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UNRAVELING KNOWLEDGE GAPS ABOUT CYANOBACTERIAL BLOOMS AND PROPOSING AN ALTERNATIVE FOR LAKE RESTORATION

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Contents

Abstract

Artificial eutrophication has been considered a problem of major concern in aquatic ecosystems around the world. Since 1960, scientific advances have been made in order to develop techniques that mitigate the effects of eutrophication. Several physical, chemical and biological procedures can be used and combined to recover lakes from cyanobacterial blooms, such as the application of a flocculant combined with natural or modified clay. However, the efficacy of local dryland soils in mitigating blooms is unknown for Brazilian manmade lakes. In this paper, we present a bibliometric analysis of the evolution of publications about cyanobacterial blooms and identify records that directly aim to overcome the occurrence of these blooms. Also, we evaluate, through laboratory experiments, the effect of the combined use of flocculent polyaluminum chloride (PAC) and a local soil from the lake catchment (LS), as ballast, in controlling cyanobacterial bloom in a shallow lake of the semiarid region of Brazil. The bibliometric research was conducted with the "Web of Science" database through the search function "TS = ((cyanobacteri* or blue green algae or cyanoprokariote or cyanophyceae) and (mass accumulation or bloom or domina*))", from 1969 to June 2016. We performed a keyword frequency analysis and quantified the number of records with a restoration approach. Besides, three sets of experiments were performed in three sampling occasions with different bloom compositions and biomass in Armando Ribeiro Gonçalves Reservoir. Our study revealed that studies about cyanobacterial blooms increased exponentially and their quantitative impact on the aquatic sciences increased significantly along the years ($F = 97.52$; $p < 0.0001$). The USA stands out as the most productive nation, followed by China and European countries. China has impressively increased its contribution to this area, surpassing the USA in the last five years. Studies about *Microcystis* and toxins, such as microcystins, are trends in research, due to their ubiquitousness and historical negative consequences. We also emphasize the need for more studies aiming at developing techniques to solve and/or mitigate the issue of blooms. In view of this, our experiments revealed that the use of PAC and LS had a remarkable effect on cyanobacterial biomass in the water column in all samplings, reducing up to 90% top chlorophyll-*a* concentration. The use of LS alone was inefficient to settle blue-green-algal biomass. In two samplings, the combination of flocculant and ballast exhibited the same efficacy as the use of solely PAC. Even so, the use of LS is important to ensure sedimentation. Combined with PAC, LS was as efficient a ballast to remove cyanobacteria as a commercially available modified clay (Phoslock®). Althugh LS *in natura* released considerable amounts of phosphorus and did not present P adsorption capacity, it managed to adsorb some dissolved phosphorus after organic matter was removed through muffling. This study shows that LS is a cheap, feasible and environment-friendly alternative to be used as a management action in reservoirs undergoing blooms in the semiarid region of Brazil.

Keywords: Restoration, cyanotoxins, eutrophication, harmful algae, *Microcystis*, “Flock and Lock”, sustainable action.

Resumo

A eutrofização artificial tem sido considerada um problema de grande preocupação nos ecossistemas aquáticos em todo o mundo. Desde 1960, os avanços científicos têm sido feitos a fim de desenvolver técnicas que atenuem os efeitos da eutrofização. Vários procedimentos físicos, químicos e biológicos podem ser usados e combinados para recuperar lagos de florações de cianobactérias, como a aplicação de um floculante combinado com argila natural ou modificada. No entanto, a eficácia dos solos de regiões áridas na mitigação de florações é desconhecida para lagos artificiais brasileiros. Neste artigo, apresentamos uma análise bibliométrica da evolução de publicações sobre florações de cianobactérias e identificamos registros que diretamente objetivam superar a ocorrência dessas florações. Além disso, avaliamos, por meio de experimentos laboratoriais, o efeito do uso combinado do floculante policloreto de alumínio (PAC) e um solo local do entorno do lago (LS), como lastro, no controle de florações de cianobactérias em um lago raso da região semiárida do Brasil. A pesquisa bibliométrica foi conduzida com o banco de dados "Web of Science" através da função de busca “TS = ((cyanobacteri* or blue green algae or cyanoprokariote or cyanophyceae) and (mass accumulation or bloom or domina*))”, de 1969 a junho de 2016. Realizamos uma análise de freqüência de palavras-chave e quantificamos o número de registros com uma abordagem de restauração. Além disso, foram realizadas três séries de experimentos em três momentos com florações diferentes em composição e biomassa no Reservatório Armando Ribeiro Gonçalves. Nossa pesquisa revelou que os estudos sobre as florações de cianobactérias aumentaram exponencialmente e seu impacto quantitativo nas ciências aquáticas aumentou significativamente ao longo dos anos ($F = 97{,}52$; $p < 0{,}0001$). Os EUA se destacam como a nação mais produtiva, seguida pela China e países europeus. A China aumentou impressionantemente sua contribuição para essa área, superando os EUA nos últimos cinco anos. Estudos sobre *Microcystis* e toxinas, tais como microcistinas, são tendências de investigação, devido a sua omnipresença e suas consequências negativas históricas. Também enfatizamos a necessidade de mais estudos com o objetivo de desenvolver técnicas para resolver e/ou mitigar a questão das florações. Em vista disso, nossos experimentos revelaram que o uso de PAC e LS teve um efeito notável na biomassa de cianobactérias da coluna de água em todas as amostragens, reduzindo até 90% a concentração de clorofila-*a*. O uso de LS sozinho foi ineficiente para remover a biomassa de algas azuis. Em duas amostragens, a combinação de floculante e lastro apresentou a mesma eficácia que o uso apenas de PAC. Mesmo assim, o uso de LS é importante para garantir a sedimentação. Combinado com PAC, o LS foi um lastro tão eficiente em remover cianobactérias quanto uma argila modificada comercialmente disponível (Phoslock®). Embora LS *in natura* tenha liberado quantidades consideráveis de fósforo e não apresentou capacidade de adsorção de P, tal argila conseguiu adsorver quantidades moderadas de fósforo dissolvido após a matéria orgânica ter sido removida por muflagem. Este estudo mostra que LS é uma alternativa economicamente viável e sustentável para ser utilizada como ação de manejo em reservatórios apresentando florações na região semiárida do Brasil.

Palavras-chave: Restauração, cianotoxinas, eutrofização, algas nocivas, *Microcystis*, “Floculação e Inativação de Nutrientes”, ação sustentável.

Introduction

During the past decades, artificial eutrophication has been considered a problem of major concern in aquatic ecosystems around the world (Xia et al., 2016). The excessive enrichment of nutrients, especially nitrogen and phosphorus, in these environments can bring negative consequences, most of which are a nuisance for the associated biota. It is straightforward the problems caused by the over-enrichment of lakes, e.g. biodiversity loss, hypolimnion hypoxia, decrease in water quality for consumption, inadequacy for bathing, implications in public health and economic issues (Smith et al. 2006).

When reservoirs become eutrophic, there is commonly a shift in the phytoplankton community towards the domination of cyanobacteria, commonly referred to as blooms. The presence and dominance of cyanoprokaryotes intensify the negative effects caused by eutrophication and these blooms are characterized as an advanced stage of the eutrophication process (Dokulil & Teubner, 2000). Some strains are well known for the production of cyanotoxins, with hepato-, dermato- and neurotoxic effects on biota and humans (Reynolds, 2006).

Cyanobacteria present some features, which enable them to become conspicuous in eutrophic environments. Several species are capable of fixing molecular nitrogen directly from the atmosphere, in a specialized cell called heterocyst. Therefore, when lakes are N-limited, these species may stand out and form persistent blooms (Beversdorf et al., 2013). Furthermore, some species are capable of storing phosphorus within their cells, making SRP not available to other phytoplankton species. Cyanobacteria are also highly adapted to warmer climates and take advantage over other species in tropical reservoirs (Kosten et al., 2011). Particularly, in tropical semiarid regions, the combination of high whole-year temperatures and light intensities boosts the establishment of cyanobacterial blooms (Bonilla et al., 2016).

Lakes constantly receive nutrients from diverse sources. Diffuse sources of nutrients are characterized by the input of allochthonous debris from the catchment into the reservoirs, especially through leaching. On the other hand, punctual sources are mainly sewage discharges and input of anthropogenic residuals in specific places (Alexander et al., 2002). As a result, reservoirs become deteriorated and unsuitable for humans and other organisms. Researchers have striven to mitigate and restore reservoirs back to meso- or oligotrophic conditions.

A fundamental procedure to minimize the occurrence of blooms and to obtain long-term results is the reduction of the external contribution of phosphorus (Søndergaard et al., 2000). However, the internal loading often makes it difficult to immediately visualize the effects of lake restoration. This phenomenon is due to the release of nutrients from the sediment as a result of, among other factors, wind resuspension (Jones & Welch, 1990) or bioturbation (Fukuhara & Sakamoto, 1987). In some temperate shallow lakes, for example, the reduction in mean annual input of phosphorus could only be verified 10 to 15 years after the application of such management action (Jeppesen et al., 2005). In addition, internal loading may return some lakes to a eutrophic state in less than 10 years (Søndergaard et al., 2007), supporting the importance of long-term monitoring studies. Therefore, effective methods must aim to minimize the effects of internal loading, by removing or immobilizing phosphorus from the water column.

Lake restoration procedures

Several oligotrophication techniques have been tested in the lab and *in situ*, encompassing physical, chemical and biological methods. Amongst the physical procedures, researchers have tested the use of dredging in preventing internal loading by removing lake sediment (Hovenkamp-Obbema & Fieggen, 1992). Even though it has been shown that lakes return to a better state, dredging is not always feasible, due to management costs.

Since advanced stages of eutrophication are characterized by hypoxia and sometimes anoxia in the hypolimnion, water circulation may be a good option in lakes to let oxygen flow through the water column. This technique also leads to nutrient dispersal and dilution, improving water quality (Hudnell et al., 2010). Limiting phytoplankton exposure to sunlight may hamper photosynthesis and cyanobacterial growth. This method can be used in small water bodies and has exhibited satisfactory results in microcosms (Zhou et al., 2014).

In the literature, restoration strategies through the use of chemical compounds are diverse. Algaecides with potential to destabilize cell integrity have been tested and resulted in decline in cyanobacterial populations (Bishop et al., 2015). However, the application of these chemicals must be cautious, as algaecides provoke cell lysis, releasing cyanotoxins to the water column. Therefore, the reckless use of chemicals may reveal devastating drawbacks, which have to be taken into account before adding these compounds into reservoirs.

Some techniques have focused on the precipitation of dissolved phosphorus through the application of iron and oxygen in the hypolimnion. In oxic conditions, iron binds to phosphorus, forming either ferric phosphate or iron hydroxide, which are not soluble in water and ultimately precipitates towards the bottom of the lake. This procedure has been applied in some developed countries, but it demands high application costs.

An old and feasible management action that has been applied is the use of flocculants to agglomerate phosphate particles. Since cyanobacteria are microscopic and cannot be sieved, flocculants help these particles to precipitate so that they can be easily removed. Flocculants based on aluminum, such as polyaluminum chloride and aluminum sulfate, have already been tested (Wang et al., 2014). The use of metal-based compounds in water bodies is controversial, as aluminum is acidic waters and in high concentrations can be toxic to the biota (Andren & Rydin, 2012). Organic compounds, like chitosan, are an alternative, even though its efficacy is not always clear (Magalhães et al., 2016).

In addition to commercial flocculants, some natural clays naturally have the potential to adsorb SRP, making it unavailable to the biota (Bahia Filho et al., 1983). In order to increase their adsorption capacities, several studies have modified these clays with flocculants, like chitosan and polyaluminum chloride (Pan et al., 2011; Zou et al., 2006). The advantage of this technique is that both dissolved and particulate phosphorus is removed from the water column and directed to the sediment, as clays serve as ballast. A commercially-available lanthanum-modified bentonite (LMB) has been created in Australia to be used as a restoration technique. Popularly known as Phoslock®, LMB has been applied in developed countries with successful results (Lürling & Faassen, 2012). However, LMB is costly and therefore may not be viable as a curative measure in countries with lacking financial support.

In order to promote a more effective sedimentation of particulate phosphorus, a promising technique is the combination of a flocculant, e.g. polyaluminum chloride, with a ballast. This combination hampers the resuspension of phosphorus stored in the sediment through the internal loading (Lürling & van Oosterhout, 2013). For instance, a study has demonstrated that the use of either PAC or LMB alone did not effectively decrease filaments of *Cylindrospermopsis raciborskii*, but the combined application of such compounds exhibited promising results (Araújo et al., 2015).

Biomanipulation, like the removal of zooplanktivorous fish or the addition of piscivorous fish, have also been tested with successful results (Kasprzak et al., 2003). Other biological methods include the addition of mussels or parasites (Waajen et al., 2016). These techniques cause cascading effects through top-down control, which ultimately decrease cyanobacterial biomass. Even though there are several restoration methods, recent studies emphasize the importance of combining practices, like the reduction of external phosphorus input and biomanipulation (Starling & Rocha, 1990).

Despite the great importance of the study on eutrophication and cyanobacteria blooms, there are few records of eutrophic lakes that have been successfully restored, mainly in tropical environments. For instance, in Brazil, Lake Paranoá, formerly dominated by cyanoprokaryotes, is one of the few cases of reservoirs that have been successfully restored in the country, as a result of the combination of sewage discharge reduction and biomanipulation (Starling & Rocha, 1990). In semiarid environments, the few records of restored lakes rises an even more serious issue, since the low amount of rainfall, high evaporation and high retention time are factors that contribute to the increase in nutrient concentration and, consequently, the eutrophic state of shallow lakes and the occurrence of cyanobacteria (Bouvy et al., 2003). Is the negligible attention eutrophic lakes have been given a result of lack of studies on oligotrophication techniques? Analyzing the actual panorama on the publications about cyanobacterial blooms and quantifying the studies on lake restoration may be useful to elucidate this question.

Aims

In this master thesis, we aimed to identify the current knowledge trends about cyanobacterial blooms, and to point out which topics and priorities future studies should focus on through a bibliometric perspective. In addition, we quantified the research publications that directly intend to overcome and to mitigate the occurrence of harmful blooms. Subsequently, we evaluated the effect of flocculant (PAC) and natural clay (local soil from the catchment; LS) addition, alone and combined, in reducing cyanobacterial biomass from three different blooms of a shallow lake in Brazilian semiarid region. We also investigate the efficacy of LS in removing dissolved phosphorus from the water column. We compared the efficiency of LS as ballast and as a P-sorbent with LMB.

CHAPTER I. Cyanobacterial blooms in eutrophic waters: a bibliometric analysis

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Abstract

Artificial eutrophication has been considered a problem of major concern in aquatic ecosystems around the world. Since 1960, scientific advances have been made in order to develop techniques that mitigate the effects of eutrophication. Several physical, chemical and biological procedures can be used and combined to recover lakes from cyanobacterial blooms. In this paper, we present a bibliometric analysis of the evolution of publications about cyanobacterial blooms and identify records that directly aim to overcome the occurrence of these blooms. The research was conducted with the "Web of Science" database through the search function "TS = ((cyanobacteri* or blue green algae or cyanoprokariote oy cyanophyceae) and (mass accumulation or bloom or domina*))", from 1969 to June 2016. We performed a keyword frequency analysis and quantified the number of records with a restoration approach. Our study revealed that studies about cyanobacterial blooms increased exponentially and their quantitative impact on the aquatic sciences increased significantly along the years ($F = 97.52$; $p < 0.0001$). The USA stands out as the most productive nation, followed by China and European countries. China has impressively increased its contribution to this area, surpassing the USA in the last five years. Studies about *Microcystis* and toxins, such as microcystins, are trends in research, due to their ubiquitousness and historical negative consequences. We also emphasize the need for more studies aiming at developing techniques to solve and/or mitigate the issue of blooms.

Keywords: Restoration, cyanotoxins, eutrophication, harmful algae, *Microcystis*.

Introduction

The enrichment of terrestrial and inland waters with phosphorus and nitrogen has doubled in the past century, following the increase of fossil fuel and fertilizer use by humans. The stratigraphic markers of this enrichment are one of the criteria supporting the formalization of a new geological epoch, named Anthropocene, distinct from the current Holocene epoch (Waters et al., 2016). The proposal of the Anthropocene epoch revels the impact of humans on Earth, among which eutrophication is included. In fact, artificial eutrophication, defined as the enrichment of an aquatic ecosystem with excess nutrients such as phosphorus and/or nitrogen, has been considered a problem of major concern (Jeppesen & Sammalkorpi, 2002).

Several negative consequences have already been reported as a result of nutrient over-enrichment in lakes, e.g. biodiversity loss, hypoxia, decrease in water quality, implications for public health and economic issues (Smith et al., 2006). In addition, blooms of certain phytoplankton species may cause a nuisance for the associated biota and humans. Cyanobacteria are photoautotrophic prokaryotes ubiquitous in fresh, marine and brackish waters. Many planktonic cyanobacteria are bloom-forming species and may proliferate profusely in eutrophic freshwater ecosystems (Chorus et al., 2000).

Even though warmer temperatures favor the dominance of cyanobacteria, some studies revealed that these organisms have expanded their geographic distribution, reaching temperate environments due to their high invasiveness (Affan et al., 2016; Svircev et al., 2016). For instance, a recent study has shown that the genus *Microcystis* alone can be found in nearly 108 countries (Harke et al., 2016). Moreover, some species, like *Cylindrospermopsis raciborskii*, thought to be tropical and subtropical can now be found even in the cold waters of Canada (Kling, 2009).

It has been demonstrated that phosphorus, instead of nitrogen, is the major nutrient involved with the eutrophication process (Schindler, 1971; Schindler, 2006). Furthermore, cyanobacteria are frequently associated with lakes presenting high concentrations of total phosphorus; its presence intensify the negative consequences of eutrophication as some strains produce toxins, which can have hepato- and neurotoxic effects (Reynolds, 2006). Under these conditions of nutrient profusion, cyanobacteria increase considerably their biomass, causing harmful blooms (Whitton & Potts, 2000; Reynolds, 2006; Beversdorf et al., 2013).

Since 1960, scientific advances have been made in order to develop techniques that mitigate the effects of eutrophication or promote lake oligotrophication. Today several physical, chemical and biological procedures can be used and combined to recover lakes from cyanobacterial blooms (Peretyatko et al., 2012; Lürling & van Oosterhout, 2013; Gołdyn et al., 2013). Therefore, it would be useful to investigate the global scientific status with regard to the studies about blue-green algal blooms and lake restoration.

Bibliometrics, a sub-area in the field of scientometrics, is a valuable tool that uses quantitative and statistical methods to extract information from published studies in order to obtain a better panorama of the current research fields (Verbeek et al., 2002). There can already be found in the literature bibliometric studies about the research trends in eutrophication and drinking water quality (Gao et al., 2015; Fu et al., 2013). A study in China, for instance, has shown through a bibliometric approach that future research on Lake Taihu should focus, among other topics, on the relationship between eutrophication and climate change, and the monitoring and prediction of cyanobacterial blooms (Zhang et al., 2016). Hence, bibliometrics can be used to unravel potential gaps in the scientific knowledge and serve as a guideline to identify hotspots for future studies.

In this paper, we present a bibliometric analysis of the evolution of publications about cyanobacterial blooms. The purpose of this study is to evaluate the current trend in research on blue-green algal blooms and to identify the records that directly aim to overcome the occurrence of these blooms. Despite the potential impacts of cyanobacterial blooms and the scientific relevance of the issue, as far as we concern, this is the first attempt to present a bibliometric survey on this subject.

Material and Methods

Data source and quantitative analysis

The research was conducted in July 2016, using the online version of Science Citation Index Expanded (SCI-Expanded) databases of the Web of Science from Thomson Reuters. The search function was defined as "TS = ((cyanobacteri* or blue green algae or cyanoprokariote or cyanophyceae) and (mass accumulation or bloom or domina*))", where TS stands for *Topic* and includes title words, abstracts and author keywords. A filter was applied, selecting the following documents: articles, letters, meeting abstracts, notes, proceeding papers and reviews. The search period was restricted from January 1969 to June 2016, since the sole publication prior to this period did not fit the scope of this study and was considered irrelevant.

In order to investigate the importance of the studies about cyanobacterial blooms within the aquatic sciences over time, the number of publications and citations in all aquatic research areas from 1969 to June 2016 was quantified and compared with the documents and citations about cyanobacterial blooms. This quantification of aquatic studies was obtained from Web of Science through the function defined as "SU = (Fisheries OR Marine & Freshwater Biology OR Water Resources OR Oceanography)", where SU stands for *Research Area.* The same filters used in the previous search were applied. The number of citations were also obtained from Web of Science databases. The publication years were then divided into eight time intervals (periods) of six years each. Subsequently, we performed an analysis of covariance (ANCOVA), adopting the research area (either cyanobacterial blooms or aquatic sciences) as a qualitative explanatory variable and the time intervals (period) as a covariate. The period was also set as a random factor (block). A significant interaction between the qualitative variable and the covariate would be interpreted as a positive or negative quantitative impact of the studies about cyanobacterial blooms in the aquatic sciences over the years. Conversely, a non-significant result would represent no change in the contribution of the studies about cyanobacterial blooms in the aquatic sciences over time. The same procedure was conducted with regard to the number of citations. All statistics were performed using R software (R Core Team 2014) with $\alpha = 0.05$. The data was previously log-transferred in order to fulfill the normality and homoscedasticity assumptions of the test. Such assumptions were investigated through analysis of the distribution of residuals.

In order to evaluate the contribution of the studies on cyanobacterial blooms to the increase in the number of publications on aquatic sciences along the years, we calculated the effect sizes of these studies, using the following formula:

$$ES = \log[(a_t/\beta_t)\ /\ (a_t'/\beta_t')],$$

where a_t and a_t' are the number of publications within the aquatic sciences and on cyanobacterial blooms at time t (year), respectively, and β_t and β_t' are the number of all studies published within the aquatic sciences and on cyanobacterial blooms until time t (year), respectively. A positive effect size indicates that the studies on cyanobacterial blooms of a year increased more than the studies on aquatic sciences, taking into account the chronological trend. We included in this analysis only the documents published from 1991 onwards, when the number of records on cyanobacterial blooms became substantial.

Word frequency exploration

The results from the database search were compiled, processed and standardized using the bibliometric tools provided by the Web of Science website, and frequency analyses of title words, author keywords and KeyWords Plus® were conducted using Bibexcel, Version 2014-03-25, developed by Olle Persson (Persson et al., 2009). Author keywords account for keywords provided by the author in the abstract, present in documents since 1991, whereas KeyWords Plus®, a tool created by Thomson Reuters, incorporates terms originated from the title of the publications cited by the author. Since there are similar keywords written in different ways (e.g. written as singular/plural nouns), the top 200 words were manually standardized in order to select the top 20 title words, author keywords and KeyWords Plus® (Gao et al., 2015). This result provides valuable information about the main topics in scientific research. In addition, co-occurrence analysis was used to reveal topics that are studied more frequently together. In order to investigate local research interests, the recent trends in research of the most representative countries in each continent were identified through the frequency analysis of author keywords since 2011.

"Restoration approach" investigation

We evaluated how much research have been done on approaches aiming to directly develop tools to overcome the occurrence of cyanobacterial blooms, by quantifying the number of records published on "restoration approach". Documents that included the following selected author keywords were regarded as "restoration approach": restoration, biomanipulation, oligotrophication, resilience, recovery, management, mitigation, remediation, control. These keywords were chosen based on a list of author keywords found in the most cited or most recently published papers within the approach "restoration", such as Mehner et al. (2002), Jeppesen et al. (2005, 2007), Søndergaard et al. (2007), Schindler (2008), Smith and Schindler (2009), Attayde et al. (2010), Jacquet et al. (2014), Zamparas et al. (2014). Variations on selected keywords were also considered, as “oligotrophication” and “re-oligotrophication” or “remediation” and “bioremediation”, for instance. As we aimed to identify those documents that have devoted a substantial effort to provide a protocol or measures to counteract cyanobacterial blooms, we excluded those that have not reached this goal despite the contribution of such publications to the knowledge of plankton dynamics.

The publications identified as belonging to the “restoration approach” category, according to author keywords, were manually inspected and classified according to the type of research (theoretical, descriptive, experimental, synthesis). Reviews and modelling articles were classified as theoretical, whereas studies about lake monitoring without human intervention were categorized as descriptive. Records about laboratory experiments and *in situ* manipulations were regarded as experimental, and syntheses of case studies were considered synthesis. Additionally, these documents (except the ones classified as theoretical) were categorized with regard to spatial scale (laboratory, mesocosm, whole lake, multiple lakes, river, coastal areas) and temporal scale (short (days to weeks), medium (six to 12 months), long (more than one year), very long (more than five years)).

Results

Quantitative results

From the 48 years included in our search, we obtained 9,592 documents, comprising 8,854 articles (92.31%), 791 proceedings paper (8.25%), 404 reviews (4.21%), 33 meeting abstracts (0.34%), 32 notes (0.33%) and 6 letters (0.06%). These records represent the effort of 21,313 authors, from 100 countries and published in 1,250 different journals.

By the end of 1970s, only 13 documents had been published (0.14%); ten years later the number of records reached 66 (0.69%). From 1991 on, the amount of documents about cyanobacterial blooms increased considerably, totalizing 1,551 (16.17%) until 2000. The period between 2001 and 2010 accounted for 3,815 (39.77%) of all publications and this number continued to increase since 2011, with a total of 4,147 (43.23%) records.

When we compared the evolution of the number of publications about cyanobacterial blooms during our research period with the evolution of published records within the aquatic sciences, it was clear that the studies about cyanobacterial blooms increased more significantly over time (F = 97.52, df = 1, $p < 0.0001$, Fig. 1A). Furthermore, the publications about this topic were progressively more cited over the years (F = 72.10, df = 1, $p < 0.0001$) if compared to the records in the aquatic sciences, since the latter did not exhibit such an increase in the number of citations as the former (Fig. 1B).

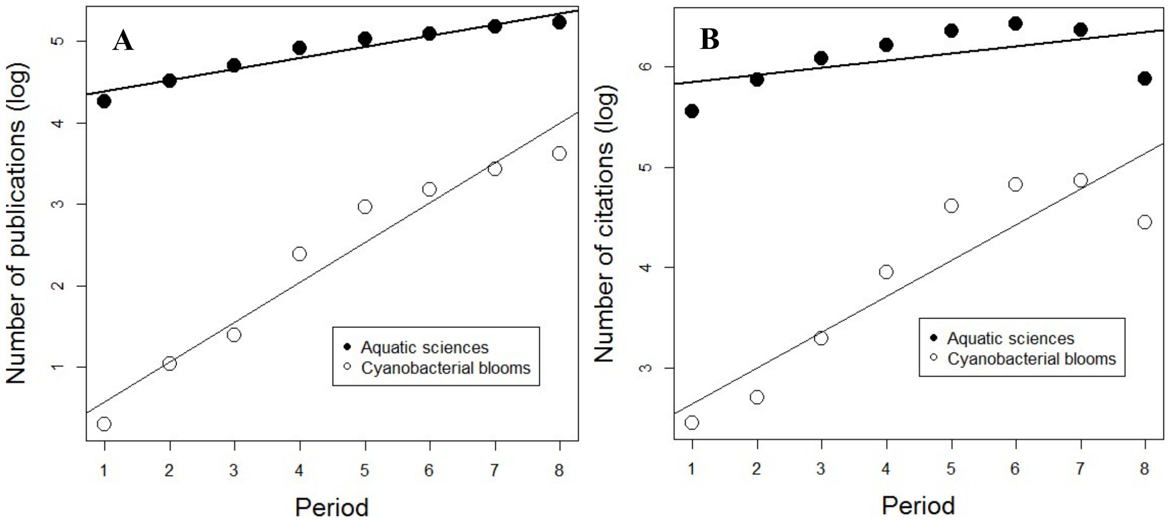

Figure 1. Relationship between the number of records with the field of study and the period of publication by the analysis of covariance. Closed circles represent the number of publications in the aquatic sciences and open circles represent the documents in the field of cyanobacterial blooms. **A.** Aquatic sciences: $\log y = 4.26 + 0.14 * \text{period}$; $r^2 = 0.93$. Cyanobacterial blooms: $\log y = 0.09 + 0.49 * \text{period}$; $r^2 = 0.94$. **B.** Aquatic sciences: $\log y = 5.77 + 0.07 * \text{period}$; $r^2 = 0.33$. Cyanobacterial blooms: $\log y = 2.29 + 0.36 * \text{period}$; $r^2 = 0.83$.

The period between 1991 and 2000 was characterized by a remarkable increase in the effect sizes, as they tended to become less negative. The studies on cyanobacterial blooms greatly increased during this period relatively to the previous years, in comparison to the increase rate of the aquatic sciences. This trend slowed down after the year of 2000 and the effect sizes tended to stabilize (Fig. 2).

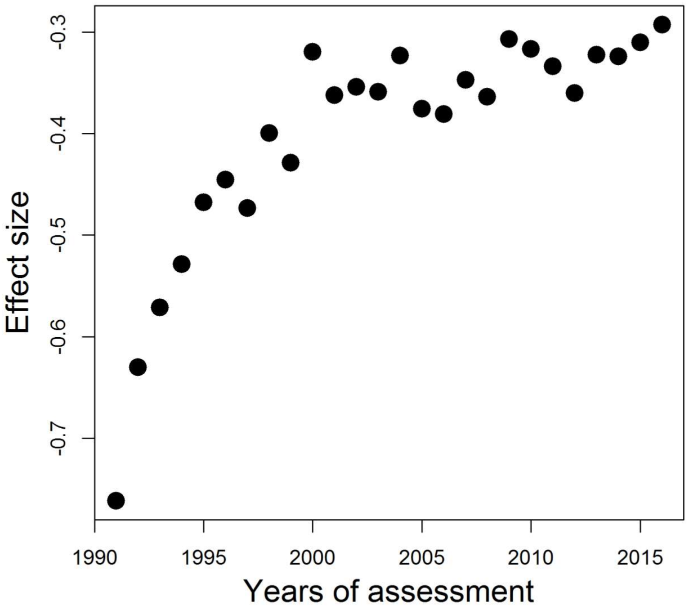

Figure 2. Effect sizes (log) of the studies within the aquatic sciences relatively to the publications on cyanobacterial blooms from January/1991 to June/2016.

Amongst all 100 countries that contributed to the study of cyanobacterial blooms in our research, 17 contributed to more than 2% of all 9,592 documents (Table 1). The United States was remarkably the most active country, collaborating with 2,341 publications, comprising 24.41% of all documents. The United States was followed by the People's Republic of China (1,454; 15.16%), Germany (883; 9.21%), Australia (700; 7.30%) and Canada (532; 5.55%). These countries were also the most productive in the last period (2011-2016), but China, with a total of 1,053 publications, surpassed the United Stated, with 959.

The United States, the People's Republic of China, Germany, Australia, Brazil and South Africa were the most representative countries in each continent. The USA dominated the number of records until 2010, but China, in particular, increased considerably its publications since the last ten years, becoming the most productive country regarding cyanobacterial blooms at the present time. Brazil and South Africa have started investigating on this topic at the end of the century, contributing moderately to the overall production (Fig. 3).

Table 1. List of the most productive countries since 1969, the number of published records and its percentage related to the 9,592 documents. Only countries that contributed to more than 2% of all 9,592 publications were analyzed. The table also provides a list of the most active countries since 2011 with their corresponding number of records. RP: recent publications (since 2011).

Rank	Country	Records	%	Rank	Country	RP
1	USA	2341	24.41	**1**	Peoples'R. of China	1053
2	People's R. of China	1454	15.16	**2**	USA	959
3	Germany	883	9.21	**3**	Germany	341
4	Australia	700	7.30	**4**	Australia	297
5	Canada	532	5.55	**5**	Canada	218
6	France	480	5.00	**6**	France	193
7	England	452	4.71	**7**	Spain	172
8	Japan	435	4.54	**8**	England	171
9	Spain	370	3.86	**9**	Brazil	142
10	Netherlands	363	3.78	**10**	Netherlands	140
11	Sweden	352	3.67	**11**	Poland	137
12	Finland	330	3.44	**12**	Japan	136
13	Brazil	285	2.97	**13**	Sweden	122
14	Poland	266	2.77	**14**	India	121
15	India	249	2.60	**15**	Italy	119
16	Italy	236	2.46	**16**	Israel	75
17	Israel	204	2.13	**17**	Finland	71

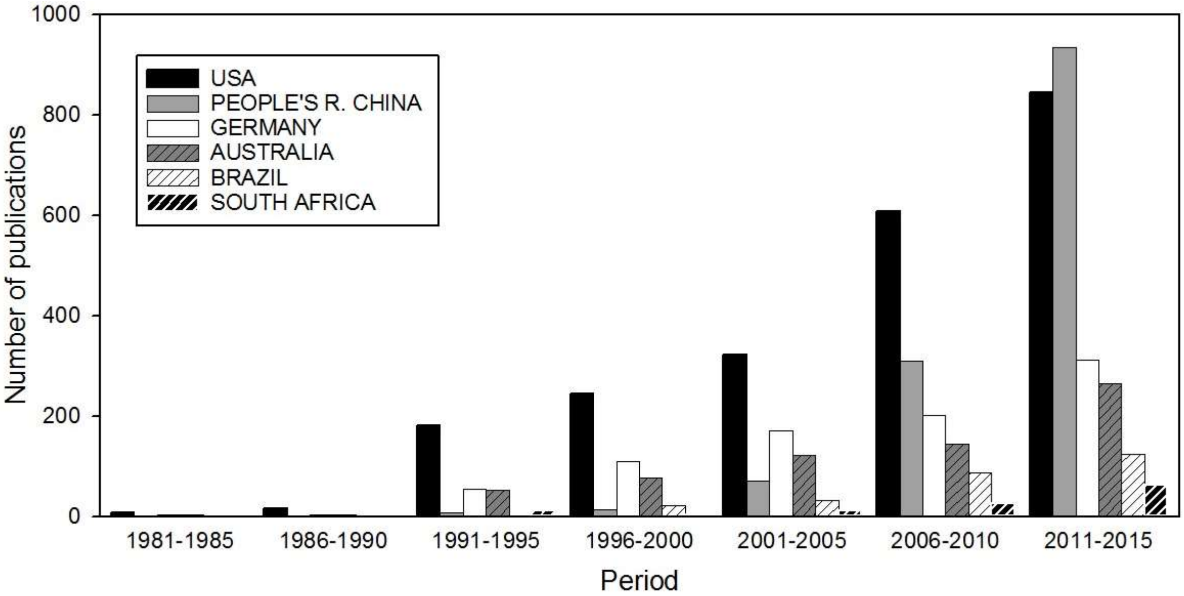

Figure 3. Number of publications of the most productive countries in each continent from 1981 to June 2016.

Word frequency output

Among 9,592 publications, it was possible to retrieve information about author keywords from 6,998 records (72.96%) and KeyWords Plus® from 9,085 documents (94.71%). This analysis clearly indicated that the cyanobacterium *Microcytis* and the cyanotoxin microcystin are highly studied. The analysis of author keywords showed that microcystin was the most frequent along with the genus *Microcystis*. The KeyWords Plus® analysis revealed that *Microcystis* was the second most recurrent word and the variant LR of microcystin was the ninth most common. In terms of title words, *Microcytis* appeared in the fifth position, microcystin was the seventh most frequent and the specific epithet *aeruginosa* (probably from *Microcystis aeruginosa*) was in the ninth position (Table 2). Similarly, the co-occurrence analysis reinforced this result, as the combination of the author keywords cyanobacteria, microcystin and *Microcystis* occurred more often (Table 3).

From this overall word frequency analysis, it is shown that (cyano)toxins are a hotspot in the study of cyanobacterial blooms (Table 2). Nutrients, such as nitrogen and phosphorus, were also frequent in title words, author keywords and KeyWords Plus®, and these words co-occurred repeatedly (Table 3). Additionally, the Baltic Sea and the word climate change were the tenth and the eighteenth most frequent author keywords, respectively. The only genus of cyanobacteria, other than *Microcystis*, that was present in our results was *Cylindrospermopsis*, in the nineteenth position of author keywords.

Table 2. List of the 20 most frequent title words, author keywords and KeyWords Plus® from January 1969 to June 2016.

Rank	**Title words**		**Author keywords**		**KeyWords Plus®**	
1	1985	lake	885	microcystin	1438	phytoplankton
2	1045	phytoplankton	713	*Microcystis*	1103	*Microcystis*
3	990	community	624	phytoplankton	834	Water
4	981	water	450	eutrophication	774	Lake
5	891	*Microcystis*	322	cyanotoxin	742	growth
6	840	effect	245	nutrient	628	Toxin
7	800	microcystin	238	phosphorus	586	diversity
8	510	reservoir	186	chlorophyll	523	community
9	493	*aeruginosa*	168	nitrogen	507	microcystin-LR
10	487	microbial	165	Baltic sea	506	fresh-water
11	477	sea	163	bacteria	485	phosphorus
12	404	growth	162	zooplankton	449	nitrogen
13	401	diversity	157	water quality	424	dynamics
14	375	dynamics	153	diatom	343	toxicity
15	374	nutrient	152	photosynthesis	322	identification
16	366	toxic	152	diversity	320	Light
17	365	change	135	nitrogen fixation	317	nitrogen-fixation
18	347	freshwater	130	climate change	313	eutrophication
19	344	shallow	123	*Cylindrospermopsis*	311	bacteria
20	341	nitrogen	121	microbial mat	282	photosynthesis

Table 3. Co-occurrence analysis of author keywords from January 1969 to June 2016.

Rank	Co-occurrent keywords	
1	cyanobacteria	microcystin(s)
2	cyanobacteria	*Microcystis*
3	microcystin(s)	*Microcystis*
4	cyanobacteria	Cyanotoxins
5	cyanobacteria	eutrophication
6	cyanobacteria	phytoplankton
7	nitrogen	Phosphorus
8	cyanobacteria	Phosphorus
9	phytoplankton	Zooplankton
10	cyanobacteria	nitrogen fixation

Expanding our examination on author keywords, we investigated the occurrence of all words related to cyanotoxins, since this topic was very relevant in our overall keyword analysis. Microcystin represented 56.37% of all toxins, whereas cylindrospermopsin accounted for 7.07% and nodularin comprised 4.97% (Table 4).

Table 4. List of the most frequent author keywords related to cyanotoxins from January 1969 to June 2016.

Rank	Author keywords	
1	885	microcystin*
2	322	Cyanotoxin
3	111	Cylindrospermopsin
4	78	Nodularin
5	67	anatoxin-a
6	55	Saxitoxin
7	52	Hepatotoxin

* The keyword “microcystin” accounts for microcystin (427). microcystins (346) and microcystin-LR (112).

Analyzing the recent contribution of the most representative countries in each continent for the author keywords since 2011, we observe that the hepatotoxin microcystin and *Microcystis* are a hotspot in all continents (Table 5). In the People’s Republic of China, there is a great deal of studies about Lake Taihu, whereas Germany has several publications about the Baltic Sea. Besides, the United States, Germany and Australia gather a substantial number of studies that investigate the effects of climate change. Our results also showed that cylindrospermopsin, another hepatotoxin, is well studied in Australia, and that saxitoxins and *Cylindrospermopsis*, a genus of cyanobacteria that produce both toxins, are highly investigated in Brazil and Australia. Data for South Africa have not been included in this analysis due to the low number of publications since 2011 (65 documents). However, it is worthy to mention that the amino acid β-N-methylamino-L-alanine (BMAA), present in cyanobacteria, was the most frequent author keyword in South Africa, followed by *Microcystis* and microcystin (data not shown).

Table 5. List of the top 10 author keywords for the most representative countries in each continent since 2011.

Rank	**USA**	**People's R. of China**	**Germany**	**Australia**	**Brazil**
1	microcystin	*Microcystis*	microcystin	Microcystin	microcystin
2	eutrophication	microcystin	phytoplankton	*Microcystis*	phytoplankton
3	*Microcystis*	Lake Taihu	eutrophication	cylindrospermopsin	*Microcystis*
4	phytoplankton	eutrophication	climate change	Cyanotoxin	cyanotoxins
5	phosphorus	phytoplankton	*Microcystis*	water quality	eutrophication
6	chlorophyll-a	bacteria	Baltic Sea	climate change	*Cylindrospermopsis*
7	nitrogen	phosphorus	nitrogen fixation	Eutrophication	reservoirs
8	cyanotoxins	diversity	Photosynthesis	Nutrients	saxitoxins
9	climate change	DGGE	Cyanotoxins	Saxitoxin	zooplankton
10	nutrients	photosynthesis	Daphnia	*Cylindrospermopsis*	seasonality

"Restoration approach" analysis

Published documents within the "restoration approach" numbered 174, accounting for 1.81% of all records obtained from our search function. From these 174 publications, the majority of them were experimental, totalizing 98 records (56.32%). Excluding the 44 theoretical documents (25,29%), the classification of the remaining 130 publications with regard to spatial scale revealed that the majority of them were performed beyond the laboratory scope, in a large spatial scale. Our analysis resulted in 57 (43.85%) encompassing whole lakes, 40 (30.77%) comprising laboratory experiments and 15 (11.54%) involving multiple lakes. Very few studies were undertaken in rivers (3) and coastal areas (2), comprising 3.85% altogether. When classified according to temporal scale, 57 (43.85%) were short studies, whereas 45 of them (34.62%) were long-term investigations with a duration of more than 5 years and 23 (17.69%) were categorized as large (Box 1).

Box 1: Records within the "restoration approach" classified according to their category (theoretical, descriptive, experimental or synthesis), spatial scale and temporal scale.

	Number of records	
Category	Theoretical	44
	Descriptive	28
	Experimental	98
	Synthesis	4
Spatial scale	Laboratory	40
	Mesocosm	13
	Whole lake	57
	Multiple lakes	15
	River(s)	3
	Coastal areas	2
Temporal scale	Short (days to weeks)	57
	Medium (six to 12 months)	5
	Large (> 1 year)	23
	Very large (> 5 years)	45

Discussion

This study shows clearly that the studies about cyanobacterial blooms intensified progressively along the years, as this is a natural trend in science (Bornmann & Mutz, 2015). More importantly, it was evident that this considerable increase in the number of publications represented a growing relevance within the aquatic sciences, especially in the 1990s. This impact in the aquatic scientific community over the years is a reflection of the increasing concern toward the bad implications of cyanobacterial blooms in aquatic environments and public health control all over the world (Merel et al., 2013).

The countries that had a more significant contribution to this topic of research were the United States and other developed countries, which was also observed in other studies (Zhou et al., 2007; Zhuang et al., 2013). The presence of the USA in the top list as the most productive country was expected, since it is a pioneer in science and technology. Furthermore, the USA is recognized by its high investments in other areas of research (Audretsch et al., 2002). In common with other developed countries, the USA has a long history of experience with science, whereas developing countries (e.g. China, Brazil and South Africa) only started publishing on cyanobacterial blooms at the end of the last century and more expressively after 2000.

With regard to the People's Republic of China, its presence as the second most productive nation is a reflection of its growing efforts to investigate cyanobacterial blooms since the end of 1990s. The topic became of relevant interest probably owing to the process of deterioration of Lake Taihu that started in the 1980s, which intensified with *Microcystis* blooms during the mid-1990s and demanded restoration procedures (Chen et al., 2003). The presence of China in the top list was also observed in other scientometric investigations in the aquatic sciences (Gao et al., 2015; Fu et al., 2013). Besides, China has increased its collaboration with other countries, such as the USA and the UK (Zhuang et al., 2013; Gao et al., 2015). In particular, the science and technology partnership between the United Stated and China, first signed in 1979 (Department of State, 2012), certainly intensified cooperation, discussions and investments in science, which may have boosted Chinese publications. In addition, it is noteworthy that this increase in the investment in science was so pronounced that China has now surpassed the USA and has taken the lead in absolute numbers regarding publications on cyanobacterial blooms.

Brazil started its contribution to the study of blue-green algal blooms in the same period as China, after an episode involving patients undergoing hemodialysis. In the late 1990s, several patients developed toxic hepatitis after hemodialysis sections, as a result of water contamination by microcistin (Azevedo et al., 2002). This outbreak of poisoning may have been the trigger for the studies on this topic.

Frequency analysis of title words, author keywords and KeyWords Plus® have been demonstrated as a useful way to identify the trending topics in scientific research (Li et al., 2009). Title words and author keywords clarify the theme of the publications whereas KeyWords Plus® is related to the theme indirectly (Garfield, 1990). Our word frequency analysis revealed that the studies about cyanobacterial blooms are mainly concentrated on cyanotoxins, primarily the hepatotoxin microcystin. The attractiveness of cyanotoxins as a focus of research is straightforward, since they are of major concern regarding water quality, loss of biodiversity and public health (Zanchett & Oliveira-Filho, 2013; EPA, 2012). Amongst all cyanotoxins, microcystin is the most common and comprises over 50 different variations that are widely distributed in eutrophic water bodies (Eguzozie et al., 2016; Oberholster et al., 2005). Particularly, microcystin-LR is of great importance in freshwater ecosystems due to its high hepatotoxicity – its oral LD_{50} is 5000μg/Kg of body weight in mice (Campos & Vasconcelos, 2010; WHO, 2003). This may explain why microcystin was one of the most frequent keywords in our analysis and is widely studied in every continent. Nevertheless, although microcystin-LR is thought to be the most abundant microcystin in aquatic systems, there is some controversy about these observations, as some authors state that the methodology to quantify this variant was the first to be used (Chorus & Bartram, 1999). Therefore, more studies on other types of microcystin could clarify and contribute more to the knowledge of cyanotoxins, their abundance and toxicity.

In addition to microcystin, the genus *Microcystis*, a producer of such a toxin, and nutrients, the direct cause of eutrophication, were also very frequent in our keyword analysis. This observation is plausible as *Microcystis aeruginosa* is the most common cyanobacterium in the world, from which cyanotoxins were first characterized (Oberholster et al., 2004). Indeed, our results show that this species is a hotspot worldwide.

Along with *Microcystis*, the second most common genus of cyanobacteria in our keyword analysis, *Cylindrospermopsis*, has recently been well studied in Brazil and Australia. The species *C. raciborskii*, for instance, is common in tropical and subtropical regions, as it stands out in polymictic and phosphorus-rich water bodies, especially during dry periods (Soares et al., 2013; Costa et al., 2016). Studies about this species in Brazil must have been intensified probably owing to recurrent blooms in its tropical reservoirs since the late 1990s, especially the semi-arid shallow lakes (Costa et al., 2016; Dantas et al., 2010; Bouvy et al., 2000). In Australia, the high interest in *C. raciborskii* and the toxin cylindrospermopsin increased after the first registered event of human poisoning from consumption of water contaminated with this cyanotoxin, which was reported in Palm Island, Queensland, Australia (Griffiths & Saker, 2002). "The Palm Island Mystery Disease", as this hepato-enteritis was popularly referred to, affected 148 adults and children and elicited a great deal of research (Hawkins et al., 1985). Even though *C. raciborskii* is common in tropical countries, its invasiveness and adaptiveness have allowed it to expand its range and to reach temperate areas (Padisák, 1997). In fact, there is evidence that cylindrospermopsin occurrence in the USA goes back to approximately 4700 years ago and is associated with European strains (Waters, 2016). However, our results indicate that more studies on this cyanotoxin and *C. raciborskiii* need to be carried out in temperate countries.

Saxitoxins, which are toxins with a neurotoxic effect, also appeared in the list of keywords from recent publications in Australia and Brazil. Although they are famous for causing the paralytic shellfish poisoning in marine environments due to dinoflagellate blooms (Costa, 2016), saxitoxins are also produced by cyanobacteria species, such as *Cylindrospermopsis* *raciborskii* (Brentano et al., 2016). However, in the keywords list related to cyanotoxins, it is shown that saxitoxins are less studied than other toxins, like nodularin and anatoxin-a.

Genetic studies have greatly encouraged scientists to investigate cyanotoxin production. Through genetic engineering, it has been possible to identify toxin-producing strains of cyanobacteria and to understand their physiology and biochemistry, particularly their ecological implications in toxic blooms (Kaebernick & Neilan, 2001; Wilson et al., 2005). Yet information concerning toxins and their consequences in cyanobacterial blooms is sparsely reported and sometimes completely absent in some countries (Mowe et al., 2015).

The Baltic Sea was considered a current trend in research in our study, especially in the European continent. Once the countries surrounding the Baltic Sea have agreed to protect it, by reducing the emissions of nutrients, in the Helsinki Commission (HELCOM), investments have been supplied in order to overcome its issues around deterioration (HELCOM, 2007). In addition, from the keyword analysis, it was also possible to note that climate change was a trending topic in the USA, Germany and Australia; therefore, we can infer that North America, Europe and Oceania present substantially more publications on climate change than the other continents. Specifically, there is a handful of studies concerning the effects of climate change on the cyanobacteria assemblage in the Baltic Sea (Hense et al., 2013; Neumann et al., 2012). Notwithstanding the considerable number of studies related to climate change in South America (Costa et al., 2016; Brasil et al., 2016; Medeiros et al., 2015) and Asia (Havens et al., 2016; Xia et al., 2016), more studies are needed, due to the significance of this topic. Based on climate models, Earth's mean surface temperature will increase between 3-5°C (prediction for 2070-2100) (Sommer et al., 2007), therefore it is of utmost importance to undertake studies on the effects, ways of prevention, and predictions of climate change.

Our results revealed that very few studies about cyanobacterial blooms focus on restoration procedures or on oligotrophication techniques. Albeit sparse, the publications were diverse, ranging from laboratory to field studies, encompassing short- and long-term observations. Laboratory experiments are of crucial importance to understand intrinsic ecological mechanisms whereas field and natural investigations give a more realistic idea of the outcome the restoration practices would provide in nature (Diamond, 1983). Therefore, the use of varied research strategies with different spatial and temporal scales may provide robust knowledge on restoration tools. Nonetheless, since toxic blooms are a nuisance worldwide and pose negative consequences on economy and public health (Smith et al., 2006), it is surprising that less than 2% of the publications in our study were regarded as “restoration approach”. However, this number does not correspond to the total amount of studies on lake restoration in the whole literature, as many publications that focus on eutrophication mitigation could not be retrieved with our search function. However, these results give us an idea of the negligible attention this important subject has received.

During our analysis, we came across a variety of biological approaches to control cyanobacteria. Some studies have shown that bacteria species and viruses (cyanophages) can either inhibit the growth or provoke lysis on several cyanobacteria (Choi et al., 2005; Wu et al., 2009). In addition, other species have been successfully tested as control agents, like protozoans (Liu et al., 2012), yeast (Kong et al., 2013) and mussels (Waajen et al., 2016). Chemical compounds have also been proved to cause oxidative damage and selective supression on cyanobacteria, like allelochemicals extracted from plants (Lu et al., 2013), hydrogen peroxide (Wang et al., 2012) and the combination of copper with algaecides (Bishop et al., 2015). In addition, a handful of studies have focused on evaluating physical methods, like sediment dredging (Hovenkamp-Obbema & Fieggen, 1992), solar shading (Zhou et al., 2014) and water transfer (Fornarelli & Antenucci, 2011).

Most of the above-mentioned techniques were tested in the laboratory with successful results, however not all of them have shown to be viable in the natural environment. For instance, a study showed that while the use of ultrasound reduces the biovolume growth and causes filament shortage on cyanobacteria, it also kills *Daphnia* (Lürling & Tolman, 2014). Also, hydrogen peroxide reduces cyanobacterial biomass, but releases toxins in the water column (Lürling et al., 2014). Therefore, it is of considerable importance to evaluate possible drawbacks of restoration measures in order not to cause negative ecological consequences.

The majority of long-term outdoor studies in our analysis used monitoring data of reservoirs that underwent external nutrient reduction (Soaresa et al., 2011; Welch, 2009). Long-term biomanipulation experiments with the removal of zooplanktivorous fish were also tested *in situ* and in combination with nutrient input reduction (Kasprzak et al., 2003). It is imperative to keep in mind that an effective long-term restoration action can only be achieved with nutrient management, especially from the catchment.

The overview on cyanobacterial blooms presented in this bibliometric study showed that this topic has progressively increased its contribution to the aquatic scientific community. Quantitatively, the USA, China and other developed nations are the most productive countries in this research topic. Furthermore, we could identify some gaps that still need further investigations, and would give a substantial contribution to the scientific knowledge. Studies on climate change and cyanotoxins other than microcystins are not equally carried out in all continents and deserve more attention. But most importantly, more studies focused on developing strategies and tools to overcome the occurrence and domination of cyanobacterial blooms are necessary.

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CHAPTER II. The efficacy of the combined use of flocculant and natural ballast on mitigating different cyanobacterial blooms of a semiarid reservoir

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Abstract

Artificial eutrophication is a common scenario in many aquatic ecosystems, affecting water quality, biodiversity and causing economic losses. Tropical semi-arid reservoirs are more vulnerable to eutrophication, due to high temperatures and light intensity, low average annual rainfall and high evapotranspiration rate. Several restoration procedures have been developed in an attempt to overcome the presence of high cyanobacterial biomass, such as the application of a flocculant combined with natural or modified clay. However, the efficacy of local dryland soils in mitigating blooms is unknown for Brazilian manmade lakes. The objective of this study is to evaluate, through laboratory experiments, the effect of the combined use of flocculent polyaluminum chloride (PAC) and a local soil from the lake catchment (LS), as a ballast, in controlling cyanobacterial bloom in a shallow lake of the semiarid region of Brazil. Three sets of laboratory experiments were performed in three sampling occasions with different bloom compositions and biomass in Armando Ribeiro Gonçalves Reservoir. The use of PAC and LS had a remarkable effect on cyanobacterial biomass in the water column in all samplings, reducing up to 90% top chlorophyll-*a* concentration. The use of LS alone was inefficient to settle blue-green-algal biomass. In two samplings, the combination of flocculant and ballast exhibited the same efficacy as the use of solely PAC. Even so, the use of LS is important to ensure sedimentation. Combined with PAC, LS was as efficient a ballast to remove cyanobacteria as a commercially available modified clay (Phoslock®). Although LS *in natura* released considerable amounts of phosphorus and did not present P adsorption capacity, it managed to adsorb some dissolved phosphorus after organic matter was removed through muffling. This study shows that LS is a cheap, feasible and environment-friendly alternative to be used as a management action in reservoirs undergoing blooms in the semiarid region of Brazil.

Keywords: Restoration; eutrophication; “Flock and Lock”; sustainable action; harmful algae.

Introduction

Due to human activities and global change, artificial eutrophication is a common scenario in many aquatic ecosystems around the globe (Xia et al., 2016; Fragoso Jr et al., 2011). Eutrophication, the excessive enrichment of water bodies, mainly with phosphorus and nitrogen, alters the community composition of lakes, ponds and rivers, and may ultimately result in an increase of microalgal and cyanobacterial populations, referred to as blooms (Schindler, 2006). Cyanobacterial and algal mass domination is of major concern, since many species produce toxins that can affect water quality, decrease fish populations and provoke human allergies (Smith et al., 2006).

Cyanobacteria present several adaptations that help them flourish even in unfavorable conditions. For instance, in nitrogen-limited ecosystems, some species of cyanobacteria are capable of fixing nitrogen directly from the atmosphere (Latysheva et al., 2012) while others have the ability to store high concentrations of phosphorus within their cells, taking advantage over other phytoplankton species (Sbiyyaa et al., 1998).

In tropical environments, the occurrence of harmful algal blooms is enhanced, since warmer temperatures favor the dominance of cyanobacteria, leading to the increase of their biomass (Kosten et al., 2011). In particular, tropical semi-arid lakes are more vulnerable to eutrophication, due to high light intensity, low average annual rainfall and high evapotranspiration rate, which increase nutrient concentration and retention time (Barbosa et al., 2012; Bouvy et al., 2003). Besides, the high drainage basin area can lead to a higher input of nutrients from the catchment, intensifying the consequences of eutrophication (Bouvy et al., 1999).

Despite the great importance of blue-green algae, few studies about cyanobacterial blooms focus on the restoration and mitigation of eutrophic ecosystems (Medeiros et al., submitted). Still, a handful of restoration procedures were developed in an attempt to overcome the presence of cyanobacteria, albeit no universal technique exists. Management actions were successfully implemented using physical techniques, such as dredging (Liu et al., 2015), application of chemicals, like hydrogen peroxide (Matthijs et al., 2012), and biological manipulation (Zhang et al., 2014). However, recent studies point towards the combination of practices, especially the reduction of external nitrogen and phosphorus input (Gołdyn et al., 2013; Beklioglu et al., 2003; Burnett et al., 2001). Even after decreasing nutrient external loading, a reduction in total phosphorus in the water column may be hampered by the internal loading, i.e. the release of phosphorus from the lake sediment (Jeppesen et al., 2005). Also, a restored lake can return to its eutrophic state in less than ten years (Søndergaard et al., 2007). Therefore, it is important to implement techniques that minimize the effects of internal loading.

Focusing on the removal of soluble reactive phosphorus (SRP) in the water column, the use of clays such as lanthanum-modified bentonite (LMB or Phoslock®) and aluminum-modified bentonite (AMB) have been extensively used in temperature lakes with successful results (Lürling & Faassen, 2012; Bishop et al., 2014; Lürling & van Oosterhout, 2012). The lanthanum is capable of strongly binding to the SRP, transporting the phosphorus to the sediment as the clay sinks, preventing internal loading (Douglas et al 2004). Therefore, modified clays are a feasible technique to recover eutrophic lakes before a cyanobacterial bloom event, as the phosphorus in the sediment becomes unavailable for the phytoplankton (Waajen et al., 2016). Nevertheless, during a bloom the concentration of SRP in the water column can get negligible levels, as it is incorporated as particulate phosphorus in the phytoplankton biomass (Yamamoto et al., 2013).

In addition to adsorbing SRP, studies have shown that modified clays are also efficient in removing the particulate phosphorus during a bloom, as it can serve as a ballast. Moreover, the sinking weight ballasts impose on phytoplankton biomass increases when a flocculant is applied (Noyma et al., 2016). The application of a flocculant combined with clay is a new technique known as “Flock and Lock” (Lürling & van Oosterhout, 2013). The efficacy of different flocculants have been tested, ranging from metal-based salts, such as polyaluminum chloride

(PAC), to organic compounds, such as chitosan (Pan et al., 2011; Zou et al., 2006). Although the use of metal-based flocculants may be controversial, studies have demonstrated that aluminum is toxic to the biota in small concentrations only when pH is lower than 6 (Baker & Schofield, 1982) or in concentrations above 10 mg L^{-1} (Jančula et al., 2011). Besides, both PAC and chitosan have been proved to be effective, even though the efficiency of the latter may not always be straightforward (Magalhães et al., 2016). Studies have also shown that PAC can remove SRP from the water column (Wang et al., 2014).

Notwithstanding its efficacy, the use of modified clays is costly (Blázquez Pallí, 2015). Owing to financial issues, tropical countries face difficulties in implementing restoration practices. For instance, in Brazil, Lake Paranoá is one of the few cases of reservoirs previously dominated by cyanobacteria that were successfully restored (Starling & Rocha, 1990; Burnett et al., 2001). As an alternative for modified clays, researchers have tested other types of clay, which could be used to lower the costs (Magalhães et al., 2016). A study in Rio de Janeiro showed that the red soil from the catchment of Reservatório do Funil basin was as efficient a ballast as LMB in removing particulate phosphorus from the water (Noyma et al., 2016). Even though the adsorption of SRP of these non-modified clays is less pronounced than LMB, in reservoirs undergoing blooms, when most of the phosphorus is particulate and orthophosphate can sometimes remain below detection level, these natural clays are promising.

The semiarid region in Brazil is characterized by a concentrated three-month rainy period and a dry season with almost complete lack of rain (MMA, 2011; Prado, 2003). The abundance of intermittent lakes, their importance for the associated municipalities and their vulnerability to eutrophication demand urgent management actions (Barbosa et al., 2012). Inasmuch as clay is constantly withdrawn from the soil by the local ceramic industry, partnerships between decision makers and local managers could enable a cheap implementation of the “Flock and Lock” technique. However, the efficacy of Brazilian dryland soils in mitigating blooms is unknown.

The aim of this study was to assess the efficacy of the “Flock and Lock” technique in laboratory experiments, with the addition of PAC and local soil (LS), alone and combined, in order to reduce cyanobacterial blooms in a manmade reservoir at Brazilian drylands. For comparison purposes, we also tested the use of LMB. We hypothesize that LS will be as efficient as LMB in removing cyanobacterial biomass from the water column. We also investigated the phosphorus adsorption capacity of LS and compared it with LMB.

Material and Methods

Study area

A series of laboratory experiments was conducted with eutrophic water collected from Armando Ribeiro Gonçalves reservoir (ARG). ARG is located in the semi-arid region of northeastern Brazil (5°47'27" S, 36°52'43" W) and is the largest reservoir in the state of Rio Grande do Norte (2.4 x 10^9 m^3, 19,200 ha, 40 m maximum depth). It is an important manmade reservoir in the region, supplying water for up to 400,000 inhabitants despite persistent eutrophication during most of the year. The reservoir undergoes blooms of several cyanobacteria species, such as the toxin-producing species *Microcystis aeruginosa* and *Planktothrix agardii* (Câmara et al., 2015; Santos & Eskinazi-Sant'Anna, 2010; Costa et al., 2006).

A total of 30 L of water was collected from the littoral zone of ARG reservoir in June September and November 2016, with blooms of *Planktothrix sp.*, co-dominance of *Planktothrix* and *Microcystis* and co-dominance of *Planktothrix*, *Microcystis* and *Sphaerocavum*, respectively. Water from ARG was concentrated using a 20-µm mesh in order to obtain an initial concentration of chlorophyll-*a* between 200–500 µg L^{-1}, mimicking common blooms in the reservoir. Water samples were maintained in the dark until the beginning of each experiment and abiotic factors were measured for characterization (Table 1).

Table 1. Characterization of water samples from Armando Ribeiro Gonçalves reservoir in June, September and November.

	June	**September**	**November**
Chlorophyll-*a* (µg L^{-1})	396.48	260.99	491.61
PT (µg L^{-1})	496.80	310.80	835.70
SRP (µg L^{-1})	3.17	10.83	10.83
Turbidity (NTU)	134,00	105.00	1130.00
pH	7.41	7.77	7.45
Alkalinity (nEq L^{-1})	22.11	22.17	22.17
Conductivity (µS cm^{-1})	318.50	318.80	387.00
Humic substances (UV_{254nm})	0.162	0.168	0.186
Total suspended solids (mg L^{-1})	92.50	54.00	54.00

PAC and ballast characterization

Local soil (LS) was taken from a local ceramic industry in the municipality of Assu, Rio Grande do Norte, Brazil (5°34'38" S, 36°54'30" W). Before starting the experiment, aliquots of LS were ground and sieved through a 500-µm mesh. Samples were sent to a specialized laboratory (EMPARN) for characterization (Table 2). The lanthanum-modified bentonite (LMB) – Phoslock® (5% La) – was obtained from HydroScience (Porto Alegre, Brazil). PAC ($Al_n(OH)_mCl_{3n-m}$) was supplied by Pan-Americana (Rio de Janeiro, Brazil).

Table 2. Characterization of LS taken from the municipality of Assu, Rio Grande do Norte, Brazil. CEC: Cation-exchange capacity. ESP: Exchangeable Sodium Percentage.

LS parameters	
pH in water	6.46
Phosphorus (mg L^{-1})	93.00
Nitrogen (mg L^{-1})	780.00
Aluminum (mg L^{-1})	0.00
Iron (mg L^{-1})	22.33
Organic matter (mg L^{-1})	7,220.00
CEC (cmol Kg^{-1})	39.74
ESP (%)	9.30
Silt-clay percentage (%)	91.40

Experimental design

The whole procedure was divided into three sets of laboratory experiments. The first experiment was to test whether the use of the flocculant PAC is effective in agglomerating cyanobacterial biomass in this reservoir, and to obtain the optimum concentration to be added in the subsequent assays. The second experiment was to evaluate whether the use of LS is efficient as a ballast, and to obtain the best dose that could precipitate the larger amount of biomass. In the third and final experiment, we evaluated the effectiveness of the use of PAC and LS, alone and combined, in removing the cyanobacterial biomass from the water column. We used the PAC dose chosen from the first experiment and the ballast dose based on the second experiment. Before each experiment, the initial chlorophyll-*a* concentration and pH were measured *in vivo* using a TD-700 Fluorometer (Turner Designs, USA) and a pHmeter, respectively. We measured pH since aluminum may change water pH and can be toxic to the biota at high concentrations and in acidic waters (Andren & Rydin, 2012; Baker & Schofield, 1982).

For the first experiment, glass tubes (25 x 300 mm) were filled with 100 mL of water from the reservoir and received different doses of PAC. We used PAC doses of 0, 2, 3, 4, 5, 6, 7 and 8 mg Al L^{-1}, in triplicates. All glass tubes were then mixed and incubated during 1 h, at room temperature. Thereafter 10-mL aliquots from the top and the bottom of the tubes were taken for *in vivo* chlorophyll-*a* quantification. We performed either linear or nonlinear regressions to test the effect of PAC dose on the top and bottom chlorophyll-*a* concentration. The flocculant dose was chosen based on a trade-off between effective flocculation, amount of PAC used and medium pH variation.

In the second experiment, glass tubes were filled with 100 mL of water from the reservoir and received different doses of LS. We used LS doses of 100, 200, 300, 400 and 500 mg L^{-1}, in triplicates. After the addition of LS, we added the PAC dose chosen from the previous experiment. The treatments were then mixed and incubated during 1 h, at room temperature. Subsequently, 10-mL aliquots from the top and the bottom of the tubes were taken for *in vivo* chlorophyll-*a* quantification, as well as pH measurement. The ballast dose was chosen based on a trade-off between effective sedimentation and amount of LS used. Higher sedimentation was determined as a decrease in the top chlorophyll-*a* concentration. The same procedure was simultaneously performed with LMB in replacement of LS for comparison purposes, as the efficacy of LMB as a ballast is well known in the literature (Magalhães et al., 2016; Noyma et al., 2016). We compared the efficiency of both ballasts in removing top cyanobacterial biomass with an ANCOVA, adopting ballast (either LS or LMB) as the qualitative explanatory variable and their concentration as the covariate. The normality and homoscedasticity assumptions of the test were investigated through analysis of the distribution of residuals.

For the third and final experiment, we filled 24 glass tubes with 100 mL of water from the reservoir and divided them into six treatments, replicated four times. The treatments were: i) absence of both flocculant and ballast (control), ii) addition of PAC alone (PAC), iii) addition of LS alone (LS), iv) addition of LMB alone (LMB), v) combination of PAC and LS (PAC+LS), and vi) combination of PAC and LMB (PAC+LMB). After 1 h of incubation at room temperature, 10-mL aliquots from the top and the bottom of the tubes were taken for *in vivo* chlorophyll-*a* quantification and pH measurement. We performed a two-way analysis of variance (two-way ANOVA), adopting flocculant (absent or present) and ballast (absent, LS or LMB) as qualitative explanatory variables, to test the efficacy of these factors, alone and combined, in removing top chlorophyll-a. LMB was tested for comparison purposes. Tukey tests were used for *a posteriori* comparisons. The normality and homoscedasticity assumptions of the test were investigated through analysis of the distribution of residuals.

All statistics were performed using R software (R Core Team, 2014) and we assumed a level of significance of 0.05.

Phosphorus adsorption experiment

We evaluated the potential amount of orthophosphate (SRP) LS could adsorb and withdraw from the water column. We dissolved KH_2PO_4 in deionized water to obtain SRP solutions with concentrations of 0, 0.5, 1, 2.5, 5, 7.5, 10, 25, 50, 75 and 100 mg L^{-1}. Subsequently, we filled 20 tubes with 50-mL aliquots of these solutions, replicated twice. We then added 100 mg of LS in each tube and incubated them for 24 h in a 12:12 light:dark rhythm at room temperature, 200 rpm orbital shaking. After the incubation period, the samples were centrifuged at 3000 rpm and filtered through 0.45 µm cellulose nitrate membrane filters (47 mm, Unifil). The SRP concentration of each filtered samples was then quantified by colorimetric method (Murphy & Rilley, 1962). The same procedure was performed with LMB in replacement of LS for comparison purposes. As LS presents a high concentration of organic matter, we removed all organic compounds by leaving aliquots of LS in a muffle furnace at 550 ºC for 4 hours and then repeated the procedure.

In order to obtain the maximum adsorption capacity of LS and LMB, we fitted adsorption isotherms with the Langmuir equation:

$$Q = \frac{Q_{max}\, b\, C_e}{1 + b\, C_e},$$

where Q is the amount of SRP adsorbed at equilibrium (mg g^{-1}), C_e is the equilibrium concentration (mg L^{-1}), Q_{max} is the maximum absorption capacity and b is the Langmuir constant (Langmuir, 1918). From non-linear regressions, Q_{max} and b values were calculated, using the *nls* function after outlier investigation (Bates & Watts, 1988).

Nutrient release experiment

In order to quantify the potential release of nutrients from LS and LMB, 12 Erlenmeyer flasks were filled with 100 mL of deionized water, 4 of which were left as controls and the remaining received 100 mg of either LS or LMB. The initial concentration of phosphorus (PO_4^{3-};

Murphy & Rilley, 1962) and nitrate (NO_3^-; Müller & Wiedemann, 1955) were determined by colorimetric method, and ammonia (NH_3; Folin & Denis, 1916) by direct nesslerization before incubation. All flasks were incubated for 24 h in a 12:12 light:dark rhythm at room temperature, 200 rpm orbital shaking. Thereafter, aliquots of 10 mL, 25 mL and 100 mL were taken for SRP, nitrate and ammonia measurements, respectively. We compared the nutrient release from the LS and LMB treatments with controls using a one-way ANOVA. We used random permutational one-way ANOVA when data failed Levene's test of homogeneity of variances assumption.

To minimize the release of phosphorus from LS, we filled 15 Erlenmeyer flasks with 100 mL of deionized and received 100 mg of LS, followed by the addition of PAC at doses 0, 2, 4, 6 and 8 mg Al L^{-1}, in triplicates. The same procedure described above was performed for SRP quantification. The effect of PAC concentration on the release of nutrients from LS to the water column was tested using a nonlinear regression model.

Results

Effect of PAC on cyanobacterial biomass

The effect of PAC on cyanobacteria biomass was different in all three samplings. In June and September, the addition of PAC decreased top chlorophyll-*a* concentration at all doses, as the flocks started to sink. In June, the reduction in top cyanobacterial biomass was significantly affected by the increase in PAC dose with a linear tendency ($F_{1,22} = 90.4$; $p < 0.001$; Fig. 1A). The reduction at the top was accompanied by a significant increase at the bottom ($F_{1,22} = 224.6$; $p < 0.001$; Fig. 1B). We selected the dose of 8 mg Al L^{-1} because the flocculation at this dose was the highest. In September, the increase in PAC concentration also significantly lowered top cyanobacterial biomass (F = 347.3; $p < 0.001$; Fig. 1C) and increased the biomass at the bottom (F = 52.7; $p < 0.001$; Fig. 1D). However, the decrease in top chlorophyll-*a* was not linear, as it stabilized around 100 µg L^{-1} after 5 mg Al L^{-1}, so we selected this dose for the next essays.

Differently from June and September, the cyanobacteria exhibited extremely high buoyancy in November, especially in the control tubes, where top chlorophyll-*a* was twice as much as the start concentration. The treatments with PAC addition presented a significantly reduction in top chlorophyll-*a* biomass (F = 467.6; $p < 0.001$; Fig. 1E), reaching a concentration lower than the initial value only at doses above 3 mg Al L^{-1}. The decrease at the top was followed by a significant increase at the bottom ($F = 145.5$; $p < 0.001$; Fig. 1F). As in September, this decrease in top chlorophyll-*a* concentration was not linear and stabilized around 95 µg L^{-1} after 6 mg Al L^{-1}. Therefore, we chose this dose to be added in the following essays. In all samplings, pH values were around 6.65, 7.33 and 7.35 in June, September and November, respectively, and they were not affected by the addition of PAC.

*Chlorophyll-*a *sedimentation after ballast addition*

The effect of increasing ballast concentration was similar in all three samplings. In general, ballast dose did not affect significantly top chlorophyll-*a* concentration (Table 3). Despite the significant interaction between flocculent dose and ballast type in June ($F_{1,26} = 6.44$; $p = 0.02$), no clear decrease in top cyanobacterial biomass could be observed in any of the samplings (Table 3; Figure 2A,C,E). Therefore, we chose the lowest (100 mg L^{-1}) concentration to be used in the final experiment in all samplings. The decrease in top chlorophyll-*a* concentration was accompanied by an increase at the bottom. In all samplings, pH was not affected, with values around 6.38, 7.58 and 7.43 in June, September and November, respectively.

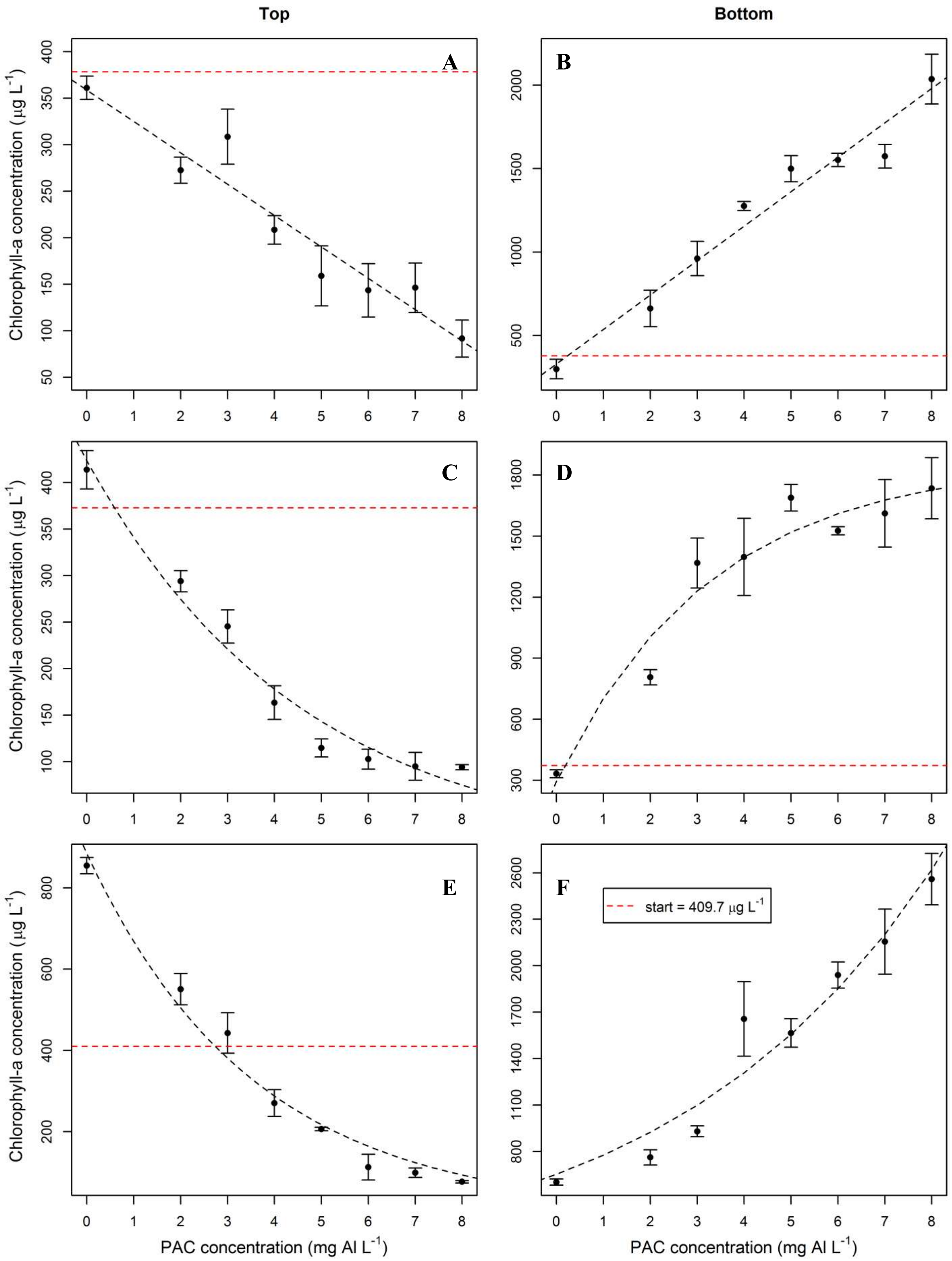

Figure 1. Effect of PAC on chlorophyll-*a* concentration at the top (left) and bottom (right) for the samplings in June (A and B), September (C and D) and November (E and F). The red dashed lines represent the initial chlorophyll-*a* concentration and the black dashed lines represent the best-fitted liner (A and B) and nonlinear (C, D, E and F) regression models. A: chl-*a* = 358.9 – 33.7 pac; B: chl-*a* = 329.6 + 206.3 pac; C: chl-*a* = 423.7 exp(– 0.21 pac); D: chl-a = 1.870 – 1.576 exp(– 0.3 pac); E: chl-*a* = 884.2 exp(– 0.28 pac); F: chl-*a* = exp(6.48 + 0.17 pac). Error bars denote SE.

Table 3. Effect of ballast dose and type of clay on top cyanobacterial biomass. Significant values are in bold (ANCOVA; n = 30).

	June		September		November	
Dose	$F_{1.26} = 0.44$	$p = 0.52$	$F_{1.26} = 3.50$	$p = 0.07$	$F_{1.26} = 3.57$	$p = 0.07$
Type of clay	$F_{1.26} = 10.36$	**$p = 0.003$**	$F_{1.26} = 8.39$	**$p = 0.001$**	$F_{1.26} = 1.38$	$p = 0.25$
Dose:Type	$F_{1.26} = 6.44$	**$p = 0.02$**	$F_{1.26} = 1.06$	$p = 0.11$	$F_{1.26} = 0.39$	$p = 0.54$

Flock and sink experiment

A significantly reduction in top chlorophyll-*a* concentration was observed after the addition of PAC combined with either LS or LMB in all samplings (Table 4), reaching a 90% reduction compared to the control in November (Fig. 3A,C,E). The use of LS or LMB alone did not settle top cyanobacterial biomass at all in June and September, and did not present a substantial reduction in November. Conversely, in all samplings, the addition of PAC alone was enough to settle significantly more cyanobacterial biomass than controls. In June and November, PAC was as efficient as the combination treatments in removing top chlorophyll-*a*. However, in September this biomass removal was more evident when either LS or LMB was used in combination with PAC (Fig. 3E). No difference was observed between LS or LMB. The decrease in top chlorophyll-*a* concentration was accompanied by an increase at the bottom and vice-versa. Values for pH were around 6.89, 7.71 and 7.42 in June, September and November, respectively, and were not affected by PAC.

Table 4. Effect of PAC and ballast on top cyanobacterial biomass. Significant values are in bold (two-way ANOVA; n = 24).

	June		September		November	
PAC	$F_{1,18} = 268.6$	**$p < 0.001$**	$F_{1,18} = 692.9$	**$p < 0.001$**	$F_{1,18} = 1490$	**$p < 0.001$**
Ballast	$F_{2,18} = 0.85$	$p = 0.44$	$F_{1,18} = 12.11$	**$p < 0.001$**	$F_{2,18} = 17.81$	**$p < 0.001$**
PAC:Ballast	$F_{2,18} = 4.53$	**$p = 0.03$**	$F_{1,18} = 9.32$	**$p = 0.002$**	$F_{2,18} = 12.33$	**$p < 0.001$**

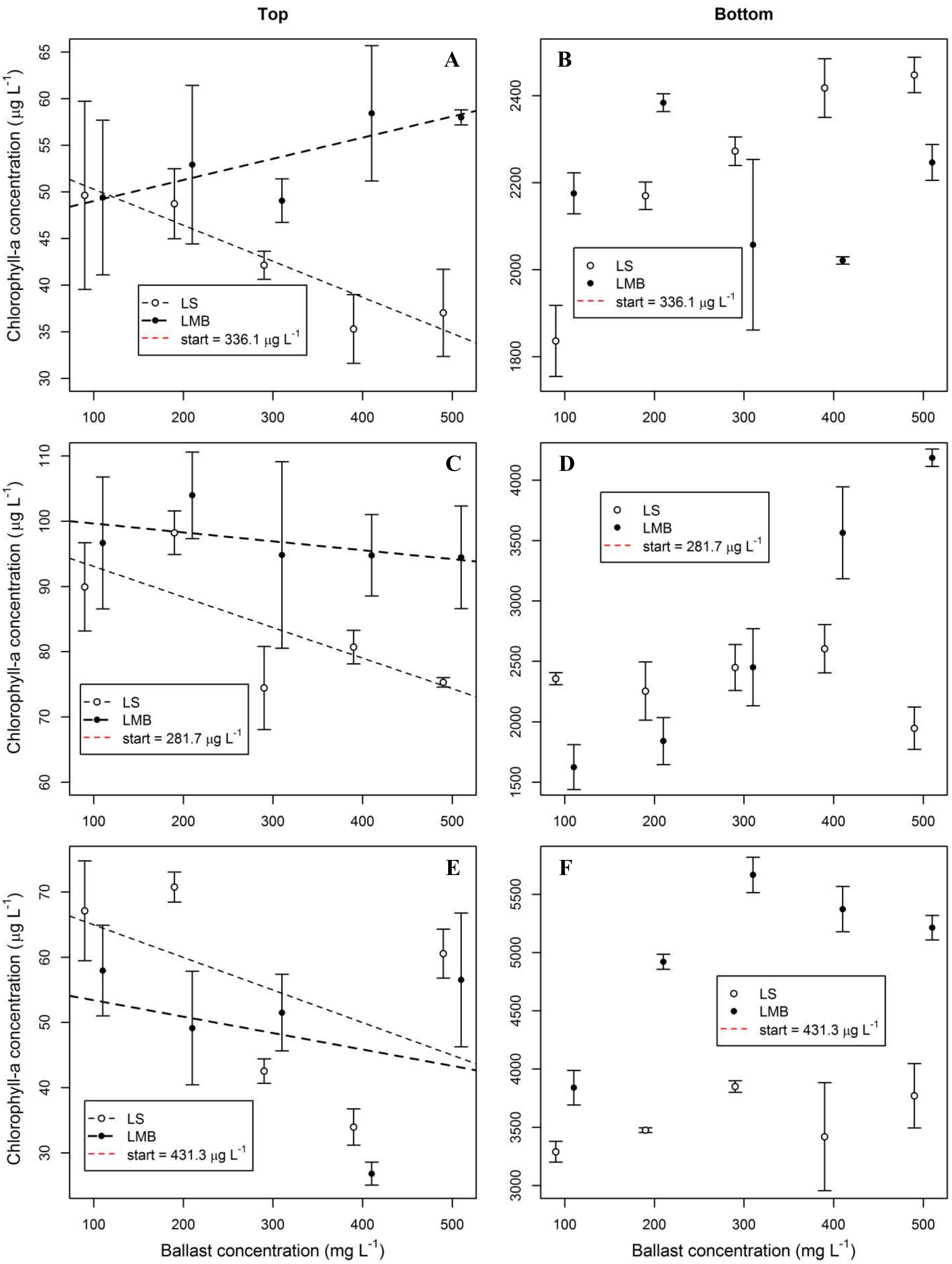

Figure 2. Effect of ballast dose on cyanobacterial biomass at the top (left) and bottom (right) for the samplings in June (A and B), September (C and D) and November (E and F). Trendlines obtained after ANCOVA for LS (open circles, thin dashed line) and LMB (filled circles, thick dashed line). Error bars denote SE.

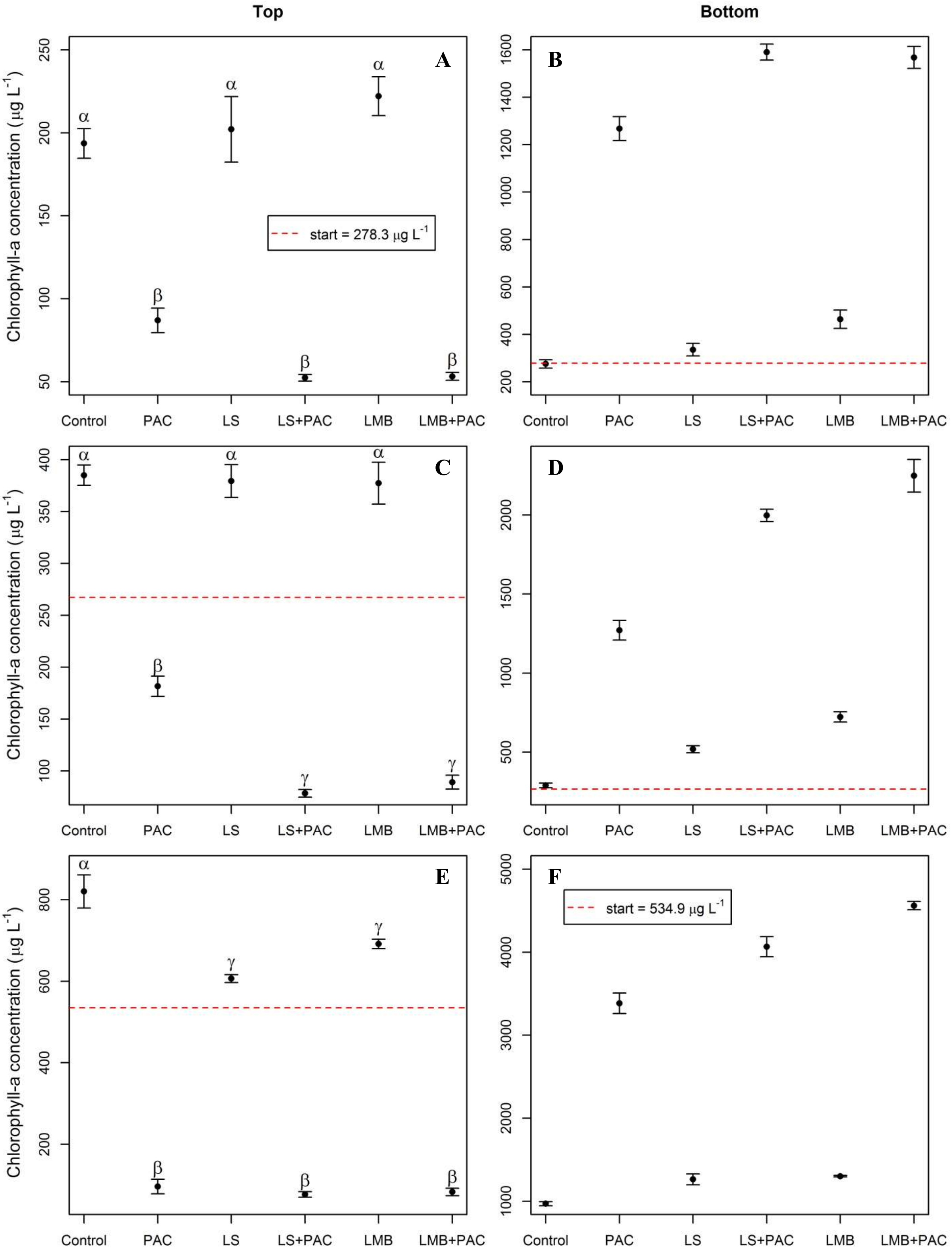

Figure 3. Effect of PAC and ballast (LS or LMB), alone and combined, on cyanobacterial biomass at the top (left) and bottom (right) for the samplings in June (A and B), September (C and D) and November (E and F). The red dashed lines indicate the start chlorophyll-*a* concentration. Error bars denote SE.

Phosphorus adsorption

The LS used in this experiment did not adsorb any SRP available in the water (Table 5; Fig. 4). On the other hand, after muffling, LS started to adsorb some SRP and presented a maximum absorption capacity in deionized water of 0.82 ± 0.09 mg g^{-1}. LMB adsorbed all SRP until the concentration of 10 mg L^{-1}, with a maximum absorption capacity estimated as 7.31 ± 2.94 mg g^{-1} (Table 5; Fig. 4).

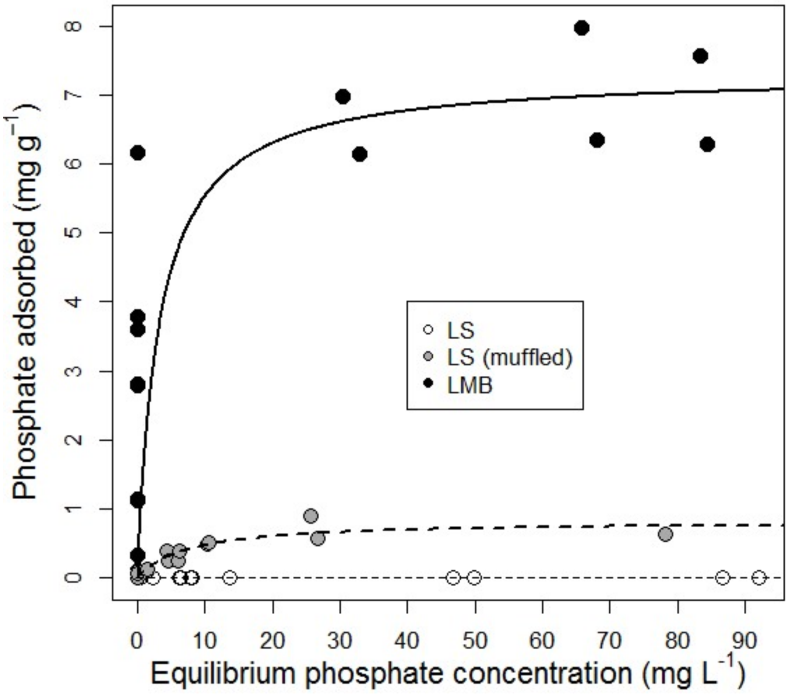

Figure 4. Langmuir isotherms of phosphate adsorption capacity (mg g^{-1}) for LS (white circles, thin dashed line), LS after muffling (gray circles, thick dashed line) and LMB (black circles, continuous line). LS (muffled) and LMB adsorption isotherms were calculated from nonlinear regression. Since LS did not adsorb phosphorus, the isotherm was zero.

Table 5. Maximum adsorption capacity (Q, mg g^{-1}) for LS, LS after muffing and LMB. Values for LS (muffled) and LMB were calculated from the Langmuir equation. Since LS did not adsorb phosphorus, the Q value was zero.

	Q (mg g^{-1})	**t-value**	**P**
LS	0	-	-
LS (muffled)	0.82 ± 0.09	8.76	< 0.001
LMB	7.31 ± 2.94	2.49	0.02

Nutrient release

In deionized water, the release of nutrients by LMB and LS was positive. The amount of SRP released by LS was significantly high, whereas LMB released a great amount of ammonia. LS and LMB also released a moderate amount of nitrate (Table 6). When PAC was added with LS, the increase in PAC concentration resulted in an exponential decline in the release of SRP in the water column (F = 3144.5; p < 0.001; Fig. 5).

Table 6. Release of nutrients from LS and LMB in deionized water (μg g^{-1}; means ± SD, *N* = 8).

	PO_4^{3-}			**NO_3^-**			**NH_3**		
	Release	**F**	**p**	**Release**	**F'**	**P**	**Release**	**F**	**p**
LS	257.5 ± 6.5	6080.0	<0.001	31.1 ± 4.0	169.0	0.024	16.6 ± 9.9	8.7	0.026
LMB	7.4 ± 1.5	44.1	<0.001	12.2 ± 3.9	17.9	0.019	120.9 ± 8.9	712.3	<0.001

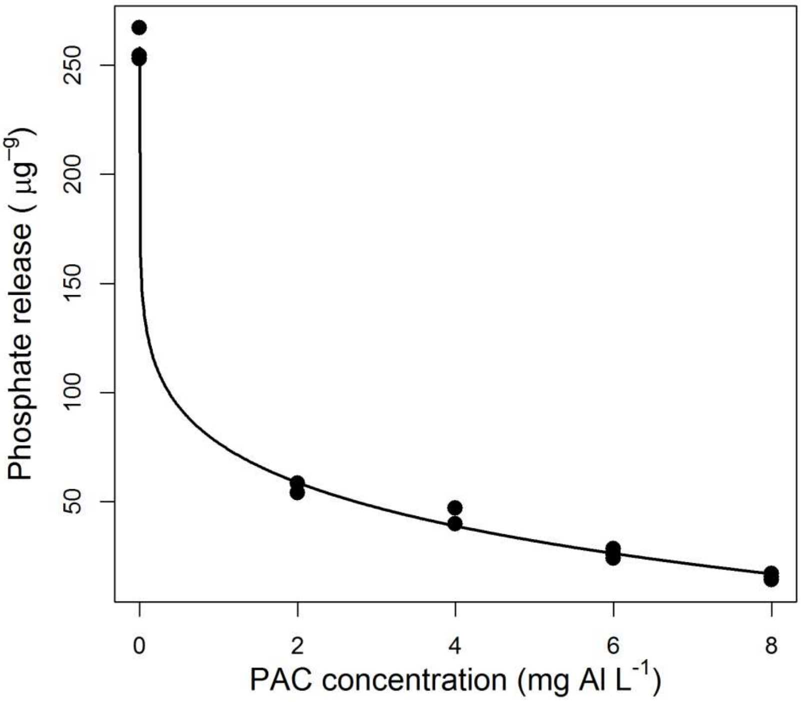

Figure 5. Effect of PAC addition on the release of SRP by LS in deionized water. srp = 258.1 – 181.2 * pac^0.1.

Discussion

This is the first study to test the efficacy of a natural clay from the semiarid region of Brazil in controlling cyanobacterial bloom of a local shallow lake. Our results support our hypothesis that LS would be as efficient a ballast as LMB in removing cyanobacteria accumulating at the surface. In all samplings, either LS or LMB, in combination with PAC, sank expressively more cyanobacteria than the application of these ballasts alone. However, the use of LS or LMB alone has been shown to be inefficient and should not be applied without a flocculant. These results are in line with previous studies using the combination of flocculants and clays (Magalhães et al., 2016; Lürling & van Oosterhout, 2013). In terms of the efficacy of PAC, its effect was not the same in all samplings. In June and November, PAC alone efficiently settled substantial cyanobacterial biomass, so the addition of either LS or LMB was not necessary. On the other hand, in September the application of any of these clays was important to decrease top chlorophyll-*a* with PAC.

The difference in the efficiency of solely PAC compared to the PAC+LS and PAC+LMB treatments among samplings may have been influenced by the PAC doses we chose from the first set of experiments. Since flocculation in all samplings presented a tendency to sedimentation, maybe the dose selection in June and November was so high that it was equivalent to the combination of PAC+LS and PAC+LMB. A study in Brazil, for instance, used 2 mg Al L^{-1} with 320 mg L^{-1} of a local red soil and obtained satisfactory results (Noyma et al., 2016). In view of this, instead of choosing the highest PAC concentrations, selecting a moderate dose and use it in combination with a ballast could also be a suitable option. Decision makers may prefer this alternative because LS is cheaper than PAC and the efficacy of PAC combined with LS is more guaranteed. Hence, it is important to find the best doses of flocculent and ballast in order to obtain the cheapest combination.

From our experiments, it was clear that the cyanobacteria from the September sampling exhibited high buoyancy. This enhanced fluctuation in September was probably due to high densities of colonial genera of cyanobacteria, like *Sphaerocavum* and *Microcystis*. Intense buoyancy and the occurrence of scums is common in *Microcystis* blooms (Almanza et al., 2016). These colonies present gas vesicles within their cells and produce mucilage, both potential features that enable *Microcystis* to exhibit intense fluctuation (Visser et al., 2005; Visser et al., 1997). Some studies have shown that PAC can stimulate cyanobacterial buoyancy instead of settling the biomass of these blooms (Noyma et al., 2016). Therefore, since the use of PAC alone does not always ensure sedimentation of biomass, its application has to be cautious. The concentration of toxins in scums can reach extremely high values (> 10 mg L^{-1}) and therefore may be harmful for the environment if not applied with a ballast (Sivonen & Jones, 1999). In spite of the different effects of PAC on cyanobacterial cells, the coupling of PAC and ballast exhibited similar results in all samplings, which highlights the importance of ballast application.

In spite of the great decrease in top cyanobacterial biomass, the combined use of flocculant and ballast did not decrease chlorophyll-*a* under mesotrophic conditions (≤ 15 µg L^{-1} chlorophyll-*a*). At the end of the incubation period, it was possible to note that there was still biomass sinking in the tubes. Besides, some cyanobacteria stuck at the walls of the tubes, since the ballasts did not impose weight enough to surpass the friction resulted from the contact with the walls. Therefore, the real reduction in cyanobacterial biomass is expected to be even more pronounced. Testing this technique on a larger scale may elucidate the real effect of these natural clays in combination with PAC. Even so, the biomass settlement of this method was notorious, reaching a removal efficacy of 90%. Hence, this technique could perfectly be used in water treatment, improving water quality to the population.

When overcoming the dominance of cyanobacteria in aquatic systems, many techniques are prone to drawbacks, such as toxin release (Dai et al., 2016). As cyanobacteria undergo lysis, the toxins accumulated within their cells may be released in the water column, imposing devastating consequences to the biota (Drobac et al., 2016). Even though the use of aluminum can rupture cyanobacterial cells, especially in high doses (Han et al., 2013), this is unlikely to happen in this study, as PAC dose was low. Therefore, the addition of PAC and LS may transport the cyanobacterial cells intact to the sediment. Another drawback from this technique is that once the huge biomass of cyanobacteria is stored in the sediment, resting cells and colonies may remain viable for long periods of time (Brunberg & Blomqvist, 2002). It has been suggested the use of calcium or hydrogen peroxide to inhibit *Microcystis aeruginosa*, but toxins may be released during this process (Lürling et al., 2014). Hence, the destiny of accumulated cells in lake sediments still needs more investigations.

Regarding the adsorption of SRP, LS *in natura* did not adsorb any phosphorus in the water column. On the contrary, LS was capable of releasing a considerable amount of orthophosphate. However, LS managed to adsorb some SRP after it was muffled and presented a maximum adsorption capacity comparable to soils from other studies (Wang & Liang, 2014; Zhu et al., 2007). Clays from the environment naturally have the ability to adsorb anions, such as orthophosphate (Bahia Filho et al., 1983). The efficacy of different non-modified clays has already been tested, showing that they present some P-sorption capacity, albeit much less pronounced than their modified counterparts (Fink et al., 2014; Wang et al., 2012). Also, some studies reported that the removal of organic matter from soils resulted in a greater P adsoption maximum (Ogunwale et al., 2006). In this study, LS was only able to adsorb phosphorus after muffling because the absence of organic matter exposed the surface of iron oxide from the soil, which can complex with phosphorus (Ogunwale, 2003; Fink et al., 2016). Aluminum oxide can also be exposed with the removal of organic matter from soils, but LS was completely devoid of aluminum, then SRP adsorption was probably a result of iron oxide complexation with phosphrus. In comparison, LMB adsorption capacity was high, which was already expected due to the complexation of lanthanum with phosphorus, as shown by previous studies (Reitzel et al., 2013; Spears et al., 2013).

Several techniques have focused on modifications of natural clays to increase their SRP adsorption. Some procedures, involving the addition of chitosan or polyacrylamide, change soil structure, increase its adsorption potential, and provide local soils with flocculant capacities (Zou et al., 2006; Pan et al., 2011). However, this could increase management costs. The soil modification used in this study to prevent the release of SRP by LS, i.e. the removal of organic matter through muffling, is cheap, fast and feasible. In addition to muffling, PAC also counteracted phosphorus release, as it is known for its adsorption capacity (Wang et al., 2014). If PAC is used with LS after muffling then the phosphorus adsorption is expected to be even more pronounced. Therefore, this technique is efficient in mitigating ongoing bloom events by removing particulate phosphorus, and it is also a good option for preventing blooms, as it can remove SRP from the water column.

Since replacing LMB with clay from the environment represents a remarkable reduction in management costs, choosing natural ballasts from the catchment, as the one tested in this study, is preferable to using modified clays or soils from elsewhere. It is important to note that allochthonous matter is naturally transported into reservoirs during episodes of rain in tropical environments (Chellappa et al., 2008). Hence, not only is it cheap but also an eco-friendly curative alternative. As the local industry extracts clay continuously from the environment to produce ceramic, it could be of considerable help to provide material for the implementation of such a technique. With more studies in this direction, long-term effects of this management procedure may be elucidated and such actions may be successfully implemented.

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Discussion and Future Perspectives

In this master thesis, our bibliometric study clearly showed that the topic about cyanobacterial blooms is important in the aquatic sciences and has greatly contributed to the aquatic scientific community. Our results state that the USA, China and other developed countries highly productive, which is already seen in other topics. Furthermore, we pointed out some knowledge gaps that demand future investigations, like studies on climate change and cyanotoxins other than microcystins. This thesis also highlights that more studies focused on developing and testing lake restoration techniques are needed, since this subject is of major importance and only few publications were retrieved in our search.

In view of the above-mentioned, on the second chapter, we tested a feasible, cheap and eco-friendly curative method in removing cyanobacterial biomass from the water column. Replacing LMB with clay from the environment represents a promising alternative, as we reduce management costs and use a ballast that is naturally from the catchment. In addition, the method presented here may encourage partnerships between decision makers and the local ceramic industry.

Even though our results were positive, in order to implement such a technique *in situ*, some questions need to be taken into account. Firstly, we did not investigate the implications of the addition of PAC and LS for the biota. As the cyanobacterial biomass sink and accumulate in the sediment, the benthonic fauna associated with the bottom of the reservoirs may be negatively affected by the amount of organic matter. Besides, as the flocks of cyanobateria may contain high concentrations of toxins, their accumulation in the sediment may be detrimental to the survival of the benthic fauna.

Secondly, the planktonic fauna in the reservoirs may also be hampered, since the cyanobacterial flocks may clog their filtration apparatus. We did not examine whether the technique presented here influences the biota, and therefore such side effects need to be evaluated.

Lastly, our experiments were performed in a restricted area, therefore the efficacy of our technique may be different on a larger scale. Blooms are not always common throughout the extension of reservoirs, especially in larger lakes, where they are concentrated in specific areas, mainly the littoral zone. Therefore, the introduction of PAC and LS should not be in the whole lake. As an alternative, the eutrophic area presenting cyanobacterial blooms may be isolated from the rest of the lake followed by the application of the technique. Mesocosm experiments may be important to obtain more realistic results of this restoration action.

It is imperative to continue evaluating the efficacy of the addition of PAC and LS as a management action, especially in larger temporal and spatial scales, so that drawbacks may be minimized. With more studies in this direction, long-term effects of this restoration procedure may be elucidated and such actions may be successfully implemented.

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