Harmful Algae 65 (2017) 27–39

Contents lists available at ScienceDirect

Harmful Algae

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

The efficiency of combined coagulant and ballast to remove harmful cyanobacterial blooms in a tropical shallow system

Marcela Miranda[a,*], Natália Noyma[b], Felipe S. Pacheco[c], Leonardo de Magalhães[b], Ernani Pinto[d], Suzan Santos[b], Maria Fernanda A. Soares[a], Vera L. Huszar[e], Miquel Lürling[f,g], Marcelo M. Marinho[b]

[a] Post-Graduate Program in Ecology, Federal University of Juiz de Fora, R. José Lourenço Kelmer, Juiz de Fora, MG 36036-330, Brazil
[b] Laboratory of Ecology and Physiology of Phytoplankton, Department of Plant Biology, University of Rio de Janeiro State, Rua São Francisco Xavier 524 – PHLC Sala 511a, 20550-900 Rio de Janeiro, Brazil
[c] Earth System Science Center (CCST), National Institute for Space Research (INPE), Av. dos Astronautas, 1.758 – Jardim da Granja, 12227-010 São José dos Campos, SP, Brazil
[d] Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580 – Bl 17, 05508-900 São Paulo, SP, Brazil
[e] Laboratory of Phycology, National Museum, Federal University of Rio de Janeiro 20940-040, Rio de Janeiro, Brazil
[f] Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands
[g] Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 26 November 2016
Received in revised form 14 March 2017
Accepted 10 April 2017
Available online 17 April 2017

Key-words:
Eutrophication control
Cyanobacteria mitigation
Microcystis
Cylindrospermopsis
Chitosan

ABSTRACT

We tested the hypothesis that a combination of coagulant and ballast could be efficient for removal of positively buoyant harmful cyanobacteria in shallow tropical waterbodies, and will not promote the release of cyanotoxins. This laboratory study examined the efficacy of coagulants [polyaluminium chloride (PAC) and chitosan (made of shrimp shells)] alone, and combined with ballast (lanthanum modified bentonite, red soil or gravel) to remove the natural populations of cyanobacteria collected from a shallow eutrophic urban reservoir with alternating blooms of *Cylindrospermopsis* and *Microcystis*. PAC combined with ballast was effective in settling blooms dominated by *Microcystis* or *Cylindrospermopsis*. Contrary to our expectation, chitosan combined with ballast was only effective in settling *Cylindrospermopsis*-dominated blooms at low pH, whereas at pH $\geq$ 8 no effective flocculation and settling could be evoked. Chitosan also had a detrimental effect on *Cylindrospermopsis* causing the release of saxitoxins. In contrast, no detrimental effect on *Microcystis* was observed and all coagulant-ballast treatments were effective in not only settling the *Microcystis* dominated bloom, but also lowering dissolved microcystin concentrations. Our data show that the best procedure for biomass reduction also depends on the dominant species.

1. Introduction

Controlling eutrophication and mitigating cyanobacteria blooms is considered a great challenge to current limnology (Smith and Schindler, 2009). The frequency, occurrence, and duration of cyanobacterial dominance is predicted to further increase because of anthropogenic eutrophication and global climate change (Paerl and Huisman, 2008, 2009; Moss et al., 2011; Pacheco et al., 2013; Paerl et al., 2014).

Blooms of potentially toxic cyanobacteria pose serious threats to the environment and public health (Chorus et al., 2000). They may generate economic damage (Steffensen, 2008; Dodds et al., 2009) because of the impossibility of using lakes, ponds and reservoirs for several purposes: as sources of drinking water, agricultural irrigation, fishing, industry water and recreation (Carmichael, 2001; Codd et al., 2005; Smith and Schindler, 2009). Thus, controlling eutrophication and mitigating nuisance cyanobacteria is an essential task (Lürling et al., 2016). Reducing the inflow of nutrients (N and P) to surface waters is a straightforward mitigation measure; however, many studies have shown that such external load reduction is not a guarantee for fast recovery as the internal loading of nutrients can delay recovery for

* Corresponding author.
E-mail address: marcela.miranda.ufjf@gmail.com (M. Miranda).

http://dx.doi.org/10.1016/j.hal.2017.04.007
1568-9883/

many years (Marsden, 1989; Jeppesen et al., 1991; Van der Molen and Boers, 1994; Søndergaard et al., 2003). Hence, in-lake measures to speed up recovery will be needed and will follow from a proper diagnosis of the water system (Lürling et al., 2016). These measures should preferably tackle both the cyanobacterial bloom and mitigate the internal loading.

Removal of cyanobacteria from the water column using a combination of coagulant and ballast is a promising technique for controlling cyanobacterial blooms (Pan et al., 2006a,b, 2011a; Noyma et al., 2016; de Magalhães et al., 2017) and can be used both in water bodies that suffer predominantly from internal loading as well as in a more curative manner in those that have ongoing external nutrient inputs (Lürling et al., 2016). An isolated bay in Lake Taihu was effectively cleared of cyanobacteria by using coagulant modified soil (Pan et al., 2011a), whereas two stratifying lakes were cleared of cyanobacteria using a coagulant and a lanthanum-modified bentonite clay (LMB) (Lürling and van Oosterhout, 2013; Waajen et al., 2016a). In this technique, cyanobacteria in the water column are flocked and the aggregates of intact cells/colonies are settled to the sediment with entrapped ballast. Polyaluminium chloride (PAC), iron chloride, *Moringa oleifera* extract or chitosan have been used as coagulants (Pan et al., 2011a,b; Li and Pan, 2013; Lürling and van Oosterhout, 2013; Waajen et al., 2016a). Polyaluminium chloride is a metallic compound, which has a higher efficiency of turbidity reduction under a wider pH range at a lower coagulant cost than other compounds (Jiang and Graham, 1998). Chitosan (CHI) is a biopolymer derived from marine shrimps and crabs. It is widely used in water and wastewater treatment and is viewed as a non-toxic, biodegradable compound with good coagulation/flocculation properties (Renault et al., 2009). As ballast, natural soils and clays are commonly used (Pan et al., 2006a,b, 2011a,b). A lanthanum-modified bentonite Phoslock® has also been tested as ballast and solid-phase Phosphorus (P) sorbent (Lürling and van Oosterhout, 2013; Copetti et al., 2016; Noyma et al., 2016; de Magalhães et al., 2017). The LMB has an advantage over soils as it can strongly adsorb P even under anoxia (Noyma et al., 2016) and thus hamper the sediment P release (Waajen et al., 2016a,b).

Most of the above mentioned flock and sink trials have been conducted with blooms of *Microcystis aeruginosa* (Pan et al., 2006a; Zou et al., 2006; Noyma et al., 2016; de Magalhães et al., 2017). *M. aeruginosa* is one of the most frequent bloom-forming cyanobacteria in freshwater waterbodies throughout the world (Harke et al., 2016). Likewise, it is one of the most common bloom-forming cyanobacteria species in Brazil, where *Cylindrospermopsis raciborskii* is also a common bloom-forming species (Soares et al., 2013). Particularly, *C. raciborskii* has shown considerable proliferation in (sub)tropical aquatic systems (Bonilla et al., 2012; Antunes et al., 2015). Up to date, no studies have examined the potential of combined coagulant and ballast to remove this nuisance species from the water.

The main reason why the above mentioned cyanobacteria are viewed as a nuisance and health risk is because they are capable of producing potent toxins. *Microcystis* is a well-known producer of microcystins (Sivonen and Jones, 1999; Harke et al., 2016), while Brazilian *C. raciborskii* strains may produce saxitoxins (Hoff-Risseti et al., 2013). It is straightforward that lowering the cyanobacterial biomass will reduce the particulate (or intracellular) toxin concentrations, because most cyanobacterial toxins are largely contained within the cyanobacterial cells. Care should, however, be taken that the intervention does not damage the cyanobacterial cells; lysis or damage could release intracellular toxins into the water (Lam et al., 1995; Steffensen et al., 1999). The release of cyanotoxins may be a serious threat to the health of humans and animals (Carmichael, 2001; Dittmann and Wiegand, 2006), and several problems caused by contact with, or consuming, water with containing cyanotoxins have been reported (Falconer, 1999; Azevedo et al., 2002; Stewart et al., 2006). For instance, toxic liver injury was found in consumers of drinking water from a reservoir in Armidale (Australia), where a *Microcystis* bloom was terminated with copper sulphate (Falconer et al., 1983). Likewise, an outbreak of hepatoenteritis occurred at Palm Island (Australia) after a *Cylindrospermopsis raciborskii* bloom was treated with copper sulphate (Hawkins et al., 1985). Hence, controlling cyanobacterial blooms not only requires strong reduction of the cyanobacterial biomass, but also of their toxins (Greenfield et al., 2014). Based on promising experimental results with natural populations of *Microcystis* from a deep freshwater reservoir (Noyma et al., 2016) and a brackish lagoon (de Magalhães et al., 2017), we hypothesized that each combination of coagulant and ballast is efficient in removing harmful cyanobacteria from water collected in a shallow tropical lake. Furthermore, although the combined coagulant and ballast technique has been applied in many studies, virtually no information exists about its effect on liberating cyanotoxins from the cells. Therefore, in this study we included an analysis of major dissolved cyanotoxins – saxitoxins in the case of *C. raciborskii* and microcystins in case of *Microcystis* – testing the hypothesis that combined coagulant and ballast, and the single components in the mixtures, will not promote the release of cyanotoxins.

2. Materials and methods

2.1. Study site

Natural cyanobacterial bloom material was collected from a small artificial pond that was created for an esthetic purpose at the Park of Mariano Procópio Museum (MAPRO), located in the urban area of the municipality of Juiz de Fora, Minas Gerais State, Brazil (21°44′S, 43°21′W, altitude 648 m). The climate conditions are wet-warm summer and dry-mild cold winter (Cwa in the Köppen's climate classification system, Alvares et al., 2014). This shallow system of 1.1 ha (maximum depth of 1.2 m), with the occurrence of cyanobacteria blooms (Fig. 1), has experienced an intense process of eutrophication in the last four years. The water source is an artesian well and rainwater, and its excess drains into the Paraibuna River, the main waterway of the city. The lake can be considered hypereutrophic, with high average values of chlorophyll-*a* ($279.4 \pm 162.8\ \mu g\ L^{-1}$), total phosphorus ($0.26 \pm 0.07$ mg L^{-1}), and total nitrogen (2.0 ± 0.7 mg L^{-1}). Its pH values range from 7.0 to 9.7 (data from two years of system monitoring).

Samples for the experiments were collected from the lake between April 2015 and March 2016, during bloom events of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju (>80% of total biomass) and *Microcystis* spp. (>95% of total biomass).

2.2. Chemicals and materials

As coagulants, we used PAC-AP (polyaluminium chloride; $Al_n(OH)_mCl_{(3n-m)}$, $\rho \approx 1.30–1.40\ kg\ dm^{-3}$, 8.9% Al, 21.0% Cl) obtained from Pan-Americana (Rio de Janeiro, Brazil), and chitosan (made of shrimp shells) obtained from Polymar Ciência e Nutrição S/A (Ceará, Brazil). Chitosan was acidified with 1% hydrochloric acid solution prior to use and diluted to a stock of $1\ g\ L^{-1}$. Three types of ballasts were tested in the experiments: i) red soil (RS), described by Noyma et al. (2016); ii) gravel (GRA) – bought in a construction store and commonly available under the regional name of "saibro roxo"; iii) lanthanum-modified bentonite Phoslock® (LMB) obtained from HydroScience (Porto Alegre, Brazil). This LMB was developed by the Australian CSIRO as a dephosphatisation technique aimed at removing dissolved phosphorus – from the

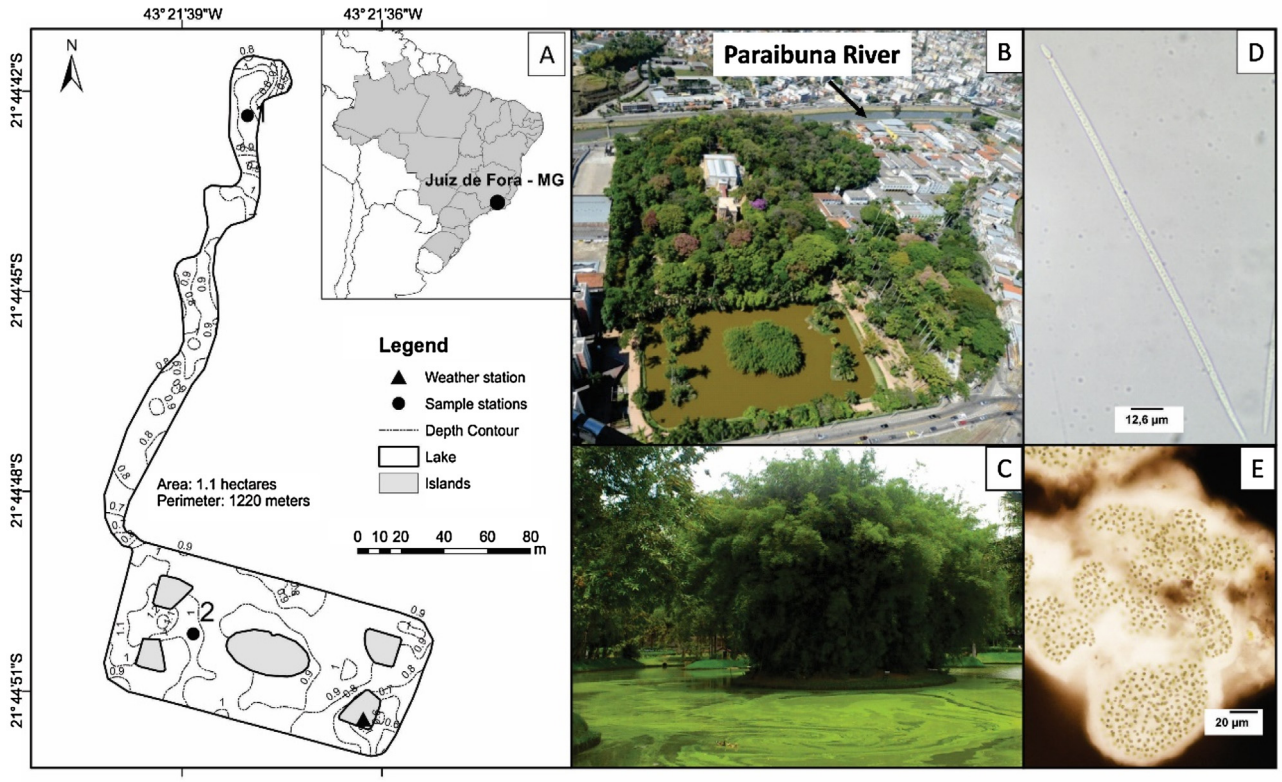

Fig. 1. Map and location of Lake Museum Mariano Procópio (A); aerial view of Park Mariano Procópio (MAPRO) Museum, located in the urban area of the municipality of Juiz de Fora, MG, Brazil and Paraibuna River (B); bloom of cyanobacteria in the MAPRO Lake (February 2016) (C); the main phytoplankton species (*C. raciborskii* and *M. aeruginosa* colonies) (D and E, respectively).

water and blocking the release of phosphorus (P) from the sediment (Douglas, 2002).

2.3. Experiments

The same procedures were used in all experiments, varying only in the type and concentration of coagulants and ballasts. Aliquots of 60 mL of cyanobacteria suspensions from the MAPRO Lake were transferred to 75 mL glass tubes (25 × 200 mm). The initial cyanobacterial chlorophyll-*a* ($\mu g\,L^{-1}$), as well as the Photosystem II efficiency (PSII), was determined using a PHYTO-PAM phytoplankton analyser (HeinzWalz GmbH, Effeltrich, Germany). The designated compound(s) were applied to the suspensions (treatment) or left untreated (controls), mixed and placed in the laboratory at 25 °C under stagnant conditions. After one or two hours, 5 mL samples were taken from both the top and the bottom of the tubes in which the chlorophyll-*a* concentrations and PSII efficiencies were measured. The pH was measured directly in the tubes after the samples were taken (Fig. 2). Experiments 1 and 2 have no replicates because they followed a regression design aimed at acquiring insights into the most appropriate coagulant dose (Experiment 1) and ballast dose (Experiment 2) to be used in Experiment 3, which was run in triplicate.

2.3.1. Experiment 1 – coagulants range

The effect was tested of different concentrations of the coagulants (PAC and CHI) on the cyanobacteria suspensions. The PAC was dosed at 0, 1, 2, 4, 8, 16 and 32 mg Al L^{-1} and the CHI at 0, 1, 2, 4, 8, 16 and 32 mg L^{-1}. Immediately after dosing, the contents in each test tube were mixed briefly using a glass rod. Tubes were left untouched for one hour after which they were sampled as described above. The initial chlorophyll-*a* of the experiment with dominance of *Cylindrospermopsis* and *Microcystis* was $334 \pm 10\ \mu g\ L^{-1}$ and $172 \pm 10\ \mu g\ L^{-1}$, respectively. The initial pH values were 7.4 ± 0.2 and 9.6 ± 0.3 respectively.

2.3.2. Experiment 2 – coagulant dose fixed and ballast range

Considering the results of Experiment 1, 4 mg Al L^{-1} of PAC and 2 mg L^{-1} of CHI were selected as working doses. The criteria used to choose the PAC dose were based on flock formation and the effects on pH and PSII efficiencies. Chitosan affected the *Cylindrospermopsis* photosystem II at all doses; a dose of 2 mg L^{-1} was selected, which is the same as has been used before by our group (Noyma et al., 2016). These fixed doses of coagulants were tested in combination with a range of RS, GRA and LMB concentrations (0, 50, 100, 200, and 400 mg L^{-1}). This range was selected based on previous studies (Lürling and van Oosterhout, 2013; Spears et al., 2013; Noyma et al., 2016). Immediately after dosing, the contents in each test tube were mixed briefly using a glass rod. The tubes were left untouched for one hour, after which they were sampled as described above. The initial chlorophyll-*a* of the experiment with dominance of *Cylindrospermopsis* and *Microcystis* was $465 \pm 10\ \mu g\ L^{-1}$ and $172 \pm 10\ \mu g\ L^{-1}$, respectively. The initial pH values were 7.9 ± 0.4 and 9.6 ± 0.3 respectively.

2.3.3. Experiment 3 – flock & sink

In the third experiment, the effects of coagulants and ballast, alone and combined, on the cyanobacterial suspensions were evaluated. The treatments were: Control, PAC, CHI, RS, GRA, LMB, PAC + RS, PAC + GRA, PAC + LMB, CHI + RS, CHI + GRA, and CHI + LMB. For each treatment, we used a fixed dose of coagulant (4 mg Al L^{-1} of PAC and 2 mg L^{-1} of CHI, based on the results of Experiment 1, as explained before) and a fixed dose of ballast determined from the results of Experiment 2 (100 mg L^{-1} of the RS, GRA and LMB). The initial chlorophyll-*a* of the experiment with dominance of *Cylindrospermopsis* and *Microcystis* was $352 \pm 3\ \mu g\ L^{-1}$ and

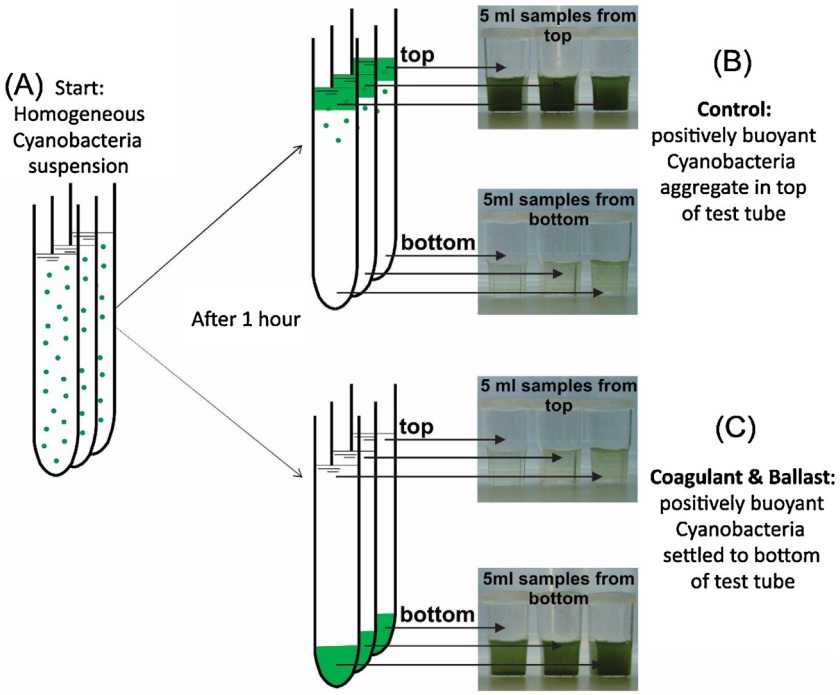

Fig. 2. Schematic design of experiments with coagulants & ballast: (A) test tubes before the beginning of the experiments with homogeneous cyanobacteria suspension; (B) test tubes with untreated water (controls) – positively buoyant cyanobacteria aggregate in the top of test tube; (C) test tubes with treated water (addition of coagulant and ballast) – cyanobacteria cell's settled to the bottom. After the incubation period (1 h) 5 mL samples from the top and bottom of each test tubes are transferred to small flasks for analysis of chlorophyll and PSII efficiency. After sampling, pH was measured inside the tubes.

382 ± 11 μg L^{-1}, respectively. The pH values were 8.11 ± 0.02 and 6.80 ± 0.01, respectively. Treatments were run in triplicate. Immediately after dosing, the contents in each test tube were mixed briefly using a glass rod. The tubes were left untouched for two hours, after which they were sampled as outlined above. Furthermore, we filtered 20 mL of water from each tube through a glass fiber membrane (GF-3, Macherey-Nagel) for the analyses of dissolved toxins (10 mL). Quantitative analyses of saxitoxins and microcystins were performed by direct competitive Enzyme-Linked Immuno Sorbent Assay (ELISA) based on polyclonal antibodies (Beacon Analytical Systems Inc., USA).

Chlorophyll-*a* concentrations and PSII efficiencies in the top and bottom of the tubes as well as pH values in the tubes, were each compared statistically running separate one-way ANOVAs in the statistical tool-pack JMP® 10.0, with treatments as the fixed factor. Differences between means were distinguished by Tukey's *post hoc* comparison test ($p = 0.05$).

3. Results

3.1. Experiment 1 – coagulants range

When exposed to PAC or CHI, clear differences appeared between the series with water dominated by *C. raciborskii* or by *Microcystis* (Figs. 3 and 4). In general, when *C. raciborskii* became flocked they settled, while *Microcystis* floated.

3.1.1. Water dominated by C. raciborskii exposed to PAC

In the PAC series with dominance of *C. raciborskii*, chlorophyll-*a* concentrations in the top 5 mL of the test tubes decreased with the increasing PAC dose; while, in the bottom 5 mL of the test tubes, chlorophyll-*a* concentrations increased with the increasing PAC dose (Fig. 3A and B). The PSII efficiency was unaffected up to 8 mg Al L^{-1} in the top (Fig. 3A) and 4 mg Al L^{-1} in the bottom (Fig. 3B) but it decreased sharply at a higher PAC dose. The pH was strongly affected only at PAC doses ≥ 16 mg Al L^{-1} (Fig. 3A).

3.1.2. Water dominated by Microcystis exposed to PAC

For *Microcystis* dominated water samples, large flocks were formed mainly at PAC doses > 8 mg Al L^{-1}. The cyanobacteria biomass accumulated in the top of these tubes (Fig. 3C). The PSII efficiency in the top was affected only at higher doses of 16 and 32 Al mg L^{-1}, and lowered from 0.49 (control) to 0.24 and 0.20, respectively. In the bottom of the tubes the PSII efficiency was unaffected and on average 0.47 (±0.01) (Fig. 3D). At higher doses of 16 and 32 mg Al L^{-1}, the pH was lowered to 6.8 and 5.4, respectively (Fig. 3C) while, in the control and the other treatments the pH remained high and was on average 9.59 (± 0.49).

3.1.3. Water dominated by C. raciborskii exposed to chitosan

When water dominated by *Cylindrospermopsis* was treated with different doses of CHI, clear flocks were formed at the highest doses of 16 and 32 mg L^{-1}. At these doses, the flocked cyanobacteria accumulated at the bottom of the tubes (Fig. 4A and B). The PSII efficiency was affected in all treatments, both in the top and at the bottom of the tubes. The PSII efficiency in the control was on average 0.55, while in the treatments it was on average 0.10 (± 0.04) in the top and 0.11 (± 0.04) in the bottom of the test tubes. The pH was rather unaffected by the CHI treatment and was on average 7.71 (± 0.34) (Fig. 4A and B).

3.1.4. Water dominated by Microcystis exposed to chitosan

In water dominated by *Microcystis* flocks were formed only at doses > 8 mg L^{-1}, with an accumulation of a *Microcystis* biomass at the surface of the tubes (Fig. 4C and D). The PSII efficiency was unaffected in all treatments and was on average 0.43 (± 0.04) in the top and 0.45 (± 0.02) in the bottom of the tubes (Fig. 4C). The pH was unaffected by the treatments and was on average 9.91 (± 0.09) (Fig. 4C).

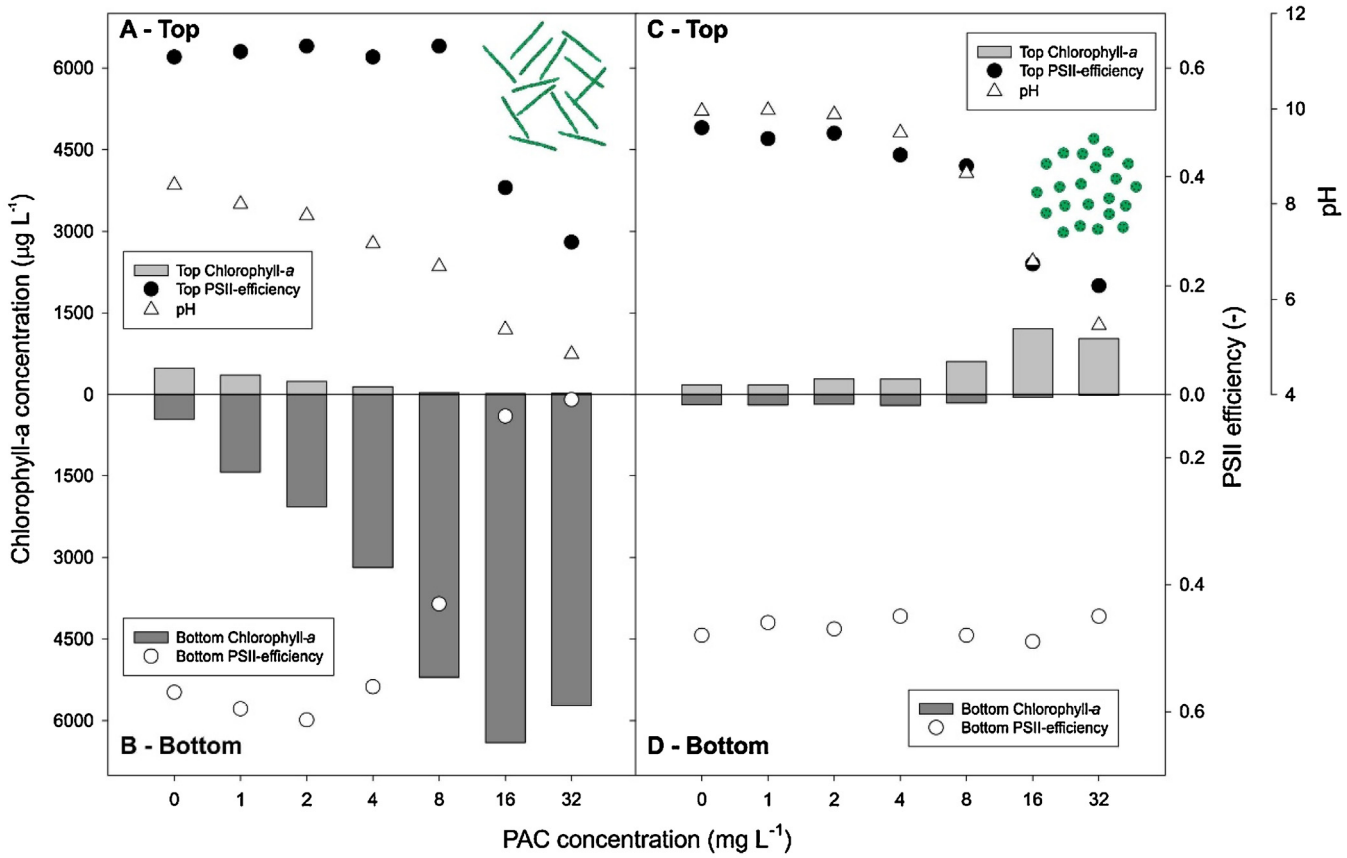

Fig. 3. Chlorophyll-a concentrations (micrograms per liter) in the top 5 mL (top light gray bars, A and C) and bottom 5 mL (lower dark gray bars, B and D) of 60 mL cyanobacteria suspensions (*Cylindrospermopsis* dominance A and B; *Microcystis* dominance C and D) incubated for 1 h in the absence or presence of different concentrations of the coagulant PAC (poly-aluminium chloride, 0–32 mg Al L^{-1}). Also included are the Photosystem II efficiencies (PSII) of the cyanobacteria collected at the water surface (filled circles) and at the bottom (open circles) as well as the pH values of the suspensions (open triangles).

3.2. Experiment 2 – coagulants dose fixed and ballast range

Cylindrospermopsis was effectively settled by PAC 4 mg Al L^{-1} alone, and especially in combination with ballast, as evidenced from strongly reduced chlorophyll-*a* concentrations in the top of tubes and increased chlorophyll-*a* concentrations at the bottom (Fig. 5A). The chlorophyll-*a* concentrations at the bottom of the tubes increased with the increasing ballast dose. The PSII efficiencies (0.59 ± 0.02) were unaffected and was lowered from initially pH 7.9 to pH 7.4 in the control and pH 6.35 (± 0.07) in the treatments with PAC (Fig. 5A).

Water with dominance of *Microcystis* also showed more aggregation in the bottom of the tubes when treated with PAC or PAC and ballast (Fig. 5B). The chlorophyll-*a* concentrations in the top of tubes were less affected by the treatments (Fig. 5B). The PSII-efficiencies were unaffected and were on average 0.46 (± 0.05), while the pH remained 9.6 in the control, but were reduced to on average pH 8.5 (± 0.54) in the PAC treatments (Fig. 5B).

When CHI was tested with different doses of ballast on water dominated by *Cylindrospermopsis*, chlorophyll-*a* concentrations in the top of the test tubes remained unaffected (Fig. 6A). The chlorophyll-*a* concentrations at the bottom of the tubes increased with higher ballast doses of either RS, GRA or LMB (Fig. 6A). The PSII efficiency was affected in all treatments and lowered considerably compared to the controls (Fig. 6A). The pH was lowered by CHI addition from pH 7.9 to on average 7.03 (± 0.10).

Chitosan alone or with different ballasts had a low effect on the chlorophyll-*a* concentrations in the top and bottom of test tubes filled with *Microcystis* dominated water (Fig. 6B). Also, the PSII efficiencies and the pH were unaffected and were on average 0.51 (± 0.04) and 8.9 (± 0.27), respectively (Fig. 6B).

3.3. Experiment 3–flock and sink assays

We found different results in the Flock and Sink assays using water dominated by *Cylindrospermopsis* or dominated by *Microcystis* (Figs. 7 and 8).

3.3.1. Water dominated by C. raciborskii

In *Cylindrospermopsis*-dominated water, PAC treatments were efficient in cyanobacterial biomass removal independently of the ballast used, while CHI alone or in combination with ballast appeared not effective (Fig. 7). One-way ANOVA indicated that chlorophyll-*a* concentrations in the top of the test tubes were significantly different ($F_{11,24} = 14.4$; $p < 0.001$). Tukey's *post-hoc* comparison revealed that chlorophyll-*a* concentrations in the top of tubes treated with PAC or with PAC and ballast were significantly lower than the controls and the other treatments (Fig. 7). Also in the bottom of the tubes chlorophyll-*a* concentrations were significantly different ($F_{11,24} = 69.7$; $p < 0.001$). Tukey's *post-hoc* comparison revealed three homogeneous groups significantly different from each other: 1) tubes treated with PAC or with PAC and RS; 2) tubes treated with PAC and either ballast (RS, GRA, LMB); and 3) controls and all other treatments with CHI, ballast, or CHI + ballast (Fig. 7).

In all treatments, except those containing CHI, PSII efficiencies remained fairly high in both the top (0.58 ± 0.02) and the bottom (0.59 ± 0.02) of the tubes. One-way ANOVA indicated that PSII

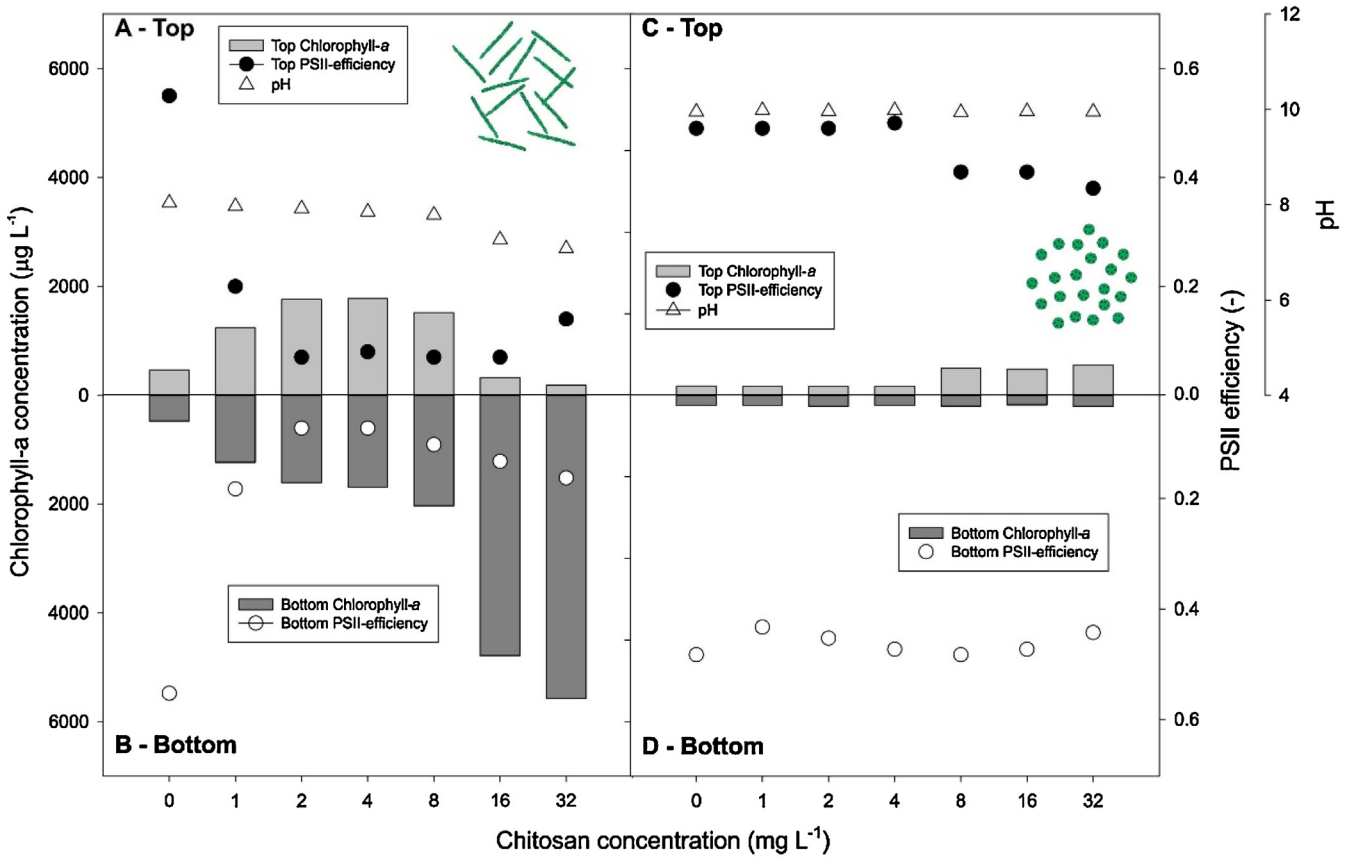

Fig. 4. Chlorophyll-a concentrations (micrograms per liter) in the top 5 mL (top light gray bars, panels A and C) and bottom 5 mL (lower dark gray bars, panels B and D) of 60 mL cyanobacteria suspensions (*Cylindrospermopsis* dominance A and B; *Microcystis* dominance C and D) incubated for 1 h in the absence or presence of different concentrations of the coagulant Chitosan (0–32 mg L^{-1}). Also included are the Photosystem II efficiencies (PSII) of the cyanobacteria collected at the water surface (filled circles) and at the bottom (open circles) as well as the pH values of the suspensions (open triangles).

efficiencies in the top of the test tubes were significantly different ($F_{11,24} = 508.1$; $p < 0.001$). Tukey's *post-hoc* comparison revealed that PSII efficiencies in the top of tubes treated with chitosan, or with CHI and ballast, were significantly lower than in the controls and the other treatments (Fig. 7). Likewise, PSII efficiencies in the bottom of the test tubes were different ($F_{11,24} = 594.3$; $p < 0.001$). Also, Tukey's test revealed that PSII efficiencies of CHI treatments differed significantly form the controls and other treatments (Fig. 7). The pH in the treatments with PAC (7.21 ± 0.09) was significantly ($F_{4,10} = 188.0$; $p < 0.001$) lower than the control (8.11 ± 0.03) and the other treatments, as revealed by a Tukey's *post-hoc* comparison.

3.3.2. *Water dominated by* Microcystis

In water dominated by *Microcystis*, all treatments with PAC and CHI (alone or combined with ballast) caused an effective removal of the cyanobacteria biomass from the water column to the bottom of the test tubes (Fig. 8). One-way ANOVA indicated that chlorophyll-*a* concentrations in the top of the test tubes were significantly different ($F_{11,24} = 122.4$; $p < 0.001$). Tukey's *post-hoc* comparison revealed four homogeneous groups: 1) chlorophyll-*a* in the top of the controls were the highest, 2) followed by tubes treated with gravel, 3) thereafter tubes treated with RS or LMB, and 4) the lowest chlorophyll-*a* concentrations in all treatments with coagulants and ballast (Fig. 8). In the bottom of the tubes significant differences were also found ($F_{11,24} = 58.0$; $p < 0.001$) with the lowest chlorophyll-*a* concentrations in the controls, and intermediate concentrations in tubes treated with only ballast. The highest chlorophyll-*a* concentrations were found in the bottom of tubes treated with solely coagulants, or coagulants combined with ballasts (Fig. 8). One-way ANOVA indicated that PSII efficiencies in the top of the test tubes were significantly different (one-way ANOVA: $F_{11,24} = 12{,}62$; $p < 0.0001$). But CHI treatments did not affect the PSII efficiencies in water dominated by *Microcystis*; PSII efficiency was on average 0.48 (± 0.08) in the top of the tubes and 0.43 (± 0.03) at the bottom. There was a significant difference between the pH of the control and the treatments (one-way ANOVA: $F_{11,24} = 4.18$; $p = 0.002$). Tukey's *post-hoc* comparison revealed three homogeneous groups: 1) CHI + Gravel, 2) Gravel, PAC, LMB, CHI + LMB, CHI + RS, PAC + LMB, PAC + RS, PAC + Gravel, 3) PAC, Control, CHI and RS.

3.3.3. *Dissolved cyanotoxins – saxitoxins and microcystins*

In the 'Flock and Sink assay' with water dominated by *Cylindrospermopsis*, significant differences in dissolved saxitoxins (STX) were found (one-way ANOVA: $F_{11,24} = 54.7$; $p < 0.001$). Tukey's test indicated that, in all treatments that included CHI, dissolved STX concentrations were significantly higher than in the controls and other treatments while, in the sole RS and LMB treatments, it was significantly lower than in the controls (Fig. 9A). Also, in water dominated by *Microcystis* significant differences in dissolved (extracellular) microcystins (MC) were found (one-way ANOVA: $F_{11,24} = 9.58$; $p < 0.001$). While extracellular MC concentrations were not affected by sole additions of coagulants or ballast, in all their combinations extracellular MC concentrations were significantly reduced (Fig. 9B).

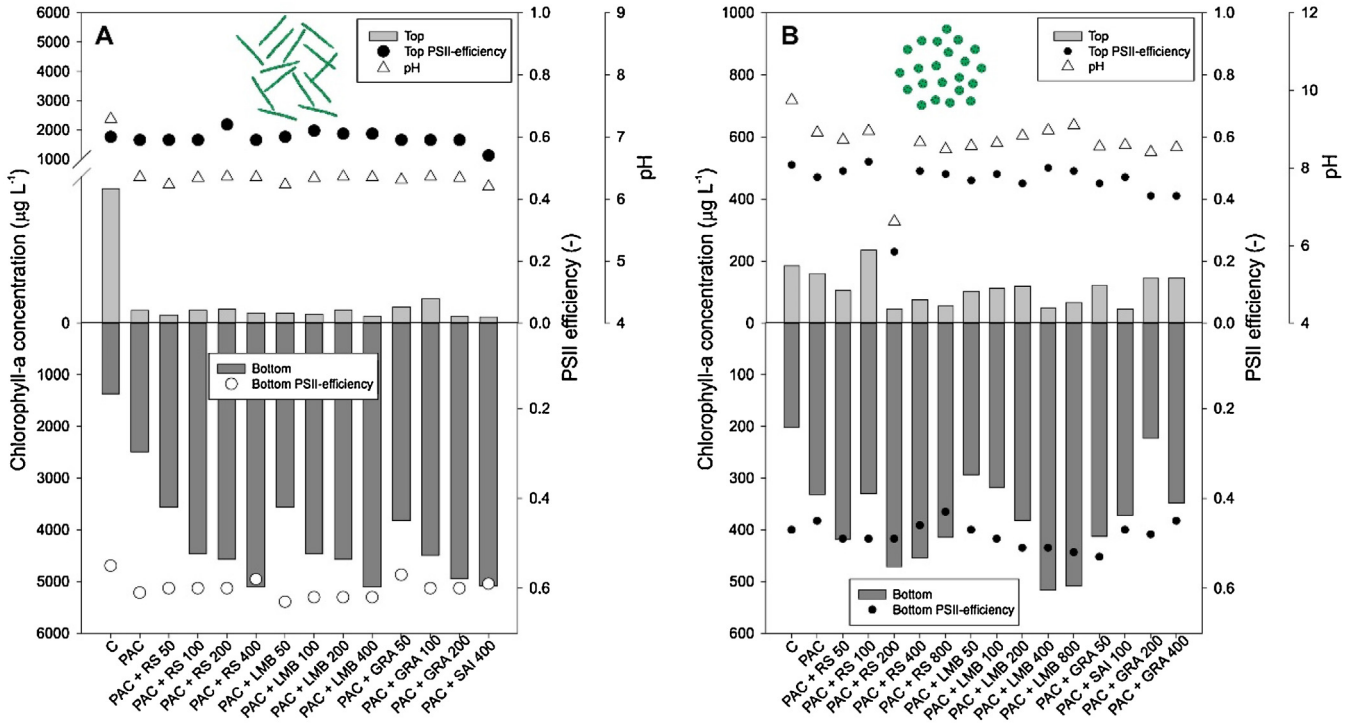

Fig. 5. Chlorophyll-*a* concentrations ($\mu g\,L^{-1}$) in the top 5 mL (top light gray bars) and bottom 5 mL (lower dark gray bars) of 60 mL cyanobacteria suspension (*Cylindrospermopsis* dominance A; *Microcystis* dominance B) incubated for one hour in the absence and presence of the coagulant PAC (poly-aluminium chloride, 4 mg Al L^{-1}) combined with different concentration (0–400 mg L^{-1}) of red soil (RS), gravel (GRA) or lanthanum modified bentonite (LMB) as ballast. Also included are the Photosystem II efficiencies (PSII) of the cyanobacteria collected at the water surface (filled circles) and at the bottom (open circles) as well as the pH values of suspensions (open triangles).

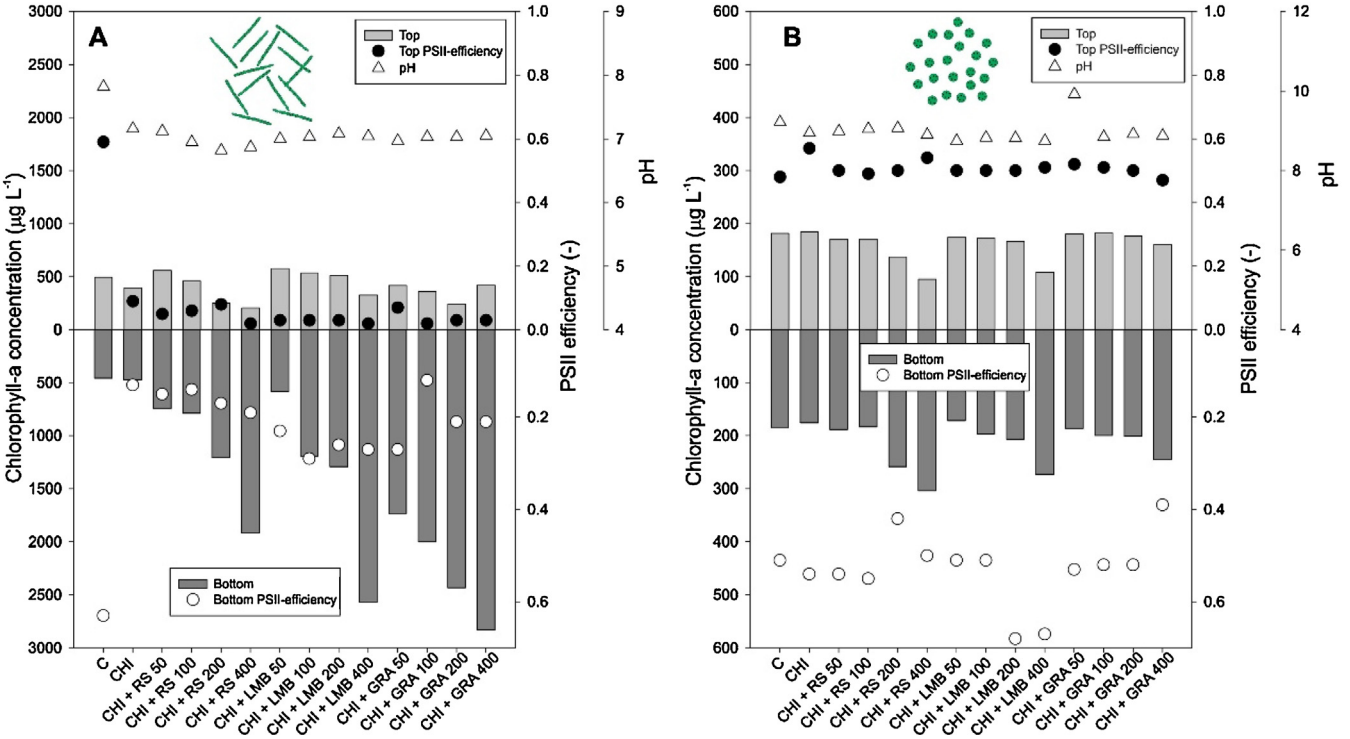

Fig. 6. Chlorophyll-*a* concentrations ($\mu g\,L^{-1}$) in the top 5 mL (top light gray bars) and bottom 5 mL (lower dark gray bars) of 60 mL cyanobacteria suspension (*Cylindrospermopsis* dominance A; *Microcystis* dominance B) incubated for one hour in the absence and presence of the coagulant chitosan (2 mg L^{-1}) combined with different concentration (0–400 mg L^{-1}) of red soil (RS), gravel (GRA) or lanthanum modified bentonite (LMB) as ballast. Also included are the Photosystem II efficiencies (PSII) of the cyanobacteria collected at the water surface (filled circles) and at the bottom (open circles) as well as the pH values of suspensions (open triangles).

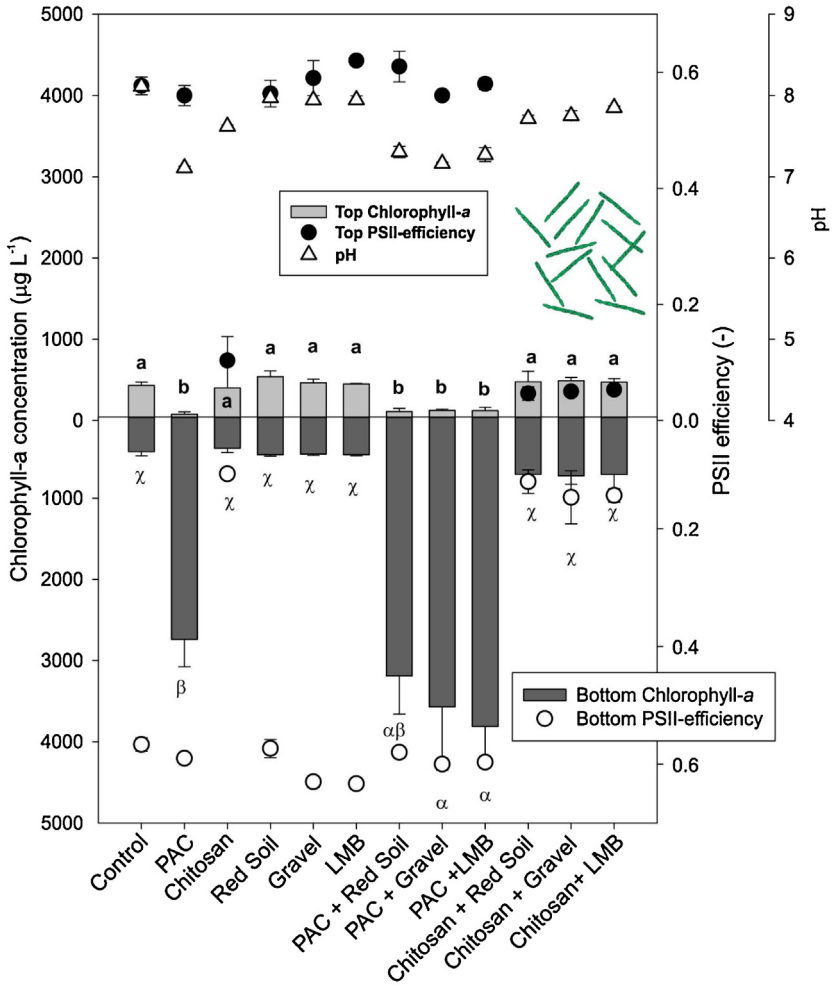

Fig. 7. Chlorophyll-a concentrations (micrograms per liter) in the top 5 mL (top light gray bars) and bottom 5 mL (lower dark gray bars) of 60 mL cyanobacteria suspensions from MAPRO lake with dominance of *C. raciborskii* incubated for two hours in the absence (control) or presence of the coagulants (poly-aluminium chloride, PAC – 4 mg Al L^{-1} or chitosan – 2 mg L^{-1}) and ballasts (red soil – RS, gravel – GRA and lanthanum modified bentonite – LMB, 100 mg L^{-1}) separately or in binary mixtures of coagulants with ballast. Also included are the Photosystem II efficiencies (PSII) of the cyanobacteria collected at the water surface (filled circles) and at the bottom (open circles) as well as the pH values of suspensions (open triangles). Error bars indicate one standard deviation (n = 3). Similar letters indicate homogeneous groups according to the Tukey method.

4. Discussion

In this paper, we tested the hypotheses that a combination of coagulant and ballast could be efficient in removing positively buoyant harmful cyanobacteria and will not promote the release of cyanotoxins in shallow tropical waterbodies. We found that positively buoyant cyanobacterial blooms can be removed out of the water column using a mixture of low dose of coagulants and ballast. Polyaluminium chloride was efficient to remove cyanobacteria regardless of the used ballast and independent on the dominant species of cyanobacteria; Chitosan was ineffective in removing *C. raciborskii* and released saxitoxins, but no detrimental effect was observed on *M. aeruginosa.* Our results indicate that positively buoyant cyanobacterial blooms can be removed from the water column using a mixture of low dose coagulants and ballast. These laboratory findings concur with whole lake experiments that effectively removed cyanobacteria out of the water column and translocated the aggregates to the sediment thereby strongly improving water quality, despite the biomass remaining in the system (Lürling and Van Oosterhout, 2013; Waajen et al., 2016a Waajen et al., 2016a). The experiments also show that, in general, PAC as a coagulant performs better than CHI. Polyaluminium chloride formed flocks in blooms dominated by either *C. raciborskii* or *M. aeruginosa* and, with ballast, effective precipitation could be achieved. In contrast, CHI only flocked at a relatively low pH and both species could be settled when the pH was around 7; however, at pH ≥ 8 no settling could be achieved, although some fluffy flocks were observed at doses higher than 8 mg L^{-1} in *Microcystis* dominated water. Such a loss of the flocculating activity of CHI at an elevated pH has also been reported elsewhere (Morales et al., 1985; Vandamme et al., 2013). A possible explanation for lower flocculation at higher pH might be that hydroxyl and other oxyanions aggregate around the protonated areas of CHI and thus prevent electrostatic interactions between the protonated amino groups of chitosan and the negatively charged cyanobacteria (Renault et al., 2009). In addition, CHI exerted a detrimental effect on *C. raciborskii*, promoting the release of saxitoxins; thus, our study suggests that broad-scale generalizations on the widespread applicability of chitosan should be regarded with caution.

4.1. Effect of coagulants on cyanobacteria

In water dominated by either *Cylindrospermopsis* or *Microcystis*, PAC was a good coagulant to flock the cyanobacteria, albeit for *Microcystis* only at the highest doses applied (i.e., ≥ 8 mg Al L^{-1}). The higher doses needed to create flocks was caused by the relatively high pH of ~ 10 in the *Microcystis* dominated water that needed higher PAC doses to lower the pH (cf. Dentel, 1991). A high

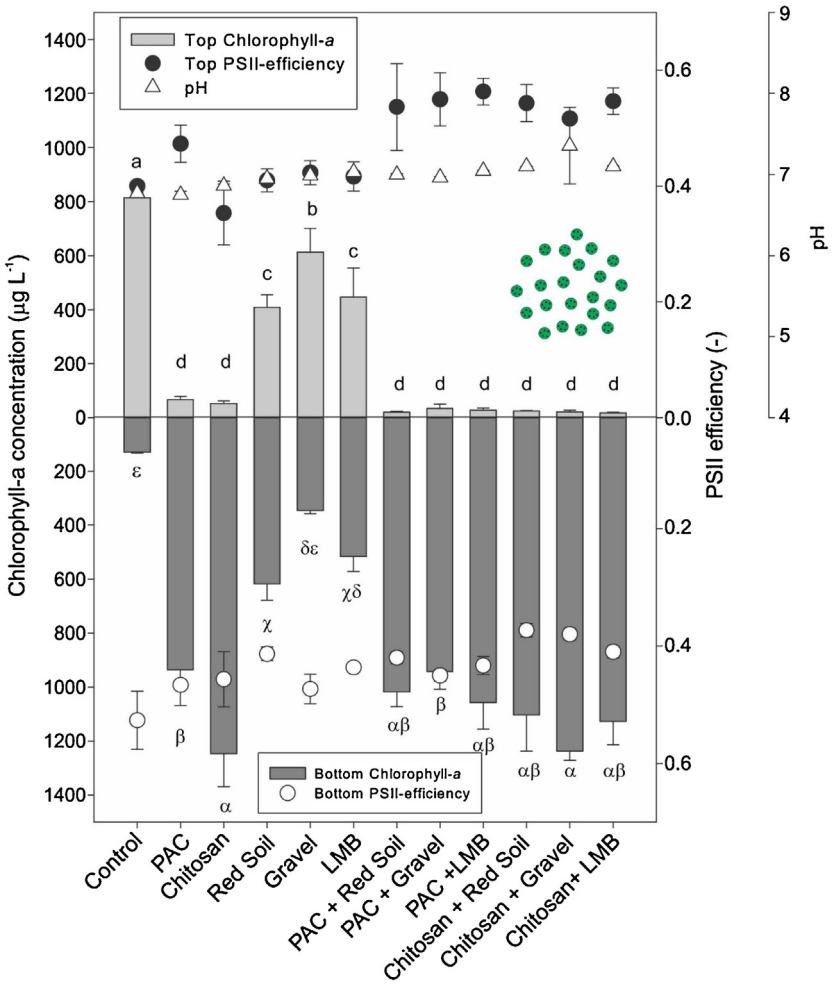

Fig. 8. Chlorophyll-a concentrations (micrograms per liter) in the top 5 mL (top light gray bars) and bottom 5 mL (lower dark gray bars) of 60 mL cyanobacteria suspensions from MAPRO lake with dominance of *Microcystis* spp. incubated for two hours in the absence (control) or presence of the coagulants (poly-aluminium chloride, PAC – 4 mg Al L^{-1} or chitosan – 2 mg L^{-1}) and ballasts (red soil – RS, gravel – GRA and lanthanum modified bentonite – LMB, 100 mg L^{-1}) separately or in binary mixtures of coagulants with ballast. Also included are the Photosystem II efficiencies (PSII) of the cyanobacteria collected at the water surface (filled circles) and at the bottom (open circles) as well as the pH values of suspensions (open triangles). Error bars indicate one standard deviation (n = 3). Similar letters indicate homogeneous groups according to the Tukey method.

pH leads to the formation of $Al(OH)_4^-$ species (Van Benschoten and Edzwald, 1990) and to increased negative charges of the cyanobacterial particles which causes less effective charge neutralization (Chen et al., 2006) and impaired flocculation. Nonetheless, the pH only needed to be lowered by about one unit, in our Experiments 1 and 2 effective flocking at lower doses (2 mg Al L^{-1} and 4 mg Al L^{-1}) was found at pH 9. Likewise, turbidity removal by PAC was found to be equally excellent in the pH range 6 to 9 (Yang et al., 2010).

It should be noted that, both in *Cylindrospermopsis* as well as in *Microcystis* dominated water, not only was the pH reduced when using the highest PAC doses of 16 and 32 mg Al L^{-1}, but also the PSII efficiency. The latter clearly points to damage of the cells and the potential release of toxins, which is also a side effect of metal based algaecides (Jones and Orr, 1994; Jančula and Maršálek, 2011; Merel et al., 2013). Hence, it is important to determine the PAC dose at which cyanobacteria can be aggregated without damage to membrane integrity; for instance, Sun et al. (2012) found an optimum dose for *M. aeruginosa* at a coagulant dose of 15 mg L^{-1} $AlCl_3$. In our study, PSII efficiencies were not, or were only marginally affected at a PAC dose of 4 mg Al L^{-1} for *Cylindrospermopsis* and 8 mg Al L^{-1} for *Microcystis*. At this dose of coagulant, the cells will not be immediately damaged (Sun et al., 2013), which makes it suitable for coagulation and combination with a ballast. In field situations, the entrapped cyanobacteria will accumulate on the sediment where, after some days, coagulant induced speeded-up lysis will occur (Sun et al., 2013; Li et al., 2015b). Usually a rich community of cyanotoxin-degrading bacteria is present in the sediment of cyanobacteria infested lakes that rapidly will degrade any released cyanotoxins (e.g., Grützmacher et al., 2010; Wu et al., 2011; Li et al., 2015a).

In general, aluminium salts are widely used in water treatment including cyanobacteria removal (Jančula and Maršálek, 2011). However, PAC has several advantages over other aluminium salt coagulants (such as alum), because there is less pH reduction, a lower dose is needed, there is less residual Al, less sulphate is added and better flocs occur at low temperature (De Julio et al., 2010; Gebbie, 2001). Particularly, a low dose of coagulant is essential to prevent cell lysis during the settling process and the liberation of cell compounds, such as intracellular toxins, into the water column. Hence, combining a low dose of PAC with a ballast is a far more promising, safer and beneficial than the use of algaecides, or a high dose of alum.

Chitosan is widely viewed as a promising non-toxic environmentally friendly coagulant that could be preferred above aluminium based coagulants in water treatment (Noyma et al., 2016; Renault et al., 2009; Yang et al., 2016), including cyanobacterial control (Pan et al., 2011a; Li and Pan, 2013).

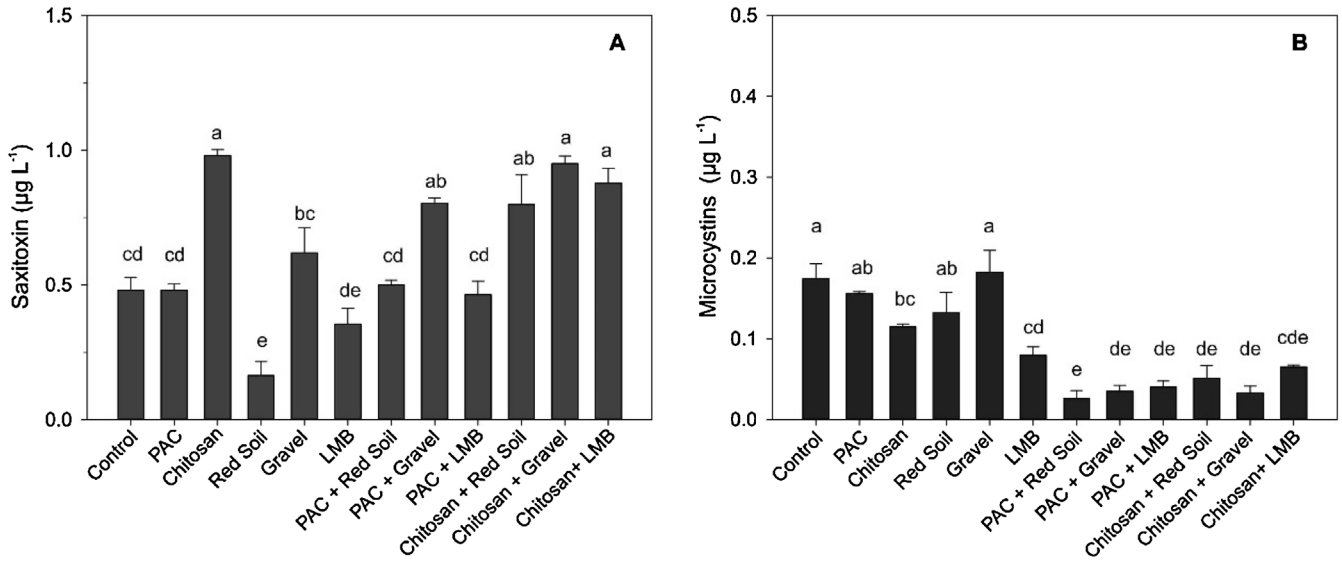

Fig. 9. Concentrations of dissolved toxins (micrograms per liter) in 60 mL cyanobacteria suspensions from MAPRO lake incubated for two hours in the absence (control) or presence of the coagulants (poly-aluminium chloride, PAC – 4 mg Al L^{-1} or chitosan – 2 mg L^{-1}) and ballasts (red soil – RS, gravel – GRA and lanthanum modified bentonite – LMB, 100 mg L^{-1}) separately or in binary mixtures of coagulants with ballast. (A) Saxitoxins – *Cylindrospermopsis* dominance and (B) microcystins – *Microcystis* dominance (B). Error bars indicate one standard deviation (n = 3). Similar letters indicate homogeneous groups according to the Tukey method (bold indicate differences from the controls).

However, our study demonstrates that this enthusiasm should be tempered by extreme care. Although chitosan could flock *Cylindrospermopsis* and *Microcystis* at a relatively high dose, a strong detrimental effect was observed on *Cylindrospermopsis* at all doses applied, as evidenced by strongly reduced PSII efficiencies. It is well-known that CHI possesses anti-microbial activities against both gram-positive and gram-negative bacteria (e.g., Liu et al., 2004; Yang et al., 2016). It is therefore not surprising to observe comparable effects against *Cylindrospermopsis*; whereas the mucous sheath around *Microcystis* colonies probably protected them from the cyanobactericidal effect of chitosan. Hence, the claim that CHI is non-toxic and ecofriendly in terms of cyanobacterial nuisance control should be refuted as it might only hold true for cyanobacteria embedded in mucus. Hence, more research with representatives of the cyanobacterial orders Nostocales, Oscillatoriales and Chroococcales are needed to shed light on the presumed widespread cyanobactericidal effect of CHI. In addition, the pH and ionic strength of the water influence chitosan performance. In circumneutral and slightly alkaline conditions the protonated amino groups of chitosan interact electrostatically with the negatively charged cyanobacteria (Renault et al., 2009); whereas different underlying mechanisms –charge neutralization, charge patching, bridging– result in flock formation, and further entrapping of particles in the settling flocs – sweeping – can occur (Yang et al., 2016). At an elevated pH, or high ionic strength, negative ions will gather around the protonated groups of chitosan and screen of charges (Qun and Ajun, 2006), which contracts the molecule (Tsaih and Chen, 1997), and leads to loss of coagulation capacity (Bilanovic et al., 1988). Consequently, CHI is not a very efficient coagulant at a high pH or high ionic strength of the water (Morales et al., 1985; Pan et al., 2011a; Vandamme et al., 2013; de Magalhães et al., 2017), which further limits its applicability. Finally, the price of CHI is much higher than that of aluminium salts (Granados et al., 2012).

4.2. Effect of different ballasts

In some of our trials, the addition of a coagulant alone was sufficient to settle aggregated cyanobacteria, but flocks were fluffy, and settling velocities were visually much higher when ballast was added. In general, adding a ballast facilitated the removal of harmful cyanobacteria from the water column, particularly for positively buoyant cyanobacteria. There was no difference among the materials used as ballast (lanthanum-modified bentonite, gravel and red soil). This is in agreement with previous findings using PAC and LMB (Lürling and van Oosterhout, 2013; Noyma et al., 2016), PAC and RS (Noyma et al., 2016; de Magalhães et al., 2017) and CHI and RS or LMB (Noyma et al., 2016). The ballast is introduced first, immediately followed by the coagulants so ballast particles will be trapped inside the formed flocks. We used 4 mg Al L^{-1} for PAC to avoid detrimental effects on the cells that, combined with ballast, was sufficient to settle *Cylindrospermopsis*, but less so for *Microcystis*, although more cyanobacteria settled in the combination with the highest ballasts dose than in the controls. We used 2 mg L^{-1} CHI that caused more *Cylindrospermopsis* to settle when combined with 400 mg L^{-1} ballast, but not at lower doses. The dose of CHI (2 mg L^{-1}) is similar to those used in other studies (Pan et al., 2012; Li and Pan, 2013), but the effective dose of ballasts is somewhat higher than the soil concentrations used in those studies (75 and 100 mg L^{-1}). In *Microcystis* dominated water, combinations of CHI and ballast did not lead to the formation of flocks and did not efficiently settle down cyanobacteria, which was probably caused by the high pH of the water, as outlined above (Morales et al., 1985; Vandamme et al., 2013). A high pH (> 8.5) also hampered flock formation of *Microcystis* in water collected from a tropical brackish coastal lagoon (de Magalhães et al., 2017).

4.3. Flocculation and sinking of cyanobacteria

As mentioned before, chitosan has been proposed as an alternative to PAC to remove cyanobacterial blooms (Pan et al., 2011a; Li and Pan, 2013). Good results have been reported for the removal of *Microcystis* from water using CHI combined with ballast (Pan et al., 2006a,b, 2012; Noyma et al., 2016; de Magalhães et al., 2017), which was partly confirmed in this study. However, CHI cannot be used as an alternative to PAC when blooms are comprised of *Cylindrospermopsis*. The CHI treatments damaged the cells of *Cylindrospermopsis* as was highlighted by the reduction

in PSII efficiency. The significant increase of dissolved saxitoxins confirmed leaking of cell constituents into the water. Although the observed concentrations of dissolved saxitoxins were low (≅ 1 $\mu g\,L^{-1}$), in all CHI treatments saxitoxin levels were twice as high as the controls and other treatments. Cyanobacterial blooms may, however, present higher saxitoxin contents (e.g, Hoeger et al., 2004). In treating such blooms, leakage may lead to exceeding the drinking water guideline of 3 μg saxitoxins L^{-1} as effected in Australia, Brazil and New Zealand (Chorus, 2012) or recreational guidelines such as the 10 $\mu g\,L^{-1}$ in Oregon (Farrer et al., 2015). Nevertheless, this negative effect was not observed for microcystins in samples with *Microcystis* dominance, probably because the mucilage provided some protection to the colonial *Microcystis*. Likewise, no negative impact of CHI on *Microcystis* has been observed in other studies (Pei et al., 2014; Noyma et al., 2016; de Magalhães et al., 2016). This seems promising as microcystins are the most frequently encountered cyanotoxins in freshwater systems (e.g., Loftin et al., 2016), sometimes at relatively high concentrations (e.g., Faassen and Lürling, 2013), whereas the common growth form of *Microcystis* is predominantly as colonies in the field. Nonetheless, more experiments are required to decipher which cyanobacteria and what growth forms are vulnerable to chitosan.

It is well-known that chitosan has antibacterial, antimicrobial and antifungal activities (e.g., Kong et al., 2010; Younes et al., 2014), where the activity against bacteria is through cell membrane damage (Liu et al., 2004). Hence, cyanobacterial membrane damage and subsequent cyanotoxin release is possible. Such cell leakage should be avoided during bloom removal, to prevent cyanotoxins release. Cell lysis, caused by algaecides to remove cyanobacteria from water has led to severe intoxication and the death of humans (Azevedo et al., 2002). Therefore, environmentally safe management strategies should not only mitigate blooms and strongly reduce cyanobacterial biomasses, but also their toxins (Greenfield et al., 2014). In this view, strategies that release toxins from the cells are less favorable as management options (Merel et al., 2013). Thus, it is strongly recommended to further study the potential damaging effect of CHI towards cyanobacteria using far more species than merely colonial *Microcystis* embedded in mucus. In addition, ecotoxicological studies should be extended towards non-target aquatic organisms; a well-founded conclusion can only then be drawn about the eco-friendliness of CHI.

The ballast compounds combined with coagulants may improve the removal of extracellular harmful algal toxins from the water (Pierce et al., 2004). Our results confirmed this potential, since a significant reduction of dissolved microcystins was observed in all coagulant + ballast treatments. This reduction can be related to the capacity of clays to bind microcystins (Couto et al., 2013). Clays and sediments can also adsorb saxitoxins (Burns et al., 2009; Pierce et al., 2004) and we were expecting some effect on treatments; however, removal capacity was not observed for saxitoxins. Saxitoxins are highly charged and polar compounds. Considering that adsorption of this class of cyanotoxins to clays is correlated with the cation exchange capacity (Burns et al., 2009), we can speculate that organic material and other compounds present in the water from the lake can interfere with and reduce the cation exchange capacity of the ballasts. There is some evidence that the used coagulants can also remove toxins (Pierce et al., 200; Shi et al., 2012). Polyaluminium chloride can be considered as efficient for saxitoxin removal, although its efficacy is dependent on dose and contact time (Shi et al., 2012) but, in the flock and sink experiment we used low dose and the contact time was short (1–2 h). Furthermore, although CHI − can be efficient in removing algae cells under laboratory experiments, it was not effective for removing extracellular toxins in the supernatant and flocs (Martínez et al., 2016).

There is a growing body of evidence showing that PAC has an overall great capacity to aggregate cyanobacteria under a wide range of conditions, while for CHI this seems to become increasingly limited. It has been postulated that "(. . .) PAC is (. . .) less efficient (or need high dosage) at low salinities in fresh waters" (Li and Pan, 2013; pp.4555). However, this statement is not supported by the study of Zou et al. (2006) referred to by Li and Pan (2013) because no PAC experiments are included in it, or by a range of studies that have shown a low dose of PAC combined with a ballast to be very effective in freshwater ecosystems (e.g., Lürling and van Oosterhout, 2013; Noyma et al., 2016; Waajen et al., 2016a, b; de Magalhães et al., 2017). Hence, there is no experimental support for the claim that "flocculation efficiency of (. . .) PAC-modified clay decreases dramatically as the water salinity decreases, making it difficult to use this technique for Cyano-HABs control in lakes and reservoirs" (Li and Pan, 2013). In contrast, our current study adds to the growing body of experimental evidence that PAC combined with ballast can be very effective in freshwater (Lürling and van Oosterhout, 2013; Noyma et al., 2016; Waajen et al., 2016a,b) and brackish environments (de Magalhães et al., 2017) to flock and settle cyanobacteria.

Our results provide further evidence that, for controlling cyanobacterial nuisance tailor made interventions are required. Chitosan-based lake restoration techniques, such as "modified local soil induced ecological restoration" (Pan et al., 2011a, 2006b) should only be applied to *Cylindrospermopsis* blooms if killing the cells is a wanted outcome. Although more controlled experiments with various strains are needed, the well-known antibacterial effect of CHI suggests a detrimental effect on all *Cylindrospermopsis* strains, and probably all cyanobacteria without protective mucous sheaths. Hence, the choice of coagulant and ballast should be based on site specific assays and a thorough underlying system diagnostics to justify the intervention (Lürling et al., 2016). Finally, larger scale experiments (mesocosms and whole lake) are needed to confirm the best technique for the removal of harmful cyanobacteria in shallow tropical systems. These experiments not only include the sediment as a potentially strong nutrient regenerating reservoir (determining the choice of ballast), but also will expose the settled cyanobacteria to ambient conditions that may either promote decay or lead to regrowth. It has been speculated that, after precipitation using a coagulant with ballast, *Microcystis* may survive for prolonged time on the sediment (cf. Brunberg and Boström, 1992) and may potentially be liberated from flocks (Noyma et al., 2016). Here, to minimize the biomass that may recolonize the water column, especially in shallow systems, permanent removal of the precipitated cyanobacteria is desirable, where killing the settled cyanobacteria could be an option (Noyma et al., 2016). Chitosan will not be so effective against *Microcystis*; calcium peroxide included in ballast was also not effective and made settled flocks rise again (Noyma et al., 2016); while killing *Microcystis* first with hydrogen peroxide and then sinking with a coagulant and ballast yielded promising results (Wang et al., 2012). Hence, we propose a gradual upscaling of experiments to determine the most efficient techniques and coagulant/ballast, or algaecide/coagulant/ballast combinations under a wide variety of conditions.

5. Conclusions

- The best technique for cyanobacterial biomass removal through flocking and sinking depends on the dominant species.
- Treatments with PAC were efficient to reduce the biomass of the water column regardless of the used ballast and independent on the dominant species of cyanobacteria.
- Chitosan was ineffective in removing a *C. raciborskii* biomass from the water of the studied lake.

- Chitosan exerted a detrimental effect on *C. raciborskii*, promoting the release of saxitoxins.
- Chitosan may be a good coagulant for blooms of *Microcystis* spp., but only at a circumneutral or slightly alkaline pH.

Acknowledgments

This study was sponsored by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through a Science without Borders grant (SWB/CNPq 400408/2014-7), CNPq Project number 473141/2013-2 and by Fundação de Apoio a Pesquisa do Estado do Rio de Janeiro (FAPERJ – 111.267/2014) and Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP 2014/50420-9 and 2014/06556-3). V. Huszar was partially supported by CNPq, This study was conducted under the flag of the CAPES (Brazil)/NUFFIC (The Netherlands) project 045/12. [CG]

References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L., Sparovek, G., 2014. Köppen's climate classification map for Brazil. Meteorol. Zeitschrift 22, 711–728.

Antunes, J.T., Leão, P.N., Vasconcelos, V.M., 2015. Cylindrospermopsis raciborskii: review of the distribution, phylogeography, and ecophysiology of a global invasive species. Front. Microbiol. 6 (473) .

Azevedo, S.M., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R., Eaglesham, G.K., 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru—Brazil. Toxicology 181, 441–446.

Bilanovic, D., Shelef, G., Sukenik, A., 1988. Flocculation of microalgae with cationic polymers – effects of medium salinity. Biomass 17, 65–76.

Bonilla, S., Aubriot, L., Soares, M.C.S., Gonzalez-Piana, M., Fabre, A., Huszar, V.L., Lürling, M., Antoniades, D., Padisák, J., Kruk, C., 2012. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? FEMS Microbiol. Ecol. 79 (3), 594–607.

Brunberg, A.-K., Boström, B., 1992. Coupling between benthic biomass of *Microcystis* and phosphorus release from the sediments of a highly eutrophic lake. Hydrobiologia 235 (1), 375–385.

Burns, J.M., Hall, S., Ferry, J.L., 2009. The adsorption of saxitoxin to clays and sediments in fresh and saline waters. Water Res. 43 (7), 1899–1904.

Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: the CyanoHABs. Hum. Ecol. Risk Assessment: Int. J. 7 (5), 1393–1407.

Chen, Z., Fan, B., Peng, X., Zhang, Z., Fan, J., Luan, Z., 2006. Evaluation of Al 30 polynuclear species in polyaluminum solutions as coagulant for water treatment. Chemosphere 64 (6), 912–918.

Chorus, I., Falconer, I.R., Salas, H.J., Bartram, J., 2000. Health risks caused by freshwater cyanobacteria in recreational waters. J. Toxicol. Environ. Health Part B: Crit. Rev. 3 (4), 323–347.

Chorus, I., 2012. Cyanotoxins: Occurrence, Causes, Consequences. Springer Science & Business Media.

Codd, G.A., Morrison, L.F., Metcalf, J.S., 2005. Cyanobacterial toxins: risk management for health protection. Toxicol. Appl. Pharmacol. 203 (3), 264–272.

Copetti, D., Finsterle, K., Marziali, L., Stefani, F., Tartari, G., Douglas, G., Reitzel, K., Spears, B.M., Winfield, I.J., Crosa, G., 2016. Eutrophication management in surface waters using lanthanum modified bentonite: a review. Water Res. 97, 162–174.

Couto, R., Guarino, A., Branco, C.W., Palermo, E., Azero, E., 2013. Application of clay minerals and polymeric resins to remove dissolved Microcystin-LR from water. Int. J. Environ. Res. 7 (2), 435–442.

de Magalhães, L., Noyma, N.P., Furtado, L.L., Mucci, M., van Oosterhout, F., Huszar, V. L., Marinho, M.M., Lürling, M., 2017. Efficacy of coagulants and ballast compounds in removal of cyanobacteria (Microcystis) from water of the Tropical Lagoon Jacarepaguá (Rio de janeiro, Brazil). Estuaries Coasts 40 (1), 121–133.

De Julio, M., Fioravante, D., De Julio, T., Oroski, F., Graham, N., 2010. A methodology for optimising the removal of cyanobacteria cells from a brazilian eutrophic water. Braz. J. Chem. Eng. 27 (1), 113–126.

Dentel, S.K., 1991. Coagulant control in water treatment. Crit. Rev. Environ. Sci. Technol. 21 (1), 41–135.

Dittmann, E., Wiegand, C., 2006. Cyanobacterial toxins-occurrence, biosynthesis and impact on human affairs. Mol. Nutr. Food Res. 50 (1), 7–17.

Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornbrugh, D.J., 2009. Eutrophication of US freshwaters: analysis of potential economic damages. Environ. Sci. Technol. 43 (1), 12–19.

Douglas, G.B., 2002. Remediation material and remediation process for sediments. Google Patents.

Faassen, E.J., Lürling, M., 2013. Occurrence of the microcystins MC-LW and MC-LF in Dutch surface waters and their contribution to total microcystin toxicity. Mar. Drugs 11 (7), 2643–2654.

Falconer, I.R., Beresford, A.M., Runnegar, M.T.C., 1983. Evidence of liver damage by toxin from a bloom of the blue–green alga, Microcystis aeruginosa. Med. J. Aust. 1, 511–514.

Falconer, I.R., 1999. An overview of problems caused by toxic blue–green algae (cyanobacteria) in drinking and recreational water. Environ. Toxicol. 14 (1), 5–12.

Farrer, D., Counter, M., Hillwig, R., Cude, C., 2015. Health-based cyanotoxin guideline values allow for cyanotoxin-based monitoring and efficient public health response to cyanobacterial blooms. Toxins 7 (2), 457–477.

Gebbie, P., 2001. Using polyaluminium coagulants in water treatment. Proc. 64th AWIEO Conference, Citeseer.

Granados, M., Acién, F., Gomez, C., Fernandez-Sevilla, J., Grima, E.M., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresour. Technol. 118, 102–110.

Greenfield, D.I., Duquette, A., Goodson, A., Keppler, C.J., Williams, S.H., Brock, L.M., Stackley, K.D., White, D., Wilde, S.B., 2014. The effects of three chemical algaecides on cell numbers and toxin content of the cyanobacteria *Microcystis aeruginosa* and *Anabaenopsis* sp. Environ. Manage. 54 (5), 1110–1120.

Grützmacher, G., Wessel, G., Klitzke, S., Chorus, I., 2010. Microcystin elimination during sediment contact. Environ. Sci. Technol. 44 (2), 657–662.

Harke, M., Steffen, M., Gobler, C., Otten, T., Wilhelm, S., Wood, S.A., Pearl, H., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis*. Harmful Algae 54, 4–20.

Hawkins, P.R., Runnegar, M.Y.C., Jackson, A.R.B., Falconer, I.R., et al., 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (Blue-Green Alga) Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl. Environ. Microbiol. 50 (5), 1292–1295.

Hoeger, S.J., Shaw, G., Hitzfeld, B.C., Dietrich, D.R., 2004. Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants. Toxicon 43 (6), 639–649.

Hoff-Risseti, C., Dörr, F.A., Schaker, P.D.C., Pinto, E., Werner, V.R., Fiore, M.F., 2013. Cylindrospermopsin and saxitoxin synthetase genes in Cylindrospermopsis raciborskii strains from Brazilian freshwater. PLoS One 8 (8), e74238.

Jančula, D., Maršálek, B., 2011. Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. Chemosphere 85 (9), 1415–1422.

Jeppesen, E., Kristensen, P., Jensen, J.P., Søndergaard, M., Mortensen, E., Lauridsen, T., 1991. Recovery resilience following a reduction in external phosphorus loading of shallow, eutrophic Danish lakes: duration, regulating factors and methods for overcoming resilience. Mem. Ist. Ital. Idrobiol. 48 (1), 127–148.

Jiang, J.-Q., Graham, N.J., 1998. Pre-polymerised inorganic coagulants and phosphorus removal by coagulation- a review. Water SA 24 (3), 237–244.

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 Res. 28 (4), 871–876.

Kong, M., Chen, X.G., Xing, K., Park, H.J., 2010. Antimicrobial properties of chitosan and mode of action: a state of the art review. Int. J. Food Microbiol. 144 (1), 51–63.

Lürling, M., van Oosterhout, F., 2013. Case study on the efficacy of a lanthanum-enriched clay (Phoslock®) in controlling eutrophication in Lake Het Groene Eiland (The Netherlands). Hydrobiologia 710 (1), 253–263.

Lürling, M., Mackay, E., Reitzel, K., Spears, B.M., 2016. Editorial–A critical perspective on geo-engineering for eutrophication management in lakes. Water Res. 97, 1–10.

Lam, A.K.-Y., Prepas, E.E., Spink, D., Hrudey, S.E., 1995. Chemical control of hepatotoxic phytoplankton blooms: implications for human health. Water Res. 29 (8), 1845–1854.

Li, L., Pan, G., 2013. A universal method for flocculating harmful algal blooms in marine and fresh waters using modified sand. Environ. Sci. Technol. 47 (9), 4555–4562.

Li, L., Zhang, H., Pan, G., 2015a. Influence of zeta potential on the flocculation of cyanobacteria cells using chitosan modified soil. J. Environ. Sci. 28, 47–53.

Li, Z., Yang, F., Yang, R., 2015b. Synthesis and characterization of chitosan derivatives with dual-antibacterial functional groups. Int. J. Biol. Macromol. 75, 378–387.

Liu, H., Du, Y., Wang, X., Sun, L., 2004. Chitosan kills bacteria through cell membrane damage. Int. J. Food Microbiol. 95 (2), 147–155.

Loftin, K.A., Graham, J.L., Hilborn, E.D., Lehmann, S.C., Meyer, M.T., Dietze, J.E., Griffith, C.B., 2016. Cyanotoxins in inland lakes of the United States: occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. Harmful Algae 56, 77–90.

Marsden, M.W., 1989. Lake restoration by reducing external phosphorus loading: the influence of sediment phosphorus release. Freshw. Biol. 21 (2), 139–162.

Martínez, T.d.C.C., Rodríguez, R.A., Voltolina, D., Morquecho, L., 2016. Effectiveness of coagulants-flocculants for removing cells and toxins of *Gymnodinium catenatum*. Aquaculture 452, 188–193.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E., Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ. Int. 59, 303–327.

Morales, J., De La Noüe, J., Picard, G., 1985. Harvesting marine microalgae species by chitosan flocculation. Aquacult. Eng. 4 (4), 257–270.

Moss, B., Kosten, S., Meerhof, M., Battarbee, R., Jeppesen, E., Mazzeo, N., Havens, K., Lacerot, G., Liu, Z., De Meester, L., 2011. Allied attack: climate change and eutrophication. Inland Waters 1 (2), 101–105.

Noyma, N.P., de Magalhães, L., Furtado, L.L., Mucci, M., van Oosterhout, F., Huszar, V. L., 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 Res. 97, 26–38.
Pacheco, F.S., Roland, F., Downing, J.A., 2013. Eutrophication reverses whole-lake carbon budgets. Inland Waters 4 (1), 41–48.
Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science 320 (5872), 57.
Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. 1 (1), 27–37.
Paerl, H.W., Hall, N.S., Peierls, B.L., Rossignol, K.L., 2014. Evolving paradigms and challenges in estuarine and coastal eutrophication dynamics in a culturally and climatically stressed world. Estuaries Coasts 37 (2), 243–258.
Pan, G., Zhang, M.-M., Chen, H., Zou, H., Yan, H., 2006a. Removal of cyanobacterial blooms in Taihu Lake using local soils. I. Equilibrium and kinetic screening on the flocculation of Microcystis aeruginosa using commercially available clays and minerals. Environ. Pollut. 141 (2), 195–200.
Pan, G., Zou, H., Chen, H., Yuan, X., 2006b. 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. Environ. Pollut. 141 (2), 206–212.
Pan, G., Chen, J., Anderson, D.M., 2011a. Modified local sands for the mitigation of harmful algal blooms. Harmful Algae 10 (4), 381–387.
Pan, G., Yang, B., Wang, D., Chen, H., Tian, B.-h., Zhang, M.-l., Yuan, X.-z., Chen, J., 2011b. In-lake algal bloom removal and submerged vegetation restoration using modified local soils. Ecol. Eng. 37 (2), 302–308.
Pan, G., Dai, L., Li, L., He, L., Li, H., Bi, L., Gulati, R.D., 2012. Reducing the recruitment of sedimented algae and nutrient release into the overlying water using modified soil/sand flocculation-capping in eutrophic lakes. Environ. Sci. Technol. 46 (9), 5077–5084.
Pei, H.-Y., Ma, C.-X., Hu, W.-R., Sun, F., 2014. The behaviors of *Microcystis aeruginosa* cells and extracellular microcystins during chitosan flocculation and flocs storage processes. Bioresour. Technol. 151, 314–322.
Pierce, R.H., Henry, M.S., Higham, C.J., Blum, P., Sengco, M.R., Anderson, D.M., 2004. Removal of harmful algal cells (*Karenia brevis*) and toxins from seawater culture by clay flocculation. Harmful Algae 3 (2), 141–148.
Qun, G., Ajun, W., 2006. Effects of molecular weight, degree of acetylation and ionic strength, 2016 strength on surface tension of chitosan in dilute solution. Carbohydr. Polym. 64, 29–36.
Renault, F., Sancey, B., Badot, P.-M., Crini, G., 2009. Chitosan for coagulation/flocculation processes–an eco-friendly approach. Eur. Polym. J. 45 (5), 1337–1348.
Søndergaard, M., Jensen, J.P., Jeppesen, E., 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506 (1-3), 135–145.
Shi, H., Ding, J., Timmons, T., Adams, C., 2012. pH effects on the adsorption of saxitoxin by powdered activated carbon. Harmful Algae 19, 61–67.
Sivonen, K., Jones, G., 1999. Cyanobacterial Toxins. Toxic Cyanobacteria in Water: a Guide to Their Public Health Consequences, Monitoring, and Management. London, E and FN Spon, UK, pp. 41–111.
Smith, V.H., Schindler, D.W., 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. 24 (4), 201–207.
Soares, M.C.S., Huszar, V.L., Miranda, M.N., Mello, M.M., Roland, F., Lürling, M., 2013. Cyanobacterial dominance in Brazil: distribution and environmental preferences. Hydrobiologia 717 (1), 1–12.
Spears, B.M., Meis, S., Anderson, A., Kellou, M., 2013. Comparison of phosphorus (P) removal properties of materials proposed for the control of sediment P release in UK lakes. Sci. Total Environ. 442, 103–110.
Steffensen, D., Burch, M., Nicholson, B., Drikas, M., Baker, P., 1999. Management of toxic blue–green algae (cyanobacteria) in Australia. Environ. Toxicol. 14 (1), 183–195.
Steffensen, D.A., 2008. Economic Cost of Cyanobacterial Blooms, Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer, pp. 855–865.
Stewart, I., Webb, P.M., Schluter, P.J., Shaw, G.R., 2006. Recreational and occupational field exposure to freshwater cyanobacteria–a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. Environ. Health 5 (1), 6.
Sun, F., Pei, H.-Y., Hu, W.-R., Ma, C.-X., 2012. The lysis of *Microcystis aeruginosa* in AlCl 3 coagulation and sedimentation processes. Chem. Eng. J. 193, 196–202.
Sun, F., Pei, H.-Y., Hu, W.-R., Li, X.-Q., Ma, C.-X., Pei, R.-T., 2013. The cell damage of *Microcystis aeruginosa* in PACl coagulation and floc storage processes. Sep. Purif. Technol. 115, 123–128.
Tsaih, M.L., Chen, R.H., 1997. Effect of molecular weight and urea on the conformation of chitosan molecules in dilute solutions. Int. J. Biol. Macromol. 20, 233–240.
Van Benschoten, J.E., Edzwald, J.K., 1990. Chemical aspects of coagulation using aluminum salts—I. Hydrolytic reactions of alum and polyaluminum chloride. Water Res. 24 (12), 1519–1526.
Van der Molen, D.T., Boers, P.C., 1994. Influence of Internal Loading on Phosphorus Concentration in Shallow Lakes Before and After Reduction of the External Loading, Nutrient Dynamics and Biological Structure in Shallow Freshwater and Brackish Lakes. Springer, pp. 379–389.
Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol. 31 (4), 233–239.
Waajen, G., van Oosterhout, F., Douglas, G., Lürling, M., 2016a. Management of eutrophication in Lake De Kuil (The Netherlands) using combined flocculant–Lanthanum modified bentonite treatment. Water Res. 97, 83–95.
Waajen, G.W., Van Bruggen, N.C., Pires, L.M.D., Lengkeek, W., Lürling, M., 2016b. Biomanipulation with quagga mussels (*Dreissena rostriformis bugensis*) to control harmful algal blooms in eutrophic urban ponds. Ecol. Eng. 90, 141–150.
Wang, Z., Li, D., Qin, H., Li, Y., 2012. An integrated method for removal of harmful cyanobacterial blooms in eutrophic lakes. Environ. Pollut. 160, 34–41.
Wu, C., Xu, X., Wang, Q., 2011. Enhanced coagulation for algae removal through the control of zeta potential with diatomite. Water Sci. Technol.: Water Supply 11 (2), 159–165.
Yang, Z., Gao, B., Yue, Q., 2010. Coagulation performance and residual aluminum speciation of $Al_2(SO_4)_3$ and polyaluminum chloride (PAC) in Yellow River water treatment. Chem. Eng. J. 165 (1), 122–132.
Yang, R., Li, H., Huang, M., Yang, H., Li, A., 2016. A review on chitosan-based flocculants and their applications in water treatment. Water Res. 95, 59–89.
Younes, I., Hajji, S., Frachet, V., Rinaudo, M., Jellouli, K., Nasri, M., 2014. Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. Int. J. Biol. Macromol. 69, 489–498.
Zou, H., Pan, G., Chen, H., Yuan, X., 2006. Removal of cyanobacterial blooms in Taihu Lake using local soils II. Effective removal of *Microcystis aeruginosa* using local soils and sediments modified by chitosan. Environ. Pollut. 141 (2), 201–205.