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# Are interactive effects of harmful algal blooms and copper pollution a concern for water quality management?

*Jennifer D. Hochmuth*, Jana Asselman, Karel A.C. De Schamphelaere*

*Laboratory of Environmental Toxicology and Aquatic Ecology, Environmental Toxicology Unit, Ghent University, J. Plateaustraat 22, B-9000 Ghent, Belgium*

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ABSTRACT

Toxicity of mixtures of stressors is one of the major challenges in water quality management. Yet until now risk assessment focuses almost exclusively on the effect characterization of individual stressors. An important concern is the potential interactive effects of cyanobacteria, sometimes referred to as harmful algal blooms, with chemical stressors. Here, we evaluated the response of two clones of the freshwater cladoceran *Daphnia magna* to the combined effects of five cyanobacteria and copper. The latter remains the most commonly applied chemical algaecide and is also often detected in eutrophic run-offs that promote harmful algal blooms. Because the different cyanobacteria studied here have known modes of action that are similar, as well as dissimilar compared to the known modes of actions of copper, we based our assessment on two widely used reference models, i.e. the Concentration Addition (CA) model for similarly acting stressors and the Independent Action (IA) model for dissimilarly acting stressors. We highlight four major findings. First, the conclusions drawn on the interaction type (non-interaction vs. synergism or antagonism) between either of the five cyanobacteria species and copper were the same for both *D. magna* clones. Second, the interaction type differed between the *Microcystis* + copper mixture (non-interaction according to CA and synergism according to IA) and the four other cyanobacteria + copper mixtures (antagonism according to CA and non-interaction according to IA). Third, both reference models provided reasonable predictions for all observed mixture toxicities. Fourth, we consistently obtained different results with the IA reference model compared to the CA model. More specifically, mixtures of Cu and *Microcystis* were synergistic with IA whereas non-interaction was observed with CA, while the remaining four cyanobacteria + copper combinations all displayed non-interaction with IA and antagonism with CA. Despite the IA reference model providing a marginally better fit to the data in general, the CA reference model delivered more conservative predictions for mixture toxicity of cyanobacteria + copper in all cases compared to the IA reference model. Thus, the CA model could serve as a conservative model to account for mixture toxicity of cyanobacteria and copper in water quality management, as it gives rise to conservative predictions of mixed stressor toxicity at sub-lethal effect levels in *D. magna*. Finally, and in accordance with other studies of cyanobacteria + chemical mixtures, we did not detect any strong synergistic effects of copper and cyanobacteria

* *Corresponding author.*
E-mail address: Jennifer.Hochmuth@ugent.be (J.D. Hochmuth).

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mixtures on *D. magna*. Consequently, based on our study with the model freshwater zooplankton species *Daphnia*, interactive effects of harmful algal blooms and copper pollution appear to be of limited concern for water quality management.
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## 1. Introduction

Harmful algal blooms pose a serious risk to environmental and human health, and the management and restoration of water quality following such a bloom can be challenging. Large scale ecosystem wide effects have been attributed to their extensive proliferation and toxin production (Davis et al., 2009; Downing et al., 2001; Falconer, 2001; Johnk et al., 2008). The application of copper-based algaecides is still one of the most common measures to eradicate freshwater phytoplankton, including cyanobacterial blooms (Garcia-Villada et al., 2004; Jancula and Marsalek, 2011). Furthermore, copper itself is listed as priority pollutant by the U.S. Environmental Protection Agency (McKnight et al., 1983), and many EU countries are developing Environmental Quality Standards (EQS) for copper in surface waters under the EU water framework directive (Comber et al., 2008). Copper pollution in surface waters can locally reach levels that may cause toxicity to aquatic species, for instance in waters affected by surface run-off from vineyards and citrus farms, where copper is still used as a biocide against fungus diseases (Banas et al., 2010; Graves et al., 2004).

As a consequence, it can be anticipated that cyanobacteria and copper pollution often co-occur in freshwater systems, either in situations where surface run-off is enriched with both copper and nutrients or where copper is actively used as a major component in chemical applications to eradicate cyanobacteria blooms. Furthermore, anthropogenic copper pollution may also act interactively with cyanobacterial stressors on aquatic biota. Interaction (e.g. synergism or antagonism) is said to occur if the level of response produced by any combination of different stressors differs from the response expected on the basis of a theoretical reference model of non-interaction (McCarty and Borgert, 2006). Considerable information already exists on interactive effects between chemical and non-chemical stressors, whereas information on the interactive effects between chemical stressors and cyanobacterial stressors is sparse (Couillard et al., 2008; Fischer et al., 2013; Holmstrup et al., 2010; Laskowski et al., 2010). Indirect "smaller than expected" effects of copper on non-target organisms could occur as copper actively eradicates the cyanobacteria, but indirect "larger than expected" effects of copper are also possible, as copper may induce cyanobacterial cell lysis, which increases external cyanobacterial toxin concentrations (Jones and Orr, 1994; Kenefick et al., 1993). Consequently an important concern is that conventional risk assessment may not be conservative enough, as it currently excludes combined and potentially interactive effects of mixtures of stressors that cannot be predicted form individual toxicities alone.

In a first approach to investigate the interactive effects of copper and cyanobacteria in an experimental context there is a need for model species that are ecologically relevant, geographically widely distributed and easy to manipulate experimentally in the laboratory. The cladoceran *Daphnia* qualifies as such a model organism as it plays a pivotal role in the food chains of freshwater ecosystems, affecting both water quality (as a primary grazer) and fish production (as a major food source) (Dodson and Hanazato, 1995). *Daphnia* is already widely used as an invertebrate model for setting water quality standards and is a recommend species according to the guidelines of the Organisation for Economic Co-operation and Development (OECD, 1998). In addition daphnids have been identified as being among the most sensitive invertebrates to copper (Brix et al., 2001; Von Der Ohe and Liess, 2004).

The mode of action of copper has been well-studied in *Daphnia*. Copper toxicity has been linked to inhibition of active sodium uptake (De Schamphelaere et al., 2007), inhibition of neuronal signal transmission and acetylcholinesterase (AChE) activity (Untersteiner et al., 2003), and oxidative stress (Barata et al., 2005; Xie et al., 2006). While it is well-known that cyanobacteria reduce the fitness of *Daphnia* sp., there appears to be no general concensus in the literature about the main mechanisms underlying this effect. Effects have mainly been associated with four factors or a combination thereof: cyanobacterial toxins (e.g. microcystins, cylindrospermopsins) (Dao et al., 2010; Demott et al., 1991; Nogueira et al., 2004; Rohrlack et al., 1999), feeding inhibition (Demott et al., 1991; Lurling, 2003), morphology (DeMott et al., 2001; Wilson et al., 2006) and the lack of essential nutrients (Martin-Creuzburg and von Elert, 2009). Although cyanotoxins exhibit high toxicity to vertebrates, including mammals (Wiegand and Pflugmacher, 2005), several studies have reported no significant differences between the effects of cyanotoxin producing and non-toxin producing cyanobacteria on zooplankton, albeit such studies have mainly focused on *Microcystis* (Tillmanns et al., 2008; Wilson et al., 2006).

Furthermore the interactive effects of cyanobacteria with chemicals have rarely been investigated, with few exceptions. In one study the insecticide carbaryl and *Microcystis aeruginosa* caused a synergistic toxicity response in *Daphnia pulicaria* (Cerbin et al., 2010). More recently, antagonistic effects were reported between carbaryl and four cyanobacteria species in *Daphnia pulex* (Asselman et al., 2013). Despite the lack of any direct influence of two polychlorinated biphenyls (PCB52 and PCB153) on the fecundity, growth and depth selection of *Daphnia longispina*, adverse effects of the filamentous cyanobacteria *Cylindrospermopsis raciborskii* on fecundity (but not on growth and on depth selection) were magnified by PCB52 in 25.8% of the clones tested and reduced in 33% of the clones,

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