Uptake and toxicity of copper and cadmium in single and mixtures exposure in the zebrafish, *Danio rerio*

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1. Introduction

The aquatic environment receives contaminants from a wide array of different sources including the industry, effluent and agricultural run-off and is highly impacted by pollution. This presence of multiple contaminants in the environment and the potential mixture effects have become a growing global concern, and therefore it is important to understand how multiple contaminants effect organisms [1]. It has been shown that the presence of the contaminants in mixtures can influence the effect of the individual compounds and result in synergistic or antagonistic toxicological effects on the organisms.

To explore the effect of single and combined exposures to Cu and Cd we have performed a series of exposure experiments with adult zebrafish under different water hardness regimes and combinations of metal concentrations. The survival of the fish was followed over 10 to 28 days and whole body metal and major cation concentrations were measured in all fish to determine whether Cu and Cd accumulation showed interactive effects and metal body burdens were predictive of toxicity. The levels of major cations were measured to determine whether loss of ion regulatory capacity was predictive of toxicity in the different single and mixture exposure scenarios.

2. Materials and methods

Four independent semi-acute (10-day) experiments and one semi-chronic (28-day) experiment including single and combined exposures to the metals were performed according to a partial factorial design. Two acute experiments were perfomed using moderately hard water, another was performed using very soft water and one using very hard water, the chronic experiment included all three water hardnesses (VS, MH, VH).

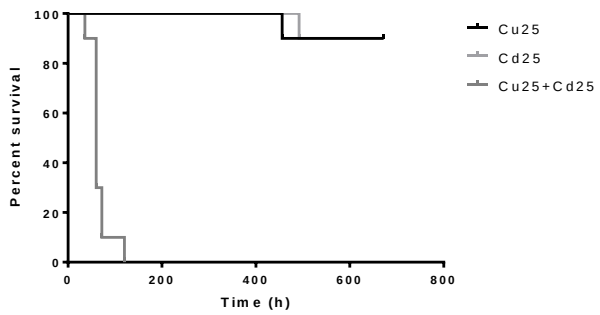
Temperature, conductivity and pH were measured daily. Water samples for organic carbon analysis and samples for metal and major ion determination were taken prior to every water change. Mortality was recorded every 12 hours and removed fish were weighed and stored for analysis. The whole body content of Cu and Cd and Na, K, Ca and Mg were determined by ICP-MS.

3. Results and discussion

3.1. Mortality

In general Cu was found to be much more toxic to zebrafish than Cd in single metal exposures. However, in the mixture scenarios the toxicity showed a large increase (Figure 1). Toxicity also strongly increased with decreasing water hardness in the single exposures and the interactive effect between the two metals was observed at each of the water hardnesses tested.

A comparison of observed mortalities with mixture toxicity predictions based on the Concentration Addition model using the 10 day EC_{10} values showed large differences between observation and prediction and was indicative of a strong synergistic effect between Cu and Cd (Figure 2).



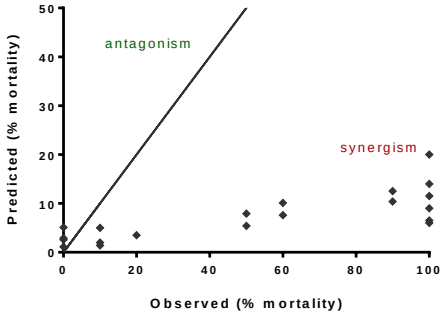
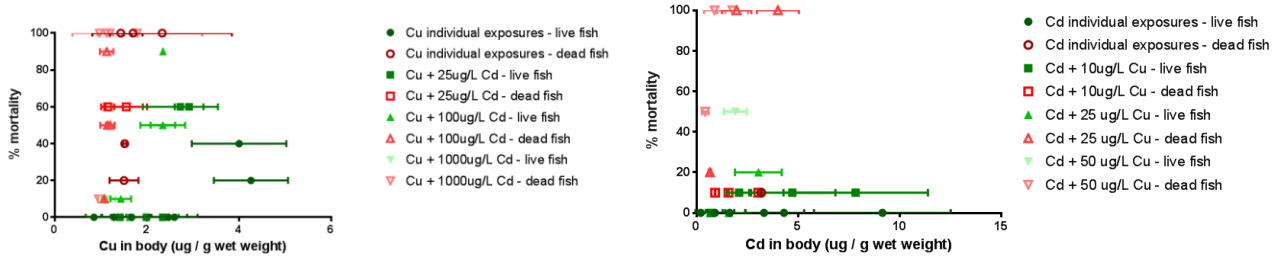


Figure 1: Illustrative case of the toxicity of single and combined exposure to Cd (25 µg/l) and/or Cu (25 µg/l) in a 28 day test with adult zebrafish.

Figure 2: Predicted versus observed toxicity (10 day EC10 mortality) on the basis of the Concentration Addition model

3.2. Effect of uptake rate and body burden on mortality

The metal accumulation data show a dose response relationship. However, neither the metal uptake rates nor body burdens of the metals in individual and mixture exposures can explain the observed mortality in the different scenarios (Figure 3a and b). Analysis of the major cations profiles shows that the significant loss of particularly Na from the body has a large impact on mortality (Figure 4). The drop in Na level resulting in mortality appears more or less the same across the different single or mixtures scenarios.



Figures 3a and b: Cu and Cd body burdens versus mortality in individual and mixture exposures in some series of the 10 day tests with adult zebrafish in MH water.

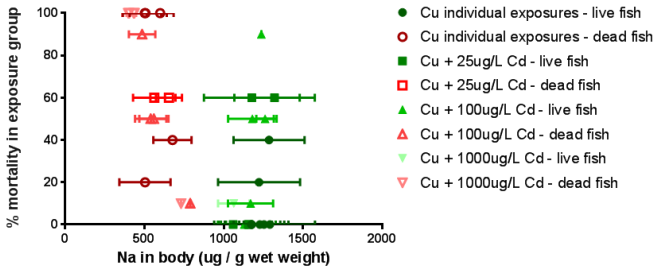


Figure 4. Sodium levels versus mortality in adult zebrafish exposed to a range of Cu concentrations at fixed Cd levels.

4. Conclusions

- Cu was more toxic than Cd to the adult zebrafish.
- Cu and Cd show a strong synergistic effect in zebrafish in reference to the CA model.
- Metal body burdens or uptake rates are poor predictors of mortality across scenarios
- A consistent relationship is observed between Na loss and toxicity

5. References

[1] Altenburger R, Backhaus T, Boedecker W, Faust M, Scholze M. 2013. Simplifying complexity: Mixture toxicity assessment in the last 20 years. Environ Toxicol Chem 32 (8): 1685-1687.

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