Protective Effects of Magnesium and Calcium Carbonate against Aluminum Toxicity in *Daphnia magna*

Amy Jiang
Science One Program
University of British Columbia
Vancouver, Canada
June 2011

Abstract

Aluminum toxicity is known to be decreased by other factors, such as the presence of silicon. In this study, acute toxicity tests were performed on *Daphnia magna* to determine if magnesium and calcium carbonate are protective against aluminum toxicity. Magnesium carbonate eliminates acute toxicity, while calcium carbonate is protective at aluminum sulphate concentrations above 40 μM. The mechanism of protection is not fully understood, but the presence of carbonates may promote the formation of aluminate ions, a less toxic species of aluminum. These results could have implications in current research studying aluminum toxicity humans.

Introduction

Aluminum is the third most abundant element in Earth's lithosphere and it is found ubiquitously in food, water, soil and consumer goods. It has no known function in living cells and organisms; however, at elevated concentrations, aluminum is a known neurotoxin. However, in some geographical regions, physiochemical properties of water cause interactions between aluminum and other elements that make the aluminum less bioavailable and therefore less toxic (Gauthier et al., 2000). For example, there is strong evidence that silicon is protective against toxicity (Birchall et al., 1989), since it causes hydroxyaluminosilicates to form (Exley et al., 1997). We speculated that the hardness of the drinking water would also decrease the bioavailability of aluminum. Hard water has a high concentration of calcium and magnesium carbonate. These compounds may interact with aluminum and change its toxicity.

In this study, we used acute toxicity tests to determine the toxic effects of aluminum sulphate on *Daphnia magna* in hard and soft water. *Daphnia magna,* more commonly known as the water flea, is a freshwater crustacean in the cladocera suborder. *Daphnia* are commonly used in toxicity tests since they are very sensitive to water quality, particularly to ion balance. We examined the viability of *Daphnia magna* when exposed to aluminum sulphate, and compared it to the viability of *Daphnia* exposed to aluminum sulphate in the presence of calcium carbonate and magnesium carbonate. We hypothesized that calcium and magnesium carbonate would both display a protective effect against aluminum toxicity; the results agreed.

Methods

The experiment tested the effect of aluminum sulphate on *Daphnia* viability in the presence of a constant amount of a saturated solution of calcium carbonate or magnesium carbonate. Chemicals used included aluminum sulphate (Fisher, A-613), calcium carbonate light (Anachemia, 18596-300) and magnesium carbonate light (Anachemia, 53478-300).

Daphnia magna were shipped in tank water containing a small amount of peat and soil from Boreal Northwest. They were kept at room temperature, out of direct light, and fed *Euglena gracilis* 24 hours before each experiment. They were used within 48 hours of delivery.

The viability of *Daphnia* was compared from four different treatments. All treatments were carried out in small borosilicate glass test tubes. The first was a range of 19 aluminum sulphate concentrations from 0 to 108 μM, increasing in increments of 6 μM. These concentrations were created by micropipetting 19 different volumes of a 2.00 g/L aluminum sulphate solution into test tubes, then mixing by inversion. Five *Daphnia* were added to each tube in one drop of tank water, and then deionized water was added until the final volume of each solution was 5 mL.

The second treatment was as a control using only deionized water. The same procedure as above was followed; however, instead of adding the 19 volumes of aluminum sulphate, 19 equivalent volumes of deionized water were added. Deionized water serves as a control for oxygen that can be dissolved in the solution through the mixing, or any possible effect that adding varying small volumes of liquid using a micropipette could have.

Super saturated calcium carbonate and magnesium carbonate solutions were created, then any undissolved solid was filtered out 24 hours later. Two more ranges of aluminum concentrations, from 0 to 108 μM, were created. In each range, 1 mL of either saturated calcium or magnesium carbonate was added. *Daphnia* were added, and the total volume adjusted to 5 mL. All samples were left undisturbed, at room temperature and out of direct light for 24 hours, and then the number of *Daphnia* alive in each tube was counted. The four treatments were repeated three times each.

Two statistical resampling methods were used to (i) find the standard error of the slope and intercept of a linear regression for all treatments and (ii) determine whether slopes and intercepts from all pairs of treatments were statistically distinct.

Results

Overall, aluminum sulphate has toxic effects on *Daphnia* viability compared to water alone; magnesium carbonate and calcium carbonate both improved *Daphnia* viability (see Figure 1). As shown previously (Anderson, 1944), we demonstrate here that aluminum sulphate has a toxic effect on *Daphnia magna*. The slope of linear regression was found to be - 0.79 ± 0.072. This was statistically distinct from the water control ($p = 0$).

We found that magnesium carbonate is strongly protective against aluminum toxicity. The slope of the linear regression of aluminum sulphate and magnesium carbonate was 0.19 ± 0.069. This was statistically distinct from the treatment with only aluminum sulphate ($p = 0$). Magnesium carbonate improved *Daphnia* viability to such an extent that its slope was comparable to the water control ($p = 0.18$).

Calcium carbonate was found to be detrimental at low concentrations of aluminum sulphate, but at aluminum sulphate concentrations of above 40 μM, they mutually eliminate their detrimental effects. The slope of the linear regression of the treatment with aluminum sulphate and magnesium carbonate was 0.21 ± 0.083. This was statistically distinct from the treatment with only aluminum sulphate ($p = 0$). However, magnesium carbonate is more strongly protective against aluminum toxicity in *Daphnia* than calcium carbonate is. While the slopes found by linear regression for the treatments involving magnesium carbonate and calcium carbonate were not statistically distinct ($p = 0.895$), their intercepts were ($p = 0.0007$). Calcium carbonate only displayed a protective effect at aluminum sulphate concentrations above 40 μM, while magnesium carbonate was protective throughout the entire concentration range.

Furthermore, it was found that aluminum sulphate decreased the pH of the solutions (see Appendix Figure 1). Adding magnesium or calcium carbonate to aluminum sulphate nearly restored the pH of the solutions to the pH of distilled water alone.

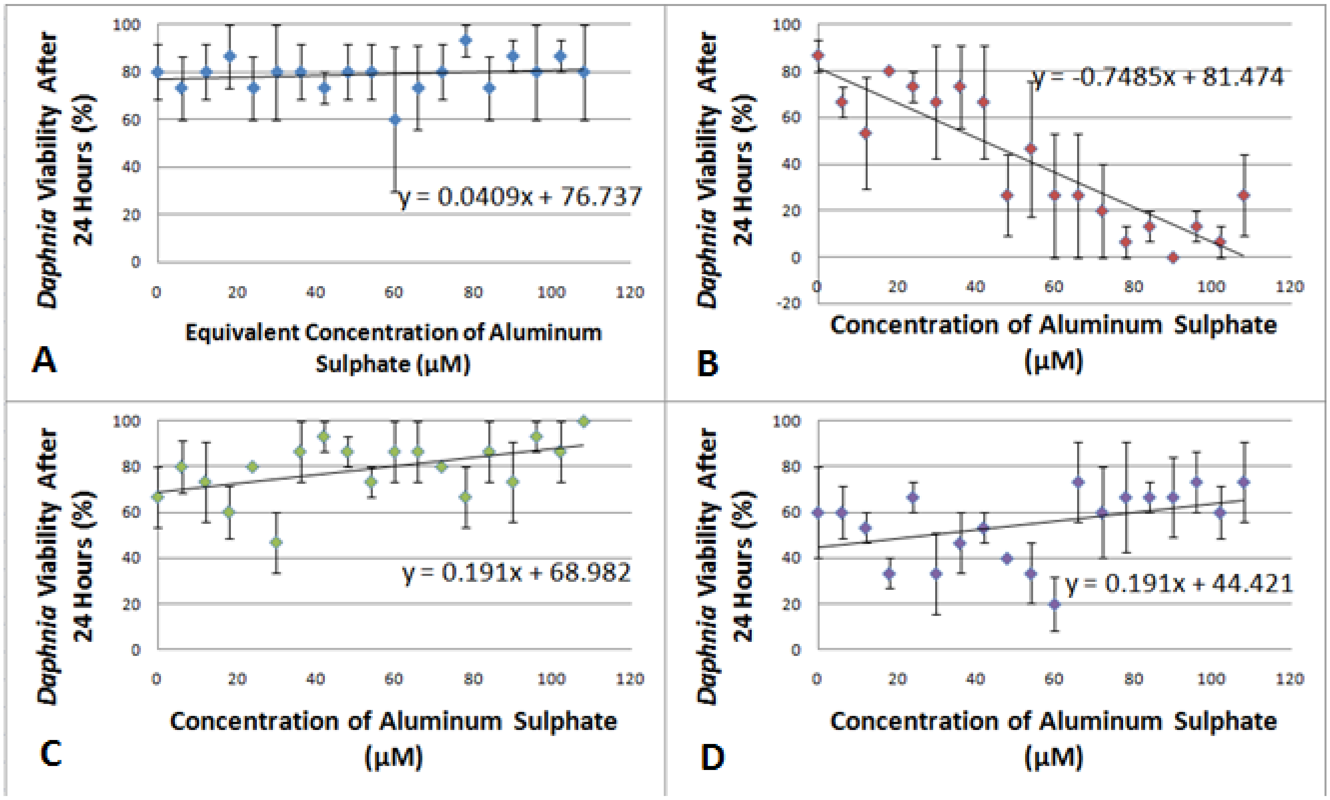

Figure 1: Effect of Various Concentration of Aluminum Sulphate on Daphnia Viability in (A) Water alone (control, no $AlSO_4$ added), (B), $AlSO_4$ alone, (C) $AlSO_4$ in the presence of $CaCO_3$ and (D) $AlSO_4$ in the presence of $MgCO_3$. Fifteen *Daphnia* were added to three different tubes at each concentration in each treatment, and viability was determined after 24 hours. Error bars represent standard error, n = 3.

Discussion

Aluminum sulphate was toxic to *Daphnia magna;* however, magnesium and calcium carbonate both restored *Daphnia* viability to some extent.

The mechanism of aluminum toxicity has been well studied in fish. Aluminum binds to certain functional groups in the gill epithelium, increasing its permeability to ions and accelerating cell death (Exley et al., 1991). The precise mechanism of aluminum toxicity in *Daphnia* is unknown, but it has been found that aluminum interferes with salt regulation, causing a loss of sodium and chloride ions from the body (Havas, 1985). *Daphnia* exposed to high levels of aluminum have been found to have aluminum bound to the maxillary glands, where ion exchange occurs (Havens, 1990). These factors considered, it is likely that *Daphnia* are affected by aluminum in the same way as fish.

Adding magnesium or calcium carbonate to water increases water hardness. It is known that water hardness affects the toxicity of various metals in many aquatic species. For example, one study found that in the freshwater crustacean, *Hyalella azteca*, aluminum is over 17 times as toxic in soft water as it is in hard water (Borgmann et al., 2005). This variation in toxicity is particularly well-studied in copper and cadmium. Calcium is known to reduce the uptake of divalent ions such as copper and cadmium by competing for binding sites (Komjarova & Blust, 2009). There is a possibility that calcium and magnesium may compete for binding sites on the *Daphnia* maxillary glands, decreasing the bioavailability of aluminum to some extent. However, since aluminum is trivalent, its toxicity may result from mechanisms distinct from those of copper or cadmium.

Adding magnesium and calcium carbonate also increased the pH of the aluminum sulphate solutions. Silicon reduces aluminum toxicity because it causes a change in the speciation of aluminum, decreasing its bioavailability (Exley et al. 1997). Changing the pH of the solution also greatly affects the speciation of aluminum (see Appendix Figure 2), which in turn affects its toxicity and bioavailability (Gensemer & Playle, 1999). In acidic waters, cationic species of aluminum are found, particularly as Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2^{+}$. These species of aluminum have been found to be extremely toxic to fish (Gensemer & Playle, 1999). However, when the pH is greater than 6.7, $Al(OH)_4^{-}$, aluminate, is the dominant species (Driscoll & Schecher ,1990). Aluminate has been found to be less toxic to fish (Poleo & Hytterod, 2003), this is possibly the case for *Daphnia* as well.

To test if increasing the pH plays a significant role in preventing aluminum toxicity, I carried out a preliminary trial exposing *Daphnia* to a range of aluminum sulphate concentrations in the presence of 0.0012 M HEPES buffer. The HEPES buffer kept all pH levels close to 7, and they also protected the *Daphnia* against aluminum toxicity (see Appendix Figure 3). This result suggests that the main mechanism of protection offered by the carbonates is through raising the pH; however, this hypothesis needs to be tested more rigorously.

Although adding magnesium carbonate and calcium carbonate raised the pH to a similar level, the two carbonates displayed different protective behaviours. Magnesium carbonate completely eliminated the toxic effects of aluminum sulphate while calcium carbonate only offered protection at higher concentrations. In fact, magnesium carbonate was found to improve *Daphnia* viability even beyond the deionized water control; however, this is likely due to biological variation in a small sample size. These findings suggest that magnesium and calcium carbonate may be using different mechanisms to protect against aluminum toxicity, and there are factors other than aluminum-hydroxide complex formation at play.

This field clearly has room for much further exploration to better understand the protective mechanism of the carbonates. More replicates should also be carried out to reduce the error due to biological variation, and to obtain a better distribution of percent viabilities at each concentration in each treatment. The speciation of aluminum should be measured in each sample, as well as the final concentration of both carbonates. Further experiments can be performed using different concentrations of magnesium and calcium carbonate to explore their potentially different protective mechanisms. The protection that a buffer such as HEPES can offer against aluminum toxicity should also be further investigated. These experiments can also be repeated using different salts of aluminum, such as aluminum chloride and aluminum nitrate as a control for the sulphate ions that were also present in each solution.

Conclusion

Magnesium and calcium carbonate, two compounds that increase to water hardness and pH, display a protective effect against aluminum toxicity in *Daphnia magna.* This may be through the formation of less toxic, anionic aluminum-hydroxide complexes; however, their respective mechanisms of protection are yet to be fully explored. A better understanding of the relationship between aluminum toxicity and water hardness could have important implications. Since hard water protected *Daphnia* against acute aluminum toxicity, it may also protect humans against aluminum's chronic neurotoxic effects.

Acknowledgements

I would like to thank Eric Cytrynbaum for his advice and for performing the data analysis on this project. Thank you to Elizabeth Straszynski and Anna Jiang for introducing me to *Daphnia*, and to John Percy for his ideas and support. Finally, I would like to thank Maire Percy for her continued guidance and dedication to this project over the past year; without her, this would not have been possible.

References

Anderson, B.G. (1944). The toxicity thresholds of various substances found in industrial wastes as determined by the use of *Daphnia magna*. *Sewage Works Journal,* 16(6), 156 – 1165.

Birchall, J.D., Exley, C., Chappell, J.S. & Phillips, M.J. (1989). Acute toxicity of aluminium to fish eliminated in silicon-rich acid waters. *Nature,* 338, 146-148.

Borgmann, U., Couillard, Y., Doyle, P. & Dixon, D.G. (2005). Toxicity of sixty-three metals and metalloids to *Hyalella azteca* at two levels of water hardness. *Environmental Toxicology and Chemistry,* 24, 641-652.

Exley, C., Chappell, J.S. & Birchall, J.D. (1991). A mechanism for acute aluminium toxicity in fish. *Journal of Theoretical Biology,* 151, 417-428.

Exley, C., Pinnegar, J.L. & Taylor, H. (1997). Hydroxyaluminosilicates and acute aluminum toxicity in fish. *Journal of Theoretical Biology,* 189, 133 – 139.

Gauthier, E., Fortier, I., Courchesne, F., Pepin, P., Mortimer, J. & Gavreau, D. Aluminum forms in drinking water and risk of Alzheimer's disease. *Environmental Research Section A,* 84, 234 – 246.

Havas, M. (1985). Aluminum bioaccumulation and toxicity to *Daphnia magna* in Soft Water at Low pH. *Canadian Journal of Fisheries and Aquatic Sciences,* 37, 1741-1748.

Havens, K.E. (1990). Aluminum Binding to Ion Exchange Sites in Sensitive Versus Acid-Tolerant Cladocerans. *Environmental Pollution,* 64, 133 – 141.

Komjarova, I. & Blust, R. (2009). Effect of Na, Ca and pH on simultaneous uptake of Cd, Cu, Ni, Pb, and Zn in the water flea Daphnia magna measured using stable isotopes. *Aquatic Toxicology,* 94, 81-86.

Poleo, A.B.S. & Hytterod, S (2003). The effect of aluminum on Atlantic salmon (*Salmo salar)* with special emphasis on alkaline water. *Journal of Inorganic Biochemistry,* 97(1), 89 – 96.

Appendix

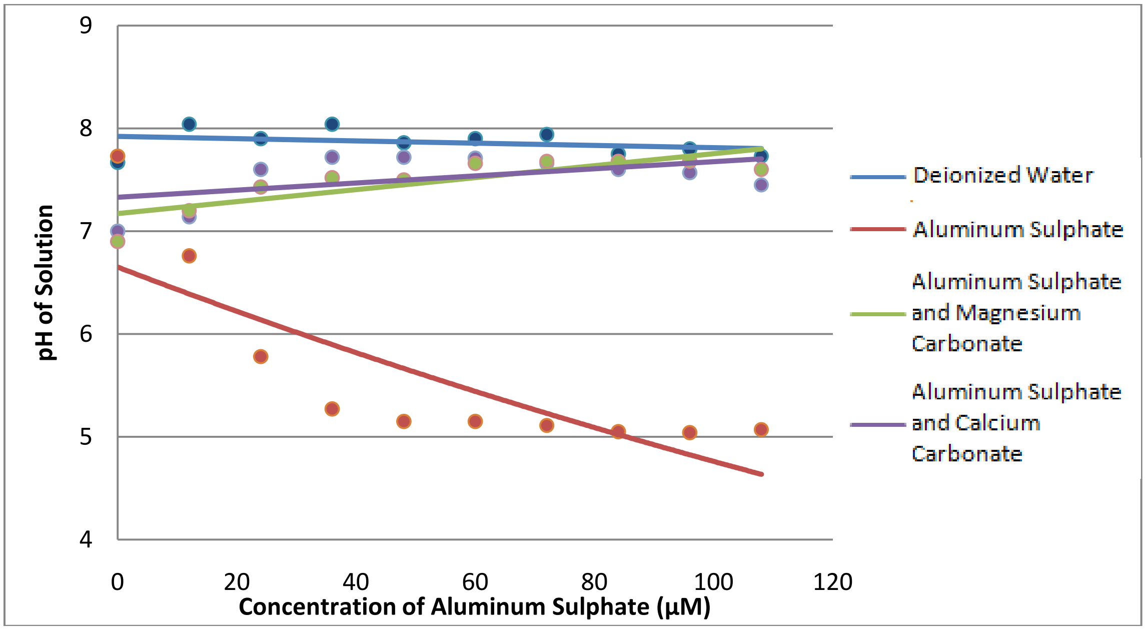

Figure 1: The pH of Solutions in Four Treatments. The pH of the aluminum sulphate solutions decreased as the aluminum sulphate concentration increased. In comparison, the pH of deionized water only, and aluminum sulphate in the presence of calcium carbonate or magnesium carbonate had a higher pH.

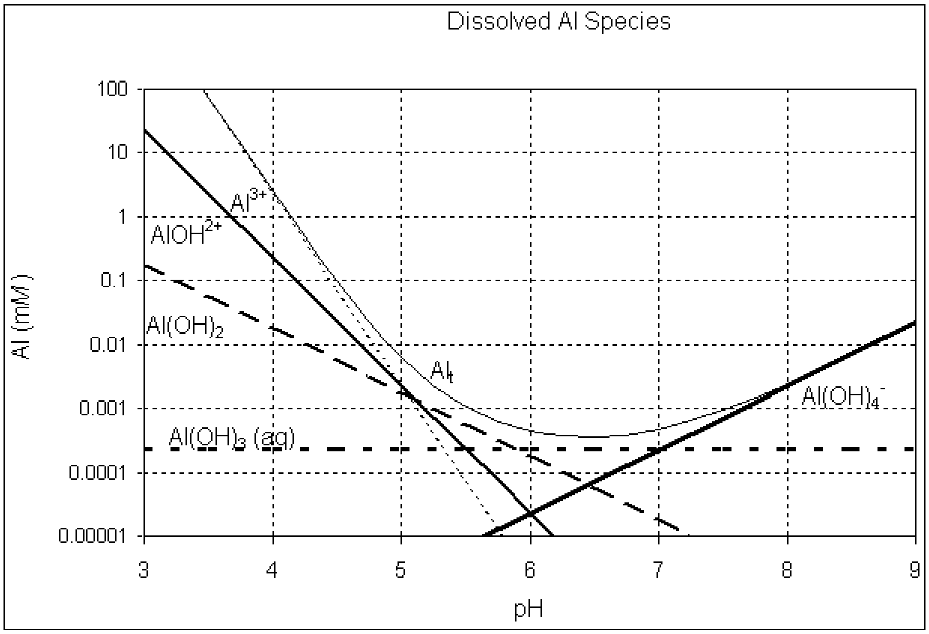

Figure 2: Dissolved Al Species at Various pH Levels (Driscoll & Schecher, 1990). Aluminate, $Al(OH)_4^-$, is the dominant species at pH>6.7.

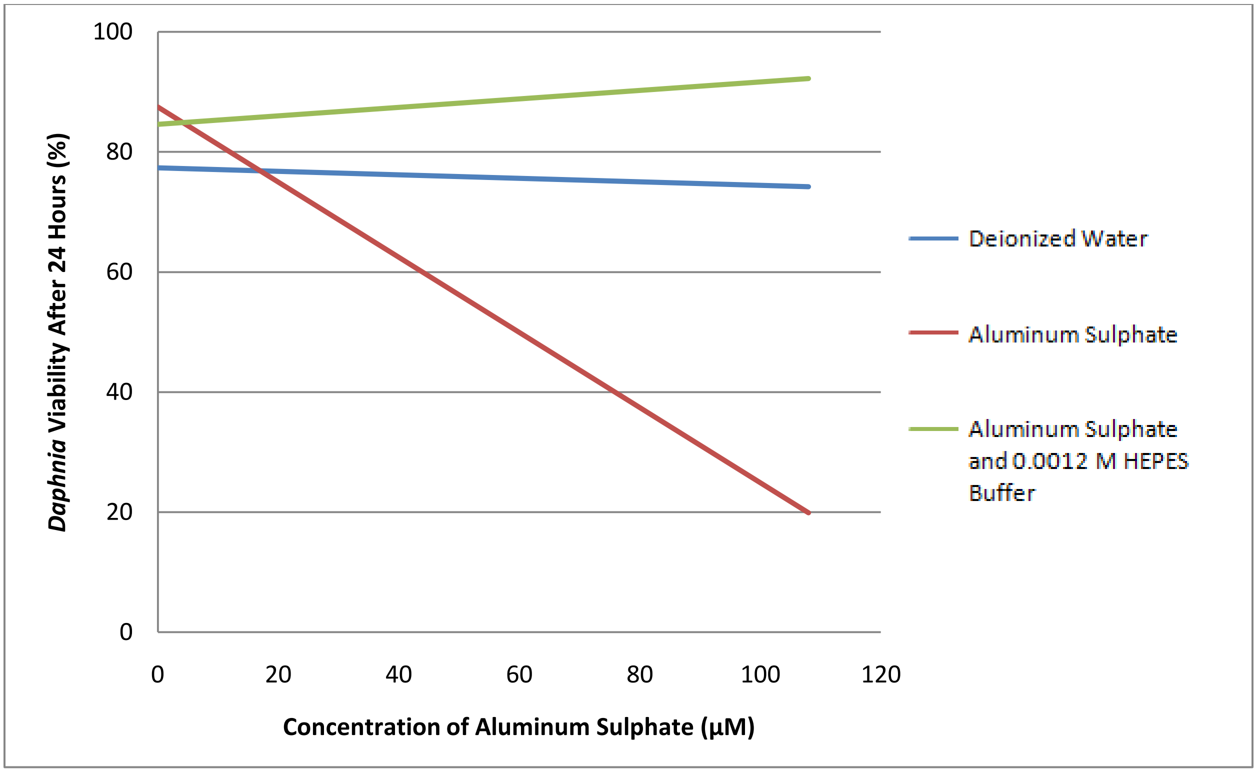

Figure 3: Viability of *Daphnia* in Deionized Water, Aluminum Sulphate Solution, and Aluminum Sulphate Solution in the Presence of 0.0012 M HEPES Buffer. In this preliminary trial, the HEPES buffer appears to have a protective effect against aluminum toxicity; n = 5.