

# Towards a Biotic Ligand Model for Green Algae: Surface-bound Copper Predicts the Effect of pH on Copper Toxicity

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## INTRODUCTION

The recently developed biotic ligand model (BLM) is successful in predicting metal toxicity to fish and invertebrate species as a function of water chemistry (Di Toro et al., 2001). The BLM predicts metal binding to the organism-water interface (the 'biotic ligand', e.g. the fish gill) based on metal speciation and competition between metal ions and cations such as  $H^+$  and  $Ca^{2+}$ . From the amount of metal bound to the biotic ligand the BLM predicts the effect, assuming that this amount determines toxicity independent of water chemistry.

To date, only limited evidence is available to justify the use of the cell surface as the biotic ligand for freshwater green micro-algae. In fact, it has only been demonstrated that surface-bound Cu predicts toxicity when dissolved organic carbon is varied (Ma et al., 2003).

Since pH is another factor which seriously affects copper toxicity to green algae (e.g., De Schamphelaere et al., 2003), it is crucial to the development of an algal BLM that surface-bound Cu does also predict Cu toxicity when pH is varied. The aim of this study was to test this hypothesis for two algae, i.e. *Chlorella* sp. and *Pseudokirchneriella subcapitata*.

## METHODS

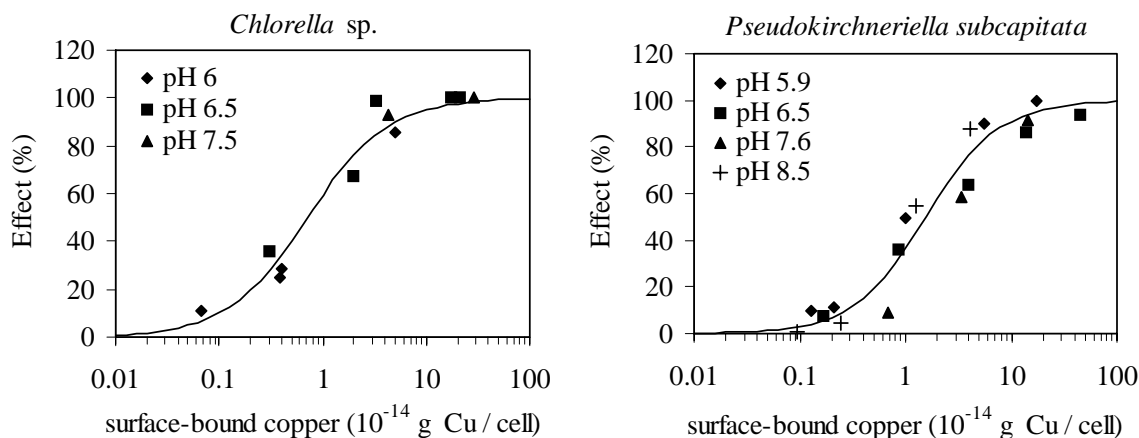
Algal toxicity tests with copper were conducted at pH 6.0, 6.5 and 7.5 with *Chlorella* sp. and at pH levels 5.9, 6.5, 7.6 and 8.5 with *P. subcapitata*. Algal growth was monitored during 48h (*Chlorella* sp.) or 72h (*P. subcapitata*). Surface-bound Cu of the algae was determined in parallel based on the copper measured in a rinsing solution of ethylene-diamine-tetra-acetic acid (0.02 M) after 20 minutes of contact with the copper-exposed algae (Franklin et al., 2002). Copper concentrations were determined using graphite furnace atomic absorption spectrometry. Median effective concentrations, resulting in 50% reduction of growth rate compared to a control were determined using a logistic regression based on calculated  $Cu^{2+}$ -activity ( $EC50_{Cu^{2+}}$ ) and surface-bound Cu ( $EC50_{\text{surface-Cu}}$ ).

## RESULTS AND DISCUSSION

Toxicity of  $Cu^{2+}$  exhibited a continuous increase with increasing pH for both species, as demonstrated by decreasing  $EC50_{Cu^{2+}}$ . For *Chlorella* sp. the 48h- $EC50_{Cu^{2+}}$  decreased from  $3.3 \times 10^{-7}$  M to  $2.8 \times 10^{-9}$  M between pH 6 and pH 7.5. For *P. subcapitata* the 72h- $EC50_{Cu^{2+}}$  decreased from  $4.7 \times 10^{-7}$  M to  $5.6 \times 10^{-10}$  M between pH 5.9 and pH 8.5. Thus, within the tested pH range  $EC50_{Cu^{2+}}$  varied 100-fold (*Chlorella* sp.) to 1,000-fold (*P. subcapitata*),

indicating that  $\text{Cu}^{2+}$  is a bad predictor of Cu-toxicity and confirming that pH is a crucial factor determining copper toxicity to green algae (e.g., De Schampelaere et al., 2003).

However, when plotting the % growth inhibition against surface-bound Cu, it is clear that data-points from different pH levels can be predicted with a single dose-response curve, suggesting that surface-bound Cu determines Cu toxicity, independent of pH (Figure 1). The latter is supported by the fact that the  $\text{EC}_{50_{\text{surface-Cu}}}$  calculated for the individual pH levels, exhibited much less variation than the  $\text{EC}_{50_{\text{Cu}^{2+}}}$ , i.e. the 72h- $\text{EC}_{50_{\text{surface-Cu}}}$  for *P. subcapitata* varied between 1.0 and  $2.8 \times 10^{-14}$  g Cu/cell (2.8-fold difference) and the 48h- $\text{EC}_{50_{\text{surface-Cu}}}$  for *Chlorella* sp. varied between 0.5 and  $1.0 \times 10^{-14}$  g Cu/cell (2-fold difference).



**Fig. 1. Relationship between surface-bound copper and inhibition of growth rate (as % effect compared to control) in *Chlorella* sp. and *Pseudokirchneriella subcapitata* at different pH levels. The solid line is the logistic dose-response curve fitted to all data.**

## CONCLUSIONS

Surface-bound Cu is capable of predicting Cu toxicity to green algae when pH (this study) and DOC (Ma et al., 2003) are varied, indicating that the cell surface may serve as the 'biotic ligand'. Thus, the final link for the establishment of an alga-BLM is a model which predicts Cu bound to the cell surface as a function of water chemistry (e.g., pH and  $\text{Cu}^{2+}$ -activity).

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