Title: Mixture toxicity of zinc and copper on the growth of the diatom *Navicula pelliculosa* and comparisons with concentration additive and independent action predictions

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Abstract

Metal mixture effects on algal growth have not often been investigated, especially for species other than green algae. Moreover, the applicability of conventional mixture toxicity prediction models, concentration additive (CA) and independent action (IA), has also not sufficiently been investigated. Here, we investigated the mixture toxicity of zinc and copper on the growth of a diatom, *Navicula pelliculosa*. A toxicity test was conducted using 96-well microplates for 72 h, and the algal growth was monitored using a fluorescence microplate reader. A 7×7 full factorial experimental design was used for the mixture test (49 combinations) in total. Tested concentrations were EC10/4, EC10/2, EC10, EC30, EC50, and EC70, which were determined by preliminary single metal toxicity tests. Free ion activities in test solutions were calculated using chemical equilibrium speciation software WHAM6. Mixture effects were predicted by CA and IA

models based on free metal ion activity-response relationships of both single metals. CA was a better predictor than IA, but CA prediction was slightly overestimation of effect. In contrast, IA prediction was underestimated the effect. This suggests that the effects of zinc and copper have the similar mode of action. That is, they share the same metal binding ligand of biological surface (biotic ligand). Moreover, we calculated the fraction of shared biotic ligand by fitting the all experimental data. The 71% of biotic ligand was shared and remained 29% of that was independent among zinc and copper.

Keywords: mixture effect, mixture model, Navicula