ELSEVIER

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal





Compounds to mitigate cyanobacterial blooms affect growth and toxicity of *Microcystis aeruginosa*

Li Kang , Maíra Mucci, Miquel Lürling

Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA, Wageningen, The Netherlands

ARTICLE INFO

Research Paper Editor: Anusuya Willis, Ph.D

Keywords: lake restoration cyanobacterial bloom controlling cyanobacteria Microcystins phosphate binding

ABSTRACT

Numerous products and techniques are used to combat harmful cyanobacterial blooms in lakes. In this study, we tested nine products, the phosphate binders Phoslock® and Aqual-PTM, the coagulant chitosan, the phosphorus binder and coagulant aluminum salts (aluminum sulphate and sodium aluminate), the copper-based algicides SeClear, Captain® XTR and CuSO₄·5H₂O, the antibiotic Streptomycin and the oxidant hydrogen peroxide (H₂O₂) on their efficiency to manage the cyanobacterium Microcystis aeruginosa (M. aeruginosa). To this end, 7 days of laboratory experiments were conducted and effects were determined on chlorophyll-a, photosystem II efficiency (PSII), soluble reactive phosphorus (SRP) and intracellular and extracellular microcystin (MC) concentrations. The algicides, chitosan and H_2O_2 were the most powerful in reducing cyanobacteria biomass. Biomass reductions compared to the controls yielded: Chitosan (99.8%) > Hydrogen peroxide (99.6%) > Captain XTR (98.2%) > SeClear (98.1%) > CuSO₄·5H₂O (97.8%) > Streptomycin (86.6%) > Phoslock® (42.6%) > Aqual- P^{TM} (28.4%) > alum (5.5%). Compounds that caused the largest reductions in biomass also strongly lowered photosystem II efficiency, while the other compounds (Phoslock®, Aqual-PTM, aluminum salts) had no effect on PSII, but strongly reduced SRP. Intracellular MC concentration followed the biomass patterns, extracellular MC was generally lower at higher doses of algicides, chitosan and H₂O₂ after one week. Recovery of PSII was observed in most algicides and chitosan, but not at the highest doses of SeClear and in all streptomycin treatments. Our results revealed that M. aeruginosa can be killed rapidly using several compounds, that in some treatments already signs of recovery occurred within one week, P fixatives are efficient in reducing SRP, and thus acting via resource suppression, which potentially may provide an addition to fast-acting algicides that kill most of the cells, but allow rapid regrowth as sufficient nutrients remain.

1. Introduction

Eutrophication is a major issue in lakes, rivers and reservoirs (Fang et al., 2022; Wurtsbaugh et al., 2019). The most common symptom in freshwaters of over-enrichment with nutrients is the formation of cyanobacteria blooms. The blooms can cause water quality problems, such as nocturnal oxygen deficiency that may lead to the death of aquatic organisms; turbid water; mal-odor and taste of the water; and due to production of toxins blooms may pose a threat to wildlife, pets and humans (Huisman et al., 2018; Natugonza et al., 2021).

Eutrophication and cyanobacterial blooms have an impact on ecosystem services and come with economic consequences, as blooms decrease commercial fisheries, aquaculture and property values, hamper recreational activities, irrigation and drinking water usage (Hamilton et al., 2014). For instance, due to intense cyanobacteria bloom in Taihu Lake (China) about 2 million residents were unable to drink water for more than a week (Guo, 2007).

Evidently, there is a great need to control eutrophication and minimize the negative impacts of nuisance blooms in which stopping excessive external nutrient inputs is the most optimal mitigation measure (Hamilton et al., 2016; Paerl et al., 2016). However, external nutrient load control may meet severe challenges; worldwide a low share of wastewater is being treated properly (WWAP, 2017) and high investments are required to improve treatment (van Loosdrecht and Brdjanovic, 2014), yet even after point sources such as wastewater effluents have been tackled, eutrophication threats may continue due to nutrient legacies in the lake bed and diffuse nutrient loads from agriculture (OECD, 2014, 2017; Ryding and Forsberg, 1976). Consequently,

E-mail address: li.kang@wur.nl (L. Kang).

^{*} Corresponding author.

in a growing number of water bodies short-term within-system interventions are applied to suppress cyanobacterial blooms directly by targeting the biomass or indirectly by reducing available resources (Jančula and Maršálek, 2011; Lürling et al., 2020; Lürling and Mucci, 2020).

The most common way to suppress cyanobacterial biomass is by using algicides (Jančula and Maršálek, 2011). They are viewed as a relatively fast and cost-effectively way of eradicating cyanobacteria for which an arsenal of compounds exists such as copper-based algicides, oxidants (hydrogen peroxide), herbicides, and antibiotics (Buley et al., 2021; Huang and Zimba, 2020; Iwinski et al., 2016; Jančula and Maršálek, 2011; Kibuye et al., 2021; Matthijs et al., 2012; Matthijs et al., 2016; Qian et al., 2012). Although algicides are generally highly effective and fast-acting, they may also increase dissolved nutrient concentrations (Coloma et al., 2017) and cause the release of intracellular toxins (Jones and Orr, 1994; Kenefick et al., 1993; Li et al., 2022). An alternative coagulant such as aluminium salts (aluminium sulphate and poly-aluminium chloride-PAC) can be used that aggregate the biomass and settle it to sediment, concomitantly adsorbing phosphate (P) (Cooke et al., 2005; Kang et al., 2022a). Coagulants like the organic polymer chitosan can also be combined with local soil or other ballast compounds to facilitate settling of the cyanobacteria-coagulant flocs (Noyma et al., 2016; Noyma et al., 2017; Pan et al., 2011; Pan et al., 2006). When combined with a solid P sorbent as ballast, the water column can be denuded from cyanobacteria while the P sorbent reduces the P release from the lake bed once settled on the sediment. Solid P sorbents can also be used on themselves to indirectly manage cyanobacterial biomass via strong reduction in the availability of P (Lürling et al., 2020; Van Oosterhout and Lürling, 2013).

In this study, we evaluated the efficacy of different compounds used in lake restoration to reduce rapidly cyanobacteria biomass. The compounds chosen vary in working mechanism: P binders (Phoslock®, aluminum salts and Aqual- P^{TM}), coagulants (chitosan and aluminum salts) and algicides (copper-based compounds, hydrogen peroxide and antibiotic), to test the hypothesis that algicides are the most powerful in reducing cyanobacteria biomass, while P sorbents evoke a milder response. The hypothesis was tested by running one-week exposure assays with the common cyanobacterium *Microcystis aeruginosa*.

2. Material and Methods

2.1. Chemicals

Nine products or chemicals commonly used in lake restoration projects were selected, including three copper-based algicides (SeClear, Captain XTR and $CuSO_4$ - $5H_2O$), two coagulants (Chitosan and

aluminum salts (Al-salts)), two phosphate fixatives (Phoslock® and Aqual- P^{TM}), one antibiotic (Streptomycin), and one oxidant (Hydrogen peroxide). Each compound was tested at six different concentrations and each concentration was run in triplicate (Table 1).

2.2. Experimental design

Microcystis aeruginosa (strain PCC 7820) was obtained from the Pasteur Culture Collection of Cyanobacteria (PCC) and cultured on a modified WC-medium (Lurling and Beekman, 2006) in 500 mL Erlenmeyer flasks placed at 22 °C, at a light intensity of 35 µmol quanta m⁻¹s⁻¹ provided in a 16:8 h light:dark cycle. Aliquots of *M. aeruginosa*, harvested during their exponential growth phase, were transferred to 250 mL conical flasks containing 50 mL of WC medium yielding a final concentration of 100 μ g L⁻¹ chlorophyll-a (Chl a), which indicated Microcystis biomass in this study. The inocula had a photosystem II efficiency (PSII efficiency) of 0.35. Six concentrations of each of the nine compounds were used (Table 1). Each concentration had three replicates. After the addition of the compounds, the flasks were closed with a cellulose plug, mixed and placed in an incubator (Gallenkamp Orbital shaker) with 50 rpm under the same conditions at which M. aeruginosa had been cultured. After 2, 24, 72 and 168 hours, samples were taken from the middle of the flasks to measure Chl a concentrations and PSII efficiencies using a PHYTO-PAM analyzer (Heinz Walz GmbH, Effeltrich, Germany). At the end of the experiment, the pH in each flask was measured using a WTW Inolab pH 7110 meter and subsamples were taken and filtered through 0.45 µm unit filters (GF/C, Whatman, Germany) to analyze orthophosphate with a Skalar SAN⁺ segmented flow analyzer. Also, both intracellular and extracellular MCs concentrations were measured. Hereto, 7.5 mL samples were filtered through glass fiber filters (GF/C, Whatman®, VWR International B.V., Amsterdam, The Netherlands), the filters were frozen at -20°C and subsequently extracted with 75% v/v methanol/water (Lürling and Faassen, 2013). Filter extracts were transferred to 8 mL glass tubes for intracellular microcystin (MC) analysis. The GF/C filtrates were also transferred in 8 mL glass tubes for dissolved extracellular MC analysis. The extracts and filtrates were dried in a SpeedVac concentrator (SavantTM SPD121P, Thermo Fisher Scientific, Asheville, NC, USA) and reconstituted using 900 µL methanol (J.T. Baker®, 97%, VWR International B.V., Amsterdam, The Netherlands). Afterward, the samples were transferred to a vial with a $0.22~\mu m$ cellulose-acetate spin centrifuge tube filters and centrifuged for 5 minutes at 16,000 \times g. The filtrates were then transferred to amber glass vials and analyzed using LC-MS/MS (Agilent 6410A QQQ, Waldbronn, Germany) for eight MC variants according to Lürling and Faassen (2013). The MC concentrations in this study are presented as extracellular MC concentrations ($\mu g L^{-1}$), intracellular MC

 Table 1

 The information of nine materials tested on M. aeruginosa.

Product	Description	Manufacturer	Dosage	Price (USD \$)	References dosage
Phoslock®	Ballast; Clay; Lanthanum- modified bentonite, LMB	Phoslock® Europe GmbH (Zug, Switzerland)	0, 50, 100, 300, 600, 1000 mg L ⁻¹	0.0025/g	(Spears et al., 2013)
Aqual-P TM	Ballast; aluminum -modified zeolite, AMZ	Blue Pacific Minerals (Tokoroa, New Zealand)	0, 50, 100, 300, 600 1000 mg L ⁻¹	0.0027/g	(Mucci et al., 2017)
Chitosan	Coagulant; CHI	Sigma, USA	0, 0.5, 1, 2, 4, 8 mg L^{-1}	0.001-0.095 /g	(Mucci et al., 2017)
Aluminium sulphate + Sodium Aluminate	Metal-based coagulant; Al-salts	Kemira (Helsinki, Finland) + Sigma- Aldrich (Darmstadt, Germany)	0, 1, 3, 10, 20, 30 mg Al L^{-1}	0.0002+0.04/ g	(Georgantas and Grigoropoulou, 2007)
SeClear	Metal-based algicides; Copper sulphate pentahydrate	SePRO Corporation, Carmel, IN, USA	0, 0.05, 0.1, 0.25, 0.5, 1 mg Cu L ⁻¹	0.005/mL	Advised dose 0.15-1.0 mg Cu L^{-1} by SePro
Captain® XTR	Metal-based algicides; Copper ethanolamine complex	SePRO Corporation, Carmel, IN, USA	0, 0.05, 0.1, 0.25, 0.5, 1 mg Cu L ⁻¹	0.014/mL	Advised dose 0.15-1.0 mg Cu ${\rm L}^{-1}$ by SePRO
CuSO ₄ ·5H ₂ O	Metal-based algicides; analysed pure	Sigma, USA	0, 0.05, 0.1, 0.25, 0.5, 1 mg Cu L ⁻¹	0.17/g	(Viriyatum and Boyd, 2016)
Streptomycin	Antibiotics; Str, inhibitor of protein synthesis	Sigma, USA	0, 0.1, 0.2, 0.4, 0.8, 1.6 mg L ⁻¹	1.3/g	(Qian et al., 2012)
Hydrogen peroxide	Oxidant; H ₂ O ₂ , liquid	Merck KGaA, Germany	0, 0.1, 0.3, 1, 3, 10 mg $\rm L^{-1}$	0.1/mL	(Matthijs et al., 2012)

concentrations (μ g L $^{-1}$), and the ratio between extracellular MC and Chl a concentrations (μ g extracellular MC per μ g Chl a), the ratio between intracellular MC and Chl a concentrations (μ g intracellular MC per μ g Chl a).

2.3. Growth rates

Growth rates were estimated from initial and final Chl *a* concentrations assuming exponential growth using the equation (Fawaz et al., 2018; Gojkovic et al., 2019):

$$\mu = \frac{\ln Chl \ a_{end} - \ln Chl \ a_{start}}{t_j - t_i}$$

where: μ is growth rates; t_i is experimental start time (days); t_j is experimental end time (days). Only initial and final Chl a data were used as cell leakage may temporarily increase Chl a concentrations determined by fluorescence (Mucci et al., 2017).

2.4. Statistical analysis

All graphs were created using Sigmaplot 14.0 version. The differences between growth rates, SRP concentrations, intracellular, extracellular MCs, intra and extracellular MCs/Chl α calculated, were tested using one-way ANOVA or a non-parameter test (Kruskal-Wallis One Way analysis of variance on ranks) when normality tests (Shapiro-Wilk) failed. EC50 values (material concentration that caused a 50% reduction in PSII relative to the control) were calculated based on the PSII value by a four-parameter logistic equation. The results section has been written

following the suggestion of using evidence language (Muff et al., 2022).

3. Results

3.1. Chlorophyll-a concentrations

Chlorophyll-a (Chl a) concentrations of the M. aeruginosa cultures were affected differently by the different compounds (Fig. 1). The Chl a increased over time at all the LMB, AMZ and Al concentrations tested. There was, however, a clear dependency of Chl a and LMB dose where Chl a increased less with higher doses of LMB (Fig. 1a). In the highest LMB, AMZ and Al concentrations used the Chl a concentrations were 42.6 %, 28.4 % and 5.5 % less than in the corresponding controls at the end of the experiment, respectively (Fig. 1a,b,d). Chitosan (CHI) caused a rapid increase in Chl a concentrations within 2 and 24 hours in all concentrations tested, however after 72 and 168 hours Chl a concentrations in the highest dose (8 mg CHI L^{-1}) were 23.6 % and 99.8 % less compared to the controls (Fig. 1c). A typical response of rapid cell lysis could be confirmed by analysis of 0.45 µm filtered culture medium (Table S1). The Chl a concentrations gradually declined in higher chitosan dosages because of the breakdown of the pigments, whereas the Chl a concentration increased in controls as a result of population growth (Fig. 1c).

The copper-based materials (Captain XTR, SeClear and $CuSO_4 \cdot 5H_2O$) showed similar trends, a strong reduction in Chl a concentration when 0.5 and 1 mg Cu L^{-1} was applied (Fig. 1e, f, g). SeClear caused a rapid increase (after 2 h) in Chl a concentrations in the highest dose, which was due to the release of cell constituents (Table S1). Shortly after the

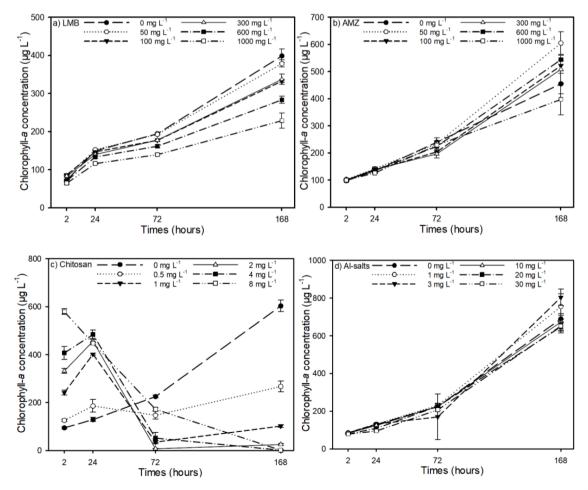


Fig. 1. Effect of nine compounds on the chlorophyll-a concentration of M. aeruginosa exposed for 2, 24, 72 and 168 h. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) CuSO₄·5H₂O, h) Streptomycin and i) H₂O₂. The initial chlorophyll-a concentration was 100 μ g L⁻¹.

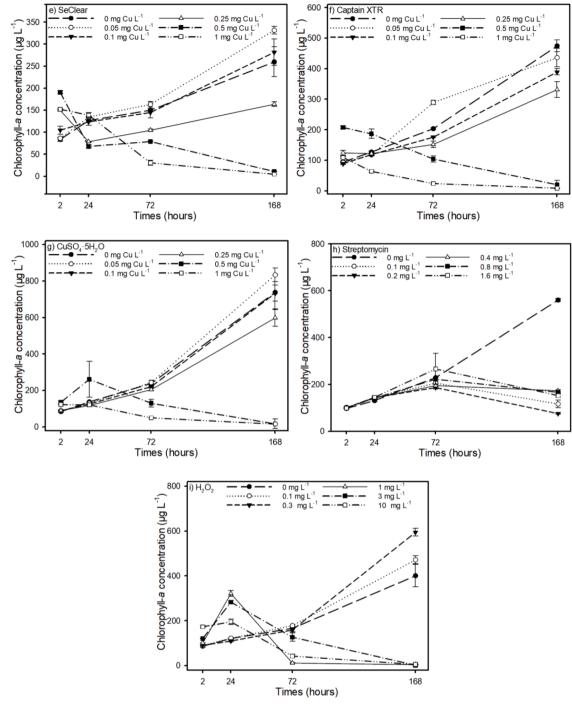


Fig. 1. (continued).

addition of Streptomycin, Chl a concentrations increased in all the concentrations tested, however by the end of the experiment Chl a concentrations were strongly reduced (69-87 %) compared to the controls (Fig. 1h). After the addition of H_2O_2 , Chl a concentrations rapidly increased in the higher H_2O_2 doses as a result of cell lysis, which was followed by a subsequent decline due to the breakdown of the pigment (Fig. 1i). In the control and H_2O_2 treatments below 0.3 mg H_2O_2 L⁻¹, Chl a concentrations were similar and showed a continuous increase reflecting M. aeruginosa growth. After one-week, Chl a concentrations in the higher H_2O_2 treatments were up to 99.5 % lower than in the controls (Fig. 1i).

3.2. Growth rates

The difference between the initial (100 μ g L⁻¹) and final Chl a concentrations in controls and each treatment were used to calculate growth rates. Growth rates of M. aeruginosa declined with increasing amounts of LMB dosed (Fig. 2a). A one-way ANOVA provided very strong evidence that growth rates were different ($F_{5, 12} = 61.2$; p < 0.001) and Tukey's test showed that growth rates in the 1000 mg LMB L⁻¹ treatments were lower (p < 0.001) than in the controls (0 mg LMB L⁻¹). Although this pattern was less clear in the AMZ treatments (Fig. 2b), the one-way ANOVA provided very strong evidence of differences in growth rates ($F_{5, 12} = 9.0$; p < 0.001) and a Tukey's test showed that growth rates in the 1000 mg AMZ L⁻¹ treatments were lower (p = 0.004) than in the

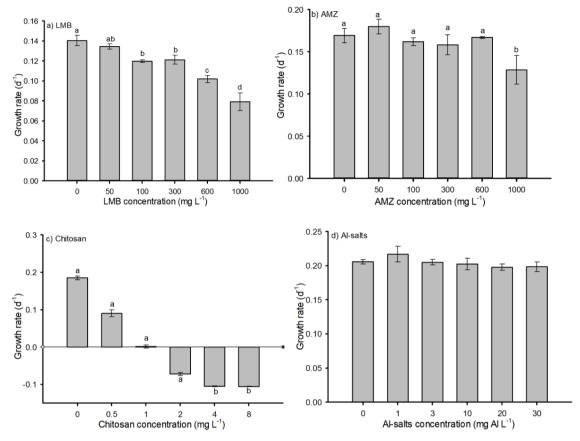


Fig. 2. Growth rate of *M. aeruginosa* at different chemical concentrations after 168 hours. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) CuSO₄·5H₂O₄, b) Streptomycin and i) H₂O₂. Similar letters (a, b, c, d) in each panel indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other (p > 0.05).

controls (0 mg AMZ L^{-1}).

In the chitosan treatments, strong evidence was found ($H_5 = 16.11$; p = 0.007) that growth rates of M. aeruginosa decreased with increased chitosan dosages (Fig. 2c). Growth rates declined strongly at a dose of 0.5 mg CHI L⁻¹, was almost zero at 1 mg CHI L⁻¹, and became negative at doses > 1 mg CHI L⁻¹ indicating a decline/death of the M. aeruginosa populations (Fig. 2c). There was no evidence that Al, at any dose tested, had an effect on growth rates of M. aeruginosa ($F_{5, 12} = 2.883$; p = 0.062) (Fig. 2d).

Compared to controls, growth rate declined 201 %, 156 % and 139 % in the highest dosages (1 mg Cu L^{-1}) of SeClear ($F_{5,\ 12}=852.92;\ p<0.001$), Captain XTR ($F_{5,\ 12}=338.86;\ p<0.001$) and CuSO₄·5H₂O ($F_{5,\ 12}=252.19;\ p<0.001$), respectively. All three compounds resulted in negative growth rates (between -0.094 and -0.075 d $^{-1}$) when dosed at 0.5 mg Cu L^{-1} or higher (Fig. 2e,f,g).

The experiment with streptomycin yielded strong evidence that streptomycin affected *M. aeruginosa* growth rate ($H_5=16.58;\ p=0.005$). A Tukey's test provided very strong evidence that growth rates in the 0.2 mg STR L⁻¹ treatments were lower (p<0.001) than in the controls (0 mg STR L⁻¹) (Fig. 2h).

There was no evidence that hydrogen peroxide affected M. aeruginosa growth rates when dosed in the range 0-0.3 mg L⁻¹, however, in doses > 1 mg H₂O₂ L⁻¹, very strong evidence was obtained that M. aeruginosa growth rates were reduced ($F_{5, 12} = 1208.78$; p < 0.001). Tukey's post hoc test revealed two homogeneous groups among the six H₂O₂ treatments: 1) positive, unaffected growth in controls and the 0.1 and 0.3 mg H₂O₂ L⁻¹ treatments, and 2) negative growth in the 1, 3 and 10 mg H₂O₂ L⁻¹ treatments (Fig. 2i).

3.3. EC₅₀ values

The EC50 values were calculated based on the PSII efficiencies (Fig. S1) of the M. aeruginosa cultures. Estimated EC50 values of the P binders LMB and AMZ exceeded the highest dose used (1000 mg L^{-1}). Likewise, EC₅₀ values of the Al coagulant were higher than the highest dose used (30 mg Al L⁻¹), indicating no or a weak effect of the compound on the physiological health of the M. aeruginosa cells. It should be noted, however, that after 7 days the PSII efficiencies in the highest dose of Al were lower than in the other treatments (Fig. S1). For chitosan, the other coagulant tested, EC₅₀ values were initially $< 1 \text{ mg L}^{-1}$ and increased over time (Table 2). EC50 values of the three copper-based chemicals were similar and as low as 0.25-0.28 mg Cu L⁻¹ after two hours exposure; they remained low during the experiment (Table 2). The antibiotic streptomycin was also effective in damaging M. aeruginosa and EC₅₀ values at the end of the experiment (after 168 hours) were as low (0.09 mg L^{-1}) at the lowest dose of STR tested (0.1 mg L^{-1}) . The EC₅₀ values of the oxidizer hydrogen peroxide (H₂O₂) dropped from 2.6 mg L^{-1} after two hours to 0.84-0.99 mg L^{-1} in the period 1-7 days (Table 2).

3.4. Phosphate concentrations

Phosphate (soluble reactive phosphorus, SRP) concentrations were measured at the end of the experiment. The SRP concentrations revealed strong evidence that higher doses of LMB resulted in less SRP ($H_5 = 16.31$; p = 0.006) (Fig. 3a). Similarly, for AMZ there was very strong evidence that higher doses of AMZ affected SRP negatively ($F_{5,12} = 101.53$; p < 0.001) (Fig. 3a), and also for Al strong evidence was found of Al negatively affecting SRP concentrations ($H_5 = 16.88$; p = 0.005)

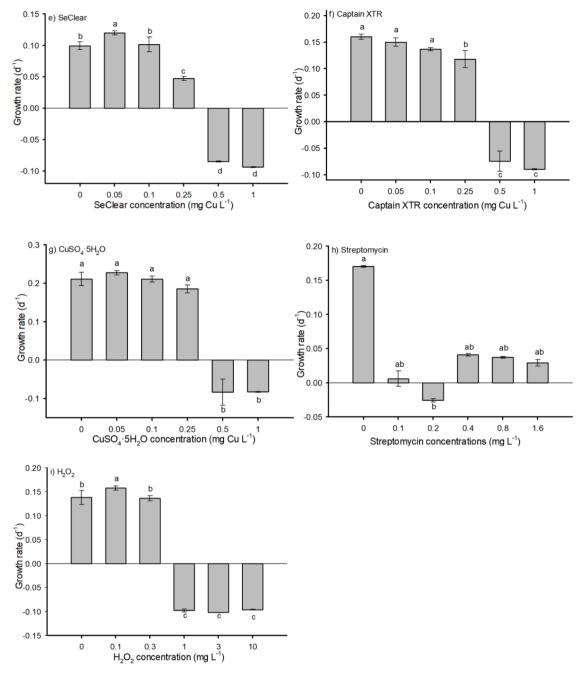


Fig. 2. (continued).

(Fig. 3b). In the series with chitosan, there was a weak evidence of lower SRP concentrations in the controls than in the CHI treatments ($H_5 = 16.75$; p = 0.06).

There was a weak evidence that SRP concentrations were lower in the controls and low doses of SeClear than at higher doses of this copperbased algicide ($H_5=9.48; p=0.091, {\rm Fig.~3c}$), while there was moderate evidence that this was the case for the other two copper-based algicides (Captain® XTR, $F_{5,12}=5.02; p=0.010; {\rm CuSO_4\cdot5H_2O}, H_5=13.71; p=0.018$) due to uptake by *M. aeruginosa* (Fig. 3c). Similar, there was weak evidence that SRP concentrations in the controls of the streptomycin series were lower than in STR treatments ($H_5=15.25; p=0.060$) (Fig. 3d). The data of the H_2O_2 series yielded weak evidence that the SRP concentrations were lower in doses 0-0.3 mg $H_2O_2 L^{-1}$ and higher in the >1 mg $H_2O_2 L^{-1}$ treatments ($H_5=17.69; p=0.070$) (Fig. 3d).

3.5. MC concentrations

Microcystins (MC) present in the WC medium were quantified at the end of the experiment (Fig. 4). Four variants (MC-LW, LY, LR and dmLR) were detected of which the variant MC-LR was the most abundant one.

The total extracellular MC concentrations in the LMB series ranged from 4.9 to5.9 μ g L⁻¹ (Fig. 4a). There was strong evidence that MC concentrations differed ($F_{5,12}=4.65; p=0.014$), a Tukey's test revealed that extracellular MC concentrations in the 600 mg LMB L⁻¹ treatment were higher than in the controls (Fig. 4a). There was strong evidence that intracellular MC concentrations declined with LMB dose ($F_{5,12}=104.64; p<0.001$), the intracellular MC concentration in the control group was 8 times higher than in the 1000 mg LMB L⁻¹ treatment (Fig. 5a). The decline was most prominent in the more hydrophilic MC variants dmMC-LR and MC-LR (Fig. 5a). Extracellular MC concentrations/Chl a was lowest in the control group and it increased with

Table 2 Mean EC_{50} values (values insides brackets represent the standard deviation (SD), n=3) of the compounds used at each time point.

Chemicals	EC_{50} -2 hours (mg L ⁻¹ , mg Al L ⁻¹ , mg Cu L ⁻¹)	EC_{50} -24 hours(mg L^{-1} , mg Al L^{-1} , mg Cu L^{-1})	EC_{50} -72 hours(mg L^{-1} , mg Al L^{-1} , mg Cu L^{-1})	EC_{50} -168 hours(mg L ⁻¹ , mg Al L ⁻¹ , mg Cu L ⁻¹)
LMB	>1000	>1000	>1000	>1000
AMZ	>1000	>1000	>1000	>1000
Chitosan	0.86 (0.09)	0.59 (0.003)	2.41 (0.02)	4.3 (0.028)
Al-salts	>30	>30	>30	>30
SeClear	0.28 (0.03)	0.45 (0.012)	0.32 (0.01)	0.39 (0.02)
Captain® XTR	0.25 (0.09)	0.3 (0.02)	0.33 (0.005)	0.42 (0.005)
CuSO ₄ ·5H ₂ O	>1	0.4 (0.01)	0.28 (0.01)	0.5 (0.005)
Streptomycin	>1.6	>1.6	0.13 (0.02)	0.09 (<0.001)
H_2O_2	2.64 (0.008)	0.84 (0.005)	0.93 (0.007)	0.99 (0.02)

increasing LMB concentrations (Fig. S2a), while intracellular MC/Chl α was the highest in the control group and decreased with increasing LMB dosages (Fig. S3a).

In the AMZ series, there was very strong evidence that extracellular MC concentrations in presence of AMZ were lower than in the control $(F_{5,12}=9.4;p<0.001)$ (Fig. 4b). The MC data yielded no evidence that intracellular MC concentrations were affected by AMZ $(F_{5,12}=1.19;p=0.371)$ (Fig. 5b). There was, however, a clear tendency of a higher share of the more hydrophobic MC variants MC-LW and MC-LY and less of the more hydrophilic dmMC-LR and MC-LR at higher doses of AMZ (Fig. 5b).

Extracellular MC data yielded strong evidence ($F_{5,12} = 28.75$; p < 0.001) that extracellular MC concentrations in presence of chitosan were higher than in absence of chitosan (Fig. 4c). The ratio of extracellular

MC/Chl a increased with the chitosan dosages (Fig. S2c). There was very strong evidence that intracellular MC concentrations in the chitosan series declined with increased chitosan dose ($F_{5,12}=336$; p<0.001) (Fig. 5c).

In the Al series, there was very strong evidence that extracellular MC concentrations increased with increasing dose of Al coagulants (Fig. 4d). Also the extracellular MC/Chl a ratio showed a similar pattern (Fig. S2d). One-Way ANOVA revealed very strong evidence that Al concentrations affected intracellular MC concentrations ($F_{5,12}=3.45; p<0.001$) (Fig. 5d), a Tukey's test showed that intracellular MC concentration in the 30 mg Al L $^{-1}$ was lower than in the control (Fig. 5d). The intracellular MC/Chl a ratio in 30 mg Al L $^{-1}$ was much higher than the ratios found in the other Al concentrations (Fig. S3d).

No evidence was found that the copper-based algicide SeClear $(F_{5,12}=0.38; p=0.85)$ had an influence on the extracellular MC concentration (Fig. 4e). In contrast, very strong evidence was found that extracellular MC contents were elevated at the highest doses at Captain® XTR $(F_{5,12}=399.2; p<0.001)$ and copper sulphate (CuSO₄·5H₂O, $F_{5,12}=31.82; p<0.001)$ (Fig. 4f,g). The extracellular MC/Chl a ratio increased at higher doses of all three copper-based algicides (Fig. S2e,f,g), while the intracellular MC/Chl a ratios gradually declined with increasing dose of each of the three copper-based algicides tested (Fig. S3e,f,g).

Evidence was obtained that streptomycin caused an increase in extracellular MC concentrations at all doses tested ($F_{5,12} = 25.83$; p < 0.001) (Fig. 4h). This was mirrored by a strong decline in intracellular MC concentrations ($F_{5,12} = 374.96$; p < 0.001) (Fig. 5h). The extracellular MC/Chl a ratio at 0.2 mg L⁻¹ were around 15 times than that at control (Fig. S2h), while intracellular MC/Chl a ratio gradually decreased as increasing Str concentration (Fig. S3h).

In the hydrogen peroxide series, despite relatively large variability found in some of the treatments (Fig. 4i), moderate evidence was

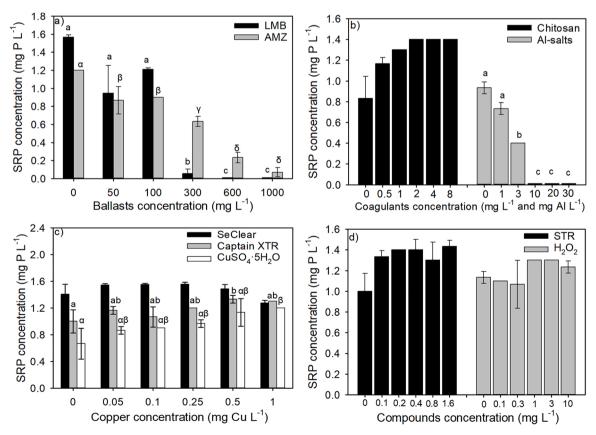


Fig. 3. SRP concentrations at six chemical exposure concentrations of nine compounds. a) LMB and AMZ, b) Chitosan and Al-salts, c) SeClear, Captain XTR and CuSO₄·5H₂O, d) Streptomycin and H₂O₂. Similar symbols (a, b, c; α , β , γ , δ) above bars indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other (p > 0.05; Tukey's post hoc comparison tests).

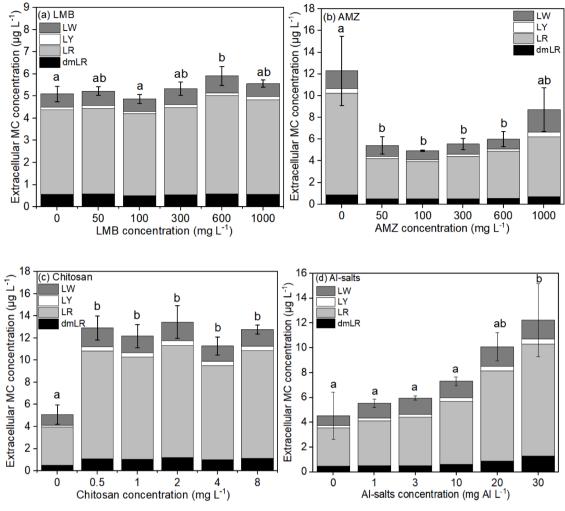


Fig. 4. Extracellular MC in all different chemicals concentrations after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) $CuSO_4 \cdot SH_2O$, h) Streptomycin and i) H_2O_2 . Similar symbols (a, b, c) above bars indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other (p > 0.05; Tukey's post hoc comparison tests).

obtained that extracellular MC concentrations were affected by $\rm H_2O_2$ ($F_{5,12}=3.97; p=0.025$). There was very strong evidence that $\rm H_2O_2$ had an effect on intracellular MC concentrations ($F_{5,12}=123.6; p<0.001$), and clearly two groups could be identified: 1) 0-0.3 mg $\rm H_2O_2\,L^{-1}$ with mean MC concentrations of 150-180 µg $\rm L^{-1}$, and 2) 1-10 mg $\rm H_2O_2\,L^{-1}$ with MC concentrations close 0 µg $\rm L^{-1}$ (Fig. 5i).

4. Discussion

This study determined the short-term (7 d) effects on *M. aeruginosa* of nine different compounds commercially available and used to mitigate cyanobacterial blooms. The compounds chosen have different modes of action, and as such different short-term effects were expected on Chl *a* concentrations, PSII efficiency, growth rates, SRP concentrations and extra/intracellular MC concentrations. Rapid negative effects on *M. aeruginosa* biomass indicators and physiological health were expected from those compounds compromising membrane integrity, such as algicides, while slow or no effects were expected from compounds that do not target cells directly, but act indirectly via reduction of available phosphate.

In line with our expectations, the two solid-phase P fixatives (LMB and AMZ) exerted a weak effect on *M. aeruginosa* biomass. Growth rates were reduced with increasing LMB doses, and given the strong depletion of SRP, this can be attributed to P limitation, but it by no means implies

that relatively high *M. aeruginosa* biomass was mitigated. Similar observations have been made in short-term laboratory experiments and field mesocosms sealed at the bottom (Buley et al., 2021). PSII efficiencies were only marginally reduced at the highest doses of P fixatives, which might have been caused by higher turbidity, or indicated minor effects on cell membrane integrity.

The extracellular MC/Chl a ratio in the LMB series increased with increasing the LMB, which might indicate relatively more release of MCs, but could also be a result of MCs not being broken down in the artificial medium within the 7 day experimental period as in lake and river water breakdown of MCs might already take this time (Edwards et al., 2008). No evidence was found that extracellular MC concentrations declined with LMB in our study. A recent study showed that LMB dosed at 50, 100, and 150 ppm decreased extracellular MC concentrations by 61 %, 86 %, and 75 % relative to the controls at a MC-LR concentration of 500 ppb, respectively, while LMB had no effect on the MC-LR concentration at lower concentrations of 100 ppb and 50 ppb (Laughinghouse et al., 2020). Hence, our results - with extracellular MC concentrations less than 20 ppb - are in line with those of Laughinghouse et al. (2020). The intracellular MC/Chl a ratio declined with increasing LMB dose and this study is the first that reports such finding as an effect of LMB. This might be related to less light available in higher doses of LMB that might lead to lower MC cell quota in M. aeruginosa (Wiedner et al., 2003). Evidently, M. aeruginosa became less toxic when

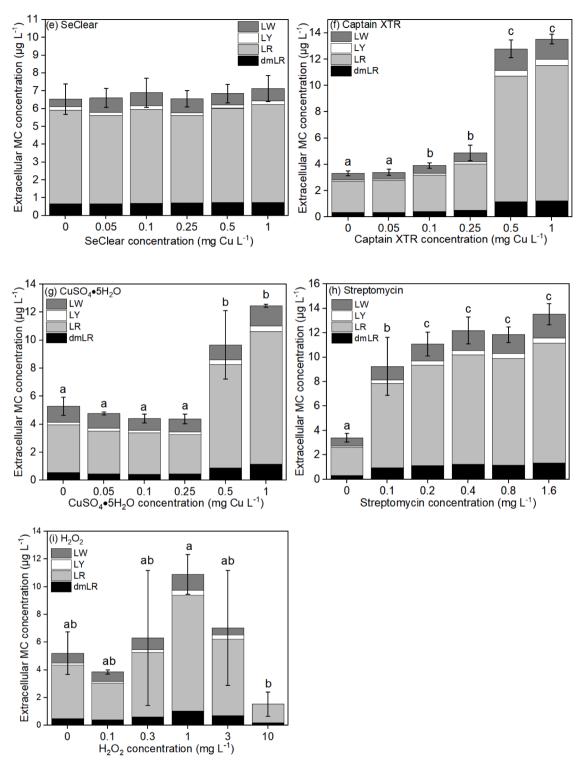


Fig. 4. (continued).

reared in presence of LMB, because intracellular MC concentrations declined 8 times at the highest LMB dose, while Chl a was only halved.

The variants dmMC-LR and MC-LR seemed to decline faster than MC-LY and MC-LW. Interestingly, the latter contain aromatic amino acids (Tyrosine, Y, and tryptophan, W), and particularly the higher amounts of these MC variants in the higher AMZ doses could suggest stimulation of the shikimate pathway, whilst reducing non-aromatic amino-acid synthesis. This finding is, however, opposite to findings that under P limitation more N-rich variants of MC are being produced (Krüger et al.,

2012). The observed increase in more hydrophobic MC variants at a higher dose of AMZ urges for care in the timing of adding such solid-phase P binders, since a higher share of those MC variants also implies higher toxicity (Fischer et al., 2010; Vesterkvist et al., 2012).

The results obtained also underpin that solid-phase P fixatives are not meant as quick fix agents eradicating cyanobacterial blooms; they are measures to reduce internal P load (Copetti et al., 2016; Douglas et al., 2016; Gibbs and Hickey, 2018). Combined with a coagulant, however, those solid phase P fixatives can be highly effective in settling

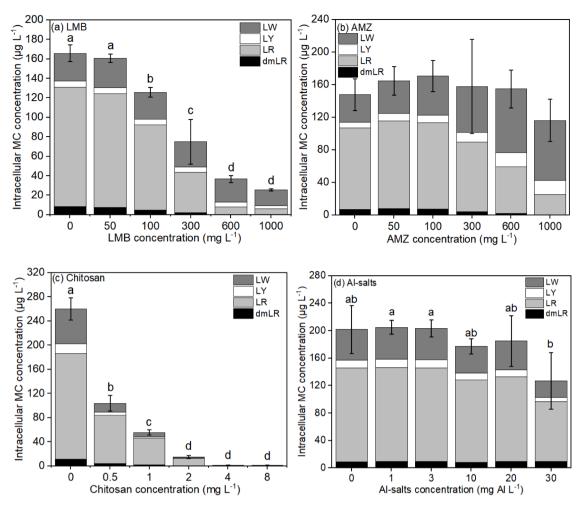


Fig. 5. Intracellular MC in all different chemicals concentrations after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) CuSO₄·5H₂O, h) Streptomycin and i) H₂O₂. Similar symbols (a, b, c) above bars indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other (p > 0.05; Tukey's post hoc comparison tests).

cyanobacterial biomass on the sediment (Lürling et al., 2020; Noyma et al., 2017), a combination that was not tested in this study.

The two coagulants tested had distinct effects on M. aeruginosa. Alsalts did not affect M. aeruginosa growth and only weakly PSII efficiencies in the highest dose, which is comparable to other studies that found alum caused hardly cell damage (Lam et al., 1995), had no growth-inhibiting effect, and did not cause M. aeruginosa cell lysis or release of MCs in the water in short term experiments (Chow et al., 1999). However, when Al-salts treated M. aeruginosa was incubated longer, severe cell damage and subsequent leakage of MCs were observed, especially in the high dose (i.e. 48 mg L⁻¹) treatment (Han et al., 2016). Hence, the elevated extracellular MC concentrations at the highest dose tested in our study (30 mg L^{-1}), the concomitant lower intracellular MC and lower PSII efficiency strongly indicate cell damage after 7 days at this Al dose. Alum also strongly reduced SRP concentrations as expected (Kang et al., 2022a). The SRP binding, particularly intercepting SRP released from P-loaded sediments, is the prime mechanism through which aluminium based compounds may control eutrophication and the development of cyanobacterial blooms (Augustyniak et al., 2019; Kibuye et al., 2021). In contrast, chitosan (CHI) did not reduce SRP concentrations, but caused a strong decline in M. aeruginosa growth and PSII efficiency, which was most probably a result of the membrane damaging effect of CHI resulting in cell lysis (Mucci et al., 2017).

There was very strong evidence for leakage of cell constituents. MCs

and other cell constituents such as pigments remain inside the cell until the membrane is damaged and the cell is lysed (Lam et al., 1995). The high Chl a concentrations measured after two hours of exposure to CHI appeared as a result of cell constituent leakage (Table S1), leading to a significant fluorescence signal, which does not reflect an increase in biomass (Bastien et al., 2011). The same strain of M. aeruginosa (PCC7820) was sensitive to CHI in another study where after 24 hours strongly elevated extracellular MC concentrations were measured (Mucci et al., 2020). Our study is consistent with that after 7 days, compared to control groups, extracellular MC concentrations were more than doubled in the CHI treatments compared to the control. There was a strong decline in intracellular MC concentrations which paralleled the strong decrease in Chl a. No changes in the relative composition of the MCs were observed, in all treatments MC-LR remained the dominant MC-congener.

The three copper-based algicides used (SeClear, Captain XTR and CuSO₄·5H₂O) had strong impacts on *M. aeruginosa*. Copper can directly target the cells causing loss of cell membrane integrity, and destroying the photosystems through the production of ROS (Iwinski et al., 2016; Qian et al., 2010; Stevenson et al., 2013). Cationic forms of Cu (Cu²⁺, CuOH⁺, Cu₂(OH)²⁺) are also toxic to non-target aquatic organisms (Closson and Paul, 2014) and are formed rapidly when copper sulphate dissociates in water (Mastin and Rodgers Jr, 2000). To lessen unwanted side effects on non-target organisms and to increase effectiveness against cyanobacteria and algae, chelated copper-based algicides have been

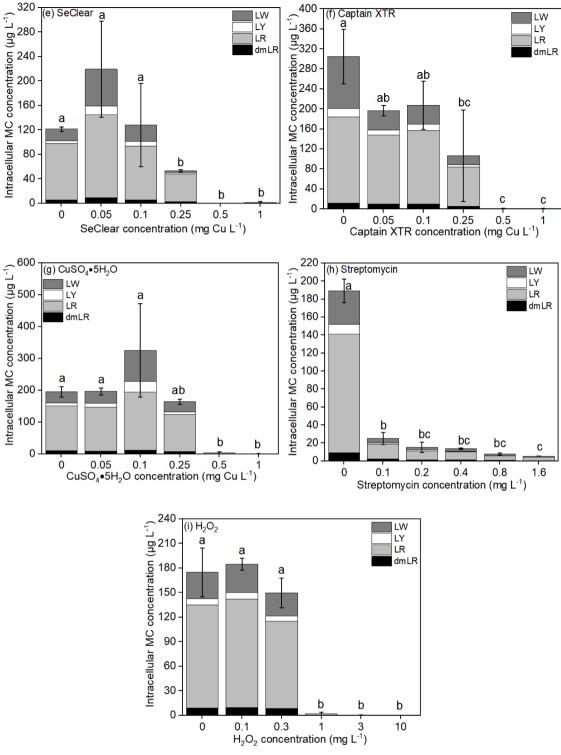


Fig. 5. (continued).

developed in which the chelator facilitates passage through cell membranes causing fast cell lysis (Closson and Paul, 2014; Kang et al., 2022b; Wagner et al., 2017). As such, the chelated copper algicide Cutrine®-Ultra was more toxic than copper sulphate to the cyanobacterium *Planktothrix agardhii* and the green alga *Pseudokirchneriella subcapitata* (Calomeni et al., 2014). In our study, the two chelated copper compounds SeClear and Captain XTR also showed faster and stronger toxicity than CuSO₄·5H₂O (*see* Table 2, EC_{50-2hrs}). Copper-induced cell lysis may also rapidly release cell constituents into the surrounding

medium, as was evidenced by the high filterable Chl a concentrations measured after two hours exposure to SeClear (Table S1). Likewise, copper-induced cell lysis may increase extracellular MC concentrations within 24 hours (Chow et al., 1999), but unexpectedly this was not observed in the SeClear treatments. Inasmuch as elevated extracellular MC concentrations were observed in the Captain XTR and CuSO $_4$ ·5H $_2$ O treatments, potential complexation of MCs with copper (Humble et al., 1997) can be excluded, as can MC breakdown (Lam et al., 1995). SeClear consists of 4.2 % copper combined with a water quality enhancer that

provides it the capacity not only to kill cyanobacteria and algae, but also to remove phosphate (information from SePRO Corporation, Carmel, IN, USA). However, in our study we did not detect a reduction in SRP concentrations; evidently more studies are needed to evaluate the potency of SeClear to lower SRP, but also to decipher if SeClear is capable of adsorbing dissolved MCs.

In the highest doses tested (0.5 and 1.0 mg Cu $\rm L^{-1}$) Chl a concentrations remained low until the end of the experiment, which is comparable to a mesocosm study that revealed strongly reduced Chl a concentrations up to 7 days, but a regrowth after 14 or 21 days (Buley et al., 2021). Here, a reduction in nutrient availability could be a welcome addition to delaying regrowth.

The antibiotic streptomycin (STR) had a growth-inhibiting effect on M. aeruginosa at all concentrations tested that had a tendency of becoming more pronounced over time. SRP remained unaffected ruling out P limitation as to the cause of growth inhibition. Streptomycin binds to the 30 S ribosome subunit in prokaryotes causing inhibition of protein synthesis (Harrass et al., 1985). Growth inhibiting concentrations of STR to M. aeruginosa of 0.28 mg L^{-1} (Harrass et al., 1985), and EC_{50} concentrations of 0.007 mg L^{-1} (Halling-Sørensen, 2000) and 0.034 mg L^{-1} (van der Grinten et al., 2010) have been found that are comparable to the low EC_{50} determined in our study. The detrimental effect of STR is also reflected in strongly reduced intracellular MC concentrations. Exposure to antibiotics may, however, increase MC release (Zhang et al., 2020), which was confirmed in our study where extracellular MC concentrations were clearly elevated in STR treatments.

After 7 days, Chl a concentrations and M. aeruginosa growth were repressed in cultures exposed to H₂O₂ concentrations of 1 mg L⁻¹ and higher (Fig. 1i and Fig. 2i). H2O2 enters cells rapidly causing intracellular damage (Zhou et al., 2018), preventing PSII electron transmission and causing detachment of phycobilisomes (PBS) from the thylakoid membranes (Drábková et al., 2007b). Leakages of pigments into the medium cause a strong increase in F₀ (Drábková et al., 2007a) that is used in the Phyto-PAM to estimate Chl a concentrations (Schreiber, 1998) and which can explain the initially elevated Chl *a* concentrations in the higher H₂O₂ doses. The decline towards the end of the experiment reflects the degradation of released pigments. The cell membrane damage will also lead to the release of MCs (Lürling et al., 2014; Sandrini et al., 2020), but this was not confirmed in our study. The lower extracellular MC concentration in the highest H2O2 dose might be caused by breakdown of MCs by H₂O₂ (Kansole and Lin, 2017), which was also observed in (Lürling et al., 2014). The use of a mostly unicellular strain as in our study may lead to lower effective H₂O₂ concentrations than when M. aeruginosa in its typical colonial form as in the field is used. Huang and Zimba (2020) found lower effective H2O2 concentrations for their laboratory strain of M. aeruginosa than when they treated a natural M. aeruginosa population in mesocosms and in a pond. In controlling natural M. aeruginosa populations a higher dose seems to be needed than when a bloom is comprised of filamentous cyanobacteria (Matthijs et al., 2016), which may point to a protective role of the mucous layer in M. aeruginosa colonies. Also other factors determine efficiency and H2O2 dose needed, such as cyanobacterial biomass (Huang and Zimba, 2020), presence of green algae (Weenink et al., 2021), light intensity (Piel et al., 2020), concentration of dissolved organic matter and reduced compounds (Matthijs et al., 2012). Hence, prior to an application tests with the natural phytoplankton community are needed, for example using enclosures to determine the H2O2 dose needed (Huang and Zimba, 2020; Matthijs et al., 2012).

Our study provides insight into the effects nine chemicals/products commonly used to mitigate cyanobacterial blooms may have on *M. aeruginosa* and MCs. As expected, algicides were most powerful in eliminating cyanobacteria biomass, while P sorbents evoked a milder response. A downside of the algicides is that MCs are liberated, the longevity of the positive effect might be short, and the cause root of the problem, over-enrichment with nutrients, remains untouched. Clearly, reducing nutrient inputs to lakes that suffer from cyanobacterial blooms

is key, yet not always feasible. Hence, combining selected algicides such as H_2O_2 or coagulants such as chitosan with a solid phase P binder might be considered to delay cyanobacteria regrowth and stretch the period of low cyanobacteria abundance (Drummond et al., 2022). A proper diagnosis of the cyanobacterial issue is recommended at each problem site followed by testing the intervention of choice on the natural cyanobacteria.

5. Conclusion

- The reduction of cyanobacteria biomass differed among the nine chemicals tested and could be ranked according to the percentage reduction compared to the controls on the 7th day as: Chitosan (99.8%) > Hydrogen peroxide (99.6%) > Captain XTR (98.2%) > SeClear (98.1%) > CuSO₄·5H₂O (97.8%) > Streptomycin (86.6%) > LMB (42.6%) > AMZ (28.4%) > Al-salts (5.5%).
- Algicides were the most powerful in reducing cyanobacteria biomass, while ballasts evoked a milder response.
- Growth rates were reduced as the chemicals' dosages increased, except for Al-salts.
- MCs are liberated under algicides treatments and the intracellular MC declined with increasing LMB dose.
- Combination of selected algicides such as H₂O₂ that is for lowing the cyanobacteria abundance, with a solid phase P binder that is for reducing nutrients and delaying cyanobacteria regrowth might be considered in the future.

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.

Data Availability

Data will be made available on request.

Acknowledgments

We thank Wendy Beekman and Frits Gillissen from Wageningen University for their assistance. This research was funded by Chinese scholarship council (CSC), grant number 201906050134.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2022.102311.

References

Augustyniak, R., Grochowska, J., Łopata, M., Parszuto, K., Tandyrak, R., Tunowski, J., 2019. Sorption properties of the bottom sediment of a lake restored by phosphorus inactivation method 15 years after the termination of lake restoration procedures. Water 11 (10), 2175.

Bastien, C., Cardin, R., Veilleux, E., Deblois, C., Warren, A., Laurion, I., 2011.Performance evaluation of phycocyanin probes for the monitoring of cyanobacteria.Journal of Environmental Monitoring 13 (1), 110–118.

Buley, R.P., Adams, C., Belfiore, A.P., Fernandez-Figueroa, E.G., Gladfelter, M.F., Garner, B., Wilson, A.E., 2021. Field evaluation of seven products to control cyanobacterial blooms in aquaculture. Environ Sci Pollut Res Int.

Calomeni, A., Rodgers, J.H., Kinley, C.M., 2014. Responses of Planktothrix agardhii and Pseudokirchneriella subcapitata to copper sulfate (CuSO4· 5H2O) and a chelated copper compound (Cutrine®-Ultra). Water, Air, & Soil Pollution 225 (12), 1–15.

Chow, C.W., Drikas, M., House, J., Burch, M.D., Velzeboer, R.M., 1999. The impact of conventional water treatment processes on cells of the cyanobacterium Microcystis aeruginosa. Water Research 33 (15), 3253–3262.

Closson, K., Paul, E., 2014. Comparison of the toxicity of two chelated copper algaecides and copper sulfate to non-target fish. Bulletin of environmental contamination and toxicology 93 (6), 660–665.

- Coloma, S.E., Dienstbier, A., Bamford, D.H., Sivonen, K., Roine, E., Hiltunen, T., 2017.Newly isolated Nodularia phage influences cyanobacterial community dynamics.Environmental Microbiology 19 (1), 273–286.
- Cooke, G.D., Welch, E.B., Peterson, S., Nichols, S.A., 2005. Restoration and management of lakes and reservoirs. CRC press.
- Copetti, D., Finsterle, K., Marziali, L., Stefani, F., Tartari, G., Douglas, G., Reitzel, K., Spears, B.M., Winfield, I.J., Crosa, G., D'Haese, P., Yasseri, S., Lürling, M., 2016. Eutrophication management in surface waters using lanthanum modified bentonite: A review. Water Research 97, 162–174.
- Douglas, G.B., Lurling, M., Spears, B.M., 2016. Assessment of changes in potential nutrient limitation in an impounded river after application of lanthanum-modified bentonite. Water Res 97, 47–54.
- Drábková, M., Admiraal, W., Maršálek, B., 2007a. Combined exposure to hydrogen peroxide and light selective effects on cyanobacteria, green algae, and diatoms. Environmental science & technology 41 (1), 309–314.
- Drábková, M., Matthijs, H., Admiraal, W., Maršálek, B., 2007b. Selective effects of H 2 O 2 on cyanobacterial photosynthesis. Photosynthetica 45 (3), 363–369.
- Drummond, E., Leite, V.B.G., Noyma, N.P., de Magalhães, L., Graco-Roza, C., Huszar, V. L., Lürling, M., Marinho, M.M., 2022. Temporal and spatial variation in the efficiency of a Floc & Sink technique for controlling cyanobacterial blooms in a tropical reservoir. Harmful Algae 117, 102262.
- Edwards, C., Graham, D., Fowler, N., Lawton, L.A., 2008. Biodegradation of microcystins and nodularin in freshwaters. Chemosphere 73 (8), 1315–1321.
- Fang, C., Song, K., Paerl, H.W., Jacinthe, P.-A., Wen, Z., Liu, G., Tao, H., Xu, X., Kutser, T., Wang, Z., Duan, H., Shi, K., Shang, Y., Lyu, L., Li, S., Yang, Q., Lyu, D., Mao, D., Zhang, B., Cheng, S., Lyu, Y., 2022. Global divergent trends of algal blooms detected by satellite during 1982–2018. Global Change Biology 28 (7), 2327–2340.
- Fawaz, E.G., Salam, D.A., Kamareddine, L., 2018. Evaluation of copper toxicity using site specific algae and water chemistry: Field validation of laboratory bioassays. Ecotoxicology and Environmental Safety 155, 59–65.
- Fischer, A., Höger, S.J., Stemmer, K., Feurstein, D., Knobeloch, D., Nussler, A., Dietrich, D.R., 2010. The role of organic anion transporting polypeptides (OATPs/ SLCOs) in the toxicity of different microcystin congeners in vitro: A comparison of primary human hepatocytes and OATP-transfected HEK293 cells. Toxicology and applied pharmacology 245 (1), 9–20.
- Georgantas, D.A., Grigoropoulou, H.P., 2007. Orthophosphate and metaphosphate ion removal from aqueous solution using alum and aluminum hydroxide. J Colloid Interface Sci 315 (1), 70–79.
- Gibbs, M.M., Hickey, C.W., 2018. Flocculants and Sediment Capping for Phosphorus Management, Lake Restoration Handbook. Springer, pp. 207–265.
- Gojkovic, Z., Lindberg, R.H., Tysklind, M., Funk, C., 2019. Northern green algae have the capacity to remove active pharmaceutical ingredients. Ecotoxicology and Environmental Safety 170, 644–656.
- Guo, L., 2007. Ecology. Doing battle with the green monster of Taihu Lake. Science 317 (5842), 1166.
- Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. Chemosphere 40 (7), 731–739.
- Hamilton, D.P., Salmaso, N., Paerl, H.W., 2016. Mitigating harmful cyanobacterial blooms: strategies for control of nitrogen and phosphorus loads. Aquatic Ecology 50 (3), 351–366.
- Hamilton, D.P., Wood, S.A., Dietrich, D.R., Puddick, J., 2014. Costs of harmful blooms of freshwater cyanobacteria. Cyanobacteria: an Economic Perspective. John Wiley & Sons, New York, pp. 247–256.
- Han, J., Jeon, B.-S., Park, H.-D., 2016. Microcystin release and Microcystis cell damage mechanism by alum treatment with long-term and large dose as in-lake treatment. Journal of Environmental Science and Health, Part A 51 (6), 455–462.
- Harrass, M., Kindig, A., Taub, F., 1985. Responses of blue-green and green algae to streptomycin in unialgal and paired culture. Aquatic Toxicology 6 (1), 1–11.
- Huang, I.S., Zimba, P.V., 2020. Hydrogen peroxide, an ecofriendly remediation method for controlling Microcystis aeruginosa toxic blooms. Journal of Applied Phycology 32 (5), 3133–3142.
- Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat Rev Microbiol 16 (8), 471–483.
- Humble, A.V., Gadd, G.M., Codd, G.A., 1997. Binding of copper and zinc to three cyanobacterial microcystins quantified by differential pulse polarography. Water Research 31 (7), 1679–1686.
- Iwinski, K.J., Calomeni, A.J., Geer, T.D., Rodgers Jr., J.H., 2016. Cellular and aqueous microcystin-LR following laboratory exposures of Microcystis aeruginosa to copper algaecides. Chemosphere 147, 74–81.
- Jančula, D., Maršálek, B., 2011. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. Chemosphere 85 (9), 1415–1422.
- Jones, G.J., Orr, P.T., 1994. Release and degradation of microcystin following algicide treatment of a Microcystis aeruginosa bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. Water Research 28 (4), 871–876.
- Kang, L., Mucci, M., Fang, J., Lürling, M., 2022a. New is not always better: Toxicity of novel copper based algaecides to Daphnia magna. Ecotoxicology and Environmental Safety 241, 113817.
- Kang, L., Mucci, M., Lürling, M., 2022b. Influence of temperature and pH on phosphate removal efficiency of different sorbents used in lake restoration. Science of The Total Environment 812, 151489.
- Kansole, M.M., Lin, T.-F., 2017. Impacts of Hydrogen Peroxide and Copper Sulfate on the Control of Microcystis aeruginosa and MC-LR and the Inhibition of MC-LR Degrading Bacterium Bacillus sp. Water 9 (4), 255.

Kenefick, S., Hrudey, S., Peterson, H., Prepas, E., 1993. Toxin release from Microcystis aeruginosa after chemical treatment. Water Science and Technology 27 (3-4), 433–440.

- Kibuye, F.A., Zamyadi, A., Wert, E.C., 2021. A critical review on operation and performance of source water control strategies for cyanobacterial blooms: Part Ichemical control methods. Harmful Algae 109, 102099.
- Krüger, T., Hölzel, N., Luckas, B., 2012. Influence of cultivation parameters on growth and microcystin production of Microcystis aeruginosa (Cyanophyceae) isolated from Lake Chao (China). Microbial ecology 63 (1), 199–209.
- Lam, A.K.Y., Prepas, E.E., Spink, D., Hrudey, S.E., 1995. Chemical control of hepatotoxic phytoplankton blooms: Implications for human health. Water Research 29 (8), 1845–1854.
- Laughinghouse, H., Lefler, F.W., Berthold, D.E., Bishop, W.M., 2020. Sorption of dissolved microcystin using lanthanum-modified bentonite clay. J. Aquat. Plant Manag 58, 72–75.
- Li, H.-y., Ye, Y.-d., Zhang, Q.-j., Du, C.-h., Li, H.-m., Yin, L., 2022. Effects of Cinnamomum camphora Leaves Extracts-Flocculants Composite Algaecide on Microcystis aeruginosa Growth and Microcystins Release. Bulletin of Environmental Contamination and Toxicology.
- Lurling, M., Beekman, W., 2006. Palmelloids formation in Chlamydomonas reinhardtii: defence against rotifer predators? Ann. Limnol. - Int. J. Lim. 42 (2), 65–72.
- Lürling, M., Faassen, E.J., 2013. Dog poisonings associated with a Microcystis aeruginosa bloom in the Netherlands. Toxins 5 (3), 556–567.
- Lürling, M., Kang, L., Mucci, M., van Oosterhout, F., Noyma, N.P., Miranda, M., Huszar, V.L.M., Waajen, G., Marinho, M.M., 2020. Coagulation and precipitation of cyanobacterial blooms. Ecological Engineering 158, 106032.
- Lürling, M., Meng, D., Faassen, E.J., 2014. Effects of hydrogen peroxide and ultrasound on biomass reduction and toxin release in the cyanobacterium, Microcystis aeruginosa. Toxins 6 (12), 3260–3280.
- Lürling, M., Mucci, M., 2020. Mitigating eutrophication nuisance: in-lake measures are becoming inevitable in eutrophic waters in the Netherlands. Hydrobiologia 847 (21), 4447–4467.
- Mastin, B., Rodgers Jr., J., 2000. Toxicity and bioavailability of copper herbicides (Clearigate, Cutrine-Plus, and copper sulfate) to freshwater animals. Archives of environmental contamination and toxicology 39 (4), 445–451.
- Matthijs, H.C., Visser, P.M., Reeze, B., Meeuse, J., Slot, P.C., Wijn, G., Talens, R., Huisman, J., 2012. Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. Water Res 46 (5), 1460–1472.
- Matthijs, H.C.P., Jančula, D., Visser, P.M., Maršálek, B., 2016. Existing and emerging cyanocidal compounds: new perspectives for cyanobacterial bloom mitigation. Aquatic Ecology 50 (3), 443–460.
- Mucci, M., Guedes, I.A., Faassen, E.J., Lürling, M., 2020. Chitosan as a Coagulant to Remove Cyanobacteria Can Cause Microcystin Release. Toxins 12 (11), 711.
- Mucci, M., Noyma, N.P., de Magalhaes, L., Miranda, M., van Oosterhout, F., Guedes, I.A., Huszar, V.L.M., Marinho, M.M., Lurling, M., 2017. Chitosan as coagulant on cyanobacteria in lake restoration management may cause rapid cell lysis. Water Res 118, 121–130.
- Muff, K., Delacoste, C., Dyllick, T., 2022. Responsible Leadership Competencies in leaders around the world: Assessing stakeholder engagement, ethics and values, systems thinking and innovation competencies in leaders around the world. Corporate Social Responsibility and Environmental Management 29 (1), 273–292.
- Natugonza, V., Musinguzi, L., Kishe, M.A., Rijssel, J.C.v., Seehausen, O., Ogutu-Ohwayo, R., 2021. The consequences of anthropogenic stressors on cichlid fish communities: revisiting Lakes Victoria, Kyoga, and Nabugabo, The behavior, ecology and evolution of cichlid fishes. Springer, pp. 217–246.
- Noyma, N.P., de Magalhães, L., Furtado, L.L., Mucci, M., van Oosterhout, F., Huszar, V.L. M., Marinho, M.M., Lürling, M., 2016. Controlling cyanobacterial blooms through effective flocculation and sedimentation with combined use of flocculants and phosphorus adsorbing natural soil and modified clay. Water Research 97, 26–38.
- Noyma, N.P., De Magalhāes, L., Miranda, M., Mucci, M., van Oosterhout, F., Huszar, V.L., Marinho, M.M., Lima, E.R., Lürling, M., 2017. Coagulant plus ballast technique provides a rapid mitigation of cyanobacterial nuisance. PLoS One 12 (6), e0178976.

OECD, 2014. Water Governance in the Netherlands.

OECD, 2017. Diffuse Pollution, Degraded Waters.

- Paerl, H.W., Gardner, W.S., Havens, K.E., Joyner, A.R., McCarthy, M.J., Newell, S.E., Qin, B.Q., Scott, J.T., 2016. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae 54, 213–222.
- Pan, G., Chen, J., Anderson, D.M., 2011. Modified local sands for the mitigation of harmful algal blooms. Harmful algae 10 (4), 381–387.
- Pan, G., Zou, H., Chen, H., Yuan, X., 2006. Removal of harmful cyanobacterial blooms in Taihu Lake using local soils III. Factors affecting the removal efficiency and an in situ field experiment using chitosan-modified local soils. Environmental Pollution 141 (2), 206–212.
- Piel, T., Sandrini, G., White, E., Xu, T., Schuurmans, J.M., Huisman, J., Visser, P.M., 2020. Suppressing Cyanobacteria with Hydrogen Peroxide Is More Effective at High Light Intensities. Toxins 12 (1), 18.
- Qian, H., Li, J., Pan, X., Sun, Z., Ye, C., Jin, G., Fu, Z., 2012. Effects of streptomycin on growth of algae Chlorella vulgaris and Microcystis aeruginosa. Environ Toxicol 27 (4), 229–237.
- Qian, H., Yu, S., Sun, Z., Xie, X., Liu, W., Fu, Z., 2010. Effects of copper sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine on oxidative stress and the expression of genes involved photosynthesis and microcystin disposition in Microcystis aeruginosa. Aquatic Toxicology 99 (3), 405–412.
- Ryding, S.-O., Forsberg, C., 1976. Six polluted lakes: a preliminary evaluation of the treatment and recovery processes. Ambio 151–156.

- Sandrini, G., Piel, T., Xu, T., White, E., Qin, H., Slot, P.C., Huisman, J., Visser, P.M., 2020.
 Sensitivity to hydrogen peroxide of the bloom-forming cyanobacterium Microcystis PCC 7806 depends on nutrient availability. Harmful Algae 99, 101916.
- Schreiber, U., 1998. Chlorophyll fluorescence: new instruments for special applications, Photosynthesis: mechanisms and effects. Springer, pp. 4253–4258.
- Spears, B.M., Lürling, M., Yasseri, S., Castro-Castellon, A.T., Gibbs, M., Meis, S., McDonald, C., McIntosh, J., Sleep, D., Van Oosterhout, F., 2013. Lake responses following lanthanum-modified bentonite clay (Phoslock®) application: an analysis of water column lanthanum data from 16 case study lakes. water research 47 (15), 5930–5942.
- Stevenson, J., Barwinska-Sendra, A., Tarrant, E., Waldron, K., 2013. Mechanism of action and applications of the antimicrobial properties of copper. Microbial pathogens and strategies for combating them: science, technology and education 2, 468–479.
- van der Grinten, E., Pikkemaat, M.G., van den Brandhof, E.-J., Stroomberg, G.J., Kraak, M.H.S., 2010. Comparing the sensitivity of algal, cyanobacterial and bacterial bioassays to different groups of antibiotics. Chemosphere 80 (1), 1–6.
- van Loosdrecht, M.C., Brdjanovic, D., 2014. Anticipating the next century of wastewater treatment. Science 344 (6191), 1452–1453.
- Van Oosterhout, F., Lürling, M., 2013. The effect of phosphorus binding clay (Phoslock®) in mitigating cyanobacterial nuisance: a laboratory study on the effects on water quality variables and plankton. Hydrobiologia 710 (1), 265–277.
- Vesterkvist, P.S., Misiorek, J.O., Spoof, L.E., Toivola, D.M., Meriluoto, J.A., 2012. Comparative cellular toxicity of hydrophilic and hydrophobic microcystins on Caco-2 cells. Toxins 4 (11), 1008–1023.

- Viriyatum, R., Boyd, C.E., 2016. Slow-release Coated Copper Sulfate as an Algicide for Aquaculture. Journal of the World Aquaculture Society 47 (5), 667–675.
- Wagner, J.L., Townsend, A.K., Velzis, A.E., Paul, E.A., 2017. Temperature and toxicity of the copper herbicide (NautiqueTM) to freshwater fish in field and laboratory trials. Cogent Environmental Science 3 (1), 1339386.
- Weenink, E.F., Matthijs, H.C., Schuurmans, J.M., Piel, T., van Herk, M.J., Sigon, C.A., Visser, P.M., Huisman, J., 2021. Interspecific protection against oxidative stress: green algae protect harmful cyanobacteria against hydrogen peroxide. Environmental Microbiology 23 (5), 2404–2419.
- Wiedner, C., Visser, P.M., Fastner, J., Metcalf, J.S., Codd, G.A., Mur, L.R., 2003. Effects of light on the microcystin content of Microcystis strain PCC 7806. Applied and environmental microbiology 69 (3), 1475–1481.
- Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. Wiley Interdisciplinary Reviews: Water 6 (5), e1373.
- WWAP, 2017. The United Nations world water development report, 2017. Wastewater: the untapped resource. UNESCO.
- Zhang, M., Steinman, A.D., Xue, Q., Zhao, Y., Xu, Y., Xie, L., 2020. Effects of erythromycin and sulfamethoxazole on Microcystis aeruginosa: Cytotoxic endpoints, production and release of microcystin-LR. Journal of Hazardous Materials 399, 123021.
- Zhou, T., Zheng, J., Cao, H., Wang, X., Lou, K., Zhang, X., Tao, Y., 2018. Growth suppression and apoptosis-like cell death in Microcystis aeruginosa by H2O2: A new insight into extracellular and intracellular damage pathways. Chemosphere 211, 1098–1108.