THE TOXICITY OF BINARY METAL MIXTURES TO *CERIODAPHNIA DUBIA* IN MINING INFLUENCED WATERS[1]

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Abstract. Revisions to current EPA regulations for surface waters containing metals, such as mining influenced waters (MIW), are under consideration. It is proposed to substitute a calculation using the Biotic Ligand Model (BLM) for the current hardness-based criteria. This approach may more accurately account for the influence of varying surface water chemistry on metal toxicity. Although the revision may be a great improvement from the traditional toxicity determination, metals are only considered individually. Most effluents from mining disturbed lands contain multiple toxic metals. Currently the approach used in this situation is to assume that metal toxicity is additive. There are reasons to believe that not all metals form additive combinations. The biotic ligand model was used to determine the LC_{50} values for copper, cadmium, nickel, and zinc individually for *Ceriodaphnia dubia* in a MIW. Binary-metal acute toxicity tests on C. *dubia* using the four metals were then conducted. Both the BLM calculations and the experiments were performed using stream water collected upstream of a mining-impacted reach of North Fork Clear Creek, Colorado. Experiments were performed in order to determine if additivity was occurring. In fact, antagonistic (less than additive) effects were seen for most binary combinations. Results indicate that, for the metal combinations tested, the proposed BLM-based regulations could be sufficiently protective if non-additive effects are accounted for in describing the toxicity of metal mixtures.

Additional Key Words: metal contamination, mining influenced waters, toxicity testing

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Introduction

Mining disturbed lands, along with other industries, frequently release mixtures of metals into surface waters. The established water quality criteria determine toxicity of a single metal in relation to the receiving water's hardness. In these criteria "relative contribution of the Ca and Mg components of total hardness as modifiers of metal toxicity is not considered" (Welsh et al., 2000). In part because calcium and magnesium protect against toxicity in different ways, the US EPA has begun to implement the biotic ligand model (BLM) for determining the toxicity of metals. The BLM of acute metal toxicity to aquatic organisms accounts for the influence of water chemistry on the concentration of metal that is complexed with the "biotic" ligand, such as the gills of fish. Mortality is directly related to the amount of metal accumulated by the "biotic" ligand (Di Toro et al., 2001). The computer program requires the input of water chemistry parameters in order to determine speciation of the metal being evaluated, and then produces an LC_{50} value, the concentration of the metal that cause 50% of the organisms to die. These LC_{50} values are produced for combinations of a single metal and a single organism. For regulation purposes, combined metal toxicity is assumed to be additive. Mixed metal toxicity has yet to be determined to an extent that can confirm if this assumption can be justified. This paper described experiments that were performed in order to determine the toxicity of binary mixtures of Cd, Cu, Ni, and Zn to *Ceriodaphnia dubia*. The experimental approach can be modified to different metal and aquatic species. Further testing avenues that will be pursued in this research are also discussed.

Toxicity of Metals

The bioavailability and toxicity of metals can be related to the concentration of the free ion form of the metal. Metals present in aquatic systems can complex with inorganic compounds, such as carbonates, hydroxides, and organic matter. The complexation of metals with these aqueous ligands is in competition with hydrogen ions and other cations (Heijerick et al., 2003). The amount of these ligands available for metals to bind with determines the concentration of free metal ions that are bioavailable to the "biotic" ligand. Competition between the metal and hardness cations for binding to the "biotic" ligand is also important. Mortality occurs when the concentration of metal bound to the biotic ligand exceeds a threshold concentration (Di Toro et al., 2001). Metals cause toxicity through different modes of action. For example, acute metal toxicity in fish has been associated with the disruption of Na^+ ion regulation. (Playle et al., 1993) The BLM takes into consideration all of the metal's interactions with other solution components in order to determine speciation and then relates the total metal concentration to the amount of metal- biotic ligand complexes that cause mortality.

When multiple metals are present it is generally assumed that their effect is additive (Neuman and Unger, 2003). This approach uses the concept of toxic units (TU) to describe acute toxicity. Generally a toxic unit of a given metal is its concentration divided by its toxic waster quality criteria value. A metal is considered at a toxic level when TU $\geq$ 1. For a mixture of metals toxicity is assumed to occur when the sum of the various TUs $\geq$ 1 as shown in equation 1.

$$TTM = \Sigma(C_i / WQC_i) \qquad\qquad (1)$$

where:

TTM = Predicted Total Toxicity of the Mixture

C_i = the concentration of the component

WQC_i = the water quality criteria for that component

The value for the WQC is currently computed using a hardness-based approach. In the future a BLM computed value for the WQC may be used. This approach is described in the following section.

Recently Norwood et al. (2003) reviewed a large number of studies of the acute toxicity of metal mixtures. They reviewed 68 publications that represented acute aquatic toxicity results for 77 biological species. These studies involved 191 tests which included 156 binary mixtures, 18 ternary mixtures, and 17 mixtures containing 4 of more metals. There analysis suggested that approximately 42 % of the results were less than additive, 28 % were additive, and 30% were more than additive. Clearly more work is required to validate the degree to which the TU approach can explain metal toxicity in mining influenced waters (MIW) where multiple metals are generally present.

The Biotic Ligand Model (BLM)

The BLM was developed to better interpret the acute toxicity of metals to aquatic organisms in relation to water chemistry parameters. Metals have been found to be toxic to fish and other aquatic organisms in the free ion form in which they are able to bind to sites on the fish gills, also known as the biotic ligand. Computational chemical speciation programs such as MINEQL (Westall et al., 1976) and MINTEQA2 (Brown and Allison, 1987) have been created to determine the concentrations of different chemical species that will occur under varying water chemistry conditions. The BLM computes metal speciation and the LC_{50} for a metal in a specific water body after temperature, pH, concentration of metal under evaluation, dissolved organic carbon (DOC), percent humic acid, calcium, magnesium, sodium, potassium, sulfate, chloride, alkalinity, and sulfide concentrations are entered into the program. These water chemistry components determine the inorganic and organic metal interactions that define the concentration of metal present in the free ion form. This concept is based on the free ion activity model (FIAM) (Morel, 1983). The BLM computation for metal interaction with organic carbon is based on the Windermere humic aqueous model, WHAM, Version 1.0, (Santore et al.,1995) and all chemical interactions are simulated by the chemical equilibrium speciation in soils and solutions model, the CHESS model. (Santore et al.,1995) The BLM also considers the competition between the metal and other cations, i.e. Ca^{2+}, H^+, Mg^{2+}, for binding with the biotic ligand in order to determine the actual amount of metal that will accumulate on the gill. The model relates mortality to a critical concentration of metal bound to the biotic ligand (Di Toro et al., 2001). The BLM determines the speciation of a metal based on the water quality conditions and the amount of metal that has to be present to cause toxicity due to the metal's affinity to the binding sites on the gill. In this way it may be applied to individual water bodies to determine water quality criteria (WQC).

The BLM is a resourceful tool; however it has some questionable components. One issue is that the percent humic acid parameter is rarely measured during evaluation of the dissolved organic carbon of a water sample. This means that a value of 10% humic acid is inputted into the model even though this amount could be incorrect. Also sulfide has been shown to influence toxicity of metals (Di Toro et al, 1990), and although the creators have recognized this by requiring an input number for sulfide concentration, this parameter has not yet been fully incorporated into the model. Another concern is that binding constants of the free ion used in the BLM to determine metal binding to gills at variable concentrations may be flawed, due to the fact that they are experimentally derived (Di Toro et al., 2001). Also there is no input parameter for standard deviation of water chemistry concentrations or error values for the final output numbers from the model. With further development of the BLM sources of error can be minimized.

Experimental Methods

Experimental Design

A set of experiments was designed in order to determine if metals have an additive toxic effect when they are present together in aquatic environments. The first step was to determine if Reconstituted laboratory water or surface water from an existing body of water should be used for testing. Reconstituted waters may bias metal toxicity results due to a difference of Ca:Mg and Na:K to that in natural waters (Welsh et al., 2001). We chose to use water from a local creek, collected upstream of mining impacts, for all metals testing.

A sensitive species, *Ceriodaphnia dubia* was chosen for the test species due to the organisms' sensitivity and the ease of culturing. Four metals that are commonly found in mining impacted waters were used in this study: Cd, Cu, Ni, and Zn. The BLM was used to estimate LC_{50} values for Cd to *C. dubia* and Cu to *C. dubia*. The BLM does not yet have programs for Zn or Ni effects of *C. dubia*. The BLM program for Zn *Daphnia Magna* was used to estimate Zn LC_{50} values for *C. dubia*. An earlier paper on Ni toxicity to *Daphnia Magna* was used to estimate Ni LC_{50} values for *C. dubia*.

Acute toxicity tests were set up in accordance with the EPA region XIII 48 hour static non-renewal acute toxicity testing protocol. The region 8 EPA lab typically uses 4 replicates of 5 neonates per concentration. Reconstituted laboratory water was used for Reference toxicity testing using 1g/L of KCl as the highest concentration to determine the LC_{50} values. The variance of those values was entered into the UCLA power calculation web page to deduce the statistical power of the data, establishing that four replicates would be statistically significant.

The single metal tests were conducted to determine the individual metal's toxic units, where 1 TU is equal to the LC_{50} value. These values were used for creating binary mixtures in which one metal was held constant at one-half of the LC_{50} value (0.5 TU) while the other was varied from 0-2.5 TU. Thus the total TU of the tests were approximately 0.5, 0.75, 1.0, 1.5, 2.5, 3.0.

Experimental Procedures

Water from North Fork Clear Creek, upstream of mining inputs, in Jefferson County Colorado was collected in two 20L cubitainers on September 7, 2004. The water was then filtered through a biological Nalgene filter (0.45 micron) into 1L small mouthed Nalgene bottles

and stored at 4 °C at the Region XIII EPA laboratory. Alkalinity was determined with a HACH titrator using the bromocresol green indicator endpoint. The pH was measured in the field. Elemental analysis was performed on acidified subsamples using a Perkin-Elmer Optima 3000 ICP-AES instrument. Hardness was computed from the sum of the Ca and Mg determined by ICP-AES. DOC was measured by UV-catalyzed persulfate oxidation using a Seivers TOC 800 Turbo instrument.

All reference and metal acute toxicity tests were performed in the same manner using different concentrations of toxicants and North Fork Clear Creek stream water as dilution water. The toxicant was diluted from the highest test concentration in a 1:1 ratio a total of four times, thus achieving concentrations of 100, 50, 25, 12.5, and 6.25 % of the original concentration. A stream water control with no toxicant was also used. In mixed metal tests, the dilution water contained 0.5TU of the constant metal. Plastic test cups were filled with 20ml of water and 5 *C. dubia* organisms. The pH, conductivity, DO, and temperature of the dilutions were measured and then the cups were covered with glass and placed in an incubator at 20 °C for 48 hr. Tests were performed using a light:dark cycke of 16:8 hours. After 48hr the samples were removed from the incubator and the surviving and dead organisms are counted. The mortality data was entered in to the Spearman-Karber program (tsk.exe) to determine the LC_{50} values.

Results and Discussion

The single metal tests were conducted to find the LC_{50} concentration for each metal. The North Fork Clear Creek water sample chemistry for the test was: Ca = 10.13 mg/L, Mg = 3.03 mg/L, Na = 4.61 mg/L, K = 1.16 mg/L, SO_4 = 12.84 mg/L, alkalinity = 15.0 (mg/L $CaCO_3$), DOC = 1.05 mg C/L, and pH = 7.5. The single metal toxicity data is presented in Figs. 1-4.
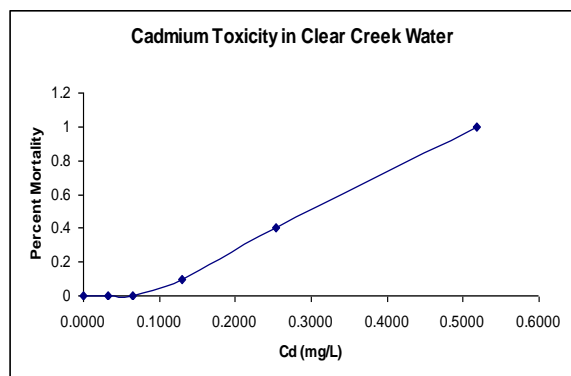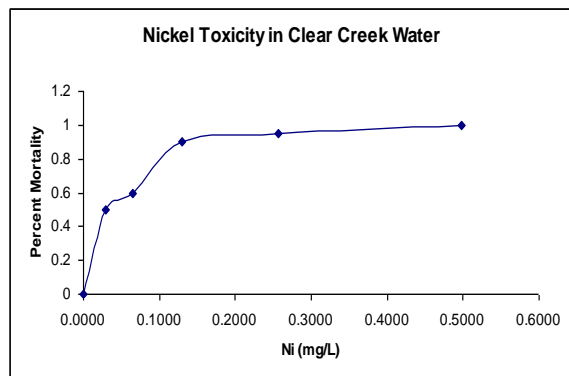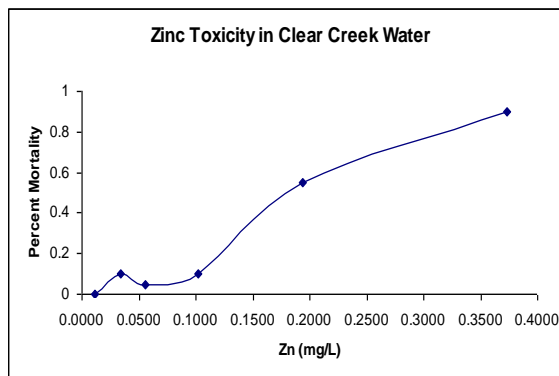

LC$_{50}$ = 0.26 mg/L Cd
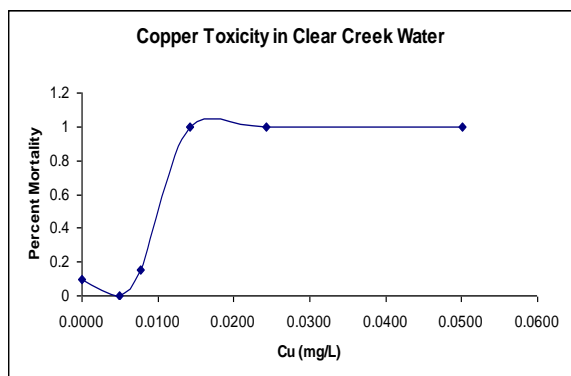
Figure 1. Cadmium Toxicity

LC$_{50}$ = 0.01 mg/L Cu

Figure 2. Copper Toxicity

Figure 3. Nickel Toxicity

Figure 4. Zinc Toxicity

The LC_{50} value for cadmium was higher than was expected from previous publications. *C. dubia* were much more sensitive to nickel than *Daphnia magna* used in prior studies. LC_{50} values for metal mixtures were calculated using toxic units. Toxic units were calculated by taking the concentration of a metal and dividing it by the LC_{50} value for that metal. These units can then be used to evaluate the metals together by adding the toxic units to find the total toxicity of the mixture. An additive mixture has an LC_{50} value = 1. For antagonistic mixtures the LC_{50} value is greater than 1 and for synergistic mixtures it is less than 1. Table 1 contains the toxicity data for two sets of mixed metal toxicity tests. Both the value of the TU for the constant metal as well as the TU for the mixture is shown.

Table 1. Results of metal mixture toxicity tests.

Constant Metal	Constant Metal TUs	Varying Metal	LC_{50} value in Toxic Units
Zinc	0.43	Cadmium	1.36
Zinc	0.44	Copper	1.51
Zinc	0.42	Nickel	1.9
Nickel	0.47	Cadmium	0.98
Nickel	0.48	Copper	1.38
Nickel	0.36	Zinc	1.34

The finding that for most mixtures the LC_{50} values are greater than 1 demonstrate a general antagonistic effect is occurring (i.e., less toxicity than predicted). This may have significant implications of MIW where multiple metals are commonly present. It would appear that in order

to protect aquatic life, water quality standards must be able to quantitatively account for non-additive effects.

Future Work

The remainder of the binary mixtures where one metal is held constant at about 0.5 TU is currently being evaluated and results will be made available in a future publication. Future evaluations will contain metal mixtures composed of one metal at a relatively low TU and greater variation of the second metal. The further testing will help verify modeling that suggests "deviations from strict additivity are due to the nonlinearity of the models". (Playle, 2004)

Acknowledgements

Literature Cited

Brown, D.S., Allison, J.D. 1987. MINTEQA1. An equilibrium metal speciation model. User's Manual. *In*: US Environmental Protection Agency, Athens, GA. EPA/600/3-87/012.

Di Toro, Dominic M., Allen, Herbert E., Meyer, Joseph S., Paquin, Paul R. 2001. Biotic ligand model of the acute toxicity of metals 1. Technical basis. p.2283-2396. In: Environmental Toxicology and Chemistry. Vol. 20, No. 10. http://dx.doi.org/10.1002/etc.5620201034.

Di Toro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Hicks, M.B., Mayr, S.M., and Redmond, M.S. 1990. Toxicity of cadmium in sediments: the role of acid volatile sulfide. p. 1487-1502. In: Environ Toxicol. Chem. Vol. 9. http://dx.doi.org/10.1002/etc.5620091208.

Heijerick, D.G., Janssen, S.R., Coen, W.M. 2003. The combined effects of hardness, pH, and dissolved organic carbon on the chronic toxicity of Zn to *D. magna*: Development of a surface response model. p. 210-217. *In*: Archies of Environmental Contamination and Toxicology.

Morel, F.M.M. 1983. Principles of Aquatic Chemistry. John Wiley, New York, NY, USA.

Newman, M.C. and Unger, M.A. 2003. Fundamentals of Ecotoxicology, 2nd Ed. Lewis Publishers, New York.

Norwood, W.P., Borgmann, U., Dixon, D.G., and Wallace, A. 2003. Effects of metal mixtures on aquatic biota: a review of observations and methods. Human and Ecological Risk Assessment, volume 9, number 4, pages 795 to 811.

Playle, Richard C. 2004. Using multiple –gill binding models and the toxic unit concept to help reconcile multiple-metal toxicity results. *In*: Aquatic Toxicology p. 359-370.

Playle, Richard C., Dixon, George D., Burnison, Kent. 1993. Copper cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. p. 2667-2677. *In*: Can. J. Fish Aquat. Sci. Vol. 50. https://doi.org/10.1139/f93-290

Santore, Robert C., Di Toro, Dominic M., Paquin, Paul R., Allen, Herbert E., and Meyer, Joseph S. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. In: Environmental Toxicology and Chemistry. Vol. 20, No. 10. http://dx.doi.org/10.1897/1551-5028(2001)020<2397:BLMOTA>2.0.CO;2 http://dx.doi.org/10.1002/etc.5620201035.

Santore, R.C., Driscoll, C.T. 1995. The CHESS model for calculating chemical equilibria in soils and solutions. p. 357-375. *In*: Loeppert R, Schwab AP, Goldberg S, eds, Chemical Equilibrium and Raction Models. Soil Science Society of America Special Publication 42. American Society of Agronomy, Madison, WI.

Welsh, Paul G., Chapman, Gary A., Hansen, James A., Lipton, Joshua. 2001. Importance of Ionic Composition of Reconstituted Laboratory Test Water in Interpreting Metal Toxicity Test Results. p. 3-15. *In*: Standard Technical Publication 1403, American Society for Testing and Materials.

Welsh, Paul G., Lipton, Joshua, Chapman, Gary A., Podrabsky, Tracy L. 2000. Relative Importance of calcium and magnesium in hardness-based modification of copper toxicity. *In*: Environmental Toxicology and Chemistry. Vol. 19, No. 6.

Westall, J.C., Zachary, J.L., Morel, F.M.M. 1976. A computer program for the calculation of chemical equilibrium composition of aqueous systems, technical note 18. *In*: Department of Civil Engineering, Massachusetts Institute of Technology, Cambridge, MA.