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Removal efficiency of phosphorus, cyanobacteria and cyanotoxins by the "flock & sink" mitigation technique in semi-arid eutrophic waters

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ABSTRACT

Geoengineering techniques have been used to control phosphorus and cyanobacteria in lakes promising greater and quicker chemical and ecological recovery. Techniques that use coagulants and clays to remove particulates and dissolved phosphorus from the water column have received great. In this study, bench-scale "flock & sink" assays were carried out to evaluate the efficiency of the coagulants aluminium sulphate (SUL), polyaluminium chloride (PAC) and chitosan (CHI), alone and combined with natural bentonite clays (BEN) and lanthanum-modified bentonite (LMB), to remove of phosphorus from a eutrophic reservoir in a semi-arid region of Brazil. In addition, the study seeks to assess the effects on the cyanobacteria density and the intra- and extracellular concentrations of cyanotoxins after the application of these geoengineering materials. The SUL and PAC coagulants effectively reduced the total phosphorus (TP), reactive soluble phosphorus (SRP), turbidity, chlorophyll-a, cyanobacteria density and intracellular microcystin, whereas CHI showed a low removal efficiency. Lanthanum-modified bentonite proved to be more effective than BEN; however, the application of the coagulants only was sufficient to successfully remove phosphorus and cyanobacteria from the water column. In addition, the efficiency of the "flock & sink" technique in cell removal varied among the cyanobacteria species. Small colonial species such as *Aphanocapsa delicatissima*, *Merismopedia glauca* and *Merismopedia tenuissima* were removed regardless of the treatment used, including those with CHI and BEN. As for the filamentous cyanobacteria, *Cylindrospermopsis raciborskii*, *Geitlerinema amphibium*, *Planktothrix agardhii* and *Pseudanabaena catenata*, removal was achieved only using PAC, SUL and LMB alone or when combined. The intracellular concentrations of saxitoxin and cylindrospermopsin and the extracellular fraction of these cyanotoxins and of microcystin were not influenced by the application of coagulants and clays. This indicates that cell lysis did not occur with the addition of the geoengineering materials. These results demonstrate that the "flock & sink" technique could be used for restoration of eutrophic waters.

1. Introduction

Eutrophication is characterized by excess nutrients in aquatic ecosystems, resulting from anthropogenic actions characterize eutrophication (Waajen et al., 2016; Araújo et al., 2018). Eutrophication promotes the growth of potentially toxic cyanobacteria and associated oxygen depletion, which pose a serious threat to human health and aquatic biota (Khan and Mohammad, 2014; Huisman et al., 2018).

Nutrients input reductions are necessary, as they have shown to be effective in improving water quality; they should be a central part cyanobacteria mitigation strategy (Paerl, 2014). Although studies indicate that an integrated approach is needed through the control of nitrogen (N) and phosphorus (P) (Hamilton et al., 2016), P control is considered more effective because it does not have a gas phase in the biogeochemical cycle nor are there biological mechanisms that compensate for its deficiency in a water body, unlike the fixation of atmospheric N (Xu et al., 2010; Waajen et al., 2016;

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Yin et al., 2016; Schindler et al., 2016). Besides that, the regulation of phosphate uptake reveals cyanobacterial bloom resilience to shifting N:P ratios, and is an efficient exploitation of limiting nutrients (Aubriot and Bonilla, 2018). Despite the importance of external sources of P in water bodies, such as those from anthropogenic activities in river basins, internal loading can contribute significantly to the total P balance and delay environmental restoration, even after reducing external loading (Lürling and van Oosterhout, 2013; Araújo et al., 2016). Internal loading originates from the processes of excretion and organic matter decomposition, from sediment resuspension or via bioturbation, which function as sources of P for the water column (Araújo et al., 2016; Lürling et al., 2016).

Studies show that the internal inputs of P seem to be more important in tropical than in temperate lakes, where the internal loading is relevant only in certain months, typically during summer (Søndergaard et al., 2003). In addition, external inputs of diffuse-source nutrients in tropical reservoirs have a lower contribution in regions with scarce rainfall, such as semi-arid regions, which are characterized by high temperatures, irregular rainfall and high evaporation rates (Tundisi et al., 2008; Barbosa et al., 2012). Thus, it is essential to reduce the external and internal P loading to mitigate the negative effects and ecological risks associated with eutrophication (Yin and Kong, 2015; Huser et al., 2016), especially in semi-arid reservoirs.

Geoengineering is an important technique in the management of eutrophication, providing a promising management method for aquatic ecosystems, if it can successfully reduce the P concentration in the water column and retain it in the sediment, in anunavailable form (Spears et al., 2013; Spears et al., 2014; Mackay et al., 2014; Lürling et al., 2016). This technique consists of the combined addition of a coagulant and a P adsorbent, which complexes with organic and inorganic matter present in the water column, forming flocs that are precipitated and settle on thesediment (Noyma et al., 2017). The most commonly used coagulants are aluminium-based salts such as aluminium sulphate and polyaluminium chloride (Yin et al., 2018). However, studies show that although they are efficient in removing P when added to water, they may promote a reduction in pH and consequently have a negative effect on biota. An additional concern with using coagulants is their toxicity through the application or re-release of aluminium in the aquatic ecosystem (Nogaro et al., 2013; Reitzel et al., 2013; Douglas et al., 2016; D'Haese et al., 2019). Thus, non-toxic and biodegradable coagulants such as chitosan (Noyma et al., 2016), a biopolymer extracted from the exoskeleton of crustaceans, have become a more ecologically viable alternative (Zou et al., 2006; Renault et al., 2009; Yang et al., 2016).

Regarding the P adsorbent, natural or metal-modified clays are the most used. Among the natural clays, the bentonite (BEN) is non-toxic, low cost and does not promote water acidification (Verspagen et al., 2006). Lanthanum-modified bentonite (LMB) (Douglas, 2002; Robb et al., 2003) has been applied to approximately 200 water bodies, but despite its widespread use (Spears et al., 2013; van Oosterhout et al., 2014; Dithmer et al., 2016; Copetti et al., 2016; Waajen et al., 2016), its application has been limited due to its cost (Spears et al., 2013). Although no acute and chronic effects of La accumulation were observed and human health risk is considered negligible, La accumulation in macrophytes, chironomids and fish following LMB applications has been reported (Spears et al., 2013; Copetti et al., 2016; Waajen et al., 2017). In fish, the highest concentrations were found in the liver, indicating depuration of La (Waajen et al., 2017). There is still a research gap in proving biomagnification in the trophic food web after LMB application.

Given this criticism of the use of metal-based coagulants and modified clays (Nogaro et al., 2013; Copetti et al., 2016; D'Haese et al., 2019), non-toxic and biodegradable compounds have been the most indicated, but some studies note that a preliminary analysis of the aquatic ecosystem is essential to better estimate the most suitable products and determine the best concentrations for P removal (Douglas et al., 2016; Lürling et al., 2016).

Another much discussed issue is the products' effects on the release of cyanotoxins produced by cyanobacteria. Recent studies by Mucci et al. (2017) and Miranda et al. (2017) showed that although chitosan, when used as a coagulant, showed high efficiency in the reduction of the cyanobacteria biomass in the water column, it caused cell lysis and consequently the release of cyanotoxins.

The objective of the present study was to evaluate the efficiency of the coagulants aluminium sulphate (SUL), polyaluminium chloride (PAC) and chitosan (CHI), alone and combined with BEN and LMB, in removaling P from eutrophic reservoir waters of the Brazilian semi-arid region and to assess the effects of these compounds on the cyanobacteria density and the intra- and extracellular cyanotoxin. The following hypotheses were tested: (i) chitosan, an organic coagulant, can be used as a substitute for chemical coagulants (SUL and PAC) in the restoration of eutrophic waters in the semi-arid region; (ii) the combined action of the coagulants with the clays (BEN or LMB) is more effective in the removal of P, and the removal capacity of LMB is greater than that of BEN; and (iii) the combined action of coagulants and clays does not cause cell lysis or the release of cyanotoxins.

2. Materials and methods

2.1. Study area and field procedures

The Argemiro de Figueiredo reservoir (7°27.5′3″ S, 35°35′52.6″ W) is located in the Itatuba municipality, Paraíba state, Brazil. This reservoir is part of the Paraíba river basin and has an average total volume of 253,000,000 m^3. This shallow system with a mean area of 2300 m^2 and maximum depth of 58 m has experienced an intense eutrophication process since its inception and presents perennial blooms of potentially toxic cyanobacteria (Cavalcante et al., 2017). The reservoir can be considered hypereutrophic with high mean concentrations of TP (945 ± 21 μg PL^{-1}) and chlorophyll-*a* (133 ± 4 μg L^{-1}), with the dominance of the species *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*. For bench-scale assays, water samples were collected from the Argemiro de Figueiredo reservoir during May 2018, near the dam, in the limnetic region at a 0.5 cm depth.

2.2. Coagulants and clays

The coagulant aluminium sulphate [$Al_2(SO_4)_3.14–18H_2O$] was obtained from the Water and Sewage Company of Paraíba (CAGEPA, for its acronym in Portuguese) and presented a purity of (98–100%) according to the hydrated form. Polyaluminium chloride ($Al_n(OH)_mCl_{3n-m}$, $\rho = 1.36$ kg.L^{-1}) was obtained from Assunção Distribuidora Ltda. (Pernambuco, Brazil), and chitosan was donated by Polymar Ciência e Nutrição S/A (Ceará, Brazil). Prior to use, the chitosan was acidified with a 1% hydrochloric acid solution and diluted to a 1 g L^{-1} stock solution, as described by Noyma et al. (2016).

A natural bentonite was supplied by Bentonisa - Bentonita do Nordeste S.A (Paraíba, Brazil), and lanthanum-modified bentonite was supplied by HydroScience (Rio Grande do Sul, Brazil).

2.3. Experimental design

A series of “flock & sink” assays were divided into three stages (with the following goals: stage 1 – determine the most efficient coagulant concentration for the removal of total phosphorus (TP) from the water column; stage 2 – determine the best concentration of clays, and stage 3 - reproduce the concentrations of coagulants and clays determined in the previous stages, on a larger scale, following the same procedures, adjusting only the type and scale of the experimental units and the types and concentrations of coagulants and clays.

Aliquots of 200 mL (stages 1 and 2), and 700 mL (stage 3) of water from the Argemiro de Figueiredo reservoir were transferred to graduated cylinders (stages 1 and 2) or glass jars (stage 3). The addition of the treatments (clays, coagulants and combinations) was according by Noyma et al. (2017). After 1 h, samples were collected from the upper (top) and lower (bottom) parts of the experimental units for further analysis, as shown in Fig. 1.

2.4. Sample processing and laboratory analysis

The pH and turbidity measured in the assays were determined on a benchtop pH meter (model MPA-210 from Poli Control) and turbidimeter (model AP, 2000 from Poli Control), respectively.

The concentrations of TP and SRP were determined according to the methodology described in Standard Methods (APHA, 2012). Chlorophyll-*a* was extracted with 96% ethanol, following the method of Jespersen and Christoffersen (1987) and determined by the spectrophotometric method of Wintermans (1965). The concentrations of TP and chlorophyll-*a* were used to determine the trophic level of the reservoir.

The samples for the phytoplankton analysis were fixed with 1% Lugol's iodine, and the species were identified through the preparation of semi-permanent slides and visualization under an optical microscope (Zeiss model Lab. A1), with the aid of specialized literature. The quantitative analysis was performed using an inverted microscope (Zeiss Axiovert 40C) with a 400x magnification, using a sedimentation chamber, as described by Uthermöl (1958). The density (cells mL^{-1}) was obtained using the equation described by Ross (1979).

The total microcystin, saxitoxin and cylindrospermopsin concentrations were determined by the Enzyme-Linked Immunosorbent Assay (ELISA) method using Abraxis, Inc. plate kits (Warminster, PA), following the manufacturer's instructions. The samples were filtered in GF/C filters (0.45 μm) to separate the intracellular and extracellular fractions. The intracellular cyanotoxin was quantified from the cells retained in the filter, while the extracellular cyanotoxin was determined from the filtered water. To extract the toxin from the cells, three freeze/thaw cycles were carried out on the samples at −40 °C. The analyses were performed using an ASYS A-5301 microplate reader (ASYS Hitech GmbH, Eugendorf, Austria).

2.5. Statistical analyses

To test for significant differences in the concentrations of TP and SRP, pH, turbidity, chlorophyll-*a*, phytoplankton density and cyanotoxins (intra- and extracellular) among the treatments tested in assays 1, 2 and 3, a one-way ANOVA was carried out, followed by Tukey's multiple comparison test. The normality and homoscedasticity were assessed using Kolmogorov-Smirnov and Levene's tests, respectively. Statistical analyses were performed considering

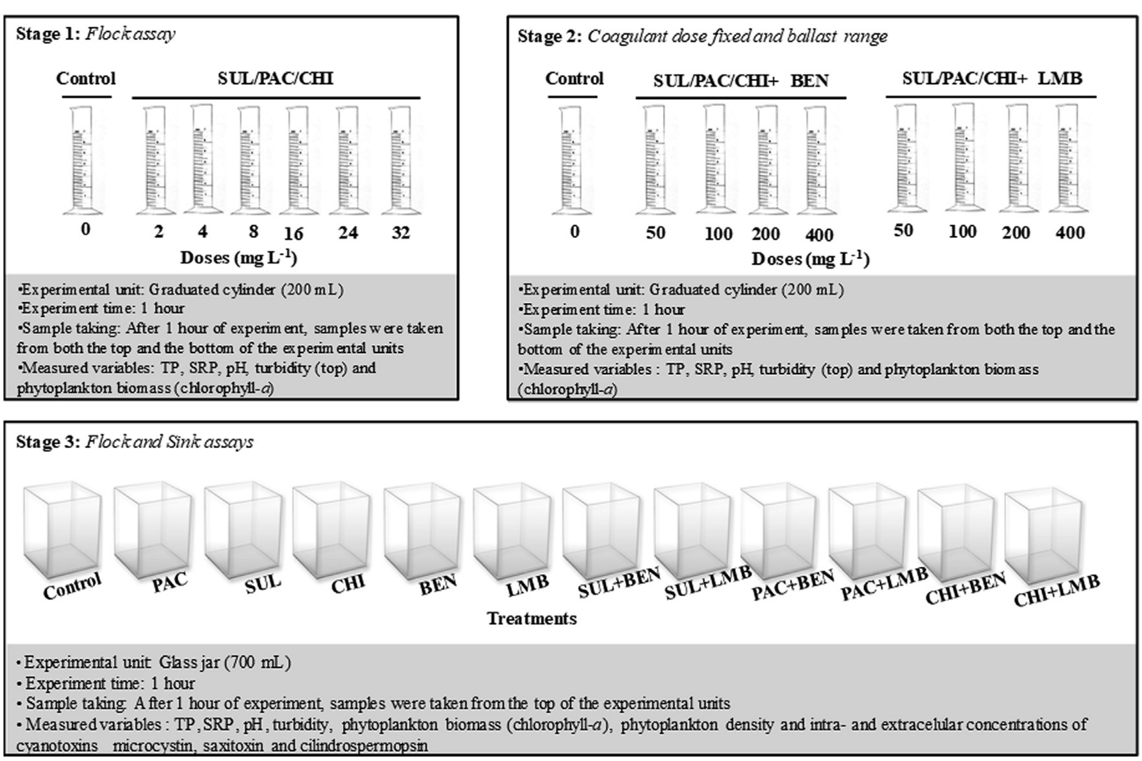

Fig. 1. Schematic representation of the experimental design. SUL, aluminium sulphate; PAC, polyaluminium chloride; CHI, chitosan, LMB, lanthanum-modified bentonite; BEN, natural bentonite.

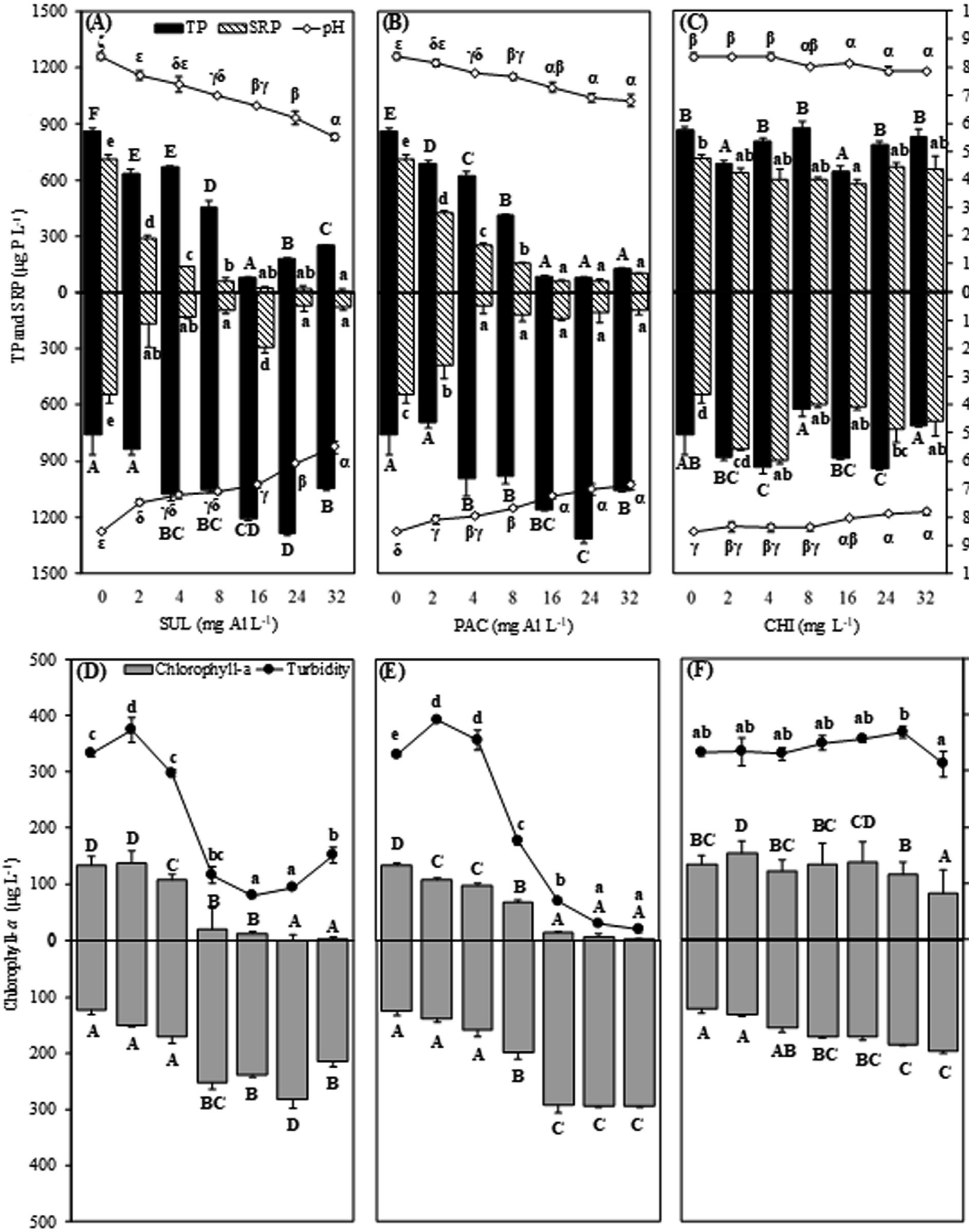

Fig. 2. Total phosphorus (TP), soluble reactive phosphorus (SRP) chlorophyll-*a*, pH and turbidity in the top (upper part of the graph) and bottom (lower part of the graph) at different concentrations of the coagulants aluminium sulphate/SUL (A and D), aluminium polychloride/PAC (B and E) and chitosan/CHI (C and F). Error bars indicate the standard deviation. Columns and rows with the same letter did not differ significantly ($p < 0.05$).

a significance level of 5% and using R *Software* for *Windows* (R Core Team, 2018).

3. Results

3.1. Physical and chemical characteristics and phytoplankton groups of the reservoir waters

The Argemiro Figueiredo reservoir exhibits an alkaline pH, high turbidity and high phosphorus and chlorophyll-*a* concentrations and is thus classified as eutrophic, according to the classification for aquatic ecosystems in semi-arid areas proposed by Thornton and Rast (1993). Cyanobacteria were the highest-density phytoplankton group, with a predominance of the species *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*.

3.2. Stage 1: flock assay

In the SUL (Fig. 2A and D) and PAC (Fig. 2B and E) series, significant reductions were observed in the water column for TP, SRP, chlorophyll-*a* and turbidity, starting at the lowest tested concentration (2 mg L^{-1}) (Table 1). Increased concentrations of coagulants caused a gradual decline in the pH, and this effect was more pronounced in the SUL, which reached 6 ± 0 at a concentration of 32 mg Al L^{-1}. In the treatments with PAC application, the pH remained at approximately 7. At the bottom of the tubes, there was a significant increase in the concentrations of the TP, SRP and chlorophyll-*a* with the increased coagulant concentrations, with the pH similar to the top.

The lowest concentration of aluminium in SUL and PAC that most reduced the TP concentration in the water column in a safe pH range (pH $\geq$ 7) was 8 mg Al L^{-1}. At this concentration, the removal efficiency of the SUL for TP, SRP and chlorophyll-*a* was 47 ± 3%, 91 ± 2% and 86 ± 2%, respectively (Table 1), and the PAC showed a removal efficiency of TP of 53 ± 1%, of SRP of 78 ± 0% and of chlorophyll-a of 49 ± 0%.

The CHI significantly reduced the TP at concentrations of 2 and 16 mg L^{-1}, with removal efficiencies of 21 ± 1 and 26 ± 5%, respectively (Fig. 2C and F; Table 1). As the pH showed no significant variation, ranging from 7 ± 1 to 8 ± 1, the 2 mg L^{-1} concentration was considered the most suitable. The SRP removal efficiency did not differ significantly between the CHI treatments, whereas the chlorophyll-*a* decreased at concentrations of 24 and 32 mg L^{-1}, with removals of 13 ± 3 and 38 ± 1%, respectively (Table 1).

3.3. Stage 2: Coagulant dose fixed and ballast range

The combination of clays (LMB or BEN) with coagulants produced significant reductions in the TP, SRP, chlorophyll-*a* and turbidity in the water column starting at the lowest concentration tested (50 mg L^{-1}) (Figs. 3 and 4; Table 2). The pH also decreased, but there was no significant difference between the different clay concentrations, as it remained above 7 ($p > 0.05$). At the bottom of the beaker, a gradual increase of the TP concentration was observed with the increase of the concentration of BEN combined with PAC and of LMB combined with SUL and with PAC, but with no significant differences in the SRP and chlorophyll-*a* concentrations (Figs. 3 and 4).

Lanthanum-modified bentonite combined with CHI did not result in the reduction of TP in the water column at the tested concentrations, while the SRP and chlorophyll-*a* significantly decreased with the application of 400 mg L^{-1} (Fig. 3C and F), showing removal efficiencies of 42 ± 4% and 15 ± 0%, respectively (Table 2). A pH reduction was observed in the water column, but it remained above 7, while the turbidity increased gradually. At the bottom of the water sample, no significant differences were observed in the TP and SRP concentrations and pH. Lanthanum-modified bentonite combined with CHI significantly reduced the TP and SRP at the 200 and 400 mg L^{-1} concentrations (Fig. 4C and F). The pH remained around 7 and the turbidity increased. At the bottom of the water sample, significant differences were observed in the SRP concentration, which decreased significantly starting at the lowest concentration tested (50 mg L^{-1}). The lowest concentrations of LMB and BEN combined with the coagulants that most reduced the TP concentration in the water column was 100 mg L^{-1}.

3.4. Stage 3: "flock & sink"

The coagulants SUL and PAC alone and combined with the LMB or BEN caused significant reductions in the TP, SRP, turbidity, chlorophyll-*a*, cyanobacteria density and intracellular concentration of microcystin in the water column (Figs. 5 and 6; Table 3). The application of BEN alone did not cause significant effects on these variables, while the LMB significantly reduced the TP, SRP, cyanobacteria and intracellular microcystin. No significant effects of the treatments were observed on the intracellular concentrations of saxitoxin and cylindrospermopsin or on the extracellular fractions of these cyanotoxins and of microcystin (Fig. 6).

The application of CHI alone caused significant reductions in the TP and chlorophyll-*a* in the water column, with removal efficiencies of 12 ± 6% and 50 ± 7%, but the combinations with BEN and LMB did not differ from the control (Fig. 5; Table 3). On the other hand, the SRP decreased significantly in the CHI + BEN and CHI + LMB treatments, with respective removal efficiencies of 17 ± 0% and 23 ± 5% (Table 3). The turbidity and cyanobacteria density did not show differences between the CHI treatment and the control but increased significantly in the CHI + BEN and CHI + LMB treatments (Fig. 5).

The removal efficiencies of the cyanobacteria species are shown in Table 4. The species *Aphanocapsa delicatissima*, *Merismopedia glauca* and *M. tenuissima* were removed with high efficiency regardless of the treatment, while *Chroococcus dispersus*,

Table 1
Removal efficiencies (%) of total phosphorus (TP), soluble reactive phosphorus (SRP) and chlorophyll-*a* (chlor-*a*) at different concentrations of aluminium sulphate (SUL), polyaluminium chloride (PAC), and chitosan (CHI). Data with the same letter did not differ significantly ($p < 0.05$).

Doses (mg L^{-1})	Removal Efficiency (%)								
	SUL			PAC			CHI		
	TP	SRP	Chlor-*a*	TP	SRP	Chlor-*a*	TP	SRP	Chlor-*a*
2	27±1^{e}	60±1^{d}	0±6^{d}	20±1^{e}	41±2^{e}	19±0^{e}	21±1^{a}	11±7^{a}	0±0^{c}
4	23±0^{e}	80±1^{c}	21±4^{c}	28±1^{d}	65±2^{d}	26±0^{d}	7±1^{bc}	15 ± 10^{a}	9±7^{bc}
8	47±3^{d}	91±2^{b}	86±2^{b}	53±1^{c}	78±0^{c}	49±0^{c}	0±3^{c}	15±2^{a}	0±6^{bc}
16	91±1^{a}	96±0^{a}	91±2^{b}	90±1^{a}	91±1^{a}	89±2^{b}	26±6^{a}	19±1^{a}	0±2^{c}
24	80±1^{b}	97±2^{a}	100±0^{a}	91±1^{a}	91±1^{a}	94±5^{ab}	9±1^{b}	7±1^{a}	13±3^{b}
32	71±0^{c}	99±1^{a}	98±1^{a}	86 ± 10^{b}	86±1^{b}	98±1^{a}	4±3^{bc}	8 ± 13^{a}	38±1^{a}

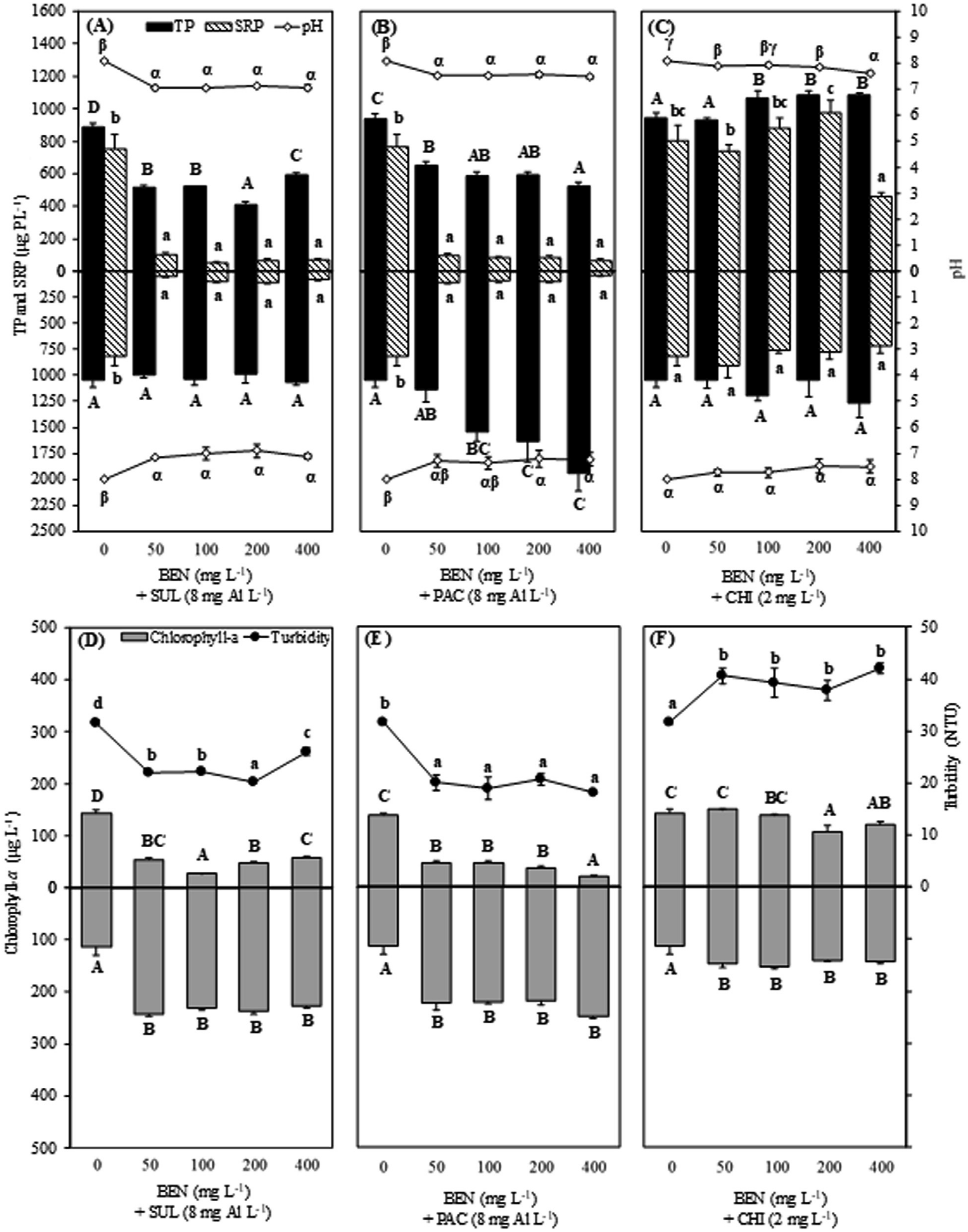

Fig. 3. Total phosphorus (TP), soluble reactive phosphorus (SRP), chlorophyll-*a* and pH at the top (upper part of the graph) and bottom (lower part of the graph) at different concentrations of natural bentonite clay (BEN) combined with aluminium sulphate (SUL, 8 mg Al L^{-1}) (A and D), polyaluminium chloride (PAC, 8 mg Al L^{-1}) (B and E) and chitosan (CHI, 2 mg L^{-1}) (C and F). Error bars indicate the standard deviation. Columns and rows with the same letter did not differ significantly ($p < 0.05$).

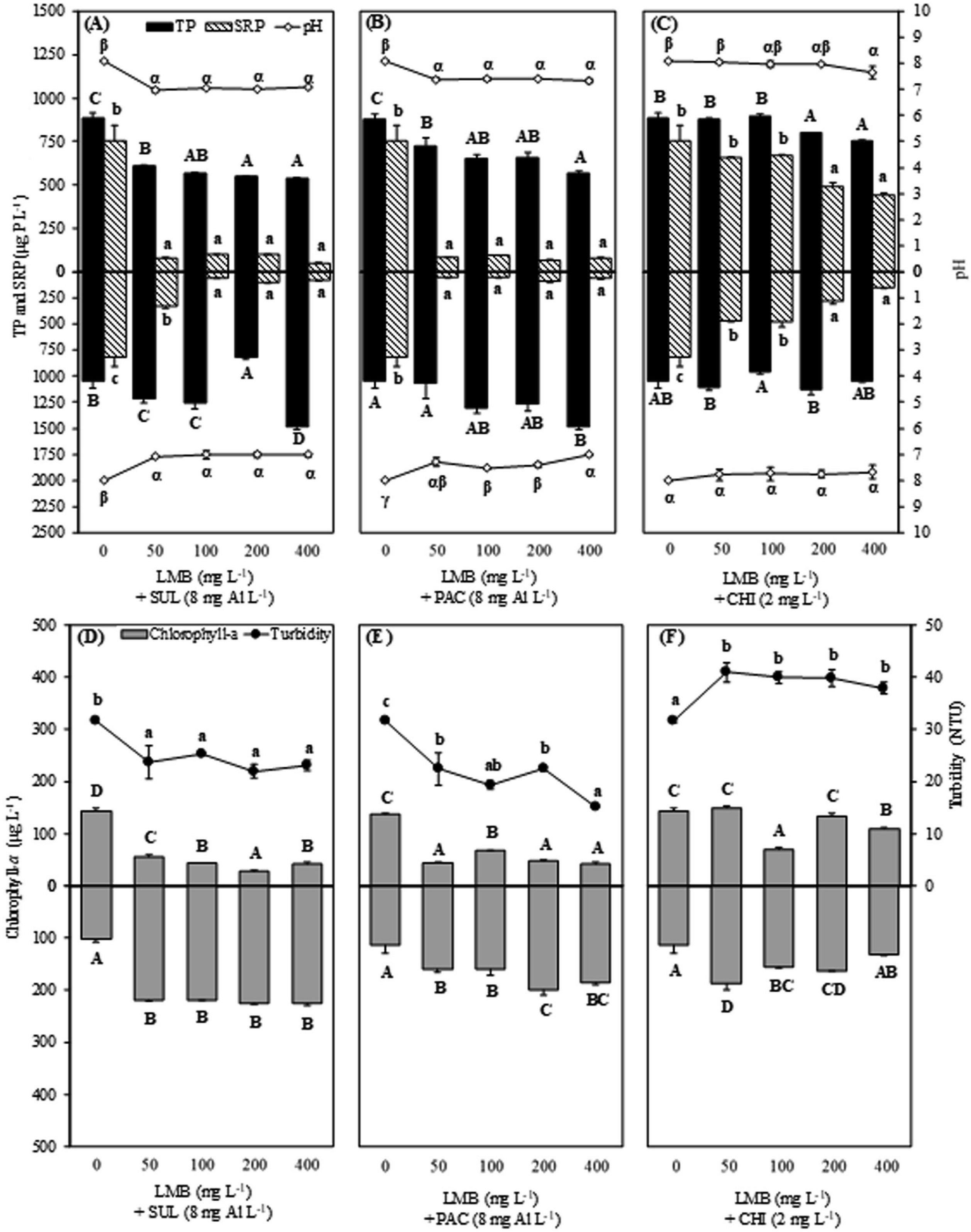

Fig. 4. Total phosphorus (TP), soluble reactive phosphorus (SRP), chlorophyll-*a*, pH and turbidity in the top (upper part of the graph) and bottom (lower part of the graph) at different concentrations of lanthanum-modified bentonite (LMB) combined with aluminium sulphate (SUL, 8 mg Al L^{-1}) (A and D), polyaluminium chloride (PAC, 8 mg Al L^{-1}) (B and E) and chitosan (CHI, 2 mg L^{-1}) (C and F). Error bars indicate the standard deviation. Columns and rows with the same letter did not differ significantly ($p < 0.05$).

Table 2
Removal efficiencies (%) of total phosphorus (TP), soluble reactive phosphorus (SRP) and chlorophyll-*a* (chlor-*a*) in the treatments with aluminium sulphate (SUL), polyaluminium chloride (PAC) and chitosan (CHI) combined with different concentrations of natural (BEN) and lanthanum-modified bentonite (LMB). Data with the same letter did not differ significantly ($p < 0.05$).

Doses (mg L^{-1})	Removal Efficiency (%)								
	BEN + SUL			BEN + PAC			BEN + CHI		
	TP	SRP	Chlor-*a*	TP	SRP	Chlor-*a*	TP	SRP	Chlor-*a*
50	42 ± 0^{b}	86 ± 3^{b}	62 ± 0^{b}	30 ± 1^{c}	87 ± 0^{a}	66 ± 3^{b}	1 ± 2^{a}	6 ± 7^{b}	0 ± 4^{b}
100	41 ± 2^{b}	93 ± 2^{a}	81 ± 2^{a}	37 ± 1^{b}	89 ± 0^{a}	66 ± 1^{b}	0 ± 1^{a}	0 ± 2^{b}	3 ± 3^{b}
200	53 ± 0^{a}	91 ± 2^{ab}	66 ± 3^{b}	37 ± 1^{b}	89 ± 0^{a}	74 ± 4^{b}	0 ± 1^{a}	0 ± 26^{b}	25 ± 11^{a}
400	33 ± 5^{c}	90 ± 2^{ab}	59 ± 3^{b}	44 ± 1^{a}	92 ± 3^{a}	85 ± 3^{a}	0 ± 2^{a}	42 ± 4^{a}	15 ± 1^{ab}
Doses (mg L^{-1})	LMB + SUL			LMB + PAC			LMB + CHI		
	TP	SRP	Chlor-*a*	TP	SRP	Chlor-*a*	TP	SRP	Chlor-*a*
50	31 ± 1^{b}	89 ± 2^{ab}	60 ± 4^{c}	18 ± 3^{c}	89 ± 2^{a}	67 ± 1^{a}	1 ± 3^{b}	11 ± 11^{abc}	0 ± 8^{c}
100	35 ± 1^{b}	86 ± 1^{b}	70 ± 1^{b}	26 ± 1^{b}	87 ± 1^{a}	51 ± 1^{b}	0 ± 2^{b}	10 ± 10^{bc}	51 ± 5^{a}
200	38 ± 2^{b}	86 ± 2^{b}	80 ± 0^{a}	25 ± 1^{bc}	91 ± 2^{a}	65 ± 2^{a}	10 ± 2^{a}	33 ± 12^{ab}	6 ± 9^{bc}
400	39 ± 2^{b}	94 ± 1^{a}	70 ± 3^{b}	37 ± 4^{a}	89 ± 2^{a}	70 ± 3^{a}	15 ± 5^{a}	40 ± 9^{a}	23 ± 6^{b}

Cylindrospermopsis raciborskii, *Geitlerinema amphibium*, *Planktothrix agardhii* and *Pseudanabaena catenata* were more efficiently removed in the treatments with the chemical coagulants (SUL and PAC) and LMB alone, as well as in the combined treatments with clays (BEN + SUL, BEN + PAC, LMB + SUL and LMB + PAC) (Table 4).

4. Discussion

In this study we have shown that CHI had low removal efficiencies of TP, SRP and cyanobacteria and is not a viable alternative for eutrophication control in semi-arid reservoirs. Lanthanum-modified bentonite, although shown to be more effective than BEN, did not increase the coagulant efficiency, indicating that the success of the “flock & sink” technique in reservoir restoration does not depend strictly on the combined action of coagulants and clays because the coagulant alone was sufficient to significantly remove P and cyanobacteria, thus improving the cost-benefit ratio of the application of this technique. The coagulants and clays did not cause increases in the extracellular cyanotoxin, suggesting that there was no cell lysis nor release of toxins into the water.

Cyanobacteria are known to have important morphological and physiological adaptations that allow them to regulate their buoyancy in the water column, such as the presence of gas vesicles (aerotopes) and a mucilaginous sheath (Reynolds et al., 1987). These characteristics may make the use of coagulants unfeasible because they prevent cell sedimentation.

Studies have suggested that the flocculation of algal cells with the best cost-benefit ratio occurs when the community is composed of organisms with small, spherical cells that are free of protruding appendages or polymeric substances (Ghernaout et al., 2010). This was observed in our experiment because small species such as *A. delicatissima, M. glauca* and *M. tenuissima* were effectively removed from the water column regardless of the treatment, including those with CHI and BEN, while cyanobacteria that form large filaments such as *C. raciborskii, G. amphibium, P. agardhii* and *P. catenata* were removed only with the application of PAC, SUL and LMB, alone or combined. Experiments with C. *raciborskii* show that this cyanobacterium has a high buoyancy potential and that its filaments tend to migrate and accumulate on the surface of the glass tubes used as experimental units. However, as observed in our study, the application of PAC combined with the LMB effectively sediments the filaments at the bottom of the tubes (Araújo et al., 2018).

The low removal efficiency of the clays observed in our experiments contrasts with studies that show high efficiencies, particularly for LMB combined with the coagulants, in controlling eutrophication. Lürling and van Oosterhout (2013) observed through laboratory experiments and in an entire lake that PAC and LMB when added separately were insufficient to sediment cyanobacteria, but induced effective sedimentation when combined.

The action of the clays is influenced by the water characteristics like pH (Lürling et al., 2014a,b; Copetti et al., 2016). Studies show that LMB achieves its maximum efficiency in the until pH 8 because the lanthanum (La) occurs as a free La^{3+} cation to intercept phosphate present in the water column. However, above this pH a series of La—OH complexes coexist with the insoluble $La(OH)_3$, potentially reducing the P removal capacity from the water column (Lürling et al., 2014a,b; D'Haese et al., 2019). The influence of pH on the performance of natural bentonite is less intense, but the mechanisms are still poorly understood (Copetti et al., 2016).

The presence of humic substances also causes an effective reduction of the P adsorption by LMB because they bind to La, forming complex chemical structures that prevent binding with P (Schnitzer, 1978; Lürling et al., 2014a,b). In the studied reservoir, the performance of LMB may have been influenced by these factors because the pH was 8.40 ± 0.13 at the beginning of the experiment and, although no analysis was performed, there were probably high concentrations of humic substances, as is commonly observed in (hyper) eutrophic lakes and reservoirs (Thurman, 1985; Lürling et al., 2014a,b).

The high pH observed in the Argemiro Figueiredo reservoir may also have influenced the low performance of CHI. This coagulant is a cationic polyelectrolyte from its amino groups, and is not protonated in basic aqueous medium (Yang et al., 2016). Protonation is a process that allows the interaction of the chitosan with the negative surface charges found in most pollutants, through the effects of charge neutralization (Renault et al., 2009; Yang et al., 2016), making it difficult for it to bind with phosphorus and cyanobacteria (Lürling et al., 2017). Studies in tropical regions (Jacarepaguá Lake, Rio de Janeiro-Brazil) - showed that chitosan was not able to form flocs, even at high concentrations (>16 mg L^{-1}), and did not efficiently sediment cyanobacteria when combined with the clays due to the high pH (9.19 ± 0.09) of the lake (de Magalhães et al., 2017). In another study (Lake of the Mariano Procópio Museum - Minas Gerais, Brazil), CHI combined with clays was effective in removing *Cylindrospermopsis*-dominated blooms at low pH, but at pH of 8, no effective flocculation or sedimentation was observed (Miranda et al., 2017). Thus, the results observed in our study confirm that CHI does not perform well at pH higher than 8, which suggests that a low pH is a prerequisite for the successful use of CHI in the “flock & sink” technique in freshwater systems.

The use of CHI has also been questioned because of the damage

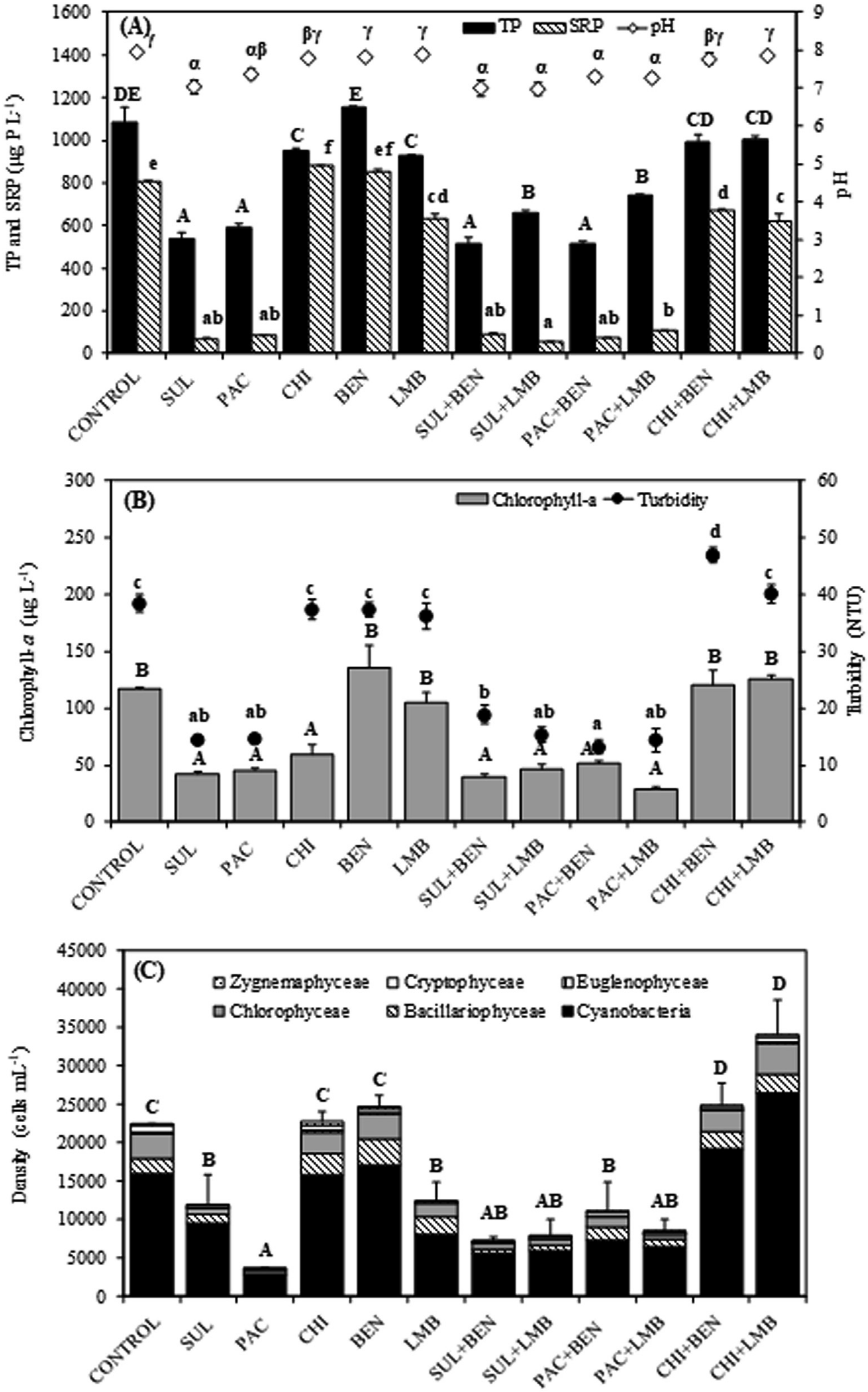

Fig. 5. Total phosphorus (TP), soluble reactive phosphorus (SRP), chlorophyll-*a*, phytoplankton density, turbidity and pH in the treatments with aluminium sulphate (SUL, 8 mg Al L^{-1}), polyaluminium chloride (PAC, 8 mg Al^{-1}), chitosan (CHI, 2 mg L^{-1}), natural bentonite (BEN, 100 mg L^{-1}) and lanthanum-modified bentonite (LMB, 100 mg L^{-1}), alone and combined. Error bars indicate the standard deviation. Columns and points with the same letter do not differ significantly ($p < 0.05$).

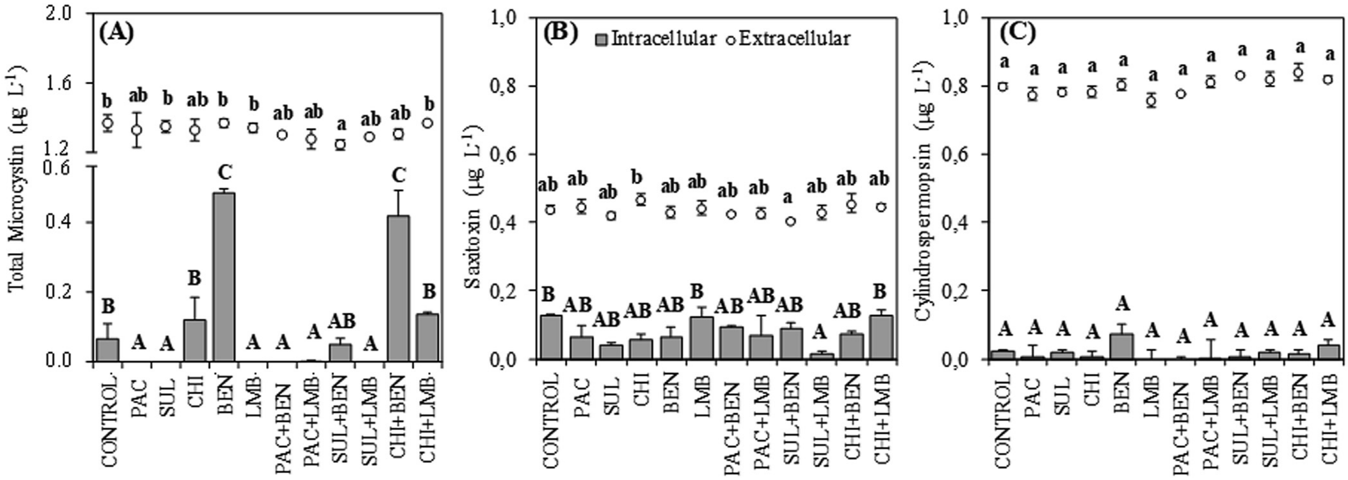

Fig. 6. Intra- and extracellular total microcystins (A), saxitoxin (B) and cylindrospermopsin (C) in the treatments with aluminium sulphate (SUL, 8 mg Al L^{-1}), polyaluminium chloride (PAC, 8 mg Al L^{-1}), chitosan (CHI, 2 mg L^{-1}), natural bentonite (BEN, 100 mg L^{-1}) and lanthanum-modified bentonite (LMB, 100 mg L^{-1}), alone and combined. Error bars indicate the standard deviation. Columns and points with the same letter do not differ significantly ($p < 0.05$).

Table 3
Removal efficiencies (%) of total phosphorus (TP), soluble reactive phosphorus (SRP) and chlorophyll-*a* (chlor-*a*) the aluminium sulphate treatment (SUL, 8 mg Al L^{-1}), polyaluminium chloride (PAC, 8 mg Al L^{-1}), chitosan (CHI, 2 mg L^{-1}), natural bentonite (BEN, 100 mg L^{-1}) and lanthanum-modified bentonite (LMB, 100 mg L^{-1}), alone and combined. Data with the same letter did not differ significantly ($p < 0.05$).

Treatments	Removal Efficiency (%)			
	TP	SRP	Chlor-*a*	Cyanobacteria
SUL	50 ± 5^{ab}	92 ± 1^{ab}	64 ± 1^{ab}	83 ± 10
PAC	45 ± 4^{ab}	90 ± 0^{ab}	61 ± 2^{ab}	40 ± 7
CHI	12 ± 6^{d}	0 ± 1^{e}	50 ± 7^{ab}	1 ± 0
BEN	0 ± 8^{d}	0 ± 1^{e}	0 ± 17^{b}	0 ± 8
LMB	14 ± 6^{d}	22 ± 2^{cd}	10 ± 8^{b}	50 ± 6
SUL + BEN	53 ± 2^{a}	89 ± 1^{ab}	66 ± 2^{ab}	65 ± 10
SUL + LMB	39 ± 4^{abc}	93 ± 0^{a}	61 ± 4^{a}	62 ± 5
PAC + BEN	53 ± 4^{a}	91 ± 0^{ab}	56 ± 2^{ab}	55 ± 8
PAC + LMB	31 ± 5^{c}	87 ± 0^{b}	76 ± 2^{ab}	60 ± 3
CHI + BEN	8 ± 3^{d}	17 ± 0^{d}	0 ± 12^{b}	0 ± 3
CHI + LMB	7 ± 5^{d}	23 ± 5^{c}	0 ± 2^{b}	0 ± 4

it causes to the cyanobacteria cell wall of, consequently leading to the release of toxins (Miranda et al., 2017; Mucci et al., 2017). In the present study, no significant effects of CHI and other coagulants on the extracellular concentrations of microcystin, saxitoxin and cylindrospermopsins were observed. The effects of the treatments were observed in the intracellular concentration only of microcystin, with a significant reduction occurring with the use of SUL and PAC alone or combined with the clays. CHI, on the other hand, was not effective in reducing the intracellular microcystin concentration, showing no significant difference from the control. This result certainly contributed to the poor efficiency of CHI compared to SUL and PAC in removing cyanobacteria cells.

Considering the above, it is important to consider the proportions of the extra- and intracellular fractions of cyanotoxins in the application of the "flock & sink" technique in the restoration of eutrophic waters because, despite its potential for removing cyanobacteria and intracellular toxins, if the extracellular fraction is not removed, a high concentration of dissolved cyanotoxins compromise water quality.

Notably, even when most of the toxins during a bloom are intracellular, flocculation/sedimentation systems efficiently remove cyanobacterial cells from the water column, and the concentrations of the toxins dissolved over time may increase (Pei et al., 2014). The algal flocs deposited in the sediment can release intracellular toxins during cyanobacterial cell decomposition or when the flocs are resuspended in water (Pei et al., 2014). Because these factors were not evaluated in this work, further studies need to be carried out to investigate the long-term effects of the flocculation and sedimentation processes of phosphorus and

Table 4
Density of cyanobacterial species in control and removal efficiencies in the treatments with aluminium sulphate (SUL, 8 mg Al L^{-1}), polyaluminium chloride (PAC, 8 mg Al L^{-1}), chitosan (CHI, 2 mg L^{-1}), natural bentonite (BEN, 100 mg L^{-1}) and lanthanum-modified bentonite (LMB, 100 mg L^{-1}).

Species	Control (cell mL^{-1})	Removal Efficiency (%)										
		SUL	PAC	CHI	BEN	LMB	SUL + BEN	SUL + LMB	PAC + BEN	PAC + LMB	CHI + BEN	CHI + LMB
Aphanocapsa delicatissima	23 ± 8	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	0 ± 0^{b}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
Chroococcus dispersus	459 ± 56	66 ± 8^{abcd}	76 ± 42^{ab}	1 ± 2^{d}	0 ± 0^{d}	1 ± 2^{d}	100 ± 0^{a}	72 ± 49^{abc}	23 ± 39^{bcd}	90 ± 17^{ab}	5 ± 9^{cd}	1 ± 2^{d}
Coelomoron tropicalis	68 ± 25	100 ± 0^{a}	100 ± 0^{a}	69 ± 53^{ab}	100 ± 0^{a}	30 ± 28^{b}	100 ± 0^{a}	100 ± 0^{a}	85 ± 27^{ab}	100 ± 0^{a}	52 ± 2^{ab}	100 ± 0^{a}
Cylindrospermopsis raciborskii	4377 ± 219	63 ± 8^{a}	49 ± 2^{ab}	8 ± 4^{cd}	0 ± 0^{d}	26 ± 6^{bc}	47 ± 13^{ab}	55 ± 18^{a}	49 ± 4^{ab}	58 ± 14^{a}	0 ± 0^{d}	0 ± 0^{d}
Dolichospermum planctonica	47 ± 16	100 ± 0^{a}	13 ± 16^{b}	0 ± 0^{b}	13 ± 16^{b}	31 ± 0^{b}	100 ± 0^{a}	0 ± 0^{b}	0 ± 0^{b}	77 ± 40^{a}	0 ± 0^{b}	0 ± 0^{b}
Geitlerinema sp.	439 ± 89	70 ± 6^{a}	79 ± 3^{a}	0 ± 0^{d}	1 ± 1^{d}	51 ± 0^{b}	55 ± 4^{b}	75 ± 1^{a}	70 ± 6^{a}	39 ± 4^{c}	0 ± 0^{d}	0 ± 0^{d}
Merismopedia glauca	23 ± 8	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
Merismopedia tenuissima	140 ± 47	100 ± 0^{a}	85 ± 27^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	81 ± 24^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	85 ± 27^{a}
Planktolyngbya limnetica	1280 ± 163	95 ± 1^{a}	26 ± 12^{d}	31 ± 4^{d}	5 ± 5^{ef}	58 ± 0^{c}	78 ± 2^{ab}	97 ± 5^{a}	62 ± 3^{bc}	20 ± 16^{de}	0 ± 0^{f}	0 ± 0^{f}
Planktothrix agardhii	4860 ± 694	86 ± 1^{a}	52 ± 1^{cd}	0 ± 0^{e}	0 ± 0^{e}	45 ± 2^{d}	57 ± 3^{bc}	52 ± 7^{cd}	66 ± 8^{b}	60 ± 3^{bc}	0 ± 0^{e}	0 ± 0^{e}
Pseudanabaena catenata	3726 ± 312	96 ± 1^{a}	33 ± 2^{e}	47 ± 9^{de}	14 ± 12^{f}	75 ± 0^{b}	80 ± 1^{b}	71 ± 1^{bc}	59 ± 6^{cd}	74 ± 3^{bc}	0 ± 0^{f}	0 ± 0^{f}
Pseudanabaena galeata	814 ± 272	100 ± 0^{a}	80 ± 35^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	97 ± 5^{a}	61 ± 8^{a}	60 ± 15^{a}	100 ± 0^{a}	67 ± 58^{a}

cyanobacteria, as well as the potential effects on the intra- and extracellular cyanotoxin fractions.

5. Conclusions

- Chitosan showed a low efficiency in the removal of phosphorus and cyanobacteria from the water column and is not a good substitute for the aluminium-based coagulants aluminium sulphate and polyaluminium chloride in the restoration of eutrophic water in semi-arid regions.
- LMB showed a more efficient removal of phosphorus and cyanobacteria from the water column compared to natural bentonite, although the addition of coagulants combined with these clays was shown to not be strictly necessary to the success of the "flock & sink" technique, which can reduce the restoration costs.
- A decrease in the intracellular microcystin concentration in the water column was observed with the addition of chemical coagulants (aluminium sulphate and polyaluminium chloride), alone and combined with lanthanum-modified bentonite, while no effects on the intracellular concentrations of saxitoxin and cylindrospermopsin were observed.
- Coagulants and clays had no effect on the extracellular cyanotoxin fraction, indicating that cyanobacterial cell lysis did not occur with the addition of these compounds.

Author contributions

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Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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