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New is not always better: Toxicity of novel copper based algaecides to *Daphnia magna*

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ABSTRACT

In this study, the effects of traditional copper ($CuSO_4.5H_2O$) and novel copper algaecides (Captain XTR, SeClear and Lake Guard Blue) were tested on *Daphnia magna* under acute (48 h) and chronic (21 d) exposure scenarios. The EC_{50} values calculated in the acute tests were between 0.5 and 0.6 mg Cu L^{-1} for all four compounds. Lake Guard Blue and $CuSO_4.5\ H_2O$ were more toxic than SeClear and Captain XTR. During the chronic test, the effects of SeClear (EC_{50}: 0.274 mg Cu L^{-1}) on reproduction and body length were larger than the effects of the other three copper-based algaecides (EC_{50}: 0.436 mg Cu L^{-1} for $CuSO_4.5\ H_2O$, 0.498 mg Cu L^{-1} for Captain XTR, and 0.295 mg Cu L^{-1} for Lake Guard Blue). Captain XTR had the strongest negative effect on body weight, whereas body weight was affected the least by $CuSO_4.5\ H_2O$. The four copper compounds affected the age at first brood significantly, which was delayed by 1.8, 2.0, 2.3 and 3.2 days for Captain XTR, $CuSO_4.5H_2O$, Lake Guard Blue and SeClear, respectively. Intrinsic rate of population increase was lowest (0.145 d^{-1}) at the highest dosage in the SeClear treatments. Chemical equilibrium modelling revealed that most copper was chelated with EDTA present in the artificial medium used. These combined results indicate that the toxicity of the novel copper algaecide SeClear to *D. magna* is greater than that of traditional copper algaecide. Prior to each Cu application, tests on the effects of Cu compounds on the organisms being targeted should be done, taking into consideration the water chemistry.

1. Introduction

Copper-based algaecides have been used extensively in freshwater, where cyanobacteria are rampant (Buley et al., 2021; Mastin et al., 2002). For instance, copper sulphate ($CuSO_4.5H_2O$) has been used to control algal blooms for already more than a century (Jančula and Maršálek, 2011). Copper is a metallic element of which its cationic forms (Cu^{2+}, $CuOH^+$, $Cu_2(OH)_2^{2+}$) are toxic to aquatic organisms (Closson and Paul, 2014). Cationic copper may form rapidly when copper sulphate dissociates in water (Mastin and Rodgers, 2000), however, how much is formed depends strongly on water composition (Jančula and Maršálek, 2011; Murray-Gulde et al., 2002). In general, ionic copper is lost rapidly from water as the result of precipitation and/or complexation, decreasing the toxicity of copper over time (Elder and Horne, 1978).

The toxicity of copper sulphate in an aqueous solution has been thoroughly studied (Elder and Horne, 1978; Kirici et al., 2017). Copper exerts toxicity to phytoplankton due to its negative effect on photosystem II (Barón et al., 1995). However, copper compounds may also affect non-target organisms such as the water flea *Daphna magna,* are among the most sensitive organisms to copper (Brix et al., 2001). Copper concentrations as low as 8.4 μg L^{-1} for acute toxicity to *D. magna* and 20.2 μg L^{-1} for chronic toxicity have been found (Brix et al., 2001). Toxicity is related to the presence of cationic forms, while complexed, chelated or precipitated Cu forms express lower toxicity to *Daphnia* (Andrew et al., 1977).

To reduce the toxicity towards non-target organisms and to increase effectiveness against target organisms, novel copper-based algaecides have been developed, which are associated with a chelator that can pass through algal cells membranes and cause cell lysis rapidly (Closson and Paul, 2014). For instance, the chelated copper formulation Cutrine®-Ultra was twice as toxic to the cyanobacterium *Planktothrix agardhii* and the green alga *Pseudokirchneriella subcapitata* as was copper sulphate, implying that less copper needs to be applied (Calomeni et al., 2014). The copper-ethanolamine complex formulations Clearigate and Cutrine-Plus were four times less toxic to the water flea *Ceriodaphnia dubia* than was copper sulphate (Murray-Gulde et al., 2002). Hence,

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toxicities of copper algaecides to non-target *Daphnia* between ionic copper and chelated copper may differ significantly because of their properties, chelator toxicity and potential formation of free hydroxyl radicals (Bishop et al., 2014, 2018; Mastin and Rodgers, 2000).

Three other novel chelated copper-based products, Captain XTR, SeClear and Lake Guard Blue have been reviewed and accepted by the U. S. Environmental Protection Agency (US EPA) in 2011, 2016 and 2018, respectively. Captain XTR (SePRO Corporation, Carmel, IN, USA) is a copper ethanolamine complex with a proprietary surfactant added designed to penetrate the protective sheath of problem algal species, and has 9.1 % metallic copper equivalent (https://sepro.com/aquatics/captain-xtr). SeClear (SePRO Corporation, Carmel, IN, USA) consists of 4.2 % copper combined with a water quality enhancer (https://sepro.com/aquatics/seclear) and it can, according to the manufacturer, both kill algae and remove phosphorus. According to the manufacturer, Captain XTR is used with infusion technology to deliver more copper to the algal cells and kill them in the early seasons. Afterwards, SeClear is coming into play for binding phosphate in the water once the algae have been diminished, but Captain XTR and SeClear could also be used separately. Lake Guard Blue (BlueGreen Water Technologies, Ltd) contains 23.9 % metallic copper equivalent that according to the manufacturer “activates a biological chain reaction in the water column, a surgical application of BlueGreen’s Lake Guard® rehabilitates entire lakes within a few short days” (https://ntcdigitaldev.co.za/). Commonly, these copper-based algaecides are applied below 1 mg Cu L^{-1} based on US EPA (EPA Reg. No. 7364-09-8959).

Despite these copper-based compounds having advantages as an algaecide (Buley et al., 2021; Iwinski et al., 2016), it is essential to assess their potential toxicity on non-target organisms before using them in lake applications. Therefore, in this study, *Daphnia magna*, which is extremely sensitive to copper (OECD, 2004, 2012), was exposed to these three novel copper-based algaecides (Captain XTR, SeClear and Lake Guard Blue) as well as to copper sulphate ($CuSO_4.5H_2O$). Laboratory experiments were conducted to: (1) evaluate and compare the toxicity (i. e., EC_{10}, EC_{20}, EC_{50} and NOEC) of the four copper-based algaecides by running acute tests (48 h) and chronic tests (21 d); (2) establish a dose-response relationship between algaecides and the growth of *Daphnia magna* based on body length and dry weight. We hypothesized that novel copper-based algaecides would cause lower mortality to *Daphnia magna* than the traditional copper-based algaecide (copper sulphate). In addition, we expected less effect on *D. magna* body size and reproduction from novel copper-based compounds than from copper sulphate.

2. Methods

2.1. Test organisms

The experiments were carried out with *Daphnia magna* Straus that has been maintained for more than 20 years in the laboratory of the Aquatic Ecology and Water Quality Management group of Wageningen University (The Netherlands). *Daphnia magna* was kept in 1 L jars containing 800 mL RT medium (Table S1) with pH 8, a conductivity of 270 µs cm^{-1} and hardness of 88 mg L^{-1} (as $CaCO_3$) (Tollrian, 1993). The jars were placed at 20 ± 2 °C, in a 12:12-h light/dark cycle. The animals were fed daily with the chlorophyte *Scenedesmus obliquus* (~ 4 mg C L^{-1}), which was grown in WC medium (Table S2) (Lürling and Beekman, 1999). Neonates born within 24 h were transferred from the stock cultures into new jars. Once the second-generation newborns (6–24 h old) were born, the experiments were conducted instantly.

2.2. Acute toxicity tests

Nine Cu solutions from each of the four algaecides were prepared from stock solutions previously made for each compound (Table 1). Subsamples from the stock solutions were filtered (Aqua 30/0.45 CA, Whatman, Germany) and analysed by ICP-OES (Thermo iCAP 6500 DV; Thermo Fisher Scientific) to check the Cu concentration from each stock solution with three replicates. Thus, the stock solutions concentration were 102.33 (± 4.84), 128.99 (± 16.5), 51.40 (± 1.03) and 167 (± 5.01) mg Cu L^{-1}, for $CuSO_4.5H_2O$, Captain XTR, SeClear and Lake Guard Blue contained respectively. The acute toxicity tests were run in triplicates, with five newborns (6–24 h old) in 100 mL jars containing 50 mL RT medium. The animals were incubated for 48 h without food, in a 12:12-h light/dark regime at 20 ± 2 °C. The acute toxicity tests were performed three times for each chemical. The 48 h-EC_{10} (effect concentration that causes acute effects to 10 % of the test population), 48 h-EC_{20} (effect concentration that causes acute effects to 20 % of the test population), 48 h-EC_{50} (effect concentration that causes acute effects to 50 % of the test population), NOEC (No Observed Effect Concentration) and LOEC (Lowest Effect Concentration) were obtained based on the immobility of the daphnids (OECD, 2004).

2.3. Chronic toxicity tests

The EC_{10} calculated from the acute test was used as the threshold for six concentrations selection in the chronic test (Table 1). This test was run in 100 mL glass tubes with 50 mL RT medium containing one newborn (6–24 h old) in each tube. The experiment was conducted using 10 replicates. Animals were fed daily with *Scenedesmus obliquus* (0.2 mg

Table 1
The total copper concentrations used in the acute tests (48 h) and in the chronic tests (21 d) for all algaecides ($CuSO_4.5H_2O$, Captain XTR, SeClear and Lake Guard Blue) used. The values in the brackets represent the standard deviation (SD).

	Algaecides	Total Copper concentrations (mg Cu L^{-1})								
Acute tests (48 h)	$CuSO_4.5H_2O$	0 (0)	0.081 (0)	0.202 (0.01)	0.323 (0.01)	0.404 (0.01)	0.485 (0.01)	0.565 (0.01)	0.646 (0.02)	0.808 (0.02)
	Captain XTR	0 (0)	0.116 (0.01)	0.290 (0.04)	0.463 (0.06)	0.579 (0.07)	0.695 (0.09)	0.811 (0.1)	0.927 (0.12)	1.159 (0.15)
	SeClear	0 (0)	0.103 (0)	0.257 (0.01)	0.411 (0.01)	0.514 (0.01)	0.617 (0.01)	0.720 (0.01)	0.823 (0.02)	1.029 (0.02)
	Lake Guard Blue	0 (0)	0.062 (0)	0.155 (0)	0.248 (0)	0.310 (0.01)	0.372 (0.01)	0.434 (0.01)	0.496 (0.01)	0.620 (0.02)
Chronic tests (21 d)	$CuSO_4.5H_2O$	0 (0)	0.091 (0)	0.181 (0.01)	0.271 (0.01)	0.362 (0.02)	0.452 (0.02)			
	Captain XTR	0 (0)	0.102 (0.01)	0.204 (0.03)	0.306 (0.04)	0.408 (0.05)	0.510 (0.07)			
	SeClear	0 (0)	0.084 (0)	0.169 (0)	0.253 (0.01)	0.337 (0.01)	0.422 (0.01)			
	Lake Guard Blue	0 (0)	0.089 (0)	0.179 (0.01)	0.268 (0.01)	0.357 (0.01)	0.446 (0.01)			

C per *Daphnia magna* per day) (OECD, 2012) and incubated for 21 days at the same conditions previously described (Section 2.2). Newborn were removed from the tubes daily and the animals were transferred to new medium containing Cu once a week.

The number of survivors, their life offspring, age at first reproduction (AFR), age at second reproduction (ASR) and age at third reproduction (ATR) were recorded daily. Fecundity was used as an endpoint to evaluate 21d-EC_{10}, 21d-EC_{20}, 21d-EC_{50} and NOEC. The intrinsic of population increase (PGR) (r) was calculated using equation (Euler, 1970; Silva et al., 2020):

$1 = \sum_{x=0}^{n} e^{-rx} l_x m_x$Where "$r$" is the rate of population increase (day^{-1}), "x" is the age class in days (0 … n) (days), "l_x" is the probability of surviving to age "x", and " m_x" is fecundity at age "x".

At the start of the experiment, the initial size of survivors (ISS) of 10 newborns were measured using a stereo-binocular microscope (Olympus SZX10, Japan) and the initial dry weight (IDW) of 20 newborns were weighed on an electronic balance (Mettler UMT 2; ± 0.1 μg). Body length was defined as the distance from the most posterior point on the eye to the base of the junction of the tail spine with the carapace (Lurling and Tolman, 2010). After 21 days, the final size of survivors (FSS) and final dry weight (FDW) of all survivors were also measured. To avoid handling stress and unforeseen damage, experimental animals were measured only at start and at the end of the experiments. The somatic growth rates (SGR) were determined as the increase in body length and dry weight separately during the 21-day period at the beginning of the experiment and on the last incubation day-day 21.

2.4. Chemical equilibrium modelling

To evaluate which copper species were prevailing in the RT medium, chemical equilibrium modelling was performed using CHEAQS Next-version 0.1.0.19 (Verweij, 2017). As input for the model we used the pH measured (pH 8) in the RT medium, the amount of copper, sulphate and the composition of RT medium (Table S3).

2.5. Statistical analysis

The EC_{10}, EC_{20} and EC_{50} values of four copper-based algaecides in the acute 48 h immobility tests were analyzed by Probit analysis in the software SPSS version 25, and those values in the chronic 21 d reproduction tests were analyzed by nonlinear regression using the three-parameter logistic curve using Statistica version 7.0 (StatSoft 2004). For chronic tests, we analyzed significant differences among 6 concentrations for age at first brood, second brood and third brood, intrinsic rate of population increase and somatic growth by running one-way ANOVA or Kruskal-Wallis One-Way Analysis of Variance on Ranks when the normality test (Shapiro-Wilk) or Equal Variance test failed followed by Tukey or Dunn's post hoc test. *Daphnia magna* images were obtained by a stereo-binocular microscope (Olympus SZX10, Japan) and were processed by ImageJ software.

3. Results

3.1. Acute toxicity of copper-based algaecides to Daphnia magna

During the acute toxicity tests with the four algaecides, 100 % survival was recorded in the control series (0 mg Cu L^{-1}). The 48 h-NOEC were quite similar between all the four compounds tested and varied from 0.40 mg Cu L^{-1} for Lake Guard Blue to 0.46 mg Cu L^{-1} for Captain XTR (Table 2). EC_{10} varied between 0.42 and 0.51 mg Cu L^{-1}, EC_{20} between 0.47 and 0.54 mg Cu L^{-1} and EC_{50} between 0.50 and 0.60 mg Cu L^{-1} for *Daphnia magna* (Table 2). The EC_{50} values were significantly different ($F_{3,104} = 28.51$; $P < 0.001$) and a Tukey's post hoc test indicated two homogenous groups: 1) Lake Guard Blue and $CuSO_4.5\,H_2O$ were more toxic (had lower EC_{50}) than 2) SeClear and Captain XTR (Table 2). The dose-response curves of the acute tests can be found in Fig. S1.

3.2. Chronic toxicity of copper-based algaecides to Daphnia magna

Survival of *D. magna* was 100 % in the control, and at each tested concentration of $CuSO_4.5H_2O$, Captain XTR and Lake Guard Blue, whereas the highest concentration of SeClear caused 40 % mortality (Table 3). The age of first reproduction (AFR) was significantly increased with higher concentrations dosed for all four algaecides, a similar, yet less prominent pattern was observed for ASR, while ATR was similar among control and treatments in each algaecide tested (Table 3). Compared with controls, AFR was delayed with 1.8, 2.0, 2.3 3.2 days in the highest dose of Captain XTR, $CuSO_4.5H_2O$, Lake Guard Blue and SeClear, respectively (Table 3).

As the copper concentration increased, the number of neonates in the first, second and third broods reduced significantly (Fig. 1; Table S4). Consequently, the total number of offspring was reduced significantly (Fig. 1; Table S4). By comparing controls and highest copper doses, the total number of neonates decreased 50 % when exposed to $CuSO_4.5H_2O$, 54 % when exposed to Captain XTR, 69 % when exposed to Lake Guard Blue and 73 % when exposed to SeClear. The EC_{50} values for total reproduction were 0.436, 0.498, 0.274 and 0.295 mg Cu L^{-1} for $CuSO_4.5H_2O$, SeClear, Captain XTR and Lake Guard Blue, respectively (Table 2). The calculated EC_{10} and EC_{20} were 0.129 and 0.202 mg Cu L^{-1} for $CuSO_4.5\,H_2O$, 0.187 and 0.268 mg Cu L^{-1} for Captain XTR, 0.076 and 0.122 mg Cu L^{-1} for SeClear, 0.045 and 0.09 mg Cu L^{-1} for Lake Guard Blue (Table 2). NOEC were below the lowest dose for the four algaecides used (Table 2).

Intrinsic rates of population increase (PGR) were positive in all $CuSO_4.5H_2O$ treatments, and declined from 0.259 d^{-1} in controls to 0.195 d^{-1} in the highest dose. Although a one-way ANOVA indicated significant differences (Table S4), this could not be confirmed by a Tukey's post hoc test (Table 3). In the series with Captain XTR, PGR decreased significantly from 0.268 d^{-1} in controls to 0.189 d^{-1} in the highest dose (Table 3; Table S4). Also in the series with SeClear, PGR decreased significantly from 0.285 d^{-1} in controls to 0.145 d^{-1} in the highest dose (Table 3; Table S4). In the series with Lake Guard Blue, PGR

Table 2
NOEC, LOEC and EC_x values calculated based on immobility to *Daphnia magna* from acute tests and fecundity from chronic tests among four copper-based algaecides. The values in the brackets represent the standard deviation (SD) ($n = 5$). 95 % CI displays 95 % confidence interval.

Tests	Treatments	NOEC	LOEC	EC_{10} (mg Cu L^{-1})	95 % CI	EC_{20} (mg Cu L^{-1})	95 % CI	EC_{50} (mg Cu L^{-1})	95 % CI
Acute tests	$CuSO_4.5H_2O$	0.404	0.485	0.452 (0.07)	0.396–0.486	0.479 (0.08)	0.432–0.505	0.521 (0.09)	0.493–0.548
	Captain XTR	0.463	0.579	0.512 (0.01)	0.424–0.553	0.542 (0.01)	0.485–0.577	0.599 (0.01)	0.561–0.636
	SeClear	0.411	0.514	0.422 (0.03)	0.224–0.493	0.471 (0.03)	0.331–0.546	0.594 (0.03)	0.512–0.675
	Lake Guard Blue	0.434	0.496	0.446 (0.06)	0.339–0.472	0.467 (0.04)	0.423–0.487	0.501 (0.02)	0.480–0.523
Chronic tests	$CuSO_4.5H_2O$	<0.091	0.09	0.129	0.09–0.168	0.202	0.161–0.244	0.436	0.396–0.476
	Captain XTR	< 0.102	0.102	0.187	0.130–0.244	0.268	0.213–0.324	0.498	0.451–0.545
	SeClear	< 0.084	0.084	0.076	0.043–0.109	0.122	0.084–0.160	0.274	0.233–0.314
	Lake Guard Blue	< 0.089	0.089	0.045	0.019–0.07	0.090	0.053–0.127	0.295	0.241–0.350

Table 3
Survival (%), intrinsic rates of population increase (PGR, d^{-1}), age at first reproduction (AFR, d^{-1}), age at second reproduction (ASR, d^{-1}), age at third reproduction (ATR, d^{-1}), the initial size of survivors (ISS, mm), the final size of survivors (FSS, mm), initial dry weight (IDW, µg), the final dry weight (FDW, µg), somatic growth rate (SGR) for body length (mm d^{-1}) and SGR for dry weight (µg d^{-1}) of *Daphnia magna* exposed to different concentrations of different copper-based algaecides ($CuSO_4.5H_2O$, Captain XTR, SeClear and Lake Guard Blue) for 21 days. Different symbols (a,., d) indicate significant differences at the 95 % level (Tukey and Dunn's post hoc comparison tests). The standard deviation (SD) are given in brackets ($n = 10$), – in the brackets indicates without SD.

	Concentration (mg Cu L^{-1})	Survival (%)	PGR (d^{-1})	AFR (d)	ASR (d)	ATR (d)	ISS (mm)	FSS (mm)	SGR (mm d^{-1}) for body length	IDW (µg)	FDW (µg)	SGR (µg d^{-1}) for dry weight
$CuSO_4.5\ H_2O$												
	0	100 (–)	0.259 (0.058)^{a}	9.2 (0.63)^{d}	13 (0.82)^{c}	19.6 (0.7)^{ab}	1.01 (0.08)	2.95 (0.07)	0.092 (0.003)	11.3 (–)	271.5 (–)	12.4 (–)
	0.091	100 (–)	0.256 (0.065)^{a}	9.1 (0.32)^{d}	12.7 (0.48)^{c}	18.9 (1)^{ab}		2.83 (0.07)	0.086 (0.003)		251.4 (–)	11.4 (–)
	0.181	100 (–)	0.236 (0.069)^{a}	9.5 (0.85)^{cd}	13.2 (0.42)^{bc}	18.7 (0.82)^{b}		2.62 (0.1)	0.077 (0.005)		249.7 (–)	11.4 (–)
	0.271	100 (–)	0.209 (0.048)^{a}	10.2 (0.79)^{bc}	13.4 (0.52)^{bc}	19.4 (0.7)^{ab}		2.58 (0.06)	0.075 (0.003)		234.7 (–)	10.6 (–)
	0.362	100 (–)	0.195 (0.072)^{a}	10.9 (0.33)^{ab}	13.9 (0.33)^{ab}	19.2 (0.67)^{ab}		2.5 (0.07)	0.070 (0.004)		218.9 (–)	9.8 (–)
	0.452	100 (–)	0.183 (0.065)^{a}	11.2 (0.63)^{a}	14.6 (1.07)^{a}	19.7 (0.48)^{a}		2.41 (0.1)	0.066 (0.005)		203.7 (–)	9.2 (–)
Captain XTR												
	0	100 (–)	0.268 (0.053)^{a}	9 (0)^{c}	12.8 (0.42)	19.5 (0.71)	1.04 (0.07)	2.93 (0.07)	0.090 (0.003)	11.1 (–)	264.2 (–)	12.1 (–)
	0.102	100 (–)	0.25 (0.066)^{ab}	9.6 (1.27)^{bc}	13.4 (1.65)	19 (1.15)		2.73 (0.07)	0.081 (0.004)		240.2 (–)	10.9 (–)
	0.204	100 (–)	0.24 (0.075)^{ab}	9.5 (0.71)^{bc}	12.9 (0.32)	19 (1.052)		2.66 (0.06)	0.077 (0.003)		236 (–)	10.7 (–)
	0.306	100 (–)	0.217 (0.063)^{ab}	9.7 (0.68)^{bc}	13 (0.47)	19.2 (0.92)		2.58 (0.10)	0.073 (0.005)		222.3 (–)	10.0 (–)
	0.408	100 (–)	0.205 (0.040)^{ab}	10.6 (0.52)^{ab}	13.5 (0.53)	19.7 (0.48)		2.47 (0.09)	0.068 (0.005)		164 (–)	7.2 (–)
	0.510	100 (–)	0.189 (0.033)^{b}	10.8 (0.92)^{a}	13.6 (0.52)	18.9 (1)		2.32 (0.07)	0.061 (0.003)		173.6 (–)	7.7 (–)
SeClear												
	0	100 (–)	0.285 (0.095)^{a}	9.1 (0.32)^{c}	13.1 (1.2)^{c}	18 (0)b	0.98 (0.02)	2.88 (0.14)	0.091 (0.007)	7.3 (–)	292.3 (–)	13.6 (–)
	0.084	100 (–)	0.281 (0.078)^{a}	9 (0)^{c}	12.9 (1)^{c}	18 (0)^{b}		2.85 (0.13)	0.089 (0.003)		309.1 (–)	14.4 (–)
	0.169	100 (–)	0.242 (0.055)^{ab}	9.5 (0.53)^{c}	13.8 (1.3)^{bc}	18.1 (0.3)^{ab}		2.66 (0.13)	0.080 (0.006)		294.3 (–)	13.7 (–)
	0.253	100 (–)	0.209 (0.039)^{ab}	10.8 (1)^{b}	14.8 (0.46)^{b}	18.5 (0.76)^{ab}		2.50 (0.09)	0.072 (0.004)		276.3 (–)	12.8 (–)
	0.337	100 (–)	0.202 (0.062)^{ab}	11.6 (0.88)^{ab}	15.3 (0.71)^{ab}	18.6 (0.53)^{ab}		2.40 (0.06)	0.068 (0.003)		251.9 (–)	11.6 (–)
	0.422	60 (–)	0.145 (0.039)^{b}	12.3 (1.89)^{a}	16.9 (1.86)^{a}	19 (1.41)^{a}		2.26 (0.05)	0.061 (0.002)		221.8 (–)	10.2 (–)
Lake Guard Blue												
	0	100 (–)	0.263 (0.036)	10 (0)^{c}	12.7 (0.52)^{b}	19 (0)^{b}	0.98 (0.06)	2.86 (0.09)	0.09 (0.004)	8.6 (–)	242.5 (–)	11.1 (–)
	0.089	100 (–)	0.225 (0.078)	10.3 (0.52)^{c}	13.3 (0.82)^{b}	19 (0)^{ab}		2.61 (0.06)	0.078 (0.003)		245.7 (–)	11.3 (–)
	0.179	100 (–)	0.217 (0.048)	10.6 (0.79)^{bc}	13.9 (1.07)^{b}	18.6 (0.79)^{ab}		2.60 (0.07)	0.077 (0.004)		246.3 (–)	11.3 (–)
	0.268	100 (–)	0.212 (0.062)	11 (1)^{bc}	14.8 (1.98)^{b}	19 (0.53)^{ab}		2.54 (0.09)	0.074 (0.004)		245.9 (–)	11.3 (–)
	0.357	100 (–)	0.192 (0.062)	11.6 (0.89)^{ab}	15 (1.4)^{b}	19.2 (0.45)^{ab}		2.46 (0.13)	0.071 (0.006)		257 (–)	11.8 (–)
	0.446	100 (–)	0.167 (0.047)	12.3 (0.46)^{a}	18.3 (1.03)^{a}	19.7 (0.58)^{a}		2.30 (0.07)	0.063 (0.003)		154 (–)	7.0 (–)

declined from 0.263 d^{-1} in controls to 0.167 d^{-1} in the highest dose, however this difference was not statistically significant (Table 3; Table S4). Compared to the corresponding control, the PGR in the highest dose of copper sulphate was 29 % less, in Captain XTR it was 49 % lower, in SeClear it was reduced by 48 % and in Lake Guard Blue the population growth was 37 % lower.

3.3. Effects of the algaecides on the somatic growth

The *Daphnia magna* at the start of the experiment had a body length of 1.01 (± 0.08) mm and a body weight of 11.3 µg ($CuSO_4.5H_2O$), 1.04 (± 0.07) mm and 11.1 µg (Captain XTR), 0.98 (± 0.02) mm and 7.3 µg (SeClear), and 0.98 (± 0.06) mm and 8.6 µg (Lake Guard Blue) ($n = 10$ for body length, $n = 20$ for body weight). After 21 days, animals reached 2.95 (± 0.07) mm, 2.93 (± 0.07) mm, 2.88 (± 0.14) mm and 2.86 (± 0.09) mm in controls of the $CuSO_4.5H_2O$, Captain XTR, SeClear and Lake Guard Blue series, respectively (Fig. 2; Table 3). The animals' body length was reduced with increased copper concentrations in all algaecides treatments (Fig. 2; Table 3). Somatic growth rates, based on body length, were significantly lower at concentrations ≥ 0.181 mg Cu L^{-1} ($CuSO_4.5H_2O$), ≥ 0.102 mg Cu L^{-1} (Captain XTR) ≥ 0.169 mg Cu L^{-1} (SeClear) and ≥ 0.089 mg Cu L^{-1} (Lake Guard Blue) (Fig. 2; Table S4).

The adult animals reached a body weight of 271.5, 264.2, 292.3 and 242.5 µg in the controls of the $CuSO_4.5H_2O$, Captain XTR, SeClear and

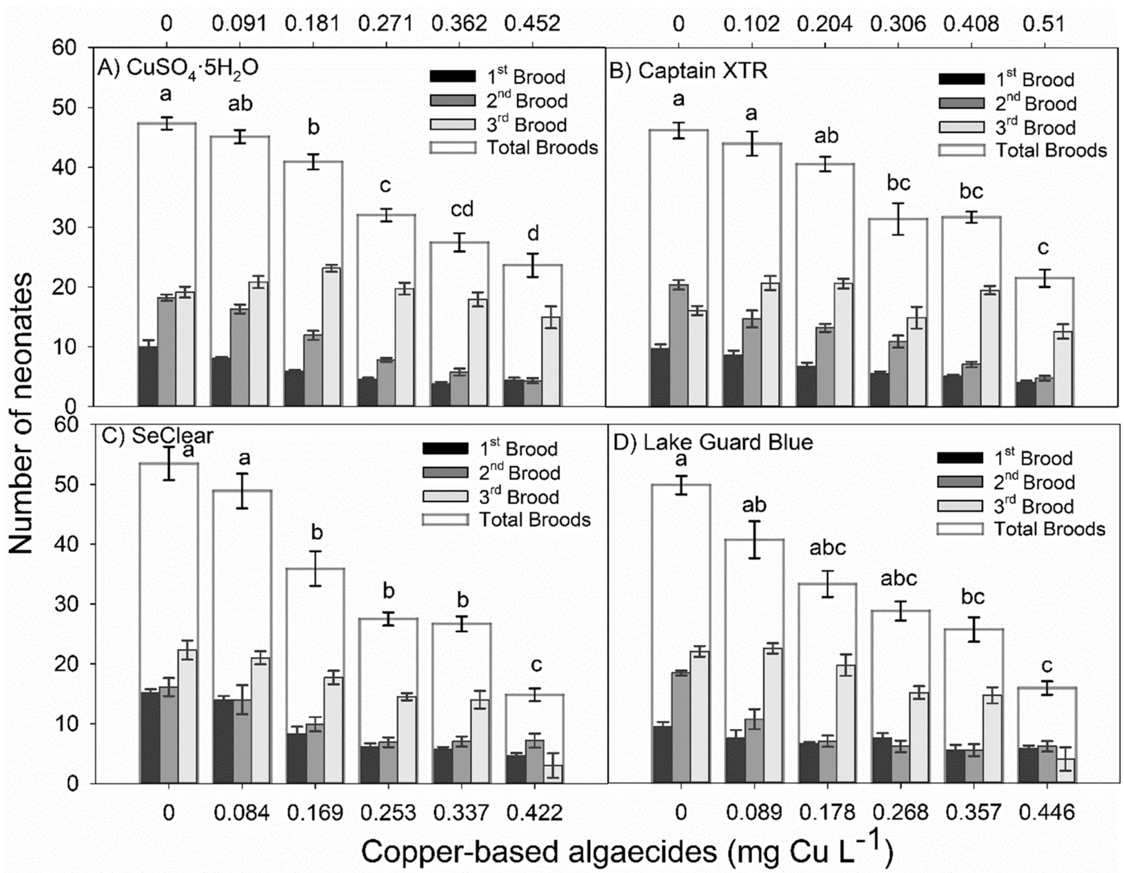

Fig. 1. Number of neonates per female *Daphnia magna* exposed to six copper concentrations of four algaecides ($CuSO_4.5H_2O$, Captain XTR, SeClear and Lake Guard Blue) for the first three consecutive broods. Different symbols (a,., d) above total broods bars indicate significant differences at the 95 % level (Tukey and Dunn's post hoc comparison tests). Error bars represent the standard deviation ($n = 10$).

Lake Guard Blue series, respectively (Table 3). In the copper sulphate series, the Captain XTR and the SeClear series, females were lighter in treatments with higher copper concentrations (Table 3). At the highest copper sulphate concentration, the animals became 25 % lighter than the control (Table 3). In the Captain XTR treatments, the lightest females were found in the 0.408 mg Cu L^{-1} treatment (164 µg), which implies a body weight loss of 38 % compared to controls. In the SeClear treatments, body weight had dropped by 24 % from 292.3 µg in controls to 221.8 µg in the highest dose (Table 3). In Lake Guard Blue treatments, dry weights were similar (242.5–257 µg) in the treatments from 0 to 0.357 mg Cu L^{-1}, yet, dry weight was, compared to the control, reduced by 36.5 % at the highest Cu concentration used (Table 3). SGR based on the dry weight were significantly different between the different Cu concentration used for all the compounds except for Lake Guard Blue series ($H_5 = 10.8$; $P = 0.055$) (Fig. 3; Table S4).

3.4. Chemical equilibrium modeling

The chemical equilibrium modeling indicated that most copper was chelated with EDTA present in the RT medium. When only copper which is the part of the composition of RT medium exists in the system, the concentrations of $Cu(EDTA)^{2-}$ was 0.035 mg L^{-1} and of $Cu(EDTA)(OH)^{3-}$ was 4.378E-06 mg L^{-1}. With the increased amount of copper added, the concentrations of $Cu(EDTA)^{2-}$ also increased (Fig. S2). The regression between the copper concentration added-(Cu_{added}) and the concentrations of $Cu(EDTA)^{2-}$ yielded: $Cu(EDTA)^{2-} = 0.9995 \times Cu_{added} + 0.0354$ ($r^2 = 1.0$).

4. Discussion

4.1. Acute toxicity based on immobility of Daphnia

Daphnia magna belongs to the most sensitive genus of aquatic invertebrates towards copper and is a standard test species used in toxicity tests (Bishop et al., 2018; Mastin and Rodgers, 2000). The acute EC_{50} values for copper-based algaecides (0.501–0.599 mg Cu L^{-1}) were in agreement with values obtained by De Schamphelaere et al. (2002) who found 48 h-EC_{50} ranging from 0.0352 to 0.792 mg Cu L^{-1} in 19 natural European surface waters contaminated with copper. Yet, these acute toxicity values are higher than reported by others in the literature where for instance 48 h-LC_{50} of $CuSO_4$ for *D. magna* between 0.011 and 0.35 mg Cu L^{-1} has been found (Bishop et al., 2018; Mastin and Rodgers, 2000; Suedel et al., 1996), or even as low as 0.0065 mg Cu L^{-1} for unfed *D. magna* and 0.0185 mg Cu L^{-1} for fed *D. magna* (Dave, 1984). Differences between studies may be caused by using different clones that may vary in their sensitivity to copper (Chain et al., 2019), but also by water composition, such as hardness and presence of complexing compounds, that determines the speciation of copper (Andrew et al., 1977). Cationic copper forms (Cu^{2+}, $CuOH^{+}$, $Cu(OH)_2^{2+}$) were identified as toxic species, while complexed, chelated or precipitated Cu forms express less toxicity to *Daphnia* (Andrew et al., 1977). Thus, prior to each Cu application, tests on the effects of Cu compounds on the organisms being targeted should be done while considerating the water chemistry.

In our study, chemical equilibrium modelling indicated that most copper was chelated with EDTA present in the RT medium. Complexation of copper by EDTA resulted in significantly reduced toxicity to *Daphnia magna* (Sorvari and Sillanpää, 1996). Complexing compounds

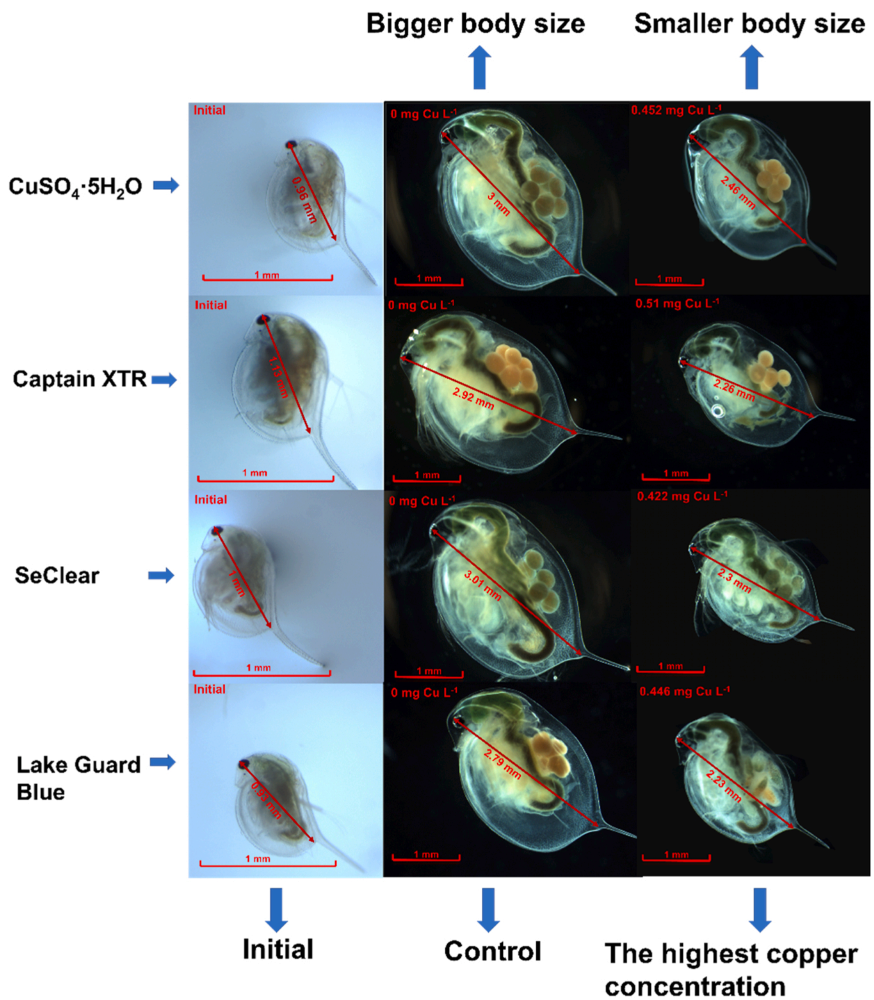

Fig. 2. Comparative body length among initial, control and the highest copper concentration for four copper algaecides ($CuSO_4$.5 H_2O, Captain XTR, SeClear and Lake Guard Blue). Red lines on *Daphnia magna* represent the body length (the distance from the most posterior point on the eye to the base of the junction of the tail spine with the carapace).

are omnipresent in aquatic ecosystems, and humic substances as DOC may easily span concentrations from 1 to 22 mg L^{-1} in surface waters (Kramer et al., 2004). Strong relation has been found between reduced copper toxicity and DOC concentrations (Kramer et al., 2004), in which humic substances reduced both the acute and chronic toxicity of copper to *Daphnia* (Winner, 1985).

Chelators are used in novel copper algaecides to enhance uptake in algae implying that less copper needs to be applied (Calomeni et al., 2014) therewith reducing risks to non-target organisms. Some chelated copper formulations (e.g., Clearigate and Cutrine-Plus) were less toxic to cladocerans than was copper sulphate (Murray-Gulde et al., 2002), another (e.g., Algimycin® PWF) had similar toxicity as copper sulphate (Johnson et al., 2008), or appeared even more toxic (Cutrine-Plus) (Mastin and Rodgers, 2000). In addition, other compounds present in formulations may contribute to observed toxicity, which was evidenced in a study comparing Captain XTR, a chelated copper formulation (9.1 % Cu) containing a surfactant, with a formulation without the surfactant (Captain) (Closson and Paul, 2014). The acute toxicity to brook trout and fathead minnows of Captain XTR was higher than that of copper sulphate, whereas the chelated formulation without surfactant was less toxic than copper sulphate (Closson and Paul, 2014).

Our study yielded that $CuSO_4$.5 H_2O and Lake Guard Blue were more acutely toxic to *D. magna* based on immobility than Captain XTR and SeClear. Although 48 h-EC_{50} were close to each other (*see* Table 2), these results are not in line with our hypothesis.

4.2. Chronic toxicity of copper-based algaecides to Daphnia magna

The highest copper concentrations dosed in the chronic tests were based on the EC_{10} values obtained from the acute toxicity tests. In all treatments in the chronic tests, 100 % survival of test animals was observed with the exception of the highest dose of SeClear that had 60 % survival. All surviving *Daphnia* were observed actively swimming in the test tubes and hence this deviated from the dosed acute-EC_{10} in the highest concentrations. This is likely caused by the presence of algae lowering the copper concentration and therewith toxicity (Bishop et al., 2018). In addition, the green algal food used was grown in a medium containing vitamins that also may have contributed to less toxicity/more resistance in *D. magna* (Winner et al., 1977).

The chronic assay was based on the OECD (2012) standard 21-day reproduction test, but included several additional life-history parameters that have been proposed as more realistic in ecotoxicity testing (Van Leeuwen et al., 1985). AFR was significantly delayed with increased copper concentrations in all four algaecides, which is comparable to the reported delayed age at maturation in cladocerans exposed to copper (Sadeq and Beckerman, 2019). Reproduction was reduced with increasing concentrations of copper which is in line with the results of a meta-analysis on the effects of copper in cladocerans and *D. magna* (Sadeq and Beckerman, 2019). Delayed AFR and reduced number of offspring may have enabled females to allocate energetic resources to growth, maintenance/detoxification rather than reproduction (DeMille

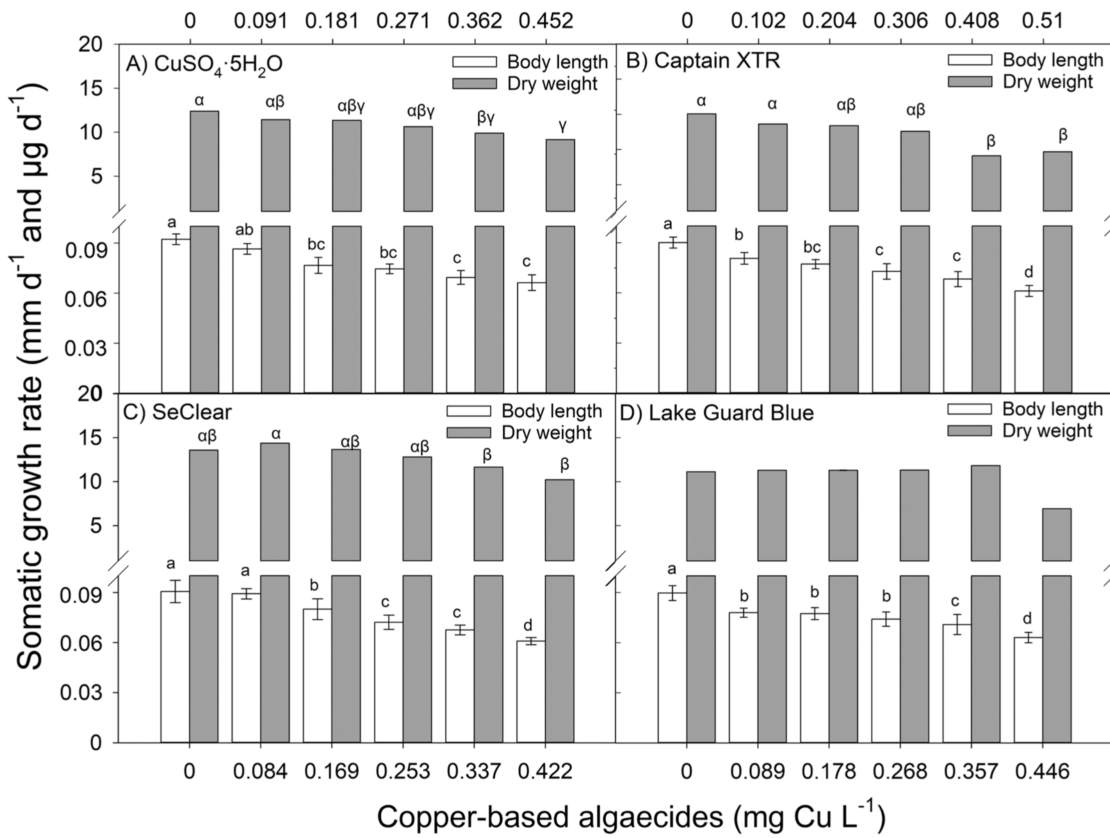

Fig. 3. Somatic growth rates of *Daphnia magna*, based on body length (white bars) and dry weight (gray bars), were exposed for 21 days to different concentrations of algaecides ($CuSO_4.5H_2O$, Captain XTR, SeClear and Lake Guard Blue). Error bars indicate standard deviation (n > 5). Different symbols indicate significant differences (a,., d for body length; α, β, γ for dry weight).

et al., 2016). However, copper exposure may also lead to reduced feeding activity in *Daphnia* (Agra et al., 2011) and therewith to fewer resources available for growth and reproduction. Copper exposed animals were smaller than non-exposed animals, which is in agreement with the results of a meta-analysis showing that with increasing copper concentrations somatic growth in *D. magna* decreased (Sadeq and Beckerman, 2019). In general, smaller *Daphnia* is most likely to produce smaller broods (Martins et al., 2017). Adult *Daphnia* mold after each brood has been released and subsequently grows in size (Anderson, 1932) until about the eleventh instar (Anderson et al., 1937), which means that females shedding the third brood are larger than those producing the first brood and in healthy *Daphnia* brood sizes increase accordingly (Lürling and Van Donk, 1997). However, the number of newborns in third broods exposed to the highest doses of SeClear and Lake Guard Blue were lower or equal to those in the first and second broods, which is indicative of chronic toxicity.

The intrinsic rate of population increase (PGR) was least in the highest copper concentrations in each of four copper algaecides (Table 3 and Fig. 1), which is in agreement with literature findings (Agra et al., 2011). PGR was 30 % lower in the highest copper sulphate and Captain XTR treatment compared to their corresponding controls, 37 % in Lake Guard Blue and 50 % lower in the highest SeClear dose compared to its control. Hence, we rejected our hypothesis that the novel-chelated copper algaecides would be less toxic to *Daphnia* than traditional copper sulphate that would have been expressed in higher population growth rates. PGR is more ecologically relevant than individual endpoints such as survival, or reproduction as it integrates effects at the population level (Forbes and Calow, 1999). Inasmuch as population growth rate and feeding rate may be correlated (Lürling and Van Donk, 1997), effects of copper-induced reduced feeding rates on PGR (Agra et al., 2011) cannot be excluded. Hence, follow up research on *Daphnia* feeding activities may be considered, which is of particular interest as these cladocerans are key organisms in lentic freshwater aquatic ecosystems capable of filtering considerable quantities of phytoplankton.

4.3. *Implication for management*

Copper-based algaecides are developed for the management of eutrophic systems that suffer from nuisance phytoplankton blooms, in particular cyanobacteria blooms (Bishop et al., 2014). Cyanobacteria may cause major problems for the use of the water and impair its ecosystem services. In addition, cyanobacteria may have strong negative effects on *Daphnia* resulting in distortion of the energy flow at the phyto-zooplankton interface causing severe ecosystem consequences (Ger et al., 2014). Hence, mitigating cyanobacterial nuisance and improving water quality is a key issue for water managers, and in cases where algaecides may be applied, these should preferably not exert irreversible negative effects on non-target organisms such as *Daphnia*. Both copper sulphate and three formulations of chelated copper caused a reduction in reproduction and PGR, but mostly at the higher doses of 0.4–0.5 mg Cu L^{-1}. Application of Cu-based compounds are not allowed in certain countries (Codd et al., 2005), however others permit Copper application in surface water, for instance USA Environmental Protection Agency allows Cu application up to 1 mg L^{-1}, which is twice as the EC_{50} found here (EPA Reg. No. 7364-09-8959). In the chronic exposure test, the water was renewed every week meaning three pulses of copper (at the start, after one week and after two weeks), which is likely more than a standard one-time application *in situ* in which copper will be bound rapidly to algae and transported to sediment reducing effects on nontarget *Daphnia* (Bishop et al., 2018; Willis and Bishop, 2016). Although the risk to non-target species, such as *Daphnia*, can be significant (Bishop et al., 2014), it can be reduced by choosing the copper formulation which has the strongest effect on the target algae using the effective dose (Bishop et al., 2018).

In decision-making, besides efficacy and side-effects, also costs and longevity of the intervention need to be included (Lurling et al., 2016). The SeClear formulation showed the strongest PGR reduction in *Daphnia* (50 % lower in the highest SeClear dose compared to its control), but SeClear, according to the manufacturer, also contains a phosphate binder, and thus may reduce the regrowth of nuisance phytoplankton such as cyanobacteria considerably. As in general copper concentrations are reduced rapidly following an application (Willis and Bishop, 2016), up scaled *in situ* experiments with these chelated copper compounds can be considered that will provide more insight into efficacy, side effects and longevity. Given that catchment nutrient control measures are often still absent or insufficient, the use of copper-based algaecides remains an option as fast emergency control of nuisance phytoplankton.

5. Conclusions

- Acute toxicity based on immobilization was rather similar and 48 h-EC_{50}'s ranged from 0.50 mg Cu L^{-1} in Lake Guard Blue to 0.60 mg Cu L^{-1} in Captain XTR.
- Chronic toxicity based on PGR was reduced at the highest doses tested (0.4–0.5 mg Cu L^{-1}) in all four formulations tested. PGR was maximally reduced by 30 % in copper sulphate and Captain XTR, 37 % in Lake Guard Blue and 50 % in SeClear.
- Most copper was chelated with EDTA present in the RT-medium.
- Novel chelated copper formulations (ethanolamine complexes) may not be necessarily less toxic to nontarget *Daphnia* than traditional copper sulphate.
- Up scaled *in situ* experiments with the chelated copper compounds will provide more insight into efficacy, side effects and longevity.

CRediT authorship contribution statement

Li Kang: Conceptualization, Investigation, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Maíra Mucci:** Supervision, Writing – review & editing. **Jingyi Fang:** Investigation, Methodology, Data curation. **Miquel Lürling:** Conceptualization, Resource, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113817.

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