival) thus the potential of dissolved organics from added food to complex Cd was alleviated.

Ca, Mg, Na, and K solution concentrations were analyzed by flame AA using a Perkin-Elmer 5000 spectrometer (Perkin-Elmer, Norwalk, CT.), Cd was analyzed by graphite furnace AA using a Perkin-Elmer 4100ZL spectrometer with Zeeman

background correction (Perkin-Elmer, Norwalk, CT.). In all cases solution concentrations were within 5% of nominal value. pH of the solutions was measured at the start and end of the toxicity tests. No attempt was made to specifically control the pH of the test solutions; however, all solutions were equilibrated with atmospheric conditions before Cd addition, and the pH of all solutions was 7.0 ±0.2 at the start and end of the test. The equilibrium speciation model MINTEQA2 (Allinson et al., 1991) was used to calculate free cation concentrations and activities for each solution. LC50s were calculated using the trimmed Spearman-Karbor method (Hamilton et al., 1987). The SAS statistical computer program was used to perform multiple linear regression analyses. The logit transformation of the mortality data was used in the regression analysis according to the formula:

$$\text{logit } P = \ln P/(1-P)$$

where P is the proportion of dead animals.

A further three 96-h toxicity tests were conducted in solutions of varying ionic strength and complexing ability for Cd. A base water (6.8 mg L^{-1} $NaHCO_3$, 4 mg L^{-1} KCl, 6 mg L^{-1} $MgSO_4$, and 96 mg L^{-1} $CaCl_2 \cdot H_2O$) was used as the low ionic strength solution. Two further solutions were created by adding equimolar amounts (10^{-2}M) of either $NaNO_3$ or NaCl to the base water. To these three solutions, five increasing concentrations of Cd were added (including a control of 0 Cd). The 96-h toxicity tests were carried out using the procedure described above. Analytical procedures for solution cation concentration and statistical procedures for regression analysis of data were performed as above.

RESULTS AND DISCUSSION

Increasing the Ca concentration of the solution had a pronounced effect on the toxicity of Cd to *H. azteca* (Table 1). In the solution with the lowest Ca concentration (2 mg L^{-1}) 98% mortality was observed at a Cd concentration of 10 µg L^{-1}. In comparison, 36% of animals survived at a Cd concentration of 75 µg L^{-1} in the solution with the greatest Ca concentration (150 mg L^{-1}). Because Ca was increased by adding the Cl salt to a stock solution of the lowest Ca treatment there was an increasing amount of Cd speciation as $CdCl^+$ at higher Ca concentrations. In addition, increasing Ca concentrations result in solutions of increasing ionic strength and, therefore, lower Cd^{2+} activity. Both these factors could influence the observed mortality, by decreasing apparent Cd toxicities at higher Ca concentration. In view of these facts MINTEQA2 was used to calculate values for free Cd^{2+} concentrations and ion activities that were used to calculate LC50 values. LC50 values calculated on a free ion concentration and activity basis still showed a significant increase with increasing Ca concentration in solution. If complexation was the primary mechanism for the reduction of Cd toxicity then LC50 values calculated on a free Cd^{2+} basis should be relatively constant in the different solution conditions, whereas if competition for uptake by Ca was the primary mechanism for reduction of toxicity then LC50 values calculated on a free Cd^{2+} basis should increase as the solution Ca concentration increases. Clearly, competition by Ca was the primary mechanism for the reduction in Cd toxicity observed in this experiment.

Table 1. Cd LC50 values and 95% confidence intervals in solutions of varying Ca concentration

Total Ca	Total Cd	Free Cd concentration	Free Cd activity
mg L^{-1}	μg L^{-1}		
2	3.8 (3.1 – 4.7)[1]	3.7 (3 – 4.5)	3.3 (2.7 – 4.1)
30	12.1 (10.5 – 14)	10.6 (9.2 – 12.2)	8.5 (7.4 – 9.8)
72	25.0 (22 – 28.4)	19.6 (17.3 – 22.3)	14.2 (12.5 16.1)
150	55.2 (40.8 – 74.5)	37.1 (27.2 – 50.1)	24.3 (19.8 – 29.8)

Increasing Mg concentrations also had an ameliorating affect on Cd toxicity to *H. azteca* but to a much lesser extent than for Ca (Table 2). LC50s calculated on either a free Cd^{2+} concentration or Cd^{2+} activity basis, showed a small but significant increase as Mg concentration increased. The lesser protective effect afforded by Mg was also evident from multiple linear regression analysis of the data. Single variable models, with either total Cd concentration, free Cd^{2+} concentration or Cd^{2+} activity as the independent variable and logit mortality as the dependant variable were all significant at the $P > 0.05$ level with R^2 0.55, 0.80, 0.95 respectively. However, inclusion of a second independent variable, either Mg concentration or activity, further improved the model. Both independent variables were significant and resulted in $R^2 > 0.96$. Increasing Na concentrations had no ameliorative effect on the toxicity of Cd to *H. azteca* and cadmium LC50s calculated on a total Cd basis were not significantly different in solutions of increasing Na concentration (results not shown).

Table 2. Cd LC50 values and 95% confidence intervals in solutions of increasing Mg concentration

Total Mg	Total Cd	Free Cd concentration	Free Cd activity
mg L^{-1}	μg L^{-1}		
1.21	3.8 (3.1 – 4.7)	3.7 (3 – 4.5)	3.3 (2.7 – 4.1)
19.2	6 (4.9 – 7.3)	5.2 (4.2 – 6.3)	4.1 (3.4 – 5)
28.8	7.6 (6.6 – 8.8)	6 (5.2 – 7)	4.4 (3.8 – 5.1)
83.2	12.1 (10.5 – 13.9)	8.3 (7.2 – 9.6)	5.4 (4.7- 6.3)

Results of the Ca, Mg and Na tests demonstrate that toxicity of Cd to *H. azteca* is related to the hardness of the water in which the toxicity test was conducted. More specifically, the toxicity of Cd to *H. azteca* was controlled by the Ca concentration of the test solution. Magnesium concentrations also produced a protective effect but to a much lesser extent than Ca. Regression analysis of the combined data from the three tests exemplifies this point (Table 3). Clearly the two variable model, where the individual protective effects of Ca and Mg are independently considered, provides the

1 values in parenthesis are 95% confidence intervals

best fit of the experimental data, while the magnitude of the coefficients for Ca and Mg indicate the relative magnitude of the protective effect afforded by each cation.

There is good agreement between the LC50s calculated on the basis of free Cd concentration and the Ca and Mg concentrations of the solution. However, similar agreement ($R^2 > 0.99$) exists for regression equations relating LC50s based on total Cd concentrations to Ca and Mg concentrations and LC50s based on Cd activity to Ca and Mg solution activities with R^2 values of 0.996 and 0.993, respectively.

Table 3. Results of regression analysis combining data from the Ca, Mg and Na bioassays[2]

model	equation	F value	R^2 value
(LC50) = (Ca)	(LC50) =3.85 + 0.22(Ca)	384.54	0.975
(LC50) = (Ca) + (Mg)	(LC50) = 2.866 + 0.23(Ca) + 0.063(Mg)	1687.54	0.997
(LC50) = (hardness)	(LC50) =2.5 + 0.058(hardness)	14.46	0.59

While these toxicity tests indicate the relative protective effects of Ca and Mg on ameliorating the toxicity of Cd to *H. azteca*, they do not differentiate between total Cd concentration, free Cd concentration, or Cd activity as the best predictor of toxicity. To investigate this, and to test the regression equations described above, a second series of tests were performed. A low ionic strength, low chloride solution (equivalent to the 2nd Ca bioassay above) was the base solution for one bioassay. Two further solutions, both 10^{-2} M, were made by adding $NaNO_3$ (high ionic strength/low chloride) and NaCl (high ionic strength/high chloride) to the base solution. The Cd LC50s from these tests are shown in Table 4.

The Ca and Mg concentrations in each of the three test solutions was fixed; however, based on LC50 values calculated from total Cd concentrations Cd appeared less toxic in the high Cl/high ionic strength matrix. This suggests the non-toxicity of the $CdCl^+$ ion pair. When LC50s were calculated on a free metal basis, the calculated values were not significantly different over the three test solutions. By contrast LC50 calculations based on free Cd activity predicted that Cd was more toxic in the high ionic strength solutions, which may be due to a concomitant decrease in Ca and Mg activities.

The regression equations derived from the Ca/Mg/Na bioassays were used to predict Cd LC50s for these latter bioassays (Table 4). Because Ca and Mg concentrations were fixed for all three test solutions, the predicted total Cd concentration and free Cd concentration LC50s were the same for each solution. Predicted LC50s based on total Cd overestimate the toxicity of Cd in the high ionic strength solution, predicting

2 LC50 values are calculated on a free Cd concentration in µg L^{-1}. Ca and Mg are in mg L^{-1} and hardness is mg L^{-1} as $CaCO_3$

Table 4. Cd LC50s and confidence intervals and predicted LC50s in solutions of varying ionic strength and $CdCl^+$ ion-pair formation

	Total Cd		Free Cd concentration		Free Cd activity		Cd speciation[3]	
	μg L^{-1}						% of total Cd	
	LC50	P_{LC50}[4]	LC50	P_{LC50}	LC50	P_{LC50}	Free Cd^{2+}	$CdCl^+$
Low IS Low Cl	14.4 (12.7 – 16.3)[5]	13.8 (11.3 – 16.3)	12.6 (11 – 14.3)	10.3 (8.9 – 11.7)	9.9 (8.8 – 11.2)	8.2 (6.7 – 9.6)	88	8
High IS Low Cl	13.3 (11.9 – 14.9)	13.8 (11.3 – 16.3)	11.8 (10.6 – 13.2)	10.3 (8.9 – 11.7)	7 (5.6 – 7.8)	6.7 (5.3 –8.2)	88	8
High IS High Cl	19.6 (17.1 – 22.5)	13.8 (11.3 – 16.3)	10 (8.8 – 13.5)	10.3 (8.9 – 11.7)	6.1 (5.2 – 6.7)	6.7 (5.3 –8.2)	51	46

3 Cd speciation predicted from MINTEQA2

4 Predicted LC50 based on regression equations derived from first series of bioassays

5 values in parenthesis are, respectively, 95 % confidence intervals and 95% prediction intervals for calculated and predicted LC50 values.

a lower LC50 value (13.7 µg L^{-1}) than the actual LC50 obtained from the bioassay (19.6 µg L^{-1}). The total Cd concentration model cannot account for changes in Cd speciation arising through ion pair or complex formation. However, much better agreement between experimental and predicted values was obtained when calculated and predicted LC50 values for the free Cd concentration were considered. Similarly, predicted activity-based Cd^{2+} LC50 values were also in good agreement with calculated Cd^{2+} activity LC50s.

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