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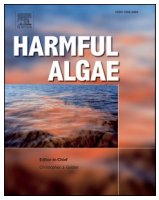

# Temporal and spatial variation in the efficiency of a Floc & Sink technique for controlling cyanobacterial blooms in a tropical reservoir

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ABSTRACT

One of the main symptoms of eutrophication is the proliferation of phytoplankton biomass, including nuisance cyanobacteria. Reduction of the external nutrient load is essential to control eutrophication, and in-lake interventions are suggested for mitigating cyanobacterial blooms to accelerate ecosystem recovery. Floc & Sink (F&S) is one such intervention technique that consists of applying a low dose of coagulants in combination with ballasts for removing cyanobacteria biomass. It is especially suitable for deep lakes with an external nutrient load that is higher than the internal load and suffers from perennial cyanobacterial bloom events. Studies showing the efficacy of the F&S technique have been published, but those testing its variation in efficacy with changes in the environmental conditions are still scarce. Therefore, we evaluated the efficiency of the F&S technique to remove cyanobacteria from water samples collected monthly from two different sites in a deep tropical reservoir (Funil Reservoir, Brazil) in the laboratory. We tested the efficacy of two coagulants, chitosan (CHI) and poly-aluminum chloride (PAC), alone and in combination with lanthanum-modified bentonite (LMB) in settling phytoplankton biomass. We hypothesized that: i) the combined treatments are more effective in removing the algal biomass and ii) the efficiency of F&S treatments varies spatially and monthly due to changes in environmental conditions. The combined treatments (PAC + LMB or CHI + LMB) removed up to seven times more biomass than single treatments (PAC, CHI, or LMB). Only the treatments CHI and LMB + CHI differed in efficiency between the sites, although all treatments showed significant variation in efficiency over the months at both the sampling sites. The combined treatments exhibited lower removal efficacy during the warm-rainy months (October–March) than during the mild-cold dry months (April–September). At high pH (pH > 10), the efficiency of the CHI and LMB + CHI treatments decreased. CHI had lower removal efficiency when single-cell cyanobacteria were abundant, while the combined treatments were equally efficient regardless of the morphology of the cyanobacteria. Hence, the combination of PAC as a coagulant with a ballast LMB is the most effective technique to precipitate cyanobacteria under the conditions that are encountered around the year in this tropical reservoir.

## 1. Introduction

Cultural eutrophication is considered the primary reason for the degradation of water quality in inland and coastal waters worldwide (Smith et al., 1999). The substantial proliferation of cyanobacteria is a key symptom of eutrophication (Huisman et al., 2018; Paerl et al., 2011), and records of the frequency and magnitude of cyanobacterial blooms have increased across the globe (Mantzouki et al., 2016; O'Neil et al., 2012; Paerl, 2018; Paerl and Paul, 2012). Cyanobacterial blooms compromise fishing, aquaculture, and drinking water production. In addition, they threaten public health because cyanobacteria may produce toxins (Díez-Quijada et al., 2019; Pearl and Paul, 2012; Steffensen,

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2008). Therefore, it is crucial to control eutrophication and mitigate cyanobacterial blooms (Lürling et al., 2016, 2020).

The first logical step in controlling eutrophication and cyanobacterial blooms is to reduce the external nutrient inputs (Cooke et al., 2005; Hilt et al., 2006; Huisman et al., 2018; Paerl et al., 2014). Although controlling the external load is straightforward, over 80% of all wastewater around the globe is still discharged without treatment (WWAP, 2017). However, even if external nutrient inputs are successfully reduced, rapid improvements in water quality may not occur (Fastner et al., 2016; Sas, 1990) due to a high internal phosphorus (P) load from the P that has been accumulated in the sediment from decades of excessive external nutrient input (Jeppesen et al., 1991; Schindler and Hecky, 2009; Søndergaard et al., 2001). Therefore, in-lake interventions have become increasingly necessary to either bring real-time relief from cyanobacterial blooms or to accelerate recovery (Lürling and Mucci, 2020). In-lake measures that focus on coagulation and precipitation of cyanobacteria and/or phosphate are promising tools for the reduction of cyanobacterial blooms (Lürling et al., 2020 a). For instance, the so-called Floc & Lock technique consists of applying a combination of a coagulant to flocculate the particulate P (Floc) and a solid phase P sorbent to lock the phosphate (Lock) into the sediment (Lürling and van Oosterhout, 2013). In this technique, different coagulants compounds can be used such poly-aluminium chloride (PAC) (Pan et al., 2011a), which have already demonstrated effectiveness in changing the water from turbid to a clear state by removing P and cyanobacteria cells from the water column, and also reduce mobile P in the sediment (Cooke et al., 2005; page 68 in Butusov and Jernelöv, 2013). However, at pH <5.5 any aluminium based coagulant can form toxic aluminium species and because Al hydrolysation may lower pH, care has to be taken when applying Al coagulants (Cooke et al., 2005). Therewith, the coagulant chitosan (CHI) (biopolymer derived from marine shrimps and crabs), has been presented as an alternative to the Al-based coagulants, given that it is a non-toxic and biodegradable material (Renault et al., 2009), and has been widely used in wastewater treatment, however, the CHI is more expensive than PAC (Granados et al., 2012; Lürling., et al. 2020 b). CHI has been tested with good results when combined with a ballast compound in settling the cyanobacterial biomass (Zou et al., 2006), although, the damage in membrane cells of cyanobacteria has already been reported for a few species (Mucci et al., 2017). The solid phase P sorbent or ballast, like lanthanum- modified bentonite (LMB), has been widely applied in water systems around the world (Copetti et al., 2016) given to its strong P binding capacities (Douglas et al., 2016; Lürling et al., 2016), on the other hand, in low-income countries, it can be considered an expensive measure if verified the necessity of repeating the application (van Oosterhout et al., 2020, 2022).

Floc & Lock is considered a good strategy in deep and stratified lakes, where the internal P load is the main reason for recurrent cyanobacterial blooms (Lürling et al., 2020 a; van Oosterhout et al., 2020, 2022). Whole-lake Flock & Lock treatments have resulted in a multiyear post-application absence of cyanobacterial blooms (van Oosterhout et al., 20202022; Waajen et al., 2016). However, in deep, stratified systems where the ongoing external nutrient input is much larger than the internal P load, the use of a solid phase P sorbent is less feasible, and a Floc & Sink (F&S) technique seems to be a more suitable approach (Lürling et al., 2020 a; Noyma et al., 2016). The F&S technique aims to remove cyanobacteria effectively from the water column to the sediment in a colder and darker hypolimnion, without the concern regarding cell resuspension (Waajen et al., 2016). The F&S technique only considers the necessary coagulant and ballast (which may be a local soil) concentration for the sedimentation of the cyanobacteria biomass (Noyma et al., 2017). The F&S technique has also proven effectiveness in shallow waters. For example, in Liaoyangyuan Bay (North Taihu Lake, China), a cyanobacterial bloom was efficiently sunk to the bottom of the lake by adding a coagulant and local soil, thereby improving water clarity, and yielding the establishment of submerged macrophytes (Pan et al., 2006, 2011a, b).

In Brazil, recent studies have shown that Floc & Lock/Sink technique is efficient in flocculating and sinking the positively buoyant natural cyanobacteria community from the Funil Reservoir (RJ), a deep and stratified system, using a combination of poly-aluminum chloride (PAC) or chitosan (CHI) with lanthanum-modified bentonite (LMB) or local red soil (LRS) in *ex situ* experiments (Noyma et al., 2016, 2017). In a laboratory experiment, cyanobacteria from a shallow system (Jacarepaguá lagoon, RJ) sank effectively as a result of the addition of PAC solely, while the efficiency improved significantly when PAC was combined with LMB (De Magalhães et al., 2017, 2019). In addition, laboratory experiments with cyanobacteria community of a shallow lake in the park of the Mariano Procópio Museum (MAPRO, MG) showed the efficiency of PAC to reduce the cyanobacteria biomass without the addition of a ballast (Miranda et al., 2017). Similar results were observed in F&S experiments using natural water samples from a deep reservoir (Argemiro de Figueiredo, RN), where highly efficient removal of cyanobacteria was achieved using only coagulants, without the need to use a ballast as well (de Lucena-Silva et al., 2019). Although all these studies tested the efficiency of various treatments, yet only with limited emphasis on environmental factors that could influence coagulation and settling efficacies.

Species-specific characteristics can affect the efficiency of coagulants when they are used alone (Lama et al., 2016) or when they are combined with a ballast (Miranda et al., 2017). Miranda et al. (2017) observed that the efficacy of the coagulant PAC or CHI varied depending on the species of cyanobacteria present in the water body and the pH of the water. CHI is less efficient at elevated values of pH and alkalinity (Lürling et al., 2017). Coagulation can also be influenced by water temperature because coagulation is faster at warmer temperatures due to the positive influence on the floc size formation (Xiao et al., 2008). The cyanobacteria biomass concentration can also influence the removal efficacy as for instance, more ballast is needed when the biomass is higher (Noyma et al., 2017). Inasmuch as all these variables may change over the year, the efficacy of the F&S treatment may also vary accordingly.

Although experiments attesting to the efficacy of the F&S technique have been published, studies testing its variation efficacy with environmental conditions are still scarce. To understand how the efficacy of F&S treatments may vary with environmental conditions, we performed year-round monthly standard F&S assays with water collected from two sites in a eutrophic and deep reservoir (Funil, southeastern Brazil). The spatial and temporal variations in the environmental conditions of this reservoir have already been reported in previous studies (Pacheco et al., 2015; Rangel et al., 2012; Soares et al., 2009). We hypothesized that: i) combined treatments are more effective in removing the algal biomass and ii) the efficiency of F&S treatment varies spatially and monthly due to changes in the environmental conditions.

## 2. Material and methods

### 2.1. Site description

The Funil Reservoir (Fig. 1) is located in the southern region of the state of Rio de Janeiro, Brazil (22° 30′ S; 44° 45′ W), at an altitude of 440 m. The reservoir is 40 $km^2$ in area, has an average depth of 22 m, a maximum depth of 77 m (close to the dam), a total volume of 890 $\times$ $10^6$ $m^3$, and has a water residence time of 25 to 80 days, regulated by power generation and precipitation (Soares et al., 2009). It is a eutrophic system with frequent cyanobacterial blooms and chlorophyll-a concentrations peaking above 500 μg $L^{-1}$ (Soares et al., 2009) and total phosphorus concentrations above 40 μg $L^{-1}$ (Rangel et al., 2012). The region is mainly characterized by two climatic periods: warm-rainy (October to March) and mild-cold dry (April to September) (Barreto, 2020). In addition, seasonal fluctuations are generally observed in limnological variables and cyanobacteria biomass (Soares et al., 2008, 2009). The highest cyanobacteria biomass is observed during the warm-rainy periods (September to February), with the cyanobacteria

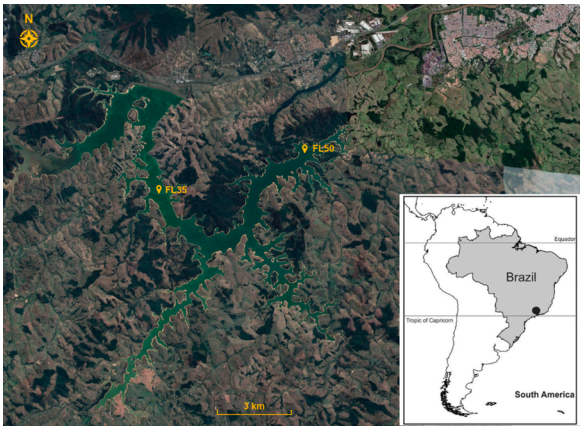


**Fig. 1.** Map and location of the Funil Reservoir showing the two sampling sites, FL35 and FL50. Source: Google Earth.

species *Microcysti aeruginosa, Dolichospermum circinalis*, and *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*) contributing the most toward the biomass amount (Rangel et al., 2016; Soares et al., 2012).

### 2.2. Sampling

Water samples were taken monthly, from February 2015 to January 2016 in two sampling sites (FL35 and FL50), located in the intermediate and limnetic zone respectively (Soares et al., 2008). Water temperature, conductivity, dissolved oxygen concentration, and pH were measured in the field using a multiparametric probe (YSI model 600 QS). Water transparency was estimated using the Secchi disk (SD), and the depth of the euphotic zone was calculated as SD × 2.7. Although the conversion coefficient used to calculate the euphotic zone can vary according to the changes in optical parameters in the water (Luhtala and Tolvanen, 2013), the coefficient of 2.7 is widely used to estimate the thickness of the euphotic zone in freshwater systems with different trophic conditions in phytoplankton ecology (Cole, 1994; de Magalhães et al., 2020; Soares et al., 2008; Zhu et al., 2013). Adding to this assumption, using the coefficient of 2.7 allows a comparison of water transparency observed in our experiments and changes in the euphotic zone observed in other phytoplankton studies (which commonly use the same "2.7″ coefficient). At each site, samples of subsurface water (0.5 m) were collected to analyze the nutrients, biomass of cyanobacteria, and eukaryotic algae. Phytoplankton samples were fixed with Lugol's solution. Also, five liters of water were collected from the subsurface of each sampling site to perform the laboratory Floc & Sink assays.

### 2.3. Sample analyses

The dissolved inorganic fractions of P (soluble reactive phosphorus, SRP) and N (nitrite, nitrate, and ammonium) and the total forms of P (TP) and N (TN) were analyzed by an automated colorimetric flow injection analysis (FIA) system equipped with an autosampler (model FIAlab-2500, FIALab Instruments Inc., Seattle, Washington, USA). The measurements were taken according to the manufacturer's protocols. Samples for dissolved nutrients were filtered through GF-3 filters (Macherey-Nagel). Samples for total nutrients were first digested with potassium persulfate and then analyzed as SRP and nitrate (Gross and Boyd, 1998). The dissolved inorganic nitrogen (DIN) was considered as the sum of $NO_2^-$-N, $NO_3^-$-N, and $NH_4^+$-N. Cyanobacteria and eukaryotic chlorophyll-a concentrations (μg $L^{-1}$) were determined using a PHYTO-PAM phytoplankton analyzer (HeinzWalzGmbH, Effeltrich, Germany).

Phytoplankton populations (organisms $mL^{-1}$) were estimated by the settling technique (Utermöhl 1958) under an inverted microscope (Zeiss Oberkochen Axiovert 10, Germany). Phytoplankton units (cells, colonies, and filaments) were enumerated in random fields (Uehlinger 1964) to at least 100 specimens of the most frequent species (Lund et al., 1958). Biovolume ($mm^3$ $L^{-1}$) was estimated from the product of the population density and the mean unit volume of each species (Hillebrand et al., 1999). We grouped cyanobacteria populations in the biovolume of the life forms (single cells, colonies, and filaments). The species of cyanobacteria considered here were those that contributed at least 2% to the total biovolume of the phytoplankton community.

### 2.4. Chemicals

The coagulant PAC (poly-aluminum chloride; $Al_n(OH)_mCl_{3n-m}$, $\rho \approx 1.37$ kg $L^{-1}$, 8.9% Al, 21.0% Cl) was obtained from Pan-Americana SA (Rio de Janeiro, Brazil) and chitosan (CHI), made of shrimp shells, was obtained from Polymar Ciência e Nutrição S/A (Ceará, Brazil). CHI was acidified with 1% hydrochloric acid before its use (Pan et al., 2006). Lanthanum-modified bentonite (LMB), Phoslock®, was obtained from HydroScience (Porto Alegre, Brazil) and was used as ballast.

### 2.5. Floc & Sink (F&S) assays

F&S assays were performed to evaluate the effects of coagulants and ballast on the sedimentation of phytoplankton biomass. Aliquots of 60 mL of water samples from both the sampling sites (FL35 and FL50) in the Funil Reservoir were transferred into 75 mL glass tubes (25 × 200 mm) and five different treatments were applied in triplicate: coagulants (CHI or PAC) and modified clay (LMB) alone, their respective combinations (LMB + CHI and LMB + PAC), and the untreated tubes (control). The doses of CHI (2 mg $L^{-1}$), PAC (2 mg Al $L^{-1}$), and LMB (400 mg $L^{-1}$) were based on previous work, in which the coagulant and ballast were tested in a single sampling of water from the Funil Reservoir (Noyma et al., 2016). After the addition of the treatments, the suspensions were homogenized and incubated in the laboratory at 25 °C under stagnant conditions.

After two hours of incubation, using a pipette, we withdraw 5 ml of sub-surface water from the top of each test tube extracting all the biomass that was flocculated in the sub-surface on the top of the tubes. We analyzed the total phytoplankton chlorophyll-a (μg $L^{-1}$) and photosynthetic efficiency (ΦPSII) of the samples using the PHYTO-PAM. Subsequently, the pH was measured using a digital pH meter (Edge Instruments, Hanna, USA). ΦPSII can be used as an indicator of stress in the photosynthetic apparatus (Parkhill et al., 2001) and can be used to determine whether the effect of the treatments can cause damage and cell lysis of cyanobacteria (Mucci et al., 2017).

The biomass removal efficiency (%) for each treatment was calculated by comparing the total chlorophyll-a concentration in the samples taken from the top of the treated test tubes and the untreated or control tubes (Eq. (1)). In the cases where we observed higher biomass in the samples taken from the top of the treated tubes than in the control, we bound the efficiency values to zero, as these indicated no biomass removal.

$$Removal\ efficiency\ (\%) = \left(\frac{control - treatment}{control}\right) * 100 \quad (1)$$

### 2.6. Statistical analyses

To evaluate the hypotheses, data were analyzed using linear mixed models (LMM) in a Bayesian framework through the R package "brms" (Bürkner, 2017; Carpenter et al., 2017). The models were run in four chains, each with 1000 iterations, a warm-up (i.e., calibration) of 1000 iterations, and weakly informative priors. For inference, we used the high-density interval (HDI) of the posterior distributions of the model parameters, considering 95% credible intervals (CI) that were estimated using Hamiltonian Monte Carlo (HMC) estimation. Model convergence

was visually checked by inspecting diagnostic plots and using the potential scale reduction statistic (Gelman et al., 2013). The significance of the explanatory variables was based on the HDI, along with the region of practical equivalence (ROPE, Kruschke and Liddell, 2018).

$$ROPE = \mp 0.1 \times sd(y)$$

To answer which treatment removed the highest amount of algal biomass when compared to the control, we employed a univariate model following a lognormal family distribution (LMM 1, Table 1). The total chlorophyll-a from the top of the test tubes at the end of the experiments was used as a dependent variable and the identity of each treatment was used as the fixed explanatory variable. To compare the treatments, we subtracted the HDI of chlorophyll-a between pairs of treatments, and we considered it significant if 95% of the differences were outside the ROPE. We included the variables months and sampling sites as random effects to control for the temporal and spatial variance in the model responses.

To answer whether there was a monthly variation in the removal efficiency and whether this variation was different between the sites, we employed a second model (LMM 2, Table 1). We first scaled the removal efficiency between zero and one, where zero denoted no biomass removal and one denoted complete biomass removal. Then, we employed a multivariate response model, following a zero-one-inflated beta distribution and using all the treatments simultaneously as the response variable, to control for the covariance among the treatment efficiencies over the months and sampling sites. Here, we included months and sampling sites as fixed effects to explicitly test the differences in the treatment efficiency on a spatial and temporal basis.

To investigate the effects of in-reservoir water quality variables and the effects of the cyanobacteria life forms on the treatment efficiency, we used two multivariate response models that followed a zero-one-inflated beta distribution. In this case, the efficiency of the five treatments (CHI, PAC, LMB, CHI + LMB, and PAC + LMB) were included as response variables. In the third model (LMM 3, Table 1), the explanatory variables were the in-reservoir total chlorophyll-a concentration, temperature, dissolved oxygen concentration, pH, alkalinity, conductivity, DIN, SRP, TN, and TP. In the fourth model (LMM 4, Table 1), the explanatory variables were the biovolume of single cells, filaments, and colonies. In both the models (LMM 3 and LMM 4), months and sampling sites were kept as random effects to control the spatial and temporal variation in the efficiency.

**Table 1**
Description of the four Bayesian linear models used in this study. The models are described according to their dependent, independent, and grouping variables. The table also shows what were the priors assigned to each model, along with the distribution family used for each model.

| | LMM 1 | LMM 2 | LMM 3 | LMM_4 |
|---|---|---|---|---|
| Dependent variable | Chlorophyll-a from the top of the test tubes at the end of the experiments (univariate model) | Removal efficiency (univariate model) | Removal efficiency (multivariate model) | Removal efficiency (multivariate model) |
| Independent variable | Treatment identity | Month + Sites | In-reservoir total chlorophyll-a concentration + temperature + dissolved oxygen concentration + pH + alkalinity + conductivity + DIN + SRP + TN + TP | biovolume of single cells + biovolume of filaments + biovolume of colonies |
| Grouping variable | Variable Intercept for each site in each month | — | Variable Intercept for each site in each month | Variable Intercept for each site in each month |
| Priors | Default | Default | Default | Default |
| Model family | Hurdle lognormal | Zero-one-inflated beta distribution | Zero-one-inflated beta distribution | zero-one-inflated beta distribution |

## 3. Results

### 3.1. In-reservoir water quality variables

During the study period, the surface water temperature at FL35 and FL50 ranged between 21.6 °C and 31.9 °C (Table 2). The average dissolved oxygen concentration was 8 mg $L^{-1}$ at the two sampling sites; however, low values were observed in May. The mean depth of the euphotic zone was approximately 5 m at both sites. The pH values were generally circumneutral, but in some months we observed pH far above 9.0 reaching extreme values of pH 11.5 and 12.0 (Table 2), in general in the months when cyanobacteria were dominant. Alkalinity showed a mean value of 530.4 μEq $L^{-1}$ at FL35 and 516.3 μEq $L^{-1}$ at FL50. Conductivity at FL35 and FL50 were 114.1 μS $cm^{-1}$ and 113.3 μS $cm^{-1}$, respectively. Overall, we found relatively high concentrations of nitrate (mean > 450 μg $L^{-1}$) at both the sites, low concentrations of nitrite (≈ 10 μg $L^{-1}$), low concentrations of ammonium (≈ 30 μg $L^{-1}$), and low concentrations of soluble reactive phosphorus (mean 9.3 and 15.2 μg $L^{-1}$), whose concentrations remained below the detection limit of the method (< 3.0 μg $L^{-1}$) during some of the months. The total chlorophyll-a concentrations were the highest in April and September at FL35 (19.4 μg $L^{-1}$) and FL50 (25.5 μg $L^{-1}$), respectively.

### 3.2. Phytoplankton biomass and life-forms of cyanobacteria in the reservoir

In general, we observed lower total phytoplankton biomass at FL50 over the year compared to FL35 (Fig. 2a). A greater contribution of cyanobacteria biomass (chlorophyll-a), compared to eukaryotic algae, was observed at FL35 in February and March 2015 and from November 2015 to January 2016. At FL50, the cyanobacteria biomass was higher than eukaryotic algae only from October to December 2015 (Fig. 2a). During the other months, eukaryotic algae were dominant, with the

**Table 2**
Average, minimum and maximum values of limnological variables, measured from February (2015) to January (2016), at the two sampling sites in the Funil Reservoir (FL35 and FL50). Temp = water temperature, OD = dissolved oxygen, $z_{eu}$ = euphotic zone, Alk = alkalinity, Cond = electrical conductivity, nitrite = $N\text{-}NO_2^-$, nitrate = $N\text{-}NO3^-$, ammonium = $N\text{-}NH_4^-$, SRP = soluble reactive phosphorus, TP = total phosphorus, TN = total nitrogen, and Chl-*a* = total Chlorophyll-a.

| | FL35 Average (minimum–maximum) | FL50 Average (minimum–maximum) |
|---|---|---|
| Temp ( °C) | 26.2 (21.7 – 30.4) | 26.0 (21.6 – 31.9) |
| OD (mg $L^{-1}$) | 8.5 (2.6 – 14.6) | 8.1 (2.3 – 12.2) |
| $z_{eu}$ | 5.3 (2.0 – 11.2) | 5.5 (2.2 – 9.5) |
| pH | 7.91 (5.8 – 11.5) | 7.9 (6.1 – 12.0) |
| Alk (μEq $L^{-1}$) | 530.4 (397.2 – 935.0) | 516.3 (371.9 – 786.5) |
| Cond (μS $cm^{-1}$) | 114.1 (92 – 133) | 113.3 (86 – 134) |
| N-NO2$^-$ (μg $L^{-1}$) | 10.7 (2.4 – 31.8) | 9.2 (1.7 – 18.7) |
| N—NO3$^-$ (μg $L^{-1}$) | 459.4 (183.8 – 963.4) | 557.6 (209.1 – 919.9) |
| N-NH4$^-$ (μg $L^{-1}$) | 30.3 (5.7 – 73.3) | 29.2 (6.2 – 57.0) |
| SRP (μg $L^{-1}$) | 15.2 (< 3 – 49.2) | 9.3 (< 3 – 38.3) |
| TP (μg $L^{-1}$) | 56.4 (8.5 – 146.8) | 36.5 (7.0 – 78.0) |
| TN (μg $L^{-1}$) | 1302.2 (528.6 – 2325.4) | 1331.9 (348.4 – 2084.9) |
| Chl-a (μg $L^{-1}$) | 9.8 (3.1 – 19.4) | 8.75 (2.7 – 25.5) |

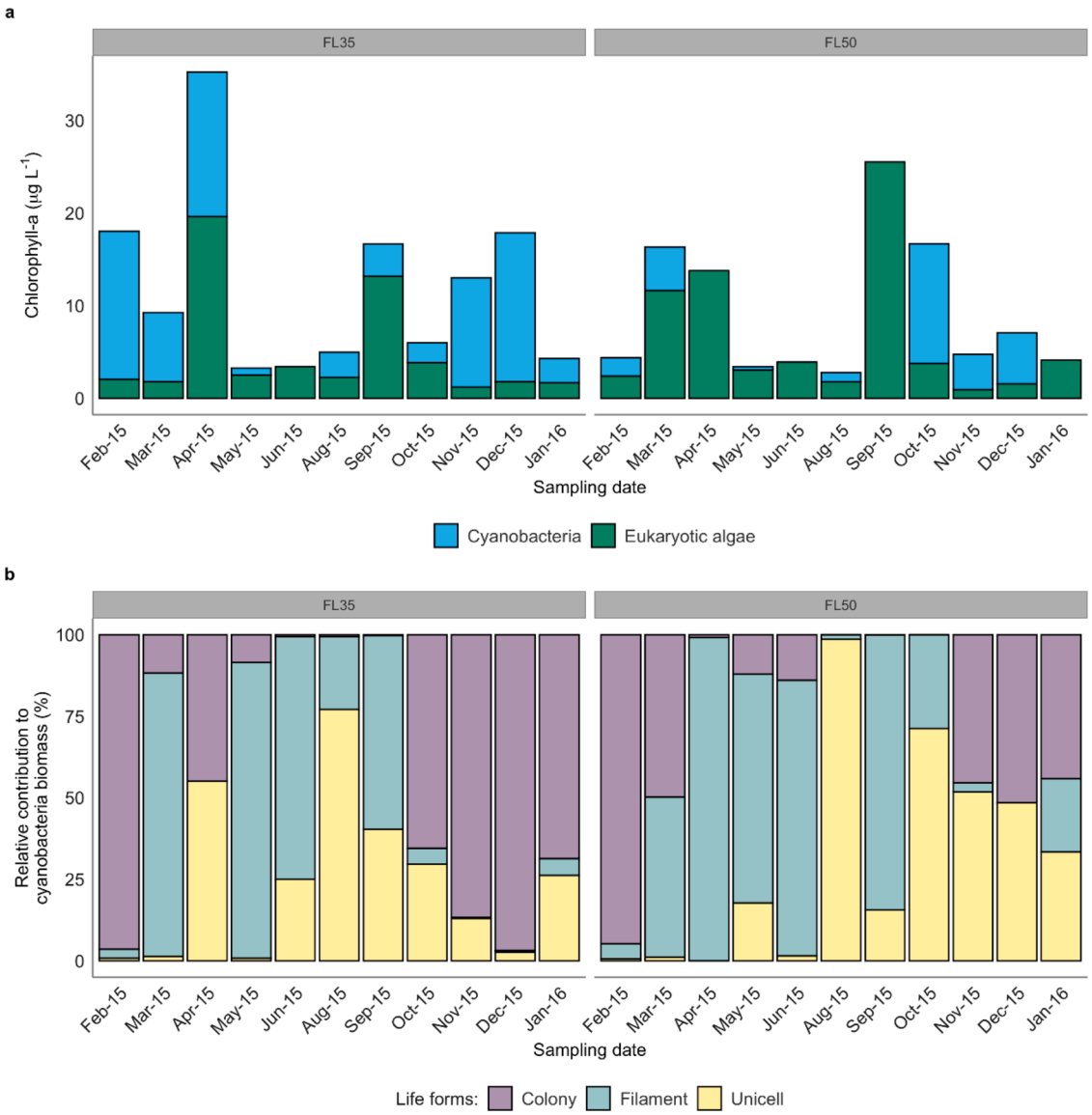


**Fig. 2.** The biomass of the phytoplankton community of the Funil Reservoir in the two sampling sites (FL35 and FL50) along the sampling months: a) chlorophyll-a concentrations of cyanobacteria and eukaryotic algae; b) relative contribution of cyanobacteria life-forms to the total cyanobacteria biomass.

highest biomass at FL50 occurring in September (25.5 µg $L^{-1}$). Regarding the life forms of cyanobacteria, high contribution of filaments (*Dolichospermum circinalis* and *Raphidiopsis raciborskii*) and unicells (mainly *Microcystis* spp.) occurred at both the sites during most months in the mild-cold dry period (March–September), whereas during the warm-rainy period (October–February), colonies of *M. aeruginosa* were dominant at both the sites (Fig 2b).

### 3.3. Floc & Sink assays

First, we tested which treatment removed the highest amount of phytoplankton biomass when compared to the control. After controlling for the variations among sampling sites and months, the Bayesian LMM 1 ($R^2$ = 0.62) indicated that the PAC, LMB + PAC, and LMB + CHI treatments removed a significant amount of the chlorophyll-a from the top of the tubes (Fig. 3a, Table 3). The LMB + PAC treatment removed the highest amount of biomass compared to the control, followed by the LMB + CHI and PAC treatments. The LMB + PAC treatment had chlorophyll-a values closer to zero (mean = 2.22 µg $L^{-1}$, 95% CI [0.86, 5.25]) by the end of the experiment. Pairwise comparisons showed that LMB + PAC, LMB + CHI, and PAC treatments differed significantly from each other, reinforcing that LMB + PAC has the best potential for biomass removal (Fig. 3b).

We also tested whether there was any variation in the efficiency of chlorophyll-a removal (hereinafter efficiency) across the two sampling sites over the months (LMM 2). All treatments showed significant variation in efficiency over the months at both the sampling sites (Fig. 4; Table S1); however, the model explained most of the variance observed in the case of the combined treatments (PAC + LMB and CHI + LMB) (Table 4). There was a significant decrease in the efficiency of the combined treatments during February, April, and November (only LMB + CHI) as shown in Fig. 4. However, the combined treatments (PAC + LMB and CHI + LMB) had the highest efficiency irrespective of the sampling site and during most of the months (Fig. 4). Regarding the spatial variation, only CHI and LMB + CHI differed in efficiency between the sites (Table S2), with a higher efficiency observed at FL50 (Fig. 4; Table S1). Therefore, the combined treatments showed significant variation in efficiency over the months at both the sampling sites. A decrease in efficiency was observed during some months that were mostly associated with the warm-rainy months (February, April, and November).

Regarding the effects of the environmental conditions on the

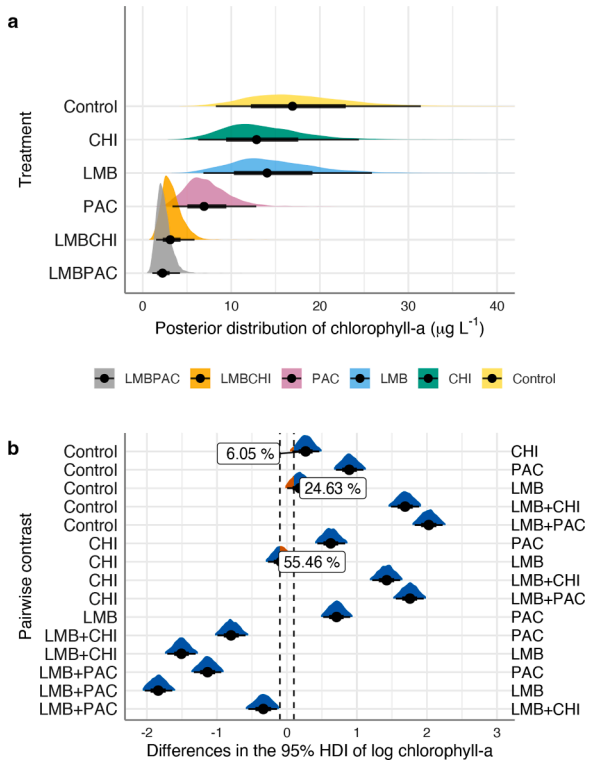


**Fig. 3.** Posterior distributions of the Lognormal Bayesian linear mixed model testing the differences in chlorophyll-a in the top of the test tubes among the treatments (LMM 1): (a) the posterior distributions of the chlorophyll-a (μg $L^{-1}$) of the control and treatments (CHI, chitosan; PAC, poly-aluminium chloride; LMB, lanthanum-modified bentonite; LMB + CHI; LMB + PAC); b) the density plots show the distribution of the differences between $\log_e$ of the posterior distributions of the chlorophyll-a among pairs of treatments (left column minus right column). Negative values indicate that the treatment in the right side of the plot has more chlorophyll-a than the treatment in the left side of the plot. Positive values indicate that the treatment in the left side of the plot has more chlorophyll-a than the treatment in the right side of the plot. Two treatments are equivalent if the differences in high-density interval (HDI) fall within the region of practical equivalence (ROPE, dashed lines). Dots represent the median difference and bars 95% credible intervals. Numbers in the white boxes show the proportion of differences within the ROPE.

**Table 3**
Coefficients of the Lognormal Bayesian linear mixed models testing the differences in chlorophyll-a (μg $L^{-1}$) in the top of the test tubes among the treatments (LMM 1). Because the model followed a lognormal distribution, the parameters are given on the log scale. The estimates of the fixed effects show the median and standard error (SE) of the posterior distribution of chlorophyll-a for the control and the differences between the median of control and each treatment. The lower and upper 95% show the boundaries of the high density interval (HDI). The estimate of the random effects shows the standard deviation (SD) of the chlorophyll-a at the controls along with months and sites.

| Treatments | Estimate (SE) | Lower 95% | Upper 95% |
|---|---|---|---|
| *Fixed effects* | | | |
| Control | 2.82 (0.34) | 2.11 | 3.45 |
| CHI | -0.27 (0.11) | -0.50 | -0.05 |
| PAC | -0.89 (0.12) | -1.12 | -0.66 |
| LMB | -0.18 (0.12) | -0.41 | 0.04 |
| LMB+CHI | -1.69 (0.12) | -1.92 | -1.45 |
| LMB+PAC | -2.02 (0.12) | -2.26 | -1.79 |
| *Random effects* | | | |
| SD(Control) months | 0.89 (0.19) | 0.60 | 1.35 |
| SD(Control) sites | 0.30 (0.16) | 0.13 | 0.69 |

efficiency of combined treatments (LMM 3), LMB + PAC had the highest explained variance ($R^2 = 0.88$; Table 4), and it was significantly related to the smallest number of variables, namely, total chlorophyll-a and pH (Table 5). After incubation, all treatments remained at a pH value above 7 for the entire period, despite a small decrease in pH when comparing the treatments to the control (Fig. S1). There was no important reduction in ΦPSII in the treatments when compared to the control. In addition, the average values of reduction in the ΦPSII were never lower than 0.2, indicating no damage to the cells (Table S3). Overall, there was strong evidence (95% support) that total chlorophyll-a positively affected all the treatments, whereas pH had a negative effect on most of the treatments (Table 5). However, LMB + CHI was less affected, and with 83% support [CI: -0.103, -0.02], the model showed that pH had a negative effect. In addition, DO, conductivity, and SRP also showed significant effects on some of the treatments (Table 5).

Finally, we also tested the effects of the cyanobacteria life forms on the removal efficiency of the treatments (LMM 4). Overall, the variance explained by the life forms was the highest for the combined treatments (Table 4). The results of the model suggest, with 92% support, that the efficiency of LMB + PAC (92% CI: 0.14, 2.83) was not affected by the life forms. The biomass of cyanobacteria unicells had a significant negative effect on the efficiency of the CHI treatment, whereas the higher biomass of colonial species increased CHI removal efficiency (Table 6). The LMM 4 model also showed, with 87% support, that higher unicellular biomass had negative effects on the LMB + CHI treatment (87% CI: −0.74, −0.03). In summary, cyanobacteria life forms did not affect LMB + PAC, but the CHI and LMB + CHI treatments were negatively affected by the presence of unicells.

## 4. Discussion

The high efficiency of biomass removal can be viewed as a successful application of a F&S treatment. Our study provided evidence that the combined treatments (LMB + PAC and LMB + CHI) had the highest efficiency and thus were more effective in removing biomass than single compound treatments (CHI, PAC, or LMB). Moreover, the efficiency of combined treatments differed in time and, to a lesser extent, between the sampling sites, mainly because of altered limnological variables and/or the composition of cyanobacterial life forms. These findings highlight the importance of understanding the variation in the environmental conditions over the year, and how they can affect the efficiency of a proposed F&S intervention.

Our monthly experiment scenario showed that the treatments combining a low dose of a coagulant with a ballast, especially LMB + PAC, removed the highest amount of chlorophyll-a. These results confirm the efficacy of combined treatments in removing cyanobacteria biomass, as reported in previous studies for the Funil Reservoir (Arruda et al., 2021; Noyma et al., 2016, 2017), Jacarepaguá lagoon (de Magalhães et al., 2017, 2019), an urban ornamental lake (Miranda et al., 2017), and in the first recorded field application in Lake Rauwbraken (Lürling and Oosterhout, 2013; van Oosterhout et al., 2020, 2022). Although both the combined treatments had the highest performance, they showed different biomass removal efficiencies. LMB + CHI treatment yielded a lower efficacy when compared to LMB + PAC. This outcome was different from the results of a previous study on the Funil Reservoir, which reported no differences in the biomass removal efficiencies of the LMB + CHI and LMB + PAC treatments (Noyma et al., 2016). This disagreement can be considered an artifact of choosing the cheapest treatment (PAC) or an environmentally friendly alternative (CHI), without testing its efficiency over time in the system of interest.

The treatment LMB + PAC showed the best cyanobacterial removal, however, the use of PAC has been questioned by its possible toxic effect on human health (Li and Pan, 2013; Renault et al., 2009). The toxicity of aluminium depends on the pH of the water (Stumm and Morgan 1996) since, $Al^{3+}$ species can be formed in pH lower than 5.5 (Driscoll and Schecher 1990; Gensemer and Playle 1999), but PAC can be considered

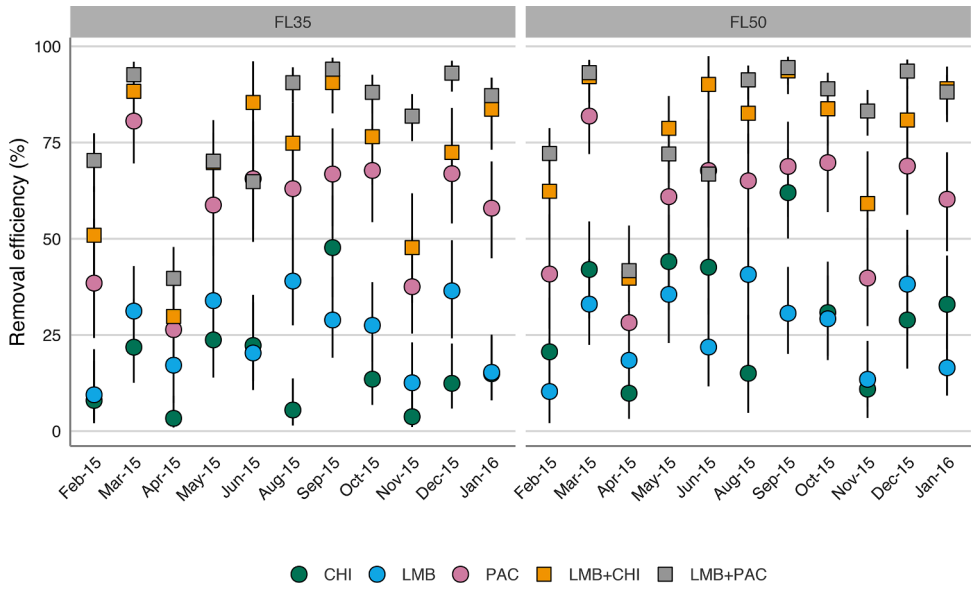


**Fig. 4.** Percentage of the monthly biomass removal efficiency (chlorophyll-a) for each treatment between February 2015 to January 2016 at FL35 and FL50 (LMM 2). Symbols represent the sample estimates (average of the efficiency based on posterior distributions). The isolated treatments are represented by circles while the combined treatments are represented by squares. Error bars are 95% credible intervals for the means.

**Table 4**
The explained variance ($R^2$) of each treatment for each of the multivariate Bayesian Linear Mixed Models. LMM 2 tested if the efficiency of treatments varies monthly and spatially, whereas LMM 3 and 4 tested the effect of the environmental conditions (in-reservoir abiotic variables + total chlorophyll-a and phytoplankton life-forms respectively) on the efficiency of treatments.

| Treatment | LMM 2 | LMM 3 | LMM 4 |
|---|---|---|---|
| CHI | 0.45 | 0.89 | 0.45 |
| LMB | 0.27 | 0.66 | 0.21 |
| PAC | 0.40 | 0.73 | 0.42 |
| LMB+PAC | 0.67 | 0.88 | 0.64 |
| LMB+CHI | 0.65 | 0.59 | 0.62 |

safe in pH ranging from 6 to 8 (Cooke et al., 2005; Jančula and Maršálek, 2011). In our experiment the pH of Funil reservoir remain alkaline for all months, therefore, no forming toxic Al species in the water was expected in the treatments with PAC.

The environmental friendliness of coagulants such as chitosan is a "biodegradable, non-toxic material" (Renault et al., 2009), and has been considered an option for the use of metal-based coagulants. Although LMB + CHI treatment yielded a lower efficacy when compared to LMB + PAC, it seems a good option for the removal of cyanobacteria biomass in the Funil Reservoir (Noyma et al., 2016). However, the use of CHI as a coagulant might be of concern in drinking water reservoirs because of its antibacterial, antimicrobial, and antifungal properties (Kong et al., 2010; Younes et al., 2014). CHI can damage the membranes of cyanobacteria, leading to cell death, which manifests as a strong reduction in the ΦPSII and a release of chlorophyll-a and toxins into the water (Miranda et al., 2017; Mucci et al., 2017). Inasmuch as we did not observe such changes in the ΦPSII when comparing treatments to the control in our study, most probably, no cell damage occurred. Similarly, F&S tests with water from another tropical reservoir yielded no indications of damage to cyanobacteria (de Lucena-Silva et al., 2019). Also, differences between the price of CHI and PAC are relevant and should be considered, given the fact that will impact the remediation cost (Lürling et al., 2016). CHI is more expensive than PAC (Granados et al., 2012; Lürling., et al. 2020 b). For instance, the compound CHI can cost around $ 39 $kg^{-1}$, while the cost of PAC is around $ 0.34 $kg^{-1}$ (averaged from https://www.alibaba.com/trade/search, accessed 18th April 2022), in other words, 115 fold more expensive than PAC.

In addition to evaluating the most efficient treatment, it is also important to verify whether the efficiencies are affected by changes in the environmental conditions of the water. Spatial and temporal variations in environmental conditions have already been reported for the Funil Reservoir (Pacheco et al., 2015; Rangel et al., 2012; Soares et al., 2008, 2009, 2012). However, no previous study has evaluated the efficiency of F&S treatments every month with water from two distinct sites. During the one-year study period, significant variation in phytoplankton removal efficiencies could be observed, and this was related to changes in the environmental conditions of both the sampling sites. This finding is in agreement with our hypotheses, reinforcing the importance of testing these treatments through time to get an insight regarding the potential variability in the efficiencies on a temporal basis. This is important because conditions may change between the time of testing and the actual application, which could affect the efficiency of cyanobacteria biomass removal.

It is important to highlight that CHI and LMB + CHI were the most affected treatments, and their efficiency decreased sharply during some months in the warm-rainy months (February, April for both treatments, and November for only LMB + CHI). Therefore, to better understand why a reduction in biomass removal efficiency occurred in these months, we looked for the environmental conditions that explained this reduction in removal efficiency.

We observed a positive relationship between total chlorophyll-a concentrations and removal efficiencies, where a higher chlorophyll-a concentration meant a better efficiency. It should be noted that overall chlorophyll-a concentrations were rather low during the year of the tests (max. 25.5 μg $L^{-1}$) and that the ballast dose used (400 mg $L^{-1}$) was found to settle 99.7% of *Microcystis* colonies from the Funil Reservoir at a low chlorophyll-a concentration of 30 μg $L^{-1}$ and 97.8% at a high chlorophyll-a concentration of 651 μg $L^{-1}$ (Noyma et al., 2017). Although the study conducted by Noyma et al. (2017) reported a high removal efficacy at both low and high amounts of phytoplankton biomass, the positive relationship observed in this study at the lower biomass end indicates that more phytoplankton favored the settling, which might be caused by a more negatively charged surface that facilitated electrostatic and polymer bridging (Bache and Gregory, 2007).

The LMM 3 model revealed that an increase in pH significantly

**Table 5**
Coefficients of the Bayesian linear mixed models testing the effects of reservoir abiotic conditions and chlorophyll-a in the biomass removal efficiency of the treatments (LMM 3). The values represent the mean and 95% credible intervals of the posterior distribution. Values in bold indicate significant relationships (95% credible intervals different than zero) and negative coefficients, decrease in removal efficiency. Lake Chl-*a* = total chlorophyll-a of the lake, Temp = water temperature, DO = dissolved oxygen, Alk = alkalinity, Cond = electrical conductivity, DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorus, TN = total nitrogen, and TP = total phosphorus. Values with more than five zeros after decimal separator were considered equal zero.

| Variables | CHI<br>Mean [95% CI] | LMB<br>Mean [95% CI] | PAC<br>Mean [95% CI] | LMB + PAC<br>Mean [95% CI] | LMB + CHI<br>Mean [95% CI] |
|---|---|---|---|---|---|
| Intercept | -3.591 [-9.395, 1.358] | -0.873 [-5.598, 3.465] | -1.794 [-7.344, 2.688] | -1.06 [-4.359, 2.34] | 0.337 [-3.192, 3.479] |
| Lake Chl-a | **0.051 [0.035, 0.067]** | **0.03 [0.014, 0.046]** | **0.037 [0.015, 0.057]** | **0.026 [0.021, 0.031]** | **0.023 [0.019, 0.027]** |
| Temp | 0.074 [-0.066, 0.236] | 0.01 [-0.103, 0.13] | -0.043 [-0.161, 0.09] | 0.016 [-0.086, 0.112] | -0.021 [-0.103, 0.083] |
| DO | -0.009 [-0.054, 0.026] | **-0.071 [-0.121, -0.025]** | **-0.058 [-0.113, -0.005]** | 0.004 [-0.016, 0.021] | **-0.034 [-0.053, -0.011]** |
| pH | **-0.16 [-0.255, -0.07]** | **-0.166 [-0.263, -0.079]** | **-0.113 [-0.219, -0.011]** | **-0.108 [-0.169, -0.053]** | -0.045 [-0.103, 0.011] |
| Alk | 0.000 [0.000, 0.000] | 0.000 [-0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [-0.000, 0.000] |
| Cond | 0.013 [-0.005, 0.034] | **0.023 [0.002, 0.045]** | **0.033 [0.008, 0.06]** | 0.008 [-0.002, 0.017] | 0.014 [0.001, 0.023] |
| DIN | 0.000 [-0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] |
| SRP | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [-0.000, 0.000] |
| TN | 0.000 [0.000, 0.000] | 0.000 [-0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [0.000, 0.000] | 0.000 [-0.000, 0.000] |
| TP | **0.01 [0.006, 0.015]** | **0.01 [0.001, 0.011]** | **0.01 [0.002, 0.012]** | 0.000 [-0.000, 0.000] | 0.000 [-0.000, 0.000] |

reduced a treatment's efficiency to remove cyanobacteria biomass. Each coagulant has an optimal range of pH that results in a high coagulation performance. For instance, a study conducted on water samples obtained from Lake Dianchi (China) reported that PAC was effective in removing *M. aeruginosa* biomass at pH values ranging from 5 to 8 (Ma et al., 2015). Additionally, the application of PAC (8 mg Al $L^{-1}$) was able to sediment natural bloom that was dominated by *M. aeruginosa* at pH > 9 in a brackish coastal lagoon (de Magalhães et al., 2017). In contrast, alkaline pH impairs the ability of CHI to bind to cyanobacteria cells due to the concentration of anions (mostly hydroxyl and carbonate anions) around the positively charged amino groups of chitosan prevents their interaction with negatively charged cyanobacteria (Renault et al., 2009). A pH above 9 reduced cyanobacteria (primarily *M. aeruginosa*) biomass removal via the combination of chitosan (2 mg $L^{-1}$) and local red soil (320 mg $L^{-1}$) in water samples from the Funil Reservoir (Lürling et al., 2017). The negative effects of a higher pH (> 9) on the capacity of CHI to sediment cyanobacteria, either alone or in combination with different types of ballasts, have also been reported for other tropical systems (de Lucena-Silva et al., 2019; De Magalhães et al., 2017; Miranda et al., 2017). Thus, the negative relationship between pH and the efficiency of CHI-containing treatments to remove cyanobacteria biomass that was observed in our work could be explained by the pH values (above 8) observed for several months throughout the study period.

Chitosan is a cationic polyelectrolyte whose amino groups are protonated in an acidic solution. Pronatations allow the interaction of chitosan with negative surface charges found on most organic compounds, through the charge neutralization effect (Yang et al., 2016). The binding capacity of the anions concentrated in the amino groups can be influenced by a few environmental variables, such as pH and alkalinity (de Magalhães et al., 2017; Lurling et al., 2017; Miranda et al., 2017). At high pH, hydroxyl and carbonate anions can shield the protonated areas hampering the electrostatic interactions between protonated amino groups of chitosan and the negatively charged cyanobacteria (Lurling et al., 2017). Contrarily, PAC is an inorganic prepolymerized hydroxyl aluminum cationic polymer containing highly positive charged Al species that provides an elevated coagulant efficiency. PAC is a very effective coagulant widely used for wastewater treatment and can be operated under a wider range of conditions, including pH variation (Zarchi et al., 2013).

In addition to limnological variables, the efficiency of biomass removal of different treatments was also related to the cyanobacteria life forms, as indicated by the LMM 4 model. Charge neutralization is proposed to yield the best flocculation when the phytoplankton population consists of spherical and small cells without polymeric substances or morphological structures such as spines (Ghernaout et al., 2010). Indeed, in F&S experiments with natural populations taken from the Argemiro Figueiredo Reservoir (northeastern Brazil), small colonial species such as *Aphanocapsa delicatissima, Merismopedia glauca*, and

**Table 6**
Coefficients from the Bayesian linear mixed model using biomass removal efficiency as a response variable and the phytoplankton life-forms as explanatory variables (LMM 4). The values represent the mean and 95% credible intervals of the posterior chlorophyll-a distribution. Values in bold indicate significant relationships (95% credible intervals differs from zero) and negative coefficients, decrease in removal efficiency. The parameters are given at the logit scale.

| Predictors | CHI<br>Estimate [95% CI] | LMB<br>Estimate [95% CI] | PAC<br>Estimate [95% CI] | LMB + CHI<br>Estimate [95% CI] | LMB + PAC<br>Estimate [95% CI] |
|---|---|---|---|---|---|
| *Fixed effects* | | | | | |
| Intercept | -0.84 [-4.79, 2.83] | -0.77 [-2.83, 1.42] | 1.71 [-0.35, 3.90] | 0.53 [-1.77, 2.84] | 1.34 [-1.01, 3.74] |
| Unicell | **-0.68 [-1.33, -0.09]** | -0.17 [-0.60, 0.25] | 0.04 [-0.37, 0.45] | -0.24 [-0.65, 0.17] | -0.37 [-0.81, 0.08] |
| Filament | 0.12 [-0.38, 0.63] | 0.01 [-0.40, 0.43] | -0.25 [-0.79, 0.32] | -0.19 [-0.67, 0.30] | -0.11 [-0.64, 0.47] |
| Colony | **0.13 [0.02, 0.25]** | 0.07 [-0.03, 0.17] | 0 [-0.08, 0.07] | 0.03 [-0.05, 0.11] | 0 [-0.09, 0.09] |
| *Random effects* | | | | | |
| SD(Intercept) Month | 1.27 [0.67, 2.2] | 0.73 [0.31, 1.38] | 0.83 [0.47, 1.37] | 1.04 [0.62, 1.7] | 1.28 [0.77, 2.13] |
| SD(Intercept) Site | 2.11 [0.55, 5.22] | 1.03 [0.03, 4.11] | 0.99 [0.02, 3.87] | 1.19 [0.04, 4.25] | 0.86 [0.01, 3.65] |

*M. tenuissima* were effectively removed from the water column in treatments with coagulants (aluminum sulfate [SUL], PAC and CHI) and ballasts (natural bentonite and LMB) and their combinations; however, large filamentous cyanobacteria (*C. raciborskii, G. amphibium, P. agardhii*, and *P. catenata*) were removed only when treated with PAC, SUL, and LMB, either alone or in combination (de Lucena-Silva et al., 2019). In contrast, our study showed that CHI treatments were much better in removing the biomass comprising colonies (*M. aeruginosa*) than they were in removing the biomass comprising unicells. This suggests that the main coagulation mechanism of chitosan (bridging) was impaired when there was a high share of unicellular cyanobacteria. It is well established that phytoplankton species may strongly determine floc size, and biopolymers in the flocs seem to especially promote coagulation by acting as polymer aids, yielding larger flocs (Gonzalez-Torres et al., 2019). Since colonial *M. aeruginosa* contains more polysaccharides than unicells and has a thicker polysaccharide envelope than unicells (Zhang et al., 2007), extracellular polysaccharides could have played an important role in coagulation and the subsequent removal efficiencies that were observed in this study.

A decrease in the removal efficiency of the combined treatments was mainly associated with the warm-rainy period in the Funil Reservoir. For instance, in April, a reduction in the efficiency of the combined treatments was probably caused by a greater contribution of unicells (*M. aeruginosa* and *S. nidulans*) at FL35, which underpins our findings. Conversely, at FL50, 99% of the cyanobacteria biomass was represented by the filaments (*Dolichospermum circinalis*). Although filamentous species can be efficiently removed from the water column with combined treatments (de Lucena-Silva et al., 2019; Miranda et al., 2017), in our study, another factor implied this reduction in efficiency. It was probably associated with the low biomass of cyanobacteria, which represented less than 5% of phytoplankton biomass (Fig. 2a). In November, a reduction in efficiency occurred only for LMB + CHI, and given that the pH was 11 at both the sites, the high pH value was probably the cause of the reduction in the efficiency, as mentioned above. Also noteworthy, another reason for this reduction in efficiency could be the lower biomass ($\approx 5\ \mu g\ L^{-1}$) and the high contribution of unicells ($> 50\%$) at FL50. Regarding the spatial variation, we found that only CHI and LMB + CHI differed in efficiency between the sites. This can also be associated with the differences in the contributions of various life forms, especially unicells, which were generally higher at FL50.

Flocculation happens in two stages. First, the coagulants alter the physical state of dissolved and suspended solids causing particles to stick together by changes in electrostatic and ionic forces. In the second stage, physical contact between particles is important to create large flocs (Auerbach et al., 2008). When the biomass is low, it can be expected that a community composed of large cells and colonies will increase the chance of collisions and create larger flocs, heavy enough to suppress the buoyance capacity of cyanobacteria. Nevertheless, the addition of ballast during the flocculation process is important to increase cyanobacterial flocs weight, and thereat, facilitates biomass sedimentation. (Lürling and van Oosterhout 2013).

In summary, our study provides further evidence that combining a coagulant with ballast is a more effective technique to precipitate cyanobacteria, compared with the usage of different compounds separately. Among the treatments, the F&S technique (mainly LMB + PAC) is appropriate for removing cyanobacterial biomass in water bodies with variations in environmental conditions on a spatial and temporal scale. Considering that a proper diagnosis of the water system is essential to determine the efficacy of F&S assays (Lürling et al., 2016, 2020), our findings encourage temporal and spatial trials to find out the best period for in-lake measures aimed to reduce cyanobacterial nuisance with the F&S technique.

## 5. Conclusion

- The F&S combined treatments (LMB + PAC and LMB + CHI) were considered efficient for the removal of cyanobacterial biomass from the water column in the Funil Reservoir (RJ).
- The combined treatments studied for the case of the Funil Reservoir should be applied in months associated with the warm-rainy months when the pH is below 8.
- LMB + PAC treatment had the highest efficiency and can be performed independently of cyanobacteria life forms, while LMB + CHI can be applied when colonial forms are dominant.
- The efficiency of the treatments can vary on a temporal and spatial scale in response to the changes in the environmental conditions and should be evaluated before deciding the best period to perform the F&S treatment.

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## Declaration of Competing Interest

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

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2022.102262.

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