Tackling Cyanobacterial Blooms:

Evaluating compounds and techniques for effective eutrophication management across different scales

Li Kang
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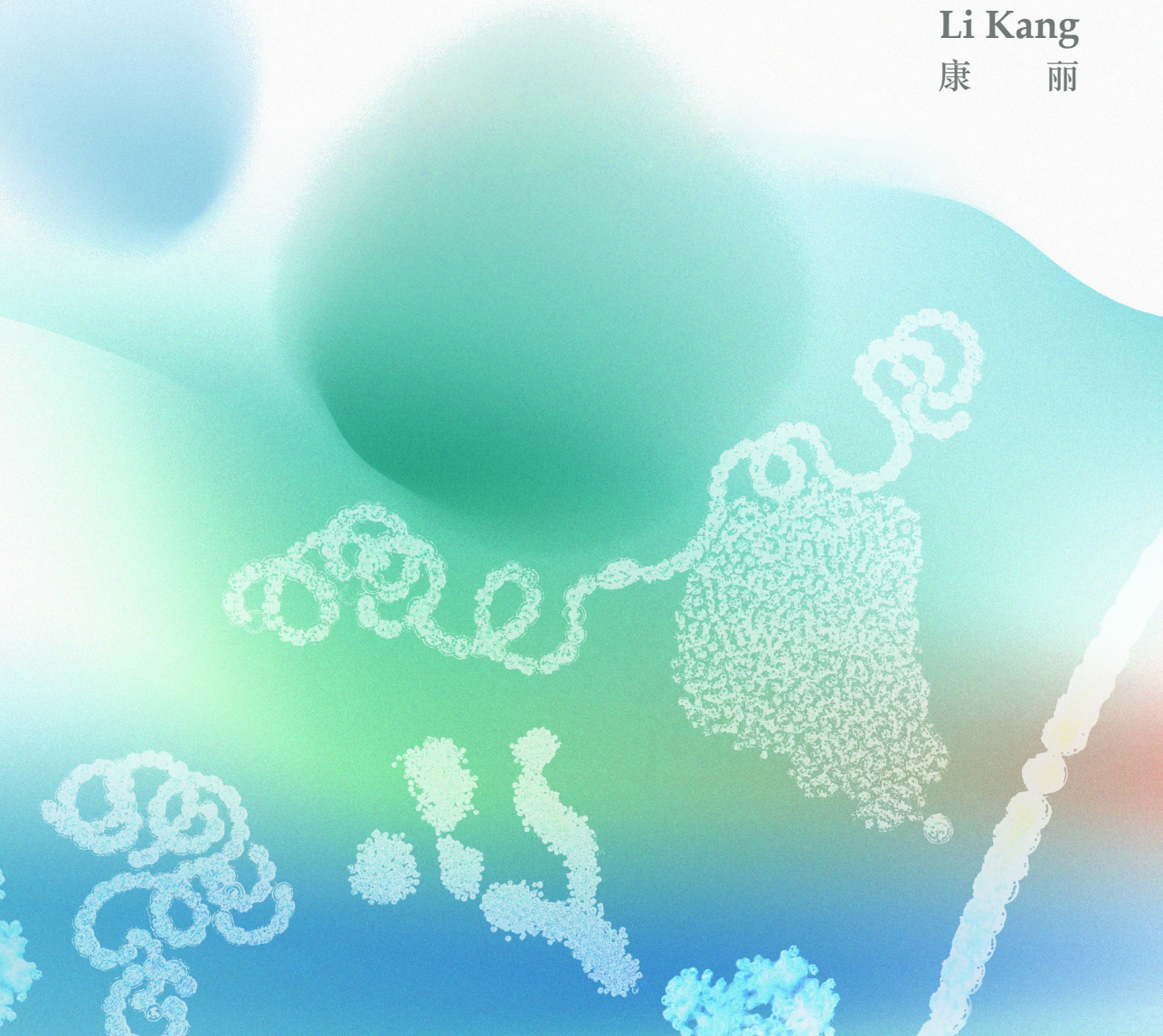

Propositions

1. System analysis of individual lakes is the crucial initial step in managing eutrophication. (this thesis)
2. Testing the effectiveness and safety of the compounds at different scales is critical before whole lake application.
   (this thesis)
3. Completion is more important than perfection for Ph.D. research.
4. Criteria beyond citations allow for a comprehensive evaluation of researchers' performance.
5. While the selective presentation of results expedites publication, it impedes scientific progress and innovation.
6. Within universities, addressing systematic discrimination is as important as excelling in educational activities.
7. Introversion is a personality tendency, not a disadvantage.

Propositions belonging to the thesis, entitled:

Tackling Cyanobacterial Blooms: Evaluating compounds and techniques for effective eutrophication management across different scales

Li Kang

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Tackling Cyanobacterial Blooms:

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Tackling Cyanobacterial Blooms:

Evaluating compounds and techniques for effective eutrophication management across different scales

Li Kang

Thesis

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Table of Contents

1

General introduction

Who kills elephants?

In May-June 2020, the shocking news appeared in the media worldwide that more than 330 African elephants *(Loxodonta africana*), which are the world's largest terrestrial mammal species, had died suddenly in the Okavango Delta, Botswana. Poaching, anthrax poisoning, and starvation were all ruled out. Instead, most of the dead elephants were discovered near watering holes, implying that the deaths were possibly related to water sources. Eventually, scientists considered that cyanotoxins produced by cyanobacteria are the most likely cause of the massive death of elephants (Wang et al., 2021). Hot and dry weather in South Africa has boosted cyanobacteria and its toxins, and 2020 was identified as an extreme year with widespread blooms (Veerman et al., 2022). Also, the shrinking water caused by the drought increased cyanotoxins concentrations and increased demand for drinking water for mammals, including other endangered species. Hence, there is considerable concern about the issue of cyanobacteria and cyanotoxins on wildlife.

What are cyanobacteria?

Over 3.5 billion years ago, when cyanobacteria first evolved on Earth, the atmosphere was very different from what it is today, with little dioxygen (O_2). Cyanobacteria, commonly known as blue-green algae, are a group of photosynthetic bacteria and oxygen-producers found in oceans, lakes, rivers and wetlands. Because of their photosynthetic activity, cyanobacteria caused a large increase in the atmospheric oxygen concentrations between 2.4 ~ 2.7 billion years ago, the great oxygenation event (Huisman et al., 2018). Cyanobacteria are natural components of (aquatic) ecosystems, they take up nutrients and carbon dioxide and under sunlight convert it into biomass. However, since the 19th and 20th centuries, the industrial revolution and growth of human population have led to an increased inflow of nutrients to aquatic ecosystems often leading to overgrowth of cyanobacteria (Fig. 1.1).

Fig. 1.1 Examples of cyanobacterial blooms in Dutch surface waters (left panel: Helmond, 2022; left-middle panel: Veghel, 2018; right-middle panel: Canal Nederwetten, 2018; right panel: Eindhoven, 2018), pictures from Miquel Lürling

The most important drivers that promote cyanobacteria blooms are rising CO_2 levels, global warming and cultural eutrophication (Huisman et al., 2018). Eutrophication is a slow and natural process in which lakes, ponds and reservoirs accumulate organic matter and nutrients over time, gradually transforming these water bodies into wetlands and eventually land. However, human activities greatly accelerated the eutrophication process, referred to as cultural eutrophication (Chislock et al., 2013). The over-enrichment of surface waters with nutrients primarily nitrogen (N) and phosphorus (P), is mostly a result of agricultural runoff, livestock activities, urban sewage and industrial wastewater discharge (Smith and Schindler, 2009), which has become one of the most important water quality issues (Downing, 2014). Eutrophication may lead to drastic ecosystem changes, such as loss of submerged plants, a decrease in water transparency, an overgrowth of algae, an increase of planktivorous and benthivorous fish, an accumulation of organic matter and a large, recyclable sediment P pool (Moss et al., 2011).

Cyanobacteria bloom is the most notorious symptom of eutrophication (Paerl et al., 2011), which may produce unpleasant odors, cause hypoxia/anoxia condition, kill fish and impair ecosystem services, such as drinking water production, irrigation, recreation, aquaculture and fisheries (Lürling et al., 2020a; Paerl and Paul, 2012). Some cyanobacteria species are capable of producing harmful toxins. For instance, *Microcystis aeruginosa* is one such species that can produce microcystins (MCs), which are hepatotoxins that can pose threats to the health of humans, pets, wildlife and other aquatic organisms.

Human population growth, climate change, and intensified anthropogenic and agricultural activities are expected to further increase eutrophication and cyanobacterial blooms in the upcoming decades (Ho et al., 2019; Jeppesen et al., 2009; Moss et al., 2011; O'Neil et al., 2012;

Paerl and Huisman, 2008; Paerl and Paul, 2012). Therefore, mitigating eutrophication and controlling cyanobacterial blooms are of high importance to authorities and water managers.

How to minimize cyanobacteria blooms?

As cyanobacteria are natural components of aquatic ecosystems, they will never disappear, however, efforts should be directed to prevent mass outbreaks and minimize cyanobacteria blooms. Since cyanobacteria blooms can be the result of a local accumulation of relatively low water column dispersed biomass or the result of eutrophication or both, adequate management starts with a proper diagnosis of the problem, a lake system analysis (Lürling et al., 2020a). Such water body specific diagnosis (Fig. 1.2) can indicate the water balance and nutrient budget, how the aquatic community deviates from the desired composition, and therewith indicates 'the buttons to be pressed' to realize the desired water quality improvement.

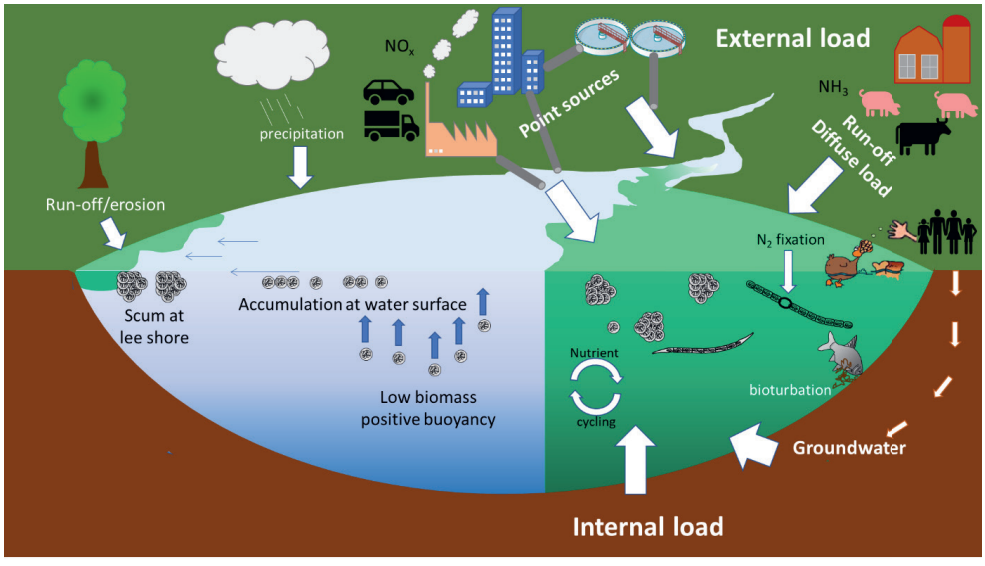

Fig. 1.2 A water body specific diagnosis

Targeting the cause of the problem is the most logical and sensible, the key in controlling eutrophication is to reduce the nutrients inputs from human activities as much as possible (Hamilton et al., 2016; Huisman et al., 2018; Paerl et al., 2016). Such external load control can be achieved by improving urban and industrial wastewater treatment, stormwater management, and reducing agricultural run-off. However, controlling external nutrient loading is not always possible (Huser et al., 2016), or it may take decades to centuries until the water system recovers (Fastner et al., 2016). This delayed recovery is because of legacies accumulated over years in

lake beds and due to ongoing diffuse pollution from mostly agricultural activities (OECD, 2014, 2017). Hence, when restoring the water systems, we cannot pin all our hopes on only reducing external nutrients inputs, especially in water bodies with high internal nutrient loading. A two-pronged approach for controlling both external and internal nutrient loading would be more sensible. Catchment measures to reduce external load that are combined or followed by in-lake interventions to reduce the internal load. In the case of a local accumulation of otherwise low concentrations of cyanobacteria, direct targeting of the cyanobacteria could be considered which is commonly done by applying algaecides (Jančula and Maršálek, 2011).

In-lake interventions

In-lake interventions are aimed to manage eutrophication and reduce cyanobacterial nuisance. A wide range of in-lake interventions is available, including biomanipulation, reservoir/lake reconstruction, dredging, water column mixing, algaecides (Lürling and Mucci, 2020). In lake interventions are designed to disrupt environmental conditions that promote cyanobacterial blooms and include dredging, hydrological manipulation, oxygenation and the construction of flow-through systems. However, they are often costly and not financially feasible.

This is probably one of the reasons why a plethora of apparently low-cost exists of actions are aimed to mitigate cyanobacterial nuisance:

1) **Public oriented measures** that provide information about risks associated with cyanobacterial blooms, increase awareness, aim to change habits (e.g., feeding fish and water fowl), and issue warnings (e.g. swimming ban).
2) **Effect oriented measures** that are aimed at reducing nuisance by preventing surface accumulations, inflow of cyanobacteria at beaches and harbors, removing (accumulated) biomass, preventing algae growth, killing cyanobacteria and mitigating foul odors. Effect-oriented measures can be subdivided into:

    - **Physical measures** such as aeration/water movement, use of ultrasound, jets, bubble screen, construction of dams, floating screens and so on.
    - **Chemical measures** such as use of algaecides, oxidants, antibiotics, coagulants, and coagulants combined with ballast.
    - **Biological measures** such as use of barley straw, *Dreissena*, mussels, effective microbes, Golden algae, plant extracts, and so on (Lürling and Mucci, 2020).

It should be noted that several of these measures, despite being heavily promoted, suffer from poor or no scientific support of efficacy in controlling cyanobacteria (Lürling and Mucci, 2020; Lürling et al., 2016).

3) **Source oriented measures** that in parallel with or following external load reduction are aimed at strong reduction of internal loading by chemical fixation of phosphorus, oxygenation, dredging, hypolimnetic withdrawal and fish, plant, cyanobacterial biomass removal (= harvesting nutrients).

Of the effect-oriented measures algaecides can be viewed as fast-acting and cost-effective; copper-based algaecides are among the most frequently used (Iwinski et al., 2016; Jančula and Maršálek, 2011). Alternatives such as hydrogen peroxide and peroxide based algaecides are gaining popularity (Matthijs et al., 2016). Coagulating cyanobacterial biomass and settling it to sediment is another possibility of rapidly removing cyanobacterial blooms. Aluminium salts (aluminium sulphate and poly-aluminium chloride-PAC) or organic polymers like chitosan can be applied to aggregate cyanobacteria. Coagulants can also be combined with a ballast compound to facilitate settling of aggregates, in addition, aluminium will also chemically inactivate phosphate depending on the dose (Cooke et al., 2005; Noyma et al., 2016; Noyma et al., 2017; Pan et al., 2011; Pan et al., 2006). These interventions can be considered in situations where rapid relief is desired, for instance to ensure safe swimming in open-water sport events. When a coagulant is combined with a solid-phase phosphate (P) sorbent as ballast, the water can simultaneously be cleared from cyanobacteria and phosphate, where subsequently the P release from the lake bed is reduced once the sorbent is settled on the sediment (Lürling et al., 2020a). Solid phase P sorbents can also be used alone without a coagulant.

Those P-fixatives are referred to as geo-engineering materials, which is defined here as "the manipulation of biogeochemical processes using a P binder" (Lürling et al., 2016; Spears et al., 2013b). P binders can be natural soils, clays, and minerals or modified/synthesized materials (Douglas et al., 2004). When a P binder is used in an aquatic system, it can adsorb the phosphate in the water column and will reduce the P-release from the sediment when settled on the lake bed. Thus, the mode of action is to prevent cyanobacteria overgrowth by strongly reducing available P in the water system. The most commonly used P binders in lake restoration are lanthanum (La) and aluminium (Al) based compounds such as lanthanum modified bentonites, commercially called Phoslock® and aluminium salts like alum (Cooke et al., 2005; Copetti et al., 2016). Some P binders can also reduce ammonium in the water, such as aluminium-modified zeolite, commercially called Aqual-P™ (Gibbs and Özkundakci, 2011; Gibbs and Hickey, 2018;

Gibbs et al., 2011).

Different in-lake interventions have their characteristics (Huser et al., 2016; Lürling et al., 2020a; Lürling et al., 2016; Mackay et al., 2014; Waajen et al., 2016). Selecting the appropriate method for restoring the water system requires careful consideration of factors such as cost, effectiveness, and ecological impact. So, how to select suitable methods for mitigating eutrophication?

Decision-making

Mohandas Karamchand Gandhi said, "No two leaves are alike, and yet there is no antagonism between them or between the branches on which they grow". No two water bodies in nature are completely alike, so the methods of restoration are different for various waterbodies. Learning the experiences from the success of water-system restoration, but copy-paste the restoring methods might lead to failures. Thus, each lake has to be studied individually before restoration measures can be applied (van Liere and Gulati, 1992). A system analysis to understand the water and nutrient fluxes and biological characteristics of a water body, should be conducted before implementing any measures. It will help identify the most effective measures to be taken (Cooke et al., 2005; Lürling et al., 2020a; Lürling et al., 2016; Mucci, 2018). At the same time, a cost-benefit analysis should be performed taking into account the desired conditions to be achieved. Now imagine yourself as a water manager seeking to implement the most effective measures to address the eutrophication and cyanobacteria nuisance in the water body. At this point, you realize that it is essential to develop a comprehensive framework, which can be divided into five aspects:

- **Whole system analysis:** To quantify water and nutrient fluxes, nutrient loading, and biological characteristics through laboratory and field research. Different materials have different properties; some adsorb P, such as LMB, while others adsorb N, such as AMZ.
- **Monitoring:** The water quality after in-lake measures over time, assessing their effectiveness and making adjustments as needed.

Also, stakeholder engagement, economics and environmental safety aspects in the decision-making process cannot be ignored.

- **Stakeholder engagement:** Understanding local cultural eutrophication and actively engaging stakeholders, including local residents, business owners, and environmental groups, can help ensure that the selected measures are acceptable to the community.
- **Economics:** Select and assess potential in-lake measures taking into account both financial and material feasibility.

- **Environmental safety:** To meet local legal requirements, evaluate the effectiveness of materials and potential ecological impacts.

Thesis Outline and Aims

The purpose of this thesis was to investigate various potential compounds and techniques to manage eutrophication and mitigate cyanobacteria blooms. This research was conducted on different scales, beginning with laboratory experiments (**Chapters 2, 3 and 4**), then processing to *in situ* enclosure study (**Chapter 5**), and ultimately culminating in a large-scale intervention in a 114 ha shallow lake (**Chapter 6**).

In-lake measures are gaining attention as complementary measures to speed up recovery in lakes and ponds suffering from nuisance eutrophication issues. In-lake measures must be efficient, easy to apply, and relatively cheap and safe, which means minimizing unwanted consequences, such as cyanotoxin releases. This thesis starts by evaluating the impact of nine commercially available products on a common bloom-forming cyanobacterium (*Microcystis aeruginosa*) and cyanotoxin (microcystins) releases (**Chapter 2).**

According to **Chapter 2**, we learned that copper-based algaecides have excellent properties for killing *M. aeruginosa*, yet they may also be toxic to non-target zooplankton grazers, such as *Daphnia magna*, which play an important role in the water ecosystem. Thus, in **Chapter 3**, we conducted experiments to assess the impact of four copper-based algaecides on *Daphnia magna*.

P binders (also called Phosphorus sorbents (PS)) as in-lake measures that immobilize the internal P pool are essential to manage eutrophication in the case. While P sorbents induced a milder response on *M. aeruginosa*, they were efficient in reducing phosphate concentration (**Chapter 2)**. As abiotic factors might affect their efficiency, PS was tested under a realistic pH and temperature range (**Chapter 4**).

Chapter 5 involved an enclosure study in a pond to investigate dredging, lanthanum-modified bentonite clay (LMB), aluminium-modified zeolite (AMZ) and iron chloride ($FeCl_2$), which was partly studied in **Chapters 2 and 4**, in mitigating nutrient release from sediment.

Building on the findings of **Chapters 2, 4, and 5**, a whole lake treatment using LMB was performed in **Chapter 6.** We collected sediment cores from the large-scale lake at pre-, post-3 months, and post-15 months LMB application and tested the sediment nutrients released under different pH.

Lastly, **Chapter 7** presents the key findings and provides a discussion on water restoration.

2

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

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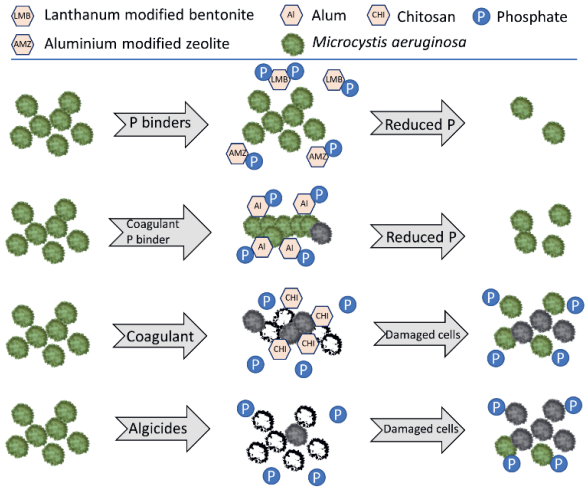


Abstract

Numerous products and techniques are used to combat harmful cyanobacterial blooms in lakes. In this study, we tested nine products, the phosphate binders Phoslock® and Aqual-P™, the coagulant chitosan, the phosphorus binder and coagulant aluminium salts (aluminium sulphate and sodium aluminate), the copper-based algaecides SeClear, Captain® XTR and $CuSO_4 \cdot 5H_2O$, the antibiotic Streptomycin and the oxidant hydrogen peroxide (H_2O_2) on their efficiency to manage the cyanobacterium *Microcystis aeruginosa* (*M. aeruginosa*). To this end, 7 days of laboratory experiments were conducted and effects were determined on chlorophyll-*a*, photosystem II efficiency (PSII), soluble reactive phosphorus (SRP) and intracellular and extracellular microcystin (MC) concentrations. The algaecides, chitosan and H_2O_2 were the most powerful in reducing cyanobacteria biomass. Biomass reductions compared to the controls yielded: Chitosan (99.8%) > Hydrogen peroxide (99.6%) > Captain XTR (98.2%) > SeClear (98.1%) > $CuSO_4 \cdot 5H_2O$ (97.8%) > Streptomycin (86.6%) > Phoslock® (42.6%) > Aqual-P™ (28.4%) > alum (5.5%). Compounds that caused the largest reductions in biomass also strongly lowered PSII, while the other compounds (Phoslock®, Aqual-P™, aluminium salts) had no effect on PSII, but strongly reduced SRP. Intracellular MC concentration followed the biomass patterns, extracellular MC was generally lower at higher doses of algaecides, chitosan and H_2O_2 after one week. Recovery of PSII was observed in most algaecides and chitosan, but not at the highest doses of SeClear and in all Streptomycin treatments. Our results revealed that *M. aeruginosa* can be killed rapidly using several compounds and that in some treatments signs of recovery occurred within one week. P fixatives are efficient in reducing SRP, and thus acting via resource suppression, which potentially may provide an addition to fast-acting algaecides that kill most of the cells, but allow rapid regrowth when sufficient nutrients remain.

Introduction

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

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

Evidently, there is a great need to control eutrophication and minimize the negative impacts of nuisance blooms in which stopping excessive external nutrient inputs is the most optimal mitigation measure (Hamilton et al., 2016; Paerl et al., 2016). However, external nutrient load control may meet severe challenges; worldwide a low share of wastewater is being treated properly (WWAP, 2017) and high investments are required to improve treatment (van Loosdrecht and Brdjanovic, 2014), yet even after point sources such as wastewater effluents have been tackled, eutrophication threats may continue due to nutrient legacies in the lake bed and diffuse nutrient loads from agriculture (OECD, 2014, 2017; Ryding and Forsberg, 1976). Consequently, in a growing number of water bodies short-term within-system interventions are being applied to suppress cyanobacterial blooms directly by targeting the biomass or indirectly by reducing available resources (Jančula and Maršálek, 2011; Lürling et al., 2020a; Lürling and Mucci, 2020).

The most common way to suppress cyanobacterial biomass is by using algaecides (Jančula and Maršálek, 2011). They are viewed as a relatively fast and cost-effectively way of eradicating cyanobacteria for which an arsenal of compounds exists such as copper-based algaecides, oxidants (hydrogen peroxide), herbicides, and antibiotics (Buley et al., 2021; Huang and Zimba, 2020; Iwinski et al., 2016; Jančula and Maršálek, 2011; Kibuye et al., 2021; Matthijs et al., 2012; Matthijs et al., 2016; Qian et al., 2012). Although algaecides are generally highly effective and fast-acting, they may also increase dissolved nutrient concentrations (Coloma et al., 2017) and cause the release of intracellular toxins (Jones and Orr, 1994; Kenefick et al., 1993; Li et al., 2022). An alternative coagulant such as aluminium salts (aluminium sulphate

and poly-aluminium chloride-PAC) can be used that aggregate the biomass and settle it to sediment, concomitantly adsorbing phosphate (P) (Cooke et al., 2005; Kang et al., 2022b). Coagulants like the organic polymer chitosan can also be combined with local soil or other ballast compounds to facilitate settling of the cyanobacteria-coagulant flocs (Noyma et al., 2016; Noyma et al., 2017; Pan et al., 2011; Pan et al., 2006). When combined with a solid P sorbent as ballast, the water column can be denuded from cyanobacteria while the P sorbent reduces the P release from the lake bed once settled on the sediment. Solid P sorbents can also be used to indirectly manage cyanobacterial biomass via strong reduction in the availability of P (Lürling et al., 2020a; van Oosterhout and Lürling, 2013).

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

Material and Methods

Chemicals

Nine products or chemicals commonly used in lake restoration projects were selected, including three copper-based algaecides (SeClear, Captain XTR and $CuSO_4 \cdot 5H_2O$), two coagulants (Chitosan and aluminium salts (Al-salts)), two phosphate fixatives (Phoslock® and Aqual-P™), one antibiotic (Streptomycin), and one oxidant (Hydrogen peroxide). Each compound was tested at six different concentrations and each concentration was run in triplicate (Table 2.1).

Table 2.1 The information of nine materials tested on *M. aeruginosa*

Product	Description	Manufacturer	Dosage	Price (USD $)	References dosage
Phoslock®	Ballast; Clay; Lanthanum-modified bentonite, LMB	Phoslock® Europe GmbH (Zug, Switzerland)	0, 50, 100, 300, 600, 1000 mg L^{-1}	0.0025/g	(Spears et al., 2013b)
Aqual-P™	Ballast; aluminium - modified zeolite, AMZ	Blue Pacific Minerals (Tokoroa, New Zealand)	0, 50, 100, 300, 600 1000 mg L^{-1}	0.0027/g	(Mucci et al., 2017)
Chitosan	Coagulant; CHI	Sigma, USA	0, 0.5, 1, 2, 4, 8 mg L^{-1}	0.001-0.095/g	(Mucci et al., 2017)

Aluminium sulphate + Sodium Aluminate	Metal-based coagulant; Al-salts	Kemira (Helsinki, Finland) + Sigma-Aldrich (Darmstadt, Germany)	0, 1, 3, 10, 20, 30 mg Al L^{-1}	0.0002+0.04/g	(Georgantas and Grigoropoulou, 2007)
SeClear	Metal-based algaecides; Copper sulphate pentahydrate	SePRO Corporation, Carmel, IN, USA	0, 0.05, 0.1, 0.25, 0.5, 1 mg Cu L^{-1}	0.005/mL	Advised dose 0.15-1.0 mg Cu L-1 by SePro
Captain® XTR	Metal-based algaecides; Copper ethanolamine complex	SePRO Corporation, Carmel, IN, USA	0, 0.05, 0.1, 0.25, 0.5, 1 mg Cu L^{-1}	0.014/mL	Advised dose 0.15-1.0 mg Cu L-1 by SePRO
$CuSO_4 \cdot 5H_2O$	Metal-based algaecides; analysed pure	Sigma, USA	0, 0.05, 0.1, 0.25, 0.5, 1 mg Cu L^{-1}	0.17/g	(Viriyatum and Boyd, 2016)
Streptomycin	Antibiotics; Str, inhibitor of protein synthesis	Sigma, USA	0, 0.1, 0.2, 0.4, 0.8, 1.6 mg L^{-1}	1.3/g	(Qian et al., 2012)
Hydrogen peroxide	Oxidant; H_2O_2, liquid	Merck KGaA, Germany	0, 0.1, 0.3, 1, 3, 10 mg L^{-1}	0.1/mL	(Matthijs et al., 2012)

Experimental design

Microcystis aeruginosa (strain PCC 7820) was obtained from the Pasteur Culture Collection of Cyanobacteria (PCC) and cultured on a modified WC-medium (Lürling and Beekman, 2006) in 500 mL Erlenmeyer flasks placed at 22 ºC, at a light intensity of 35 µmol quanta $m^{-1}s^{-1}$ provided in a 16:8 h light: dark cycle. Aliquots of *M. aeruginosa*, harvested during their exponential growth phase, were transferred to 250 mL conical flasks containing 50 mL of WC medium yielding a final concentration of 100 µg L^{-1} chlorophyll-*a* (Chl *a*), which indicated *Microcystis* biomass in this study. The inocula had a photosystem II efficiency (PSII efficiency) of 0.35. Six concentrations of each of the nine compounds were used (Table 2.1). Each concentration had three replicates. After the addition of the compounds, the flasks were closed with a cellulose plug, mixed and placed in an incubator (Gallenkamp Orbital shaker) with 50 rpm under the same conditions at which *M. aeruginosa* had been cultured. After 2, 24, 72 and 168 hours, samples were taken from the middle of the flasks to measure Chl *a* concentrations and PSII efficiencies using a PHYTO-PAM analyzer (Heinz Walz GmbH, Effeltrich, Germany). At the end of the experiment, the pH in each flask was measured using a WTW Inolab pH 7110 meter and subsamples were taken and filtered through 0.45 µm unit filters (Aqua 30/0.45 CA, Germany) to analyze orthophosphate with a Skalar SAN^+ segmented flow analyzer. Also, both intracellular and extracellular MCs concentrations were measured. Hereto, 7.5 mL samples were filtered through glass fiber filters (GF/C, Whatman®, VWR International B.V., Amsterdam,

The Netherlands), the filters were frozen at -20°C and subsequently extracted with 75% v/v methanol/water (Lürling and Faassen, 2013). Filter extracts were transferred to 8 mL glass tubes for intracellular microcystin (MC) analysis. The GF/C filtrates were also transferred in 8 mL glass tubes for dissolved extracellular MC analysis. The extracts and filtrates were dried in a SpeedVac concentrator (Savant™ SPD121P, Thermo Fisher Scientific, Asheville, NC, USA) and reconstituted using 900 µL methanol (J.T. Baker®, gradient grade, 97%, VWR International B.V., Amsterdam, The Netherlands). Afterward, the samples were transferred to a vial with a 0.22 µm cellulose-acetate spin centrifuge tube filters and centrifuged for 5 minutes at 16,000 × g. The filtrates were then transferred to amber glass vials and analyzed using LC-MS/MS (Agilent 6410A QQQ, Waldbronn, Germany) for eight MC variants according to Lürling and Faassen, 2013. The MC concentrations in this study are presented as extracellular MC concentrations (µg L^{-1}), intracellular MC concentrations (µg L^{-1}), the ratio between extracellular MC and Chl *a* concentrations (µg extracellular MC per µg Chl *a*), and the ratio between intracellular MC and Chl *a* concentrations (µg intracellular MC per µg Chl *a*).

Growth rates

Growth rates were estimates from initial and final Chl *a* concentrations assuming exponential growth using the equation (2-1) (Fawaz et al., 2018; Gojkovic et al., 2019):

$$\mu = \frac{ln\ Chl\ a_{end} - ln\ Chl\ a_{start}}{t_j - t_i} \quad (2\text{-}1)$$

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

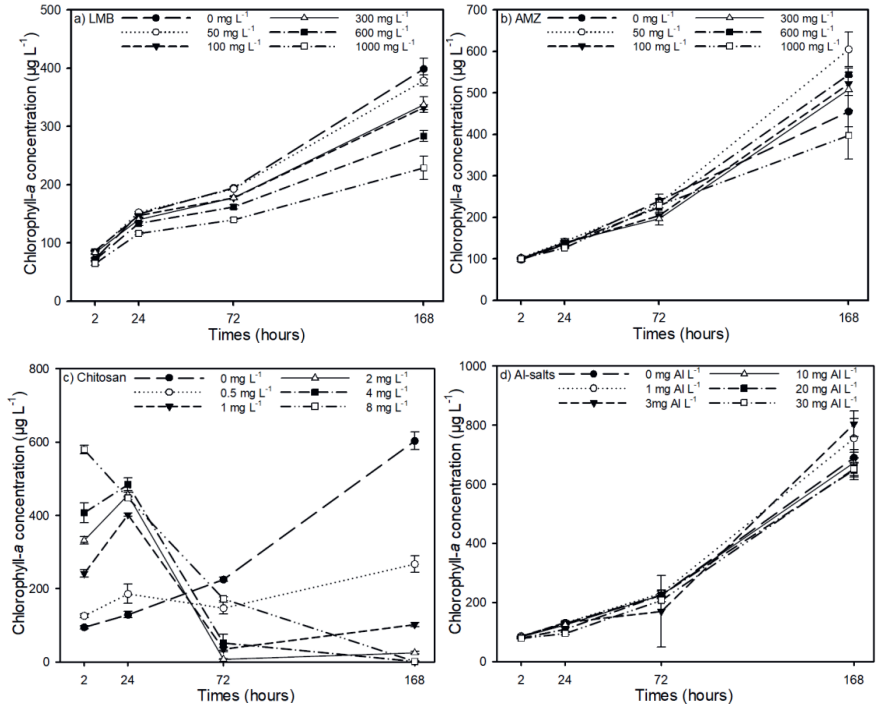
Statistical analysis

All graphs were created using Sigmaplot version 14.0. The differences between growth rates, SRP concentrations, intracellular and extracellular MCs, intra and extracellular MCs/Chl *a*, were tested using one-way ANOVA or a non-parameter test (Kruskal-Wallis One Way analysis of variance on ranks) when normality tests (Shapiro-Wilk) failed. EC_{50} values (compound concentration that caused a 50% reduction in PS-II relative to the control) were calculated based on the PSII value by a four-parameter logistic equation. The results section has been written following the suggestion of using evidence language (Muff et al., 2022).

Results

Chlorophyll-a concentrations

Chlorophyll-*a* (Chl *a*) concentrations of the *M. aeruginosa* cultures were affected differently by the different compounds (Fig. 2.1). Chl *a* increased over time at all the LMB, AMZ and Al concentrations tested. There was, however, a clear dependency of Chl *a* and LMB dose where Chl *a* increased less with higher doses of LMB (Fig. 2.1a). In the highest LMB, AMZ and Al concentrations used the Chl *a* concentrations were 42.6%, 28.4% and 5.5% less than in the corresponding controls at the end of the experiment, respectively (Fig. 2.1a,b,d). Chitosan (CHI) caused a rapid increase in Chl *a* concentrations within 2 and 24 hours in all concentrations tested, however after 72 and 168 hours Chl *a* concentrations in the highest dose (8 mg CHI L^{-1}) were 23.6% and 99.8% less compared to the controls (Fig. 2.1c). A typical response of rapid cell lysis could be confirmed by analysis of 0.45 µm (GF/C) filtered culture medium (Table 2.S1). The Chl *a* concentrations gradually declined in higher chitosan dosages because of the breakdown of the pigments, whereas the Chl *a* concentration increased in controls as a result of population growth (Fig. 2.1c).



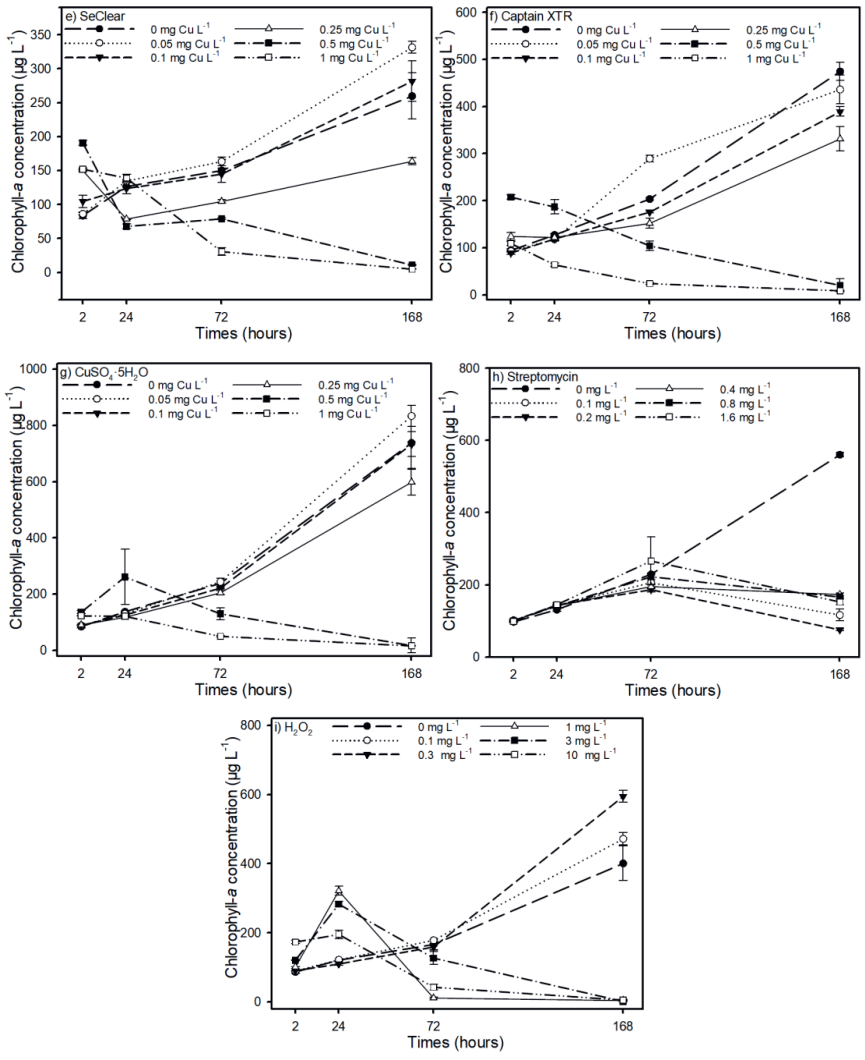


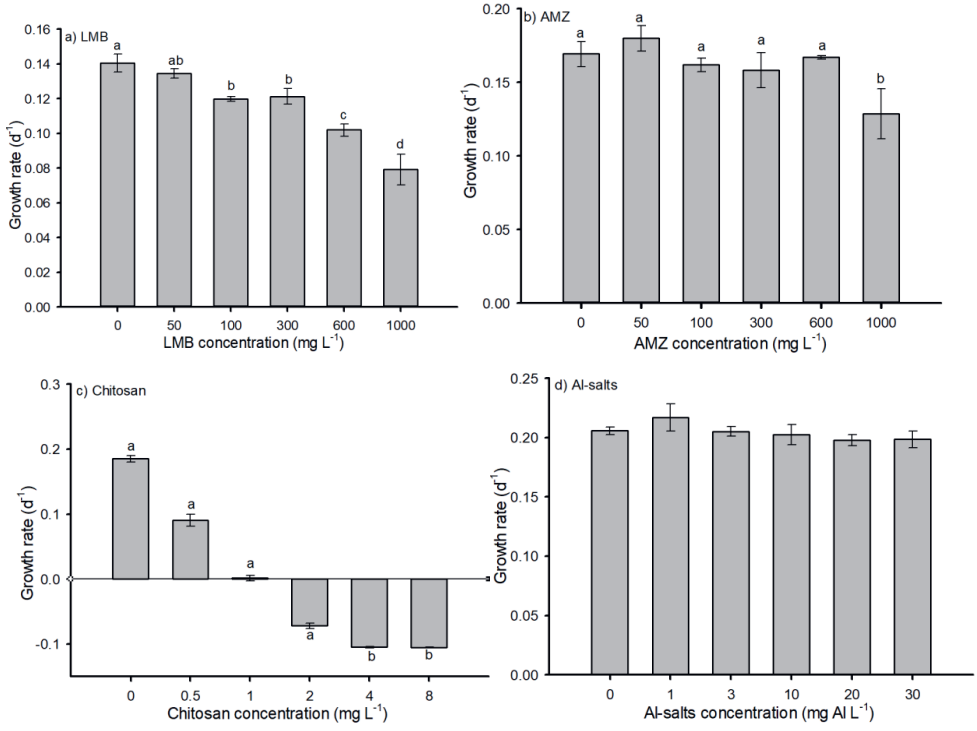
Fig. 2.1 Effect of nine compounds on the chlorophyll-*a* concentration of *M. aeruginosa* exposed for 2, 24, 72 and 168 h. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2. The initial chlorophyll-*a* concentration was 100 µg L^{-1}

The copper-based materials (Captain XTR, SeClear and $CuSO_4 \cdot 5H_2O$) showed similar trends, a strong reduction in Chl *a* concentration when 0.5 and 1 mg Cu L^{-1} was applied (Fig. 2.1e,f,g). SeClear caused a rapid increase (after 2 h) in Chl *a* concentrations in the highest dose, which was due to the release of cell constituents (Table 2.S1). Shortly after the addition of Streptomycin, Chl *a* concentrations increased in all the concentrations tested, however by the end of the experiment Chl *a* concentrations were strongly reduced (69-87%) compared to the

controls (Fig. 2.1h). After the addition of H_2O_2, Chl *a* concentrations rapidly increased in the higher H_2O_2 doses as a result of cell lysis, which was followed by a subsequent decline due to the breakdown of the pigment (Fig. 2.1i). In the control and H_2O_2 treatments below 0.3 mg H_2O_2 L^{-1}, Chl *a* concentrations were similar and showed a continuous increase reflecting *M. aeruginosa* growth. After one-week Chl *a* concentrations in the higher H_2O_2 treatments were up to 99.5% lower than in the controls (Fig. 2.1i).

Growth rates

The difference between the initial (100 µg L^{-1}) and final Chl *a* concentrations in controls and each treatment were used to calculate growth rates. Growth rates of *M. aeruginosa* declined with increasing amounts of LMB dosed (Fig. 2.2a). A one-way ANOVA provided evidence that growth rates were different ($F_{5, 12} = 61.2$; $p < 0.001$) and Tukey's test showed that growth rates in the 1000 mg LMB L^{-1} treatments were lower ($p < 0.001$) than in the controls (0 mg LMB L^{-1}). Although this pattern was less clear in the AMZ treatments (Fig. 2.2b), the one-way ANOVA provided very strong evidence of differences in growth rates ($F_{5, 12} = 9.0$; $p < 0.001$) and a Tukey's test showed that growth rates in the 1000 mg AMZ L^{-1} treatments were lower ($p = 0.004$) than in the controls (0 mg AMZ L^{-1}).



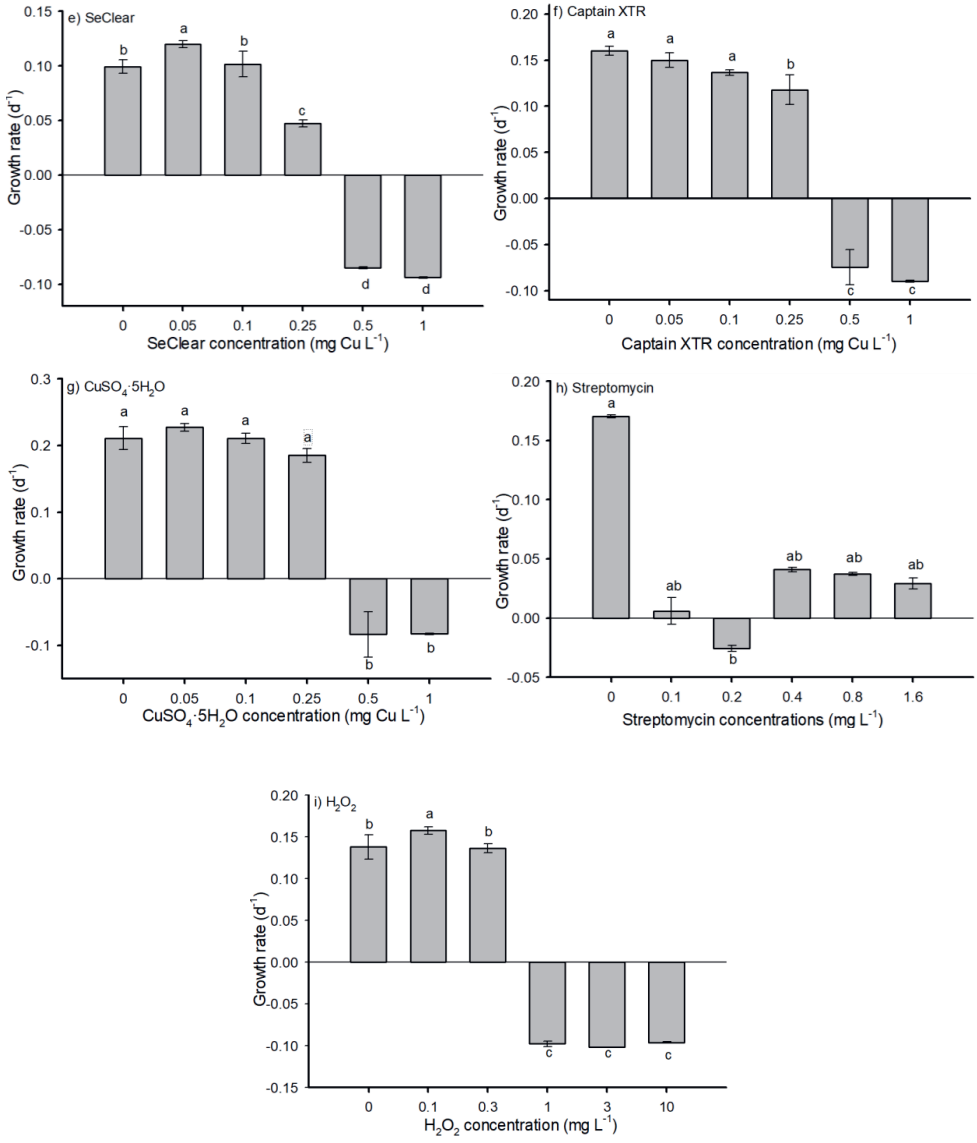


Fig. 2.2 Growth rate of *M. aeruginosa* at different chemical concentrations after 168 hours. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2. Similar letters (a, b, c, d) in each panel indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other ($p > 0.05$)

In the chitosan treatments, strong evidence was found ($H_5 = 16.11$; $p = 0.007$) that growth rates of *M. aeruginosa* decreased with increased chitosan dosages (Fig. 2.2c). Growth rates declined strongly at a dose of 0.5 mg CHI L^{-1}, was almost zero at 1 mg CHI L^{-1}, and became negative at doses > 1 mg CHI L^{-1} indicating a decline/death of the *M. aeruginosa* populations (Fig. 2.2c).

There was no evidence that Al, at any dose tested, had an effect on growth rates of *M. aeruginosa* ($F_{5, 12} = 2.883$; $p = 0.062$) (Fig. 2.2d).

Compared to controls, growth rate declined 201%, 156% and 139% in the highest dosages (1 mg Cu L^{-1}) of SeClear ($F_{5, 12} = 852.92$; $p < 0.001$), Captain XTR ($F_{5, 12} = 338.86$; $p < 0.001$) and $CuSO_4 \cdot 5H_2O$ ($F_{5, 12} = 252.19$; $p < 0.001$), respectively. All three compounds resulted in negative growth rates (between -0.094 and -0.075 d^{-1}) when dosed at 0.5 mg Cu L^{-1} or higher (Fig. 2.2e,f,g).

The experiment with streptomycin yielded strong evidence that streptomycin affected *M. aeruginosa* growth rate ($H_5 = 16.58$; $p = 0.005$). A Tukey's test provided very strong evidence that growth rates in the 0.2 mg STR L^{-1} treatments were lower ($p < 0.001$) than in the controls (0 mg STR L^{-1}) (Fig. 2.2h).

There was no evidence that hydrogen peroxide affected *M. aeruginosa* growth rates when dosed in the range 0-0.3 mg L^{-1}, however, in doses > 1 mg H_2O_2 L^{-1}, very strong evidence was obtained that *M. aeruginosa* growth rates were reduced ($F_{5, 12} = 1208.78$; $p < 0.001$). Tukey's post hoc test revealed two homogeneous groups among the six H_2O_2 treatments: 1) positive, unaffected growth in controls and the 0.1 and 0.3 mg H_2O_2 L^{-1} treatments, and 2) negative growth in the 1, 3 and 10 mg H_2O_2 L^{-1} treatments (Fig. 2.2i).

EC_{50} values

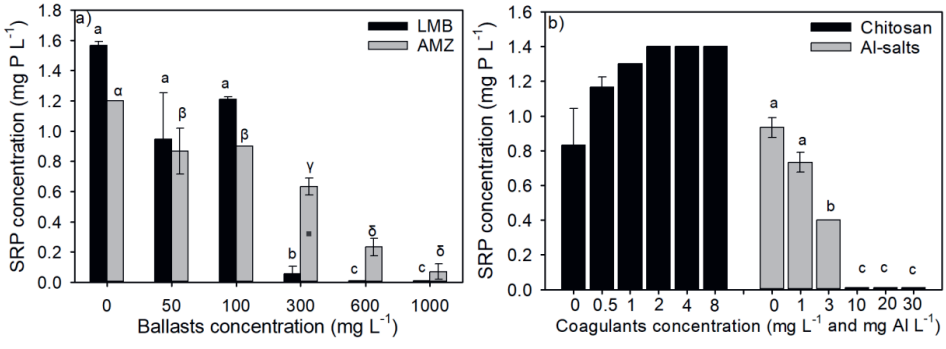
The EC_{50} values were calculated based on the PSII efficiencies (Fig. 2.S1) of the *M. aeruginosa* cultures. Estimated EC_{50} values of the P binders LMB and AMZ exceeded the highest dose used (1000 mg L^{-1}). Likewise, EC_{50} values of the Al coagulant were higher than the highest dose used (30 mg Al L^{-1}), indicating no or a weak effect of the compound on the physiological health of the *M. aeruginosa* cells. It should be noted, however, that after 7 days the PSII efficiencies in the highest dose of Al were lower than in the other treatments (Fig. 2.S1). For chitosan, the other coagulant tested, EC_{50} values were initially < 1 mg L^{-1} and increased over time (Table 2.2). EC_{50} values of the three copper-based chemicals were similar and as low as 0.25-0.28 mg Cu L^{-1} after two hours exposure; they remained low during the experiment (Table 2.2). The antibiotic streptomycin was also effective in damaging *M. aeruginosa* and EC_{50} values at the end of the experiment (after 168 hours) were low (0.09 mg L^{-1}) at the lowest dose of STR tested (0.1 mg L^{-1}). The EC_{50} values of the oxidizer hydrogen peroxide (H_2O_2) dropped from 2.6 mg L^{-1} after two hours to 0.84-0.99 mg L^{-1} in the period 1-7 days (Table 2.2).

Table 2.2 Mean EC_{50} values (values insides brackets represent the standard deviation (SD), n = 3) of the compounds used at each time point

Chemicals	EC_{50}-2 hours (mg L^{-1}, mg Al L^{-1}, mg Cu L^{-1})	EC_{50}-24 hours (mg L^{-1}, mg Al L^{-1}, mg Cu L^{-1})	EC_{50}-72 hours (mg L^{-1}, mg Al L^{-1}, mg Cu L^{-1})	EC_{50}-168 hours (mg L^{-1}, mg Al L^{-1}, mg Cu L^{-1})
LMB	>1000	>1000	>1000	>1000
AMZ	>1000	>1000	>1000	>1000
Chitosan	0.86 (0.09)	0.59 (0.003)	2.41 (0.02)	4.3 (0.028)
Al-salts	>30	>30	>30	>30
SeClear	0.28 (0.03)	0.45 (0.012)	0.32 (0.01)	0.39 (0.02)
Captain® XTR	0.25 (0.09)	0.3 (0.02)	0.33 (0.005)	0.42 (0.005)
$CuSO_4 \cdot 5H_2O$	>1	0.4 (0.01)	0.28 (0.01)	0.5 (0.005)
Streptomycin	>1.6	>1.6	0.13 (0.02)	0.09 (<0.001)
H_2O_2	2.64 (0.008)	0.84 (0.005)	0.93 (0.007)	0.99 (0.02)

Phosphate concentrations

Phosphate (soluble reactive phosphorus, SRP) concentrations were measured at the end of the experiment. The SRP concentrations revealed strong evidence that higher doses of LMB resulted in less SRP (H_5 = 16.31; p = 0.006) (Fig. 2.3a). Similarly, for AMZ there was very strong evidence that higher doses of AMZ affected SRP negatively ($F_{5,12}$ = 101.53; p < 0.001) (Fig. 2.3a), and also for Al strong evidence was found of Al negatively affecting SRP concentrations (H_5 = 16.88; p = 0.005) (Fig. 2.3b). In the series with chitosan, there was a weak evidence of lower SRP concentrations in the controls than in the CHI treatments (H_5 = 16.75; p = 0.06).



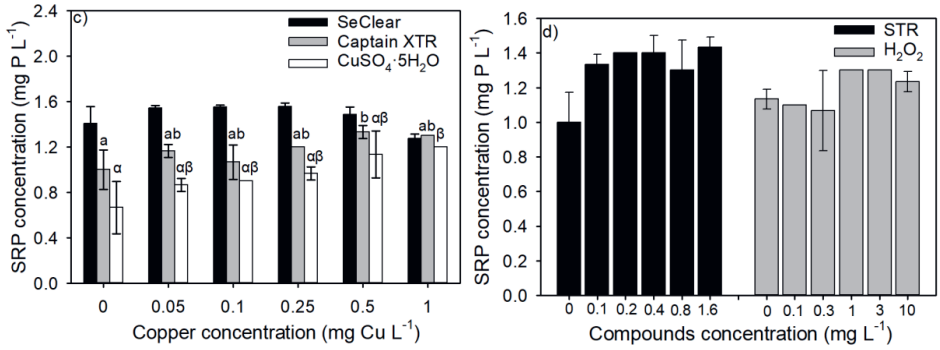


Fig. 2.3 SRP concentrations at six chemical exposure concentrations of nine compounds. a) LMB and AMZ, b) Chitosan and Al-salts, c) SeClear, Captain XTR and $CuSO_4 \cdot 5H_2O$, d) Streptomycin and H_2O_2. Similar symbols (a, b, c; α, β, γ, δ) above bars indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other ($p > 0.05$; Tukey's post hoc comparison tests).

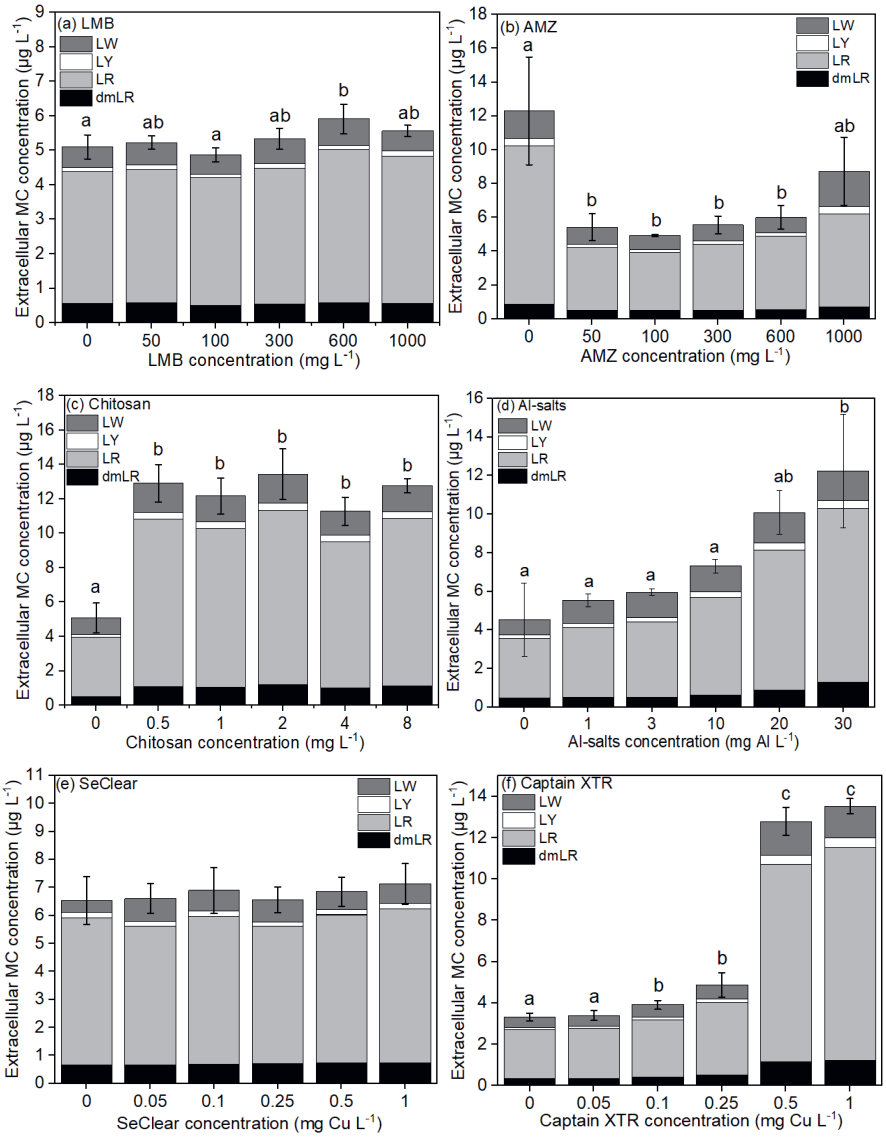
There was a weak evidence that SRP concentrations were lower in the controls and low doses of SeClear than at higher doses of this copper-based algaecide ($H_5 = 9.48$; $p = 0.091$, Fig. 2.3c), while there was moderate evidence that this was the case for the other two copper-based algaecides (Captain® XTR, $F_{5,12} = 5.02$; $p = 0.010$; $CuSO_4 \cdot 5H_2O$, $H_5 = 13.71$; $p = 0.018$) due to uptake by *M. aeruginosa* (Fig. 2.3c). Similarly, there was weak evidence that SRP concentrations in the controls were lower than in STR treatments ($H_5 = 15.25$; $p = 0.060$) (Fig. 2.3d). The data of the H_2O_2 series yielded weak evidence that the SRP concentrations were lower in doses 0-0.3 mg H_2O_2 L^{-1} and higher in the >1 mg H_2O_2 L^{-1} treatments ($H_5 = 17.69$; $p = 0.070$) (Fig. 2.3d).

MC concentrations

Microcystins (MC) present in the WC medium were quantified at the end of the experiment (Fig. 2.4). Four variants (MC-LW, LY, LR and dmLR) were detected of which the variant MC-LR was the most abundant one.

The total extracellular MC concentrations in the LMB series ranged from 4.9-5.9 µg L^{-1} (Fig. 2.4a). There was strong evidence that MC concentrations differed ($F_{5,12} = 4.65$; $p = 0.014$), a Tukey's test revealed that extracellular MC concentrations in the 600 mg LMB L^{-1} treatment were higher than in the controls (Fig. 2.4a). There was strong evidence that intracellular MC concentrations declined with LMB dose ($F_{5,12} = 104.64$; $p < 0.001$), the intracellular MC concentration in the control group was 8 times higher than in the 1000 mg LMB L^{-1} treatment (Fig. 2.5a). The decline was most prominent in the more hydrophilic MC variants dmMC-LR

and MC-LR (Fig. 2.5a). Extracellular MC concentrations/Chl *a* was lowest in the control group and it increased with increasing LMB concentrations (Fig. 2.S2a), while intracellular MC/Chl *a* was the highest in the control group and decreased with increasing LMB dosages (Fig. 2.S3a).



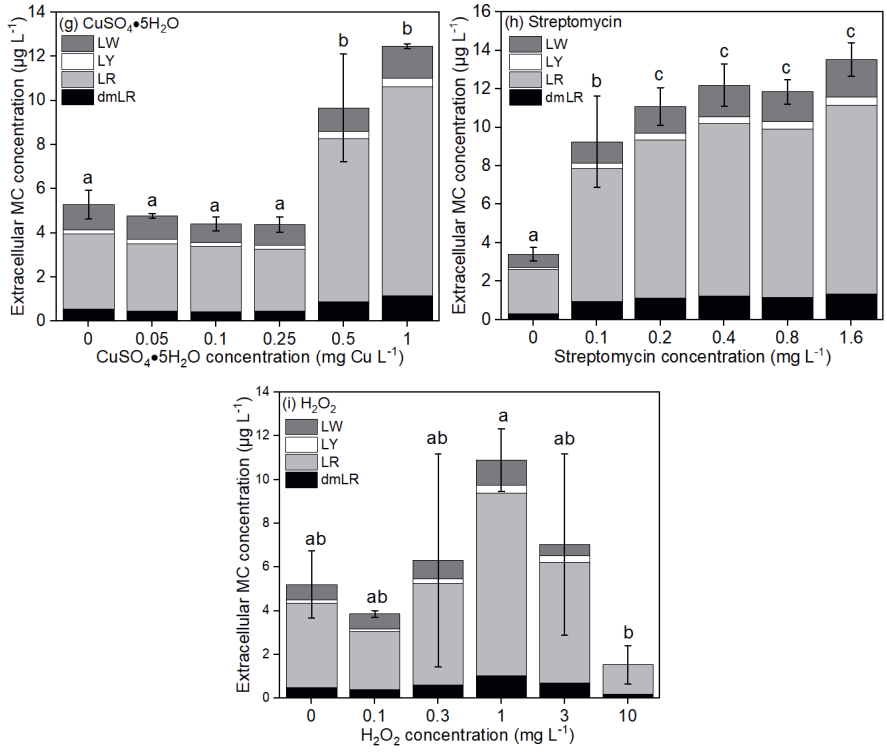
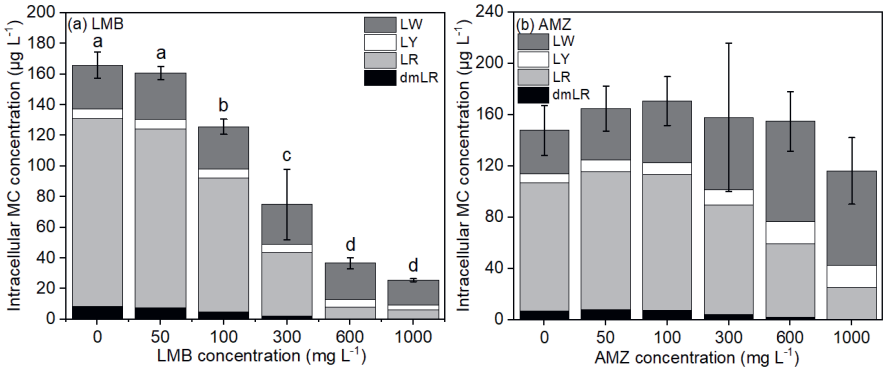
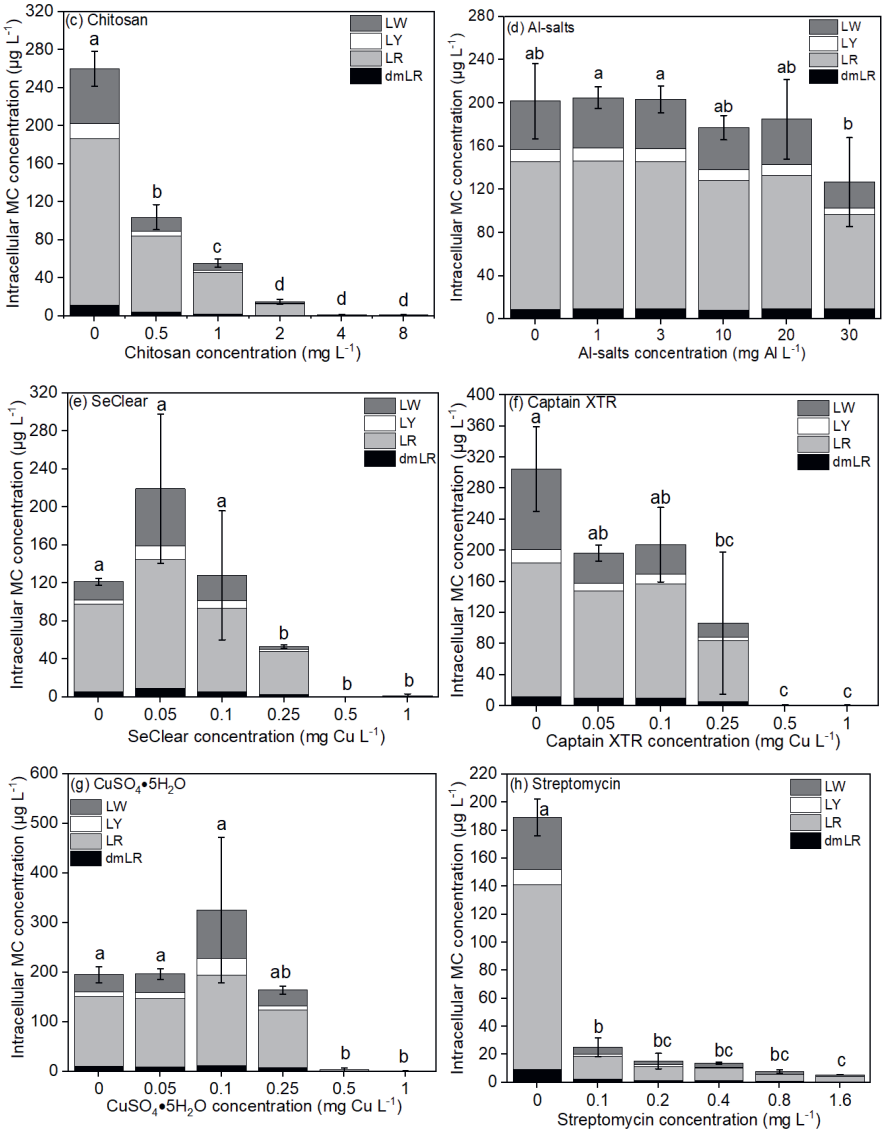


Fig. 2.4 Extracellular MC in all different chemicals concentrations after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2. Similar symbols (a, b, c) above bars indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other ($p > 0.05$; Tukey's post hoc comparison tests)




(c) Chitosan
LW
LY
LR
dmLR
Intracellular MC concentration (µg L-1)
Chitosan concentration (mg L-1)
(d) Al-salts
Al-salts concentration (mg Al L-1)
(e) SeClear
SeClear concentration (mg Cu L-1)
(f) Captain XTR
Captain XTR concentration (mg Cu L-1)
(g) CuSO4•5H2O
CuSO4•5H2O concentration (mg Cu L-1)
(h) Streptomycin
Streptomycin concentration (mg L-1)

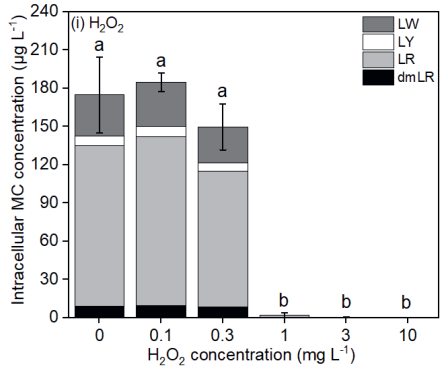


Fig. 2.5 Intracellular MC in all different chemicals concentrations after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2. Similar symbols (a, b, c) above bars indicate homogenous groups, i.e. treatments for which no evidence was found that they were different from each other ($p > 0.05$; Tukey's post hoc comparison tests)

In the AMZ series, there was very strong evidence that extracellular MC concentrations in presence of AMZ were lower than in the control ($F_{5,12} = 9.4$; $p < 0.001$) (Fig. 2.4b). The MC data yielded no evidence that intracellular MC concentrations were affected by AMZ ($F_{5,12} = 1.19$; $p = 0.371$) (Fig. 2.5b). There was, however, a clear tendency of a higher share of the more hydrophobic MC variants MC-LW and MC-LY and less of the more hydrophilic dmMC-LR and MC-LR at higher doses of AMZ (Fig. 2.5b).

Extracellular MC data yielded strong evidence ($F_{5,12} = 28.75$; $p < 0.001$) that extracellular MC concentrations in the presence of chitosan were higher than in the absence of chitosan (Fig. 2.4c). The ratio of extracellular MC/Chl *a* increased with chitosan dosages (Fig. 2.S2c). There was very strong evidence that intracellular MC concentrations in the chitosan series declined with increased chitosan dose in this study ($F_{5,12} = 336$; $p < 0.001$) (Fig. 2.5c).

In the Al series, there was very strong evidence that extracellular MC concentrations increased with increasing dose of Al coagulants (Fig. 2.4d). Also the extracellular MC/Chl *a* ratio showed a similar pattern (Fig. 2.S2d). One-Way ANOVA revealed very strong evidence that Al concentrations affected intracellular MC concentrations ($F_{5,12} = 3.45$; $p < 0.001$) (Fig. 2.5d), a Tukey's test showed that intracellular MC concentration in the 30 mg Al L^{-1} was lower than in the control (Fig. 2.5d). The intracellular MC/Chl *a* ratio in 30 mg Al L^{-1} was much higher than the ratios found in the other Al concentrations (Fig. 2.S3d).

No evidence was found that the copper-based algaecide SeClear ($F_{5,12} = 0.38$; $p = 0.85$) had an influence on the extracellular MC concentration (Fig. 2.4e). In contrast, very strong evidence was found that extracellular MC contents were elevated at the highest doses at Captain® XTR

($F_{5,12}$ = 399.2; p < 0.001) and copper sulphate ($CuSO_4 \cdot 5H_2O$, $F_{5,12}$ = 31.82; p < 0.001) (Fig. 2.4f,g). The extracellular MC/Chl *a* ratio increased at higher doses of all three copper-based algaecides (Fig. 2.S2e,f,g), while the intracellular MC/Chl *a* ratios gradually declined with increasing dose of each of the three copper-based algaecides tested (Fig. 2.S3e,f,g).

Evidence was obtained that streptomycin caused an increase in extracellular MC concentrations at all doses tested ($F_{5,12}$ = 25.83; p < 0.001) (Fig. 2.4h). This was mirrored by a strong decline in intracellular MC concentrations ($F_{5,12}$ = 374.96; p < 0.001) (Fig. 2.5h). The extracellular MC/Chl *a* ratio at 0.2 mg L^{-1} were around 15 times than that of the control (Fig. 2.S2h), while intracellular MC/Chl *a* ratio gradually decreased as increasing STR concentration (Fig. 2.S3h).

In the hydrogen peroxide series, despite relatively large variability found in some of the treatments (Fig. 2.4i), moderate evidence was obtained that extracellular MC concentrations were affected by H_2O_2 ($F_{5,12}$ = 3.97; p = 0.025). There was very strong evidence that H_2O_2 had an effect on intracellular MC concentrations ($F_{5,12}$ = 123.6; p < 0.001), and clearly two groups could be identified: 1) 0-0.3 mg H_2O_2 L^{-1} with mean MC concentrations of 150-180 µg L^{-1}, and 2) 1-10 mg H_2O_2 L^{-1} with MC concentrations close 0 µg L^{-1} (Fig. 2.5i).

Discussion

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

In line with our expectations, the two solid-phase P fixatives (LMB and AMZ) exerted a weak effect on *M. aeruginosa* biomass. Growth rates were reduced with increasing LMB doses, and given the strong depletion of SRP, this can be attributed to P limitation, but it by no means implies that relatively high *M. aeruginosa* biomass was mitigated. Similar observations have been made in short-term laboratory experiments and field mesocosms sealed at the bottom (Buley et al., 2021). PSII efficiencies were only marginally reduced at the highest doses of P fixatives, which might have been caused by higher turbidity, or indicated minor effects on cell membrane integrity.

The extracellular MC/Chl *a* ratio in the LMB series increased with increasing LMB, which might indicate relatively more release of MCs. Another possibility is MCs not being broken down in the artificial medium within the 7 day experimental period as in lake and river water breakdown of MCs might already take this time (Edwards et al., 2008). No evidence was found that extracellular MC concentrations declined with LMB in our study. A recent study showed that LMB dosed at 50, 100, and 150 ppm decreased extracellular MC concentrations by 61%, 86%, and 75% relative to the controls at a MC-LR concentration of 500 ppb, respectively, while LMB had no effect on the MC-LR concentration at lower concentrations of 100 ppb and 50 ppb (Laughinghouse et al., 2020). Hence, our results - with extracellular MC concentrations less than 20 ppb - are in line with those of Laughinghouse et al. (2020). The intracellular MC/Chl *a* ratio declined with increasing LMB dose and this study is the first that reports such finding as an effect of LMB. This might be related to less light available in higher doses of LMB that might lead to lower MC cell quota in *M. aeruginosa* (Wiedner et al., 2003). Evidently, *M. aeruginosa* became less toxic when reared in the presence of LMB, because intracellular MC concentrations declined 8 times at the highest LMB dose, while Chl *a* was only halved.

The variants dmMC-LR and MC-LR seemed to decline faster than MC-LY and MC-LW. Interestingly, the latter contain aromatic amino acids (Tyrosine, Y, and tryptophan, W), and the higher amounts of these MC variants in the higher AMZ doses could suggest stimulation of the shikimate pathway, whilst reducing non-aromatic amino-acid synthesis. This finding is, however, opposite to findings that under P limitation more N-rich variants of MC are being produced (Krüger et al., 2012). The observed increase in more hydrophobic MC variants at a higher dose of AMZ urges for care in the timing of adding such solid-phase P binders, since a higher share of those MC variants also implies higher toxicity (Fischer et al., 2010; Vesterkvist et al., 2012).

The results obtained also underpin that solid-phase P fixatives are not meant as quick fix agents eradicating cyanobacterial blooms; they are measures to reduce internal P load (Copetti et al., 2016; Douglas et al., 2016b; Gibbs and Hickey, 2018). Combined with a coagulant, however, those solid phase P fixatives can be highly effective in settling cyanobacterial biomass on the sediment (Lürling et al., 2020a; Noyma et al., 2017), a combination that was not tested in this study.

The two coagulants tested had distinct effects on *M. aeruginosa*. Al-salts did not affect *M. aeruginosa* growth and only weakly PSII efficiencies in the highest dose, which is comparable to other studies that found alum caused low levels cell damage (Lam et al., 1995), had no growth-inhibiting effect, and did not cause *M. aeruginosa* cell lysis or release of MCs in the

water in short term experiments (Chow et al., 1999). However, when Al-salts treated *M. aeruginosa* was incubated in 7 days, severe cell damage and subsequent leakage of MCs were observed, especially in the high dose (i.e. 48 mg L^{-1}) treatment (Han et al., 2016). Hence, the elevated extracellular MC concentrations at the highest dose tested (30 mg Al L^{-1}), the concomitant lower intracellular MC and lower PSII efficiency strongly indicate cell damage after 7 days. Alum also strongly reduced SRP concentrations as expected (Kang et al., 2022b). The SRP binding, particularly intercepting SRP released from P-loaded sediments, is the prime mechanism through which aluminium based compounds may control eutrophication and the development of cyanobacterial blooms (Augustyniak et al., 2019; Kibuye et al., 2021). In contrast, chitosan (CHI) did not reduce SRP concentrations, but caused a strong decline in *M. aeruginosa* growth and PSII efficiency, which was most probably a result of the membrane damaging effect of CHI resulting in cell lysis (Mucci et al., 2017).

There was very strong evidence for leakage of cell constituents. MCs and other cell constituents such as pigments remain inside the cell until the membrane is damaged and the cell is lysed (Lam et al., 1995). The high Chl *a* concentrations measured after two hours of exposure to CHI appeared as a result of cell constituent leakage (Table 2.S1), leading to a significant fluorescence signal, which does not reflect an increase in biomass (Bastien et al., 2011). The same strain of *M. aeruginosa* (PCC7820) was sensitive to CHI in another study where after 24 hours strongly elevated extracellular MC concentrations were measured (Mucci et al., 2020b). Our study is consistent with that after 7 days, compared to control groups, extracellular MC concentrations were more than doubled in the CHI treatments compared to the control. There was a strong decline in intracellular MC concentrations which paralleled the strong decrease in Chl *a*. No changes in the relative composition of the MCs were observed, in all treatments MC-LR remained the dominant MC-congener.

The three copper-based algaecides used (SeClear, Captain XTR and $CuSO_4 \cdot 5H_2O$) had strong impacts on *M. aeruginosa*. Copper can directly target the cells causing loss of cell membrane integrity, and destroying the photosystems through the production of reactive oxygen species (ROS) (Iwinski et al., 2016; Qian et al., 2010; Stevenson et al., 2013). Cationic forms of Cu (Cu^{2+}, $CuOH^{+}$, $Cu_2(OH)_2^{2+}$) are also toxic to non-target aquatic organisms (Closson and Paul, 2014) and are formed rapidly when copper sulphate dissociates in water (Mastin and Rodgers Jr, 2000). To lessen unwanted side effects on non-target organisms and to increase effectiveness against cyanobacteria and algae, chelated copper-based algaecides have been developed in which the chelator facilitates passage through cell membranes causing fast cell lysis (Closson and Paul, 2014; Kang et al., 2022a; Wagner et al., 2017a). As such, the chelated copper

algaecide Cutrine®-Ultra was more toxic than copper sulphate to the cyanobacterium *Planktothrix agardhii* and the green alga *Pseudokirchneriella subcapitata* (Calomeni et al., 2014). In our study, the two chelated copper compounds SeClear and Captain XTR also showed faster and stronger toxicity than $CuSO_4 \cdot 5H_2O$ (*see* Table 2.2, $EC_{50\text{-}2hrs}$). Copper-induced cell lysis may also rapidly release cell constituents into the surrounding medium, as was evidenced by the high filterable Chl *a* concentrations measured after two hours exposure to SeClear (Table 2.S1). Likewise, copper-induced cell lysis may increase extracellular MC concentrations within 24 hours (Chow et al., 1999), but unexpectedly this was not observed in the SeClear treatments. Inasmuch as elevated extracellular MC concentrations were observed in the Captain XTR and $CuSO_4 \cdot 5H_2O$ treatments, potential complexation of MCs with copper (Humble et al., 1997) can be excluded, as can MC breakdown (Lam et al., 1995). SeClear consists of 4.2% copper combined with a water quality enhancer that provides it the capacity not only to kill cyanobacteria and algae, but also to remove phosphate (information from SePRO Corporation, Carmel, IN, USA). However, in our study we did not detect a reduction in SRP concentrations; evidently more studies are needed to evaluate the potency of SeClear to lower SRP, but also to decipher if SeClear is capable of adsorbing dissolved MCs.

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

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

After 7 days, Chl *a* concentrations and *M. aeruginosa* growth were repressed in cultures exposed to H_2O_2 concentrations of 1 mg L^{-1} and higher (Fig. 2.1i and Fig. 2.2i). H_2O_2 enters

cells rapidly causing intracellular damage (Zhou et al., 2018), preventing PSII electron transmission and causing detachment of phycobilisomes (PBS) from the thylakoid membranes (Drábková et al., 2007b). Leakages of pigments into the medium cause a strong increase in F_0 (Drábková et al., 2007a) that is used in the Phyto-PAM to estimate Chl *a* concentrations (Schreiber, 1998) and which can explain the initially elevated Chl *a* concentrations in the higher H_2O_2 doses. The decline towards the end of the experiment reflects the degradation of released pigments. The cell membrane damage will also lead to the release of MCs (Lürling et al., 2014a; Sandrini et al., 2020), but this was not confirmed in our study. The lower extracellular MC concentration in the highest H_2O_2 dose might be caused by breakdown of MCs by H_2O_2 (Kansole and Lin, 2017), which was also observed in Lürling et al., 2014a. The use of a mostly unicellular strain as in our study may lead to lower effective H_2O_2 concentrations than when *M. aeruginosa* in its typical colonial form as in the field is used. Huang and Zimba (2020) found lower effective H_2O_2 concentrations for their laboratory strain of *M. aeruginosa* than when they treated a natural *M. aeruginosa* population in mesocosms and in a pond. In controlling natural *M. aeruginosa* populations a higher dose seems to be needed than when a bloom is comprised of filamentous cyanobacteria (Matthijs et al., 2016), which may point to a protective role of the mucous layer in *M. aeruginosa* colonies. Also other factors determine efficiency and H_2O_2 dose needed, such as cyanobacterial biomass (Huang and Zimba, 2020), presence of green algae (Weenink et al., 2021), light intensity (Piel et al., 2020), concentration of dissolved organic matter and reduced compounds (Matthijs et al., 2012). Hence, prior to an application tests with the natural phytoplankton community are needed, for example using enclosures to determine the H_2O_2 dose needed (Huang and Zimba, 2020; Matthijs et al., 2012).

Our study provides insight into the effects nine chemicals/products commonly used to mitigate cyanobacterial blooms may have on *M. aeruginosa* and MCs. As expected, algaecides were most powerful in eliminating cyanobacteria biomass, while P sorbents evoked a milder response. A downside of the algaecides is that MCs are liberated, the longevity of the positive effect might be short, and the cause root of the problem, over-enrichment with nutrients, remains untouched. Clearly, reducing nutrient inputs to lakes that suffer from cyanobacterial blooms is key, yet not always feasible. Hence, combining selected algaecides such as H_2O_2 or coagulants such as chitosan with a solid phase P binder might be considered to delay cyanobacteria regrowth and stretch the period of low cyanobacteria abundance (Drummond et al., 2022). A proper diagnosis of the cyanobacterial issue is recommended at each problem site followed by testing the intervention of choice on the natural cyanobacteria.

Conclusion

- The reduction of cyanobacteria biomass differed among the nine chemicals tested and could be ranked according to the percentage reduction compared to the controls on the 7th day as: Chitosan (99.8%) > Hydrogen peroxide (99.6%) > Captain XTR (98.2%) > SeClear (98.1%) > $CuSO_4 \cdot 5H_2O$ (97.8%) > Streptomycin (86.6%) > LMB (42.6%) > AMZ (28.4%) > Al-salts (5.5%).
- Algaecides were the most powerful in reducing cyanobacteria biomass, while ballasts evoked a milder response.
- Growth rates were reduced as the chemicals' dosages increased, except for Al-salts.
- MCs are liberated under algaecides treatments and the intracellular MC declined with increasing LMB dose.
- Combination of selected algaecides such as H_2O_2 that is for lowing the cyanobacteria abundance, with a solid phase P binder that is for reducing nutrients and delaying cyanobacteria regrowth might be considered in the future.

Acknowledgments

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

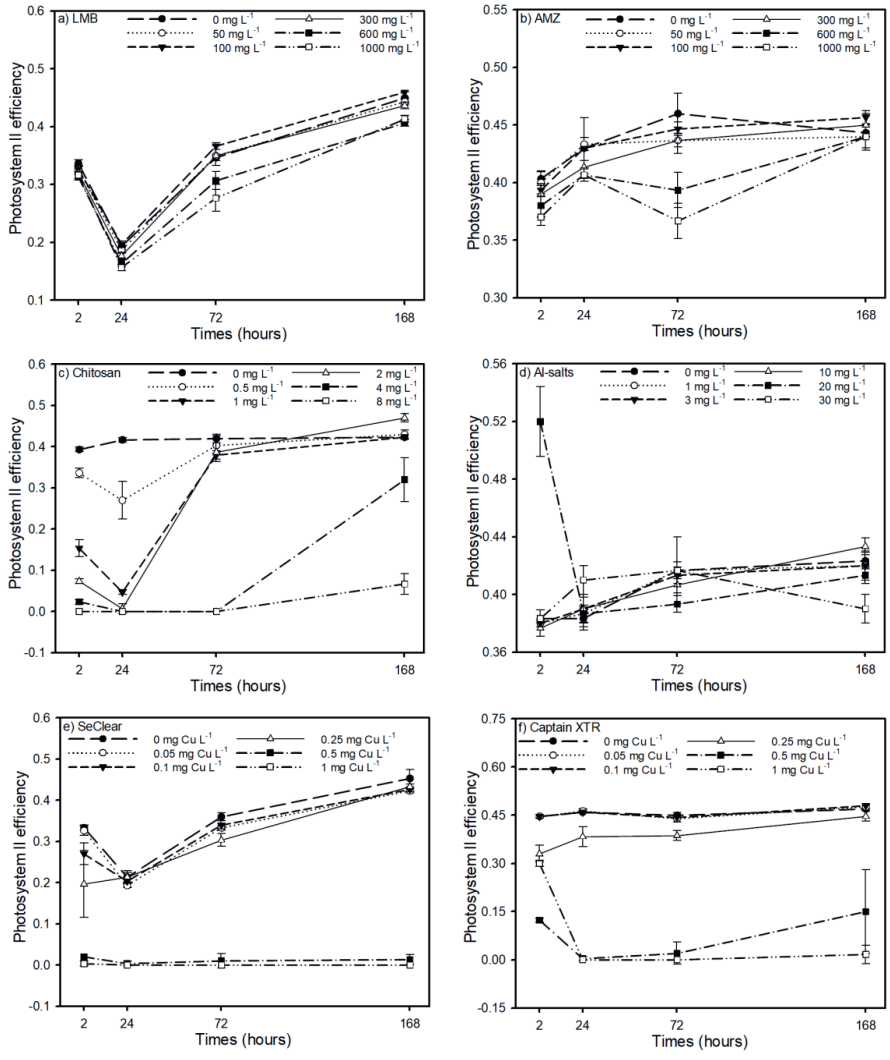
Supplementary information

List:

- Table 2.S1 Filterable chlorophyll-*a* and photosystem II (PSII) efficiency at different exposure time
- Fig. 2.S1 Effect of nine compounds on photosystem II efficiency of *M. aeruginosa* exposed for 2, 24, 72 and 168 h. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain® XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2
- Fig. 2.S2 Extracellular MC/Chl *a* in all different chemicals concentrations after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain® XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2
- Fig. 2.S3 Intracellular MC/Chl *a* in six different chemicals concentration after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain® XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2

Table 2.S1 Filterable chlorophyll-*a* and photosystem II (PSII) efficiency at different exposure time

Chemicals	Doses	2 hours		24 hours		72 hours		168 hours	
		Chlorophyll -a	PSII efficiency	Chlorophyll-a	PSII efficiency	Chlorophyll-a	PSII efficiency	Chlorophyll -a	PSII efficiency
Chitosan	0	8.69	0	0.67	0.04	1.02	0	0	0
	8	357.98	0	423.57	0	154.54	0	0	0
SeClear	0	1.25	0.01	none	none	none	none	none	None
	1	103.03	0	none	none	none	none	none	none



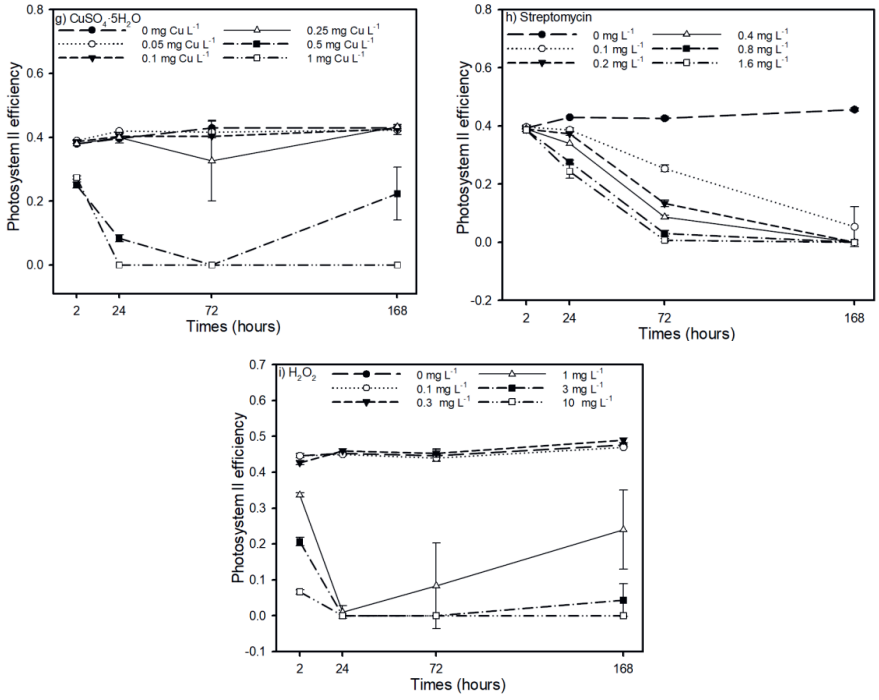
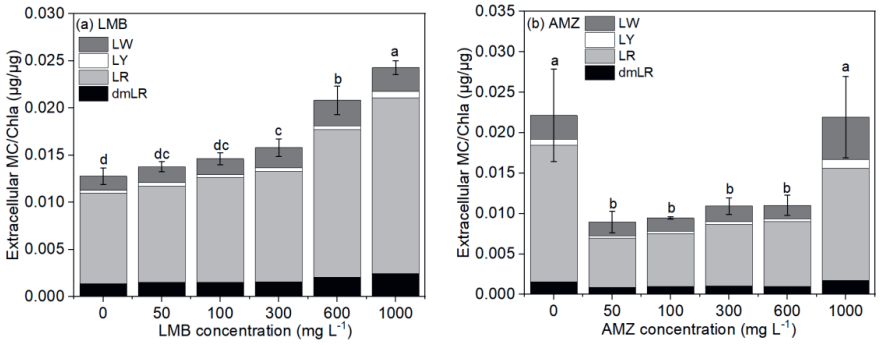


Fig. 2.S1 Effect of nine compounds on photosystem II of *M. aeruginosa* exposed for 2, 24, 72 and 168 h. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain® XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2



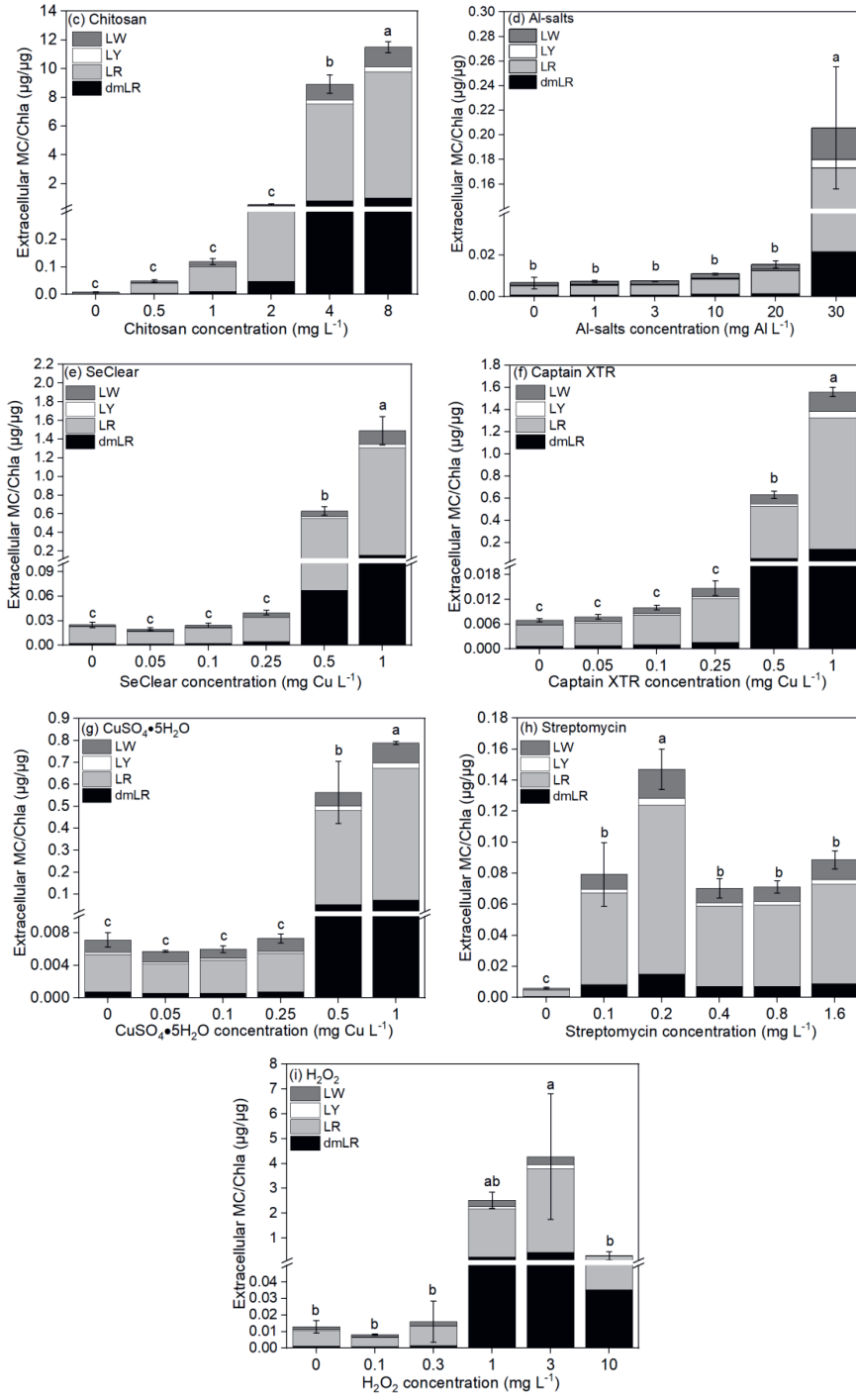
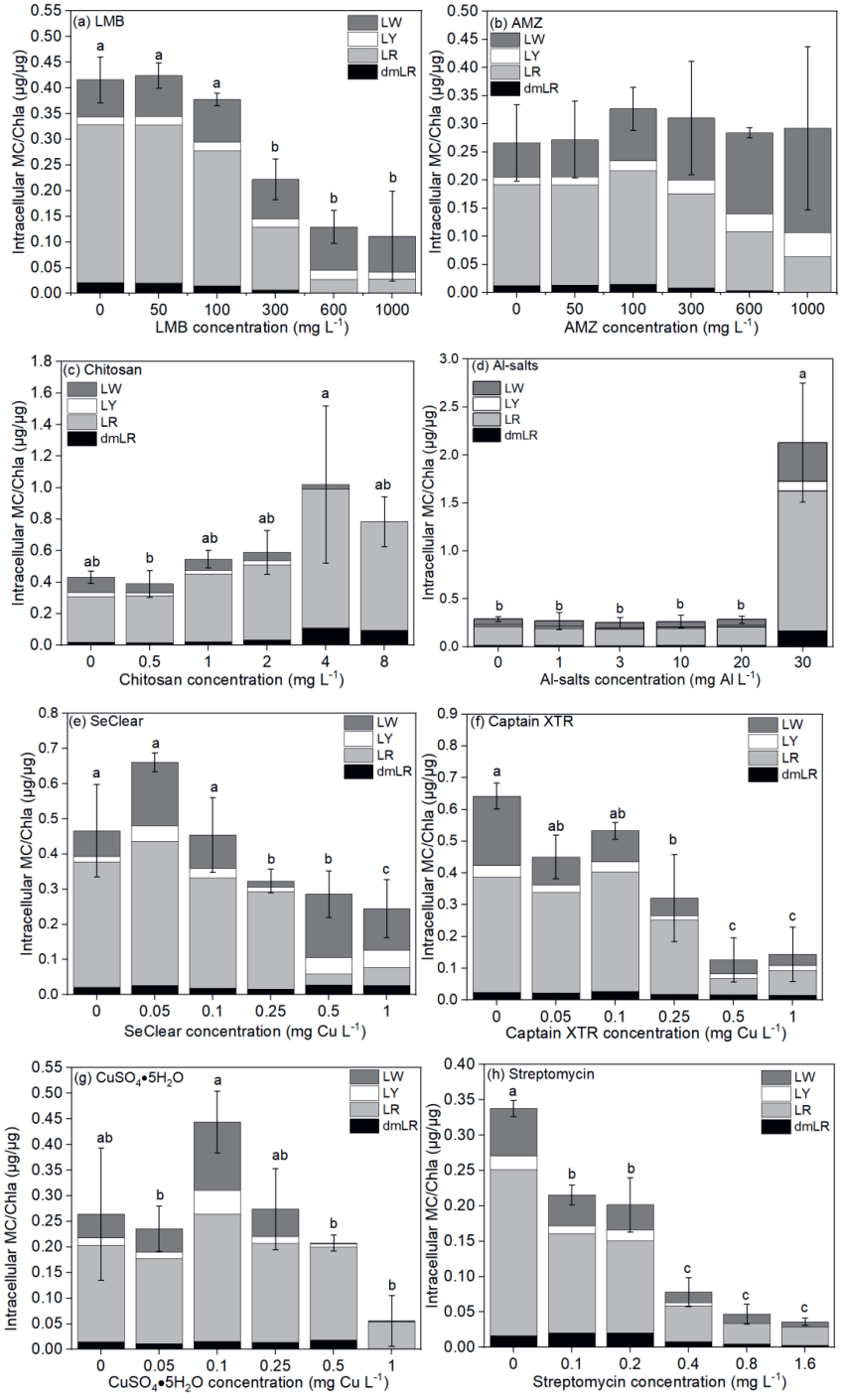


Fig. 2.S2 Extracellular MC/Chl *a* in all different chemicals concentrations after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain® XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2


(a) LMB
LW
LY
LR
dmLR
Intracellular MC/Chla (µg/µg)
LMB concentration (mg L-1)
(b) AMZ
AMZ concentration (mg L-1)
(c) Chitosan
Chitosan concentration (mg L-1)
(d) Al-salts
Al-salts concentration (mg Al L-1)
(e) SeClear
SeClear concentration (mg Cu L-1)
(f) Captain XTR
Captain XTR concentration (mg Cu L-1)
(g) CuSO4•5H2O
CuSO4•5H2O concentration (mg Cu L-1)
(h) Streptomycin
Streptomycin concentration (mg L-1)

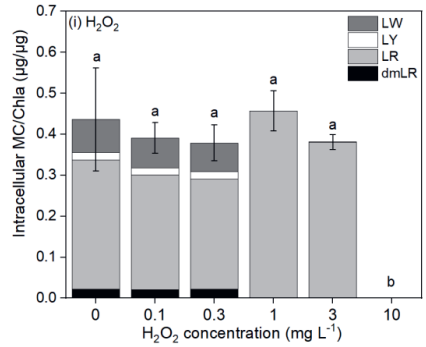


Fig. 2.S3 Intracellular MC/Chl *a* in six different chemicals concentration after 7 days. a) LMB, b) AMZ, c) Chitosan, d) Al-salts, e) SeClear, f) Captain® XTR, g) $CuSO_4 \cdot 5H_2O$, h) Streptomycin and i) H_2O_2

3

New is not always better: toxicity of novel copper based algaecides to *Daphnia magna*

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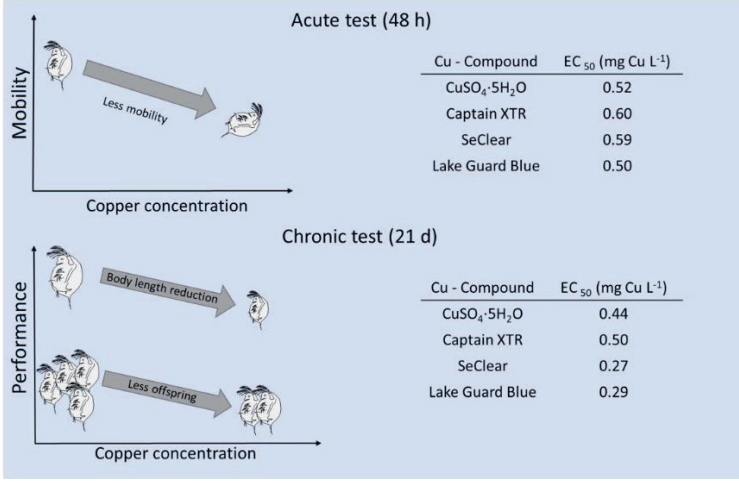

Abstract

In this study, the effects of traditional copper ($CuSO_4 \cdot 5H_2O$) and novel copper algaecides (Captain XTR, SeClear and Lake Guard Blue) were tested on *Daphnia magna* (*D. magna*) under acute (48 h) and chronic (21 d) exposure scenarios. The EC_{50} values calculated in the acute tests were between 0.5 and 0.6 mg Cu L^{-1} for all four compounds. Lake Guard Blue and $CuSO_4 \cdot 5H_2O$ were more toxic than SeClear and Captain XTR. During the chronic test, the effects of SeClear (EC_{50}: 0.274 mg Cu L^{-1}) on reproduction and body length were larger than the effects of the other three copper-based algaecides (EC_{50}: 0.436 mg Cu L^{-1} for $CuSO_4 \cdot 5H_2O$, 0.498 mg Cu L^{-1} for Captain XTR, and 0.295 mg Cu L^{-1} for Lake Guard Blue). Captain XTR had the strongest negative effect on body weight, whereas body weight was affected the least by $CuSO_4 \cdot 5H_2O$. The four copper compounds affected the age at first brood significantly, which was delayed by 1.8, 2.0, 2.3 and 3.2 days for Captain XTR, $CuSO_4 \cdot 5H_2O$, Lake Guard Blue and SeClear, respectively. Intrinsic rate of population increase was lowest (0.145 d^{-1}) at the highest dosage in the SeClear treatments. Chemical equilibrium modelling revealed that most copper was chelated with EDTA present in the artificial medium used. These combined results indicate that the toxicity of the novel copper algaecide SeClear to *D. magna* is greater than that of traditional copper algaecide. Prior to each Cu application, tests on the effects of Cu compounds on the organisms being targeted should be done, taking into consideration the water chemistry.

Introduction

Copper-based algaecides have been used extensively in freshwater, where cyanobacteria are rampant (Buley et al., 2021; Mastin et al., 2002). For instance, copper sulphate ($CuSO_4 \cdot 5H_2O$) has been used to control algal blooms for more than a century (Jančula and Maršálek, 2011). Copper is a metallic element of which its cationic forms (Cu^{2+}, $CuOH^+$, $Cu_2(OH)_2^{2+}$) are toxic to aquatic organisms (Closson and Paul, 2014). Cationic copper may form rapidly when copper sulphate dissociates in water (Mastin and Rodgers, 2000), however, how much is formed depends strongly on water composition (Jančula and Maršálek, 2011; Murray-Gulde et al., 2002). In general, ionic copper is lost rapidly from water as the result of precipitation and/or complexation, decreasing the toxicity of copper over time (Elder and Horne, 1978).

The toxicity of copper sulphate in an aqueous solution has been thoroughly studied (Elder and Horne, 1978; Kirici et al., 2017). Copper exerts toxicity to phytoplankton due to its negative effect on photosystem II (Barón et al., 1995). However, copper compounds may also affect non-target organisms such as zooplankton. Cadocerans, such as the water flea *Daphna magna* (*D. magna*), are among the most sensitive organisms to copper (Brix et al., 2001). Copper concentrations as low as 8.4 µg L^{-1} for acute toxicity to *D. magna* and 20.2 µg L^{-1} for chronic toxicity have been found (Brix et al., 2001). Toxicity is related to the presence of cationic forms, while complexed, chelated or precipitated Cu forms express lower toxicity to *Daphnia* (Andrew et al., 1977).

To reduce the toxicity towards non-target organisms and to increase effectiveness against target organisms, novel copper-based algaecides have been developed, which are associated with a chelator that can pass through algal cells membranes and cause cell lysis rapidly (Closson and Paul, 2014). For instance, the chelated copper formulation Cutrine®-Ultra was twice as toxic to the cyanobacterium *Planktothrix agardhii* and the green alga *Pseudokirchneriella subcapitata* as was copper sulphate, implying that less copper needs to be applied (Calomeni et al., 2014). The copper-ethanolamine complex formulations Clearigate and Cutrine-Plus were four times less toxic to the water flea *Ceriodaphnia dubia* than was copper sulphate (Murray-Gulde et al., 2002). Hence, toxicities of copper algaecides to non-target *Daphnia* between ionic copper and chelated copper may differ significantly because of their properties, chelator toxicity and potential formation of free hydroxyl radicals (Bishop et al., 2014; Bishop et al., 2018; Mastin and Rodgers, 2000).

Three other novel chelated copper-based products, Captain XTR, SeClear and Lake Guard Blue have been reviewed and accepted by the U.S. Environmental Protection Agency (US EPA) in

2011, 2016 and 2018, respectively. Captain XTR (SePRO Corporation, Carmel, IN, USA) is a copper ethanolamine complex with a proprietary surfactant added designed to penetrate the protective sheath of problem algal species, and has 9.1% metallic copper equivalent (https://sepro.com/aquatics/captain-xtr). SeClear (SePRO Corporation, Carmel, IN, USA) consists of 4.2% copper combined with a water quality enhancer (https://sepro.com/aquatics/seclear) and it can, according to the manufacturer, both kill algae and remove phosphorus. According to the manufacturer, Captain XTR is used with infusion technology to deliver more copper to the algal cells and kill them in the early seasons. Afterwards, SeClear is coming into play for binding phosphate in the water once the algae have been diminished, but Captain XTR and SeClear could also be used separately. Lake Guard Blue (BlueGreen Water Technologies, Ltd) contains 23.9% metallic copper equivalent that according to the manufacturer "activates a biological chain reaction in the water column, a surgical application of BlueGreen's Lake Guard® rehabilitates entire lakes within a few short days" (https://ntcdigitaldev.co.za/). Commonly, these copper-based algaecides are applied below 1 mg Cu L^{-1} based on US EPA (EPA Reg. No. 7364-09-8959).

Despite these copper-based compounds having advantages as an algaecide (Buley et al., 2021; Iwinski et al., 2016), it is essential to assess their potential toxicity on non-target organisms before using them in lake applications. Therefore, in this study, *Daphnia magna*, which is extremely sensitive to copper (OECD, 2004, 2012), was exposed to these three novel copper-based algaecides (Captain XTR, SeClear and Lake Guard Blue) as well as to copper sulphate ($CuSO_4 \cdot 5H_2O$). Laboratory experiments were conducted to: 1) evaluate and compare the toxicity (i.e., EC_{10}, EC_{20}, EC_{50} and NOEC) of the four copper-based algaecides by running acute tests (48 h) and chronic tests (21 d); 2) establish a dose-response relationship between algaecides and the growth of *Daphnia magna* based on body length and dry weight. We hypothesized that novel copper-based algaecides would cause lower mortality to *Daphnia magna* than the traditional copper-based algaecide (copper sulphate). In addition, we expected less effect on *D. magna* body size and reproduction from novel copper-based compounds than from copper sulphate.

Methods

Test organisms

The experiments were carried out with *Daphnia magna* Straus that has been maintained for more than 20 years in the laboratory of the Aquatic Ecology and Water Quality Management

group of Wageningen University (The Netherlands). *Daphnia magna* was kept in 1 L jars containing 800 mL RT medium (Table 3.S1) with pH 8, a conductivity of 270 µS cm^{-1} and hardness of 88 mg L^{-1} (as $CaCO_3$) (Tollrian, 1993). The jars were placed at 20 ± 2ºC, in a 12:12-h light/dark cycle. The animals were fed daily with the chlorophyte *Scenedesmus obliquus* (~ 4 mg C L^{-1}), which was grown in WC medium (Table 3.S2) (Lürling and Beekman, 1999). Neonates born within 24 h were transferred from the stock cultures into new jars. Once the second-generation newborns (6-24 h old) were born, the experiments were conducted instantly.

Acute toxicity tests

Nine Cu solutions from each of the four algaecides were prepared from stock solutions previously made for each compound (Table 3.1). Subsamples from the stock solutions were filtered (Aqua 30/0.45 CA, Whatman, Germany) and analysed by ICP-OES (Thermo iCAP 6500 DV; Thermo Fisher Scientific) to check the Cu concentration from each stock solution with three replicates. Thus, the stock solutions concentration were 102.33 (±4.84), 128.99 (±16.5), 51.40 (±1.03) and 167 (±5.01) mg Cu L^{-1}, for $CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue contained respectively. The acute toxicity tests were run in triplicates, with five newborns (6-24 h old) in 100 mL jars containing 50 mL RT medium. The animals were incubated for 48 h without food, in a 12:12-h light/dark regime at 20 ± 2°C. The acute toxicity tests were performed three times for each chemical. The 48 h-EC_{10} (effect concentration that causes acute effects to 10% of the test population), 48 h-EC_{20} (effect concentration that causes acute effects to 20% of the test population), 48 h-EC_{50} (effect concentration that causes acute effects to 50% of the test population), NOEC (No Observed Effect Concentration) and LOEC (Lowest Effect Concentration) were obtained based on the immobility of the daphnids (OECD, 2004).

Table 3.1 The total copper concentrations used in the acute tests (48 h) and in the chronic tests (21 d) for all algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue) used. The values in the brackets represent the standard deviation (SD)

	Algaecides	Total Copper concentrations (mg Cu L^{-1})								
Acute tests (48 h)	$CuSO_4 \cdot 5H_2O$	0 (0)	0.081 (0)	0.202 (0.01)	0.323 (0.01)	0.404 (0.01)	0.485 (0.01)	0.565 (0.01)	0.646 (0.02)	0.808 (0.02)
	Captain XTR	0 (0)	0.116 (0.01)	0.290 (0.04)	0.463 (0.06)	0.579 (0.07)	0.695 (0.09)	0.811 (0.1)	0.927 (0.12)	1.159 (0.15)
	SeClear	0 (0)	0.103 (0)	0.257 (0.01)	0.411 (0.01)	0.514 (0.01)	0.617 (0.01)	0.720 (0.01)	0.823 (0.02)	1.029 (0.02)
	Lake Guard Blue	0 (0)	0.062 (0)	0.155 (0)	0.248 (0)	0.310 (0.01)	0.372 (0.01)	0.434 (0.01)	0.496 (0.01)	0.620 (0.02)
Chronic tests (21 d)	$CuSO_4 \cdot 5H_2O$	0 (0)	0.091 (0)	0.181 (0.01)	0.271 (0.01)	0.362 (0.02)	0.452 (0.02)			
	Captain XTR	0 (0)	0.102 (0.01)	0.204 (0.03)	0.306 (0.04)	0.408 (0.05)	0.510 (0.07)			
	SeClear	0 (0)	0.084 (0)	0.169 (0)	0.253 (0.01)	0.337 (0.01)	0.422 (0.01)			
	Lake Guard Blue	0 (0)	0.089 (0)	0.179 (0.01)	0.268 (0.01)	0.357 (0.01)	0.446 (0.01)			

Chronic toxicity tests

The EC_{10} calculated from the acute test was used as the threshold for six concentration selection in the chronic test (Table 3.1). This test was run in 100 mL glass tubes with 50 mL RT medium containing one newborn (6-24 h old) in each tube. The experiment was conducted using 10 replicates. Animals were fed daily with *Scenedesmus obliquus* (0.2 mg C per *Daphnia magna* per day) (OECD, 2012) and incubated for 21 days at the same conditions previously described. Newborns were removed from the tubes daily and the animals were transferred to new medium containing Cu once a week.

The number of survivors, their live offspring, age at first reproduction (AFR), age at second reproduction (ASR) and age at third reproduction (ATR) were recorded daily. Fecundity was used as an endpoint to evaluate 21d-EC_{10}, 21d-EC_{20}, 21d-EC_{50} and NOEC. The intrinsic of population increase (PGR) (r) was calculated using equation (3-1) (Euler, 1970; Silva et al., 2020):

$$1 = \sum_{x=0}^{n} e^{-rx} l_x m_x \qquad (3\text{-}1)$$

Where "r" is the rate of population increase (day^{-1}), "x" is the age class in days (0 … n) (days), "l_x" is the probability of surviving to age "x", and " m_x" is fecundity at age "x".

At the start of the experiment, the initial size of survivors (ISS) of 10 newborns were measured using a stereo-binocular microscope (Olympus SZX10, Japan) and the initial dry weight (IDW) of 20 newborns were weighed on an electronic balance (Mettler UMT 2; ± 0.1 µg). Body length

was defined as the distance from the most posterior point on the eye to the base of the junction of the tail spine with the carapace (Lürling and Tolman, 2010). After 21 days, the final size of survivors (FSS) and final dry weight (FDW) of all survivors were also measured. To avoid handling stress and unforeseen damage, experimental animals were measured only at start and at the end of the experiments. The somatic growth rates (SGR) were determined as the increase in body length and dry weight separately during the 21-day period at the beginning of the experiment and on the last incubation day-day 21.

Chemical equilibrium modelling

To evaluate which copper species were prevailing in the RT medium, chemical equilibrium modelling was performed using CHEAQS Next-version 0.1.0.19 (Verweij, 2017). As input for the model we used the pH measured (pH 8) in the RT medium, the amount of copper, sulphate and the composition of RT medium (Table 3.S3).

Statistical analysis

The EC_{10}, EC_{20} and EC_{50} values of four copper-based algaecides in the acute 48 h immobility tests were analyzed by Probit analysis in the software SPSS version 25, and those values in the chronic 21 d reproduction tests were analyzed by nonlinear regression using the three-parameter logistic curve using Statistica version 7.0 (StatSoft 2004). For chronic tests, we analyzed significant differences among 6 concentrations for age at first brood, second brood and third brood, intrinsic rate of population increase and somatic growth by running one-way ANOVA or Kruskal-Wallis One-Way Analysis of Variance on Ranks when the normality test (Shapiro-Wilk) or Equal Variance test failed followed by Tukey or Dunn's post hoc test. *Daphnia magna* images were obtained by a stereo-binocular microscope (Olympus SZX10, Japan) and were processed by ImageJ software.

Results

Acute toxicity of copper-based algaecides to Daphnia magna

During the acute toxicity tests with the four algaecides, 100% survival was recorded in the control series (0 mg Cu L^{-1}). The 48 h-NOEC were quite similar between all compounds tested and varied from 0.40 mg Cu L^{-1} for Lake Guard Blue to 0.46 mg Cu L^{-1} for Captain XTR (Table 3.2). EC_{10} varied between 0.42 and 0.51 mg Cu L^{-1}, EC_{20} between 0.47 and 0.54 mg Cu L^{-1} and EC_{50} between 0.50 and 0.60 mg Cu L^{-1} for *Daphnia magna* (Table 3.2). The EC_{50} values were significantly different ($F_{3,104} = 28.51$; $P < 0.001$) and a Tukey's post hoc test indicated two

homogenous groups: 1) Lake Guard Blue and $CuSO_4 \cdot 5H_2O$ were more toxic (had lower EC_{50}) than 2) SeClear and Captain XTR (Table 3.2). The dose-response curves of the acute tests can be found in Fig. 3.S1.

Table 3.2 NOEC, LOEC and EC_x values calculated based on immobility to *Daphnia magna* from acute tests and fecundity from chronic tests among four copper-based algaecides. The values in the brackets represent the standard deviation (SD) (n = 5). 95% CI displays 95% confidence interval

Tests	Treatments	NOEC	LOEC	EC_{10} (mg Cu L^{-1})	95% CI	EC_{20} (mg Cu L^{-1})	95% CI	EC_{50} (mg Cu L^{-1})	95% CI
Acute tests	$CuSO_4 \cdot 5H_2O$	0.404	0.485	0.452 (0.07)	0.396-0.486	0.479 (0.08)	0.432-0.505	0.521 (0.09)	0.493-0.548
	Captain XTR	0.463	0.579	0.512 (0.01)	0.424-0.553	0.542 (0.01)	0.485-0.577	0.599 (0.01)	0.561-0.636
	SeClear	0.411	0.514	0.422 (0.03)	0.224-0.493	0.471 (0.03)	0.331-0.546	0.594 (0.03)	0.512-0.675
	Lake Guard Blue	0.434	0.496	0.446 (0.06)	0.339-0.472	0.467 (0.04)	0.423-0.487	0.501 (0.02)	0.480-0.523
Chronic tests	$CuSO_4 \cdot 5H_2O$	< 0.091	0.09	0.129	0.09-0.168	0.202	0.161-0.244	0.436	0.396-0.476
	Captain XTR	< 0.102	0.102	0.187	0.130-0.244	0.268	0.213-0.324	0.498	0.451-0.545
	SeClear	< 0.084	0.084	0.076	0.043-0.109	0.122	0.084-0.160	0.274	0.233-0.314
	Lake Guard Blue	< 0.089	0.089	0.045	0.019-0.07	0.090	0.053-0.127	0.295	0.241-0.350

Chronic toxicity of copper-based algaecides to Daphnia magna

Survival of *D. magna* was 100% in the control, and at each tested concentration of $CuSO_4 \cdot 5H_2O$, Captain XTR and Lake Guard Blue, whereas the highest concentration of SeClear caused 40% mortality (Table 3.3). The age of first reproduction (AFR) was significantly increased with higher concentrations dosed for all four algaecides, a similar, yet less prominent pattern was observed for ASR, while ATR was similar among control and treatments in each algaecide tested (Table 3.3). Compared with controls, AFR was delayed with 1.8, 2.0, 2.3 and 3.2 days in the highest dose of Captain XTR, $CuSO_4 \cdot 5H_2O$, Lake Guard Blue, and SeClear, respectively (Table 3.3).

As the copper concentration increased, the number of neonates in the first, second and third broods reduced significantly (Fig. 3.1; Table 3.S4). Consequently, the total number of offspring was reduced significantly (Fig. 3.1; Table 3.S4). By comparing controls and highest copper doses, the total number of neonates decreased 50% when exposed to $CuSO_4 \cdot 5H_2O$, 54% when exposed to Captain XTR, 69% when exposed to Lake Guard Blue and 73% when exposed to

SeClear . The EC_{50} values for total reproduction were 0.436, 0.498, 0.274 and 0.295 mg Cu L^{-1} for $CuSO_4 \cdot 5H_2O$, SeClear, Captain XTR and Lake Guard Blue, respectively (Table 3.2). The calculated EC_{10} and EC_{20} were 0.129 and 0.202 mg Cu L^{-1} for $CuSO_4 \cdot 5H_2O$, 0.187 and 0.268 mg Cu L^{-1} for Captain XTR, 0.076 and 0.122 mg Cu L^{-1} for SeClear, 0.045 and 0.09 mg Cu L^{-1} for Lake Guard Blue (Table 3.2). NOEC were below the lowest dose for the four algaecides used (Table 3.2).

Intrinsic rates of population increase (PGR) were positive in all $CuSO_4 \cdot 5H_2O$ treatments, and declined from 0.259 d^{-1} in controls to 0.195 d^{-1} in the highest dose. Although a one-way ANOVA indicated significant differences (Table 3.S4), this could not be confirmed by a Tukey's post hoc test (Table 3.3). In the series with Captain XTR, PGR decreased significantly from 0.268 d^{-1} in controls to 0.189 d^{-1} in the highest dose (Table 3.3; Table 3.S4). Also in the series with SeClear, PGR decreased significantly from 0.285 d^{-1} in controls to 0.145 d^{-1} in the highest dose (Table 3.3; Table 3.S4). In the series with Lake Guard Blue, PGR declined from 0.263 d^{-1} in controls to 0.167 d^{-1} in the highest dose, however this difference was not statistically significant (Table 3.3; Table 3.S4). Compared to the corresponding control, the PGR in the highest dose of copper sulphate was 29% less, in Captain XTR it was 49% lower, in SeClear it was reduced by 48% and in Lake Guard Blue the population growth was 37% lower.

Table 3.3 Survival (%), intrinsic rates of population increase (PGR, d^{-1}), age at first reproduction (AFR, d^{-1}), age at second reproduction (ASR, d^{-1}), age at third reproduction (ATR, d^{-1}), the initial size of survivors (ISS, mm), the final size of survivors (FSS, mm), initial dry weight (IDW, µg), the final dry weight (FDW, µg), somatic growth rate (SGR) for body length (mm d^{-1}) and SGR for dry weight (µg d^{-1}) of *Daphnia magna* exposed to different concentrations of different copper-based algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue) for 21 days. Different symbols (a, .., d) indicate significant differences at the 95% level (Tukey and Dunn's post hoc comparison tests). The standard deviation (SD) are given in brackets (n = 10), -- in the brackets indicates without SD

	Concentration (mg Cu L^{-1})	Survival (%)	PGR (d^{-1})	AFR (d)	ASR (d)	ATR (d)	ISS (mm)	FSS (mm)	SGR (mm d^{-1}) for body length	IDW (µg)	FDW (µg)	SGR (µg d^{-1}) for dry weight
$CuSO_4 \cdot 5H_2O$												
	0	100	0.259	9.2	13	19.6		2.95	0.092 (0.003)		271.5	12.4
		(--)	(0.058)^{a}	(0.63)^{d}	(0.82)^{c}	(0.7)^{ab}		(0.07)			(--)	(--)
	0.091	100	0.256	9.1	12.7	18.9		2.83	0.086 (0.003)		251.4	11.4
		(--)	(0.065)^{a}	(0.32)^{d}	(0.48)^{c}	(1)^{ab}		(0.07)			(--)	(--)
	0.181	100	0.236	9.5	13.2	18.7		2.62	0.077 (0.005)	11.3	249.7	11.4
		(--)	(0.069)^{a}	(0.85)^{cd}	(0.42)^{bc}	(0.82)^{b}	1.01	(0.1)		(--)	(--)	(--)
	0.271	100	0.209	10.2	13.4	19.4	(0.08)	2.58	0.075 (0.003)		234.7	10.6
		(--)	(0.048)^{a}	(0.79)^{bc}	(0.52)^{bc}	(0.7)^{ab}		(0.06)			(--)	(--)
	0.362	100	0.195	10.9	13.9	19.2		2.5	0.070 (0.004)		218.9	9.8
		(--)	(0.072)^{a}	(0.33)^{ab}	(0.33)^{ab}	(0.67)^{ab}		(0.07)			(--)	(--)
	0.452	100	0.183	11.2	14.6	19.7		2.41	0.066 (0.005)		203.7	9.2
		(--)	(0.065)^{a}	(0.63)^{a}	(1.07)^{a}	(0.48)^{a}		(0.1)			(--)	(--)
Captain XTR												
	0	100	0.268	9	12.8	19.5		2.93	0.090 (0.003)		264.2	12.1
		(--)	(0.053)^{a}	(0)^{c}	(0.42)	(0.71)		(0.07)			(--)	(--)
	0.102	100	0.25	9.6	13.4	19		2.73	0.081 (0.004)		240.2	10.9
		(--)	(0.066)^{ab}	(1.27)^{bc}	(1.65)	(1.15)		(0.07)			(--)	(--)
	0.204	100	0.24	9.5	12.9	19		2.66	0.077 (0.003)		236	10.7
		(--)	(0.075)^{ab}	(0.71)^{bc}	(0.32)	(1.052)	1.04	(0.06)		11.1	(--)	(--)
	0.306	100	0.217	9.7	13	19.2	(0.07)	2.58	0.073 (0.005)	(--)	222.3	10.0
		(--)	(0.063)^{ab}	(0.68)^{bc}	(0.47)	(0.92)		(0.10)			(--)	(--)
	0.408	100	0.205	10.6	13.5	19.7		2.47	0.068 (0.005)		164	7.2
		(--)	(0.040)^{ab}	(0.52)^{ab}	(0.53)	(0.48)		(0.09)			(--)	(--)
	0.510	100	0.189	10.8	13.6	18.9		2.32	0.061 (0.003)		173.6	7.7
		(--)	(0.033)^{b}	(0.92)^{a}	(0.52)	(1)		(0.07)			(--)	(--)
SeClear												
	0	100	0.285	9.1	13.1	18	0.98	2.88	0.091 (0.007)	7.3	292.3	13.6

		(--)	(0.095)[a]	(0.32)[c]	(1.2)[c]	(0)b	(0.02)	(0.14)		(--)	(--)	(--)
	0.084	100 (--)	0.281 (0.078)[a]	9 (0)[c]	12.9 (1)[c]	18 (0)[b]		2.85 (0.13)	0.089 (0.003)		309.1 (--)	14.4 (--)
	0.169	100 (--)	0.242 (0.055)[ab]	9.5 (0.53)[c]	13.8 (1.3)[bc]	18.1 (0.3)[ab]		2.66 (0.13)	0.080 (0.006)		294.3 (--)	13.7 (--)
	0.253	100 (--)	0.209 (0.039)[ab]	10.8 (1)[b]	14.8 (0.46)[b]	18.5 (0.76)[ab]		2.50 (0.09)	0.072 (0.004)		276.3 (--)	12.8 (--)
	0.337	100 (--)	0.202 (0.062)[ab]	11.6 (0.88)[ab]	15.3 (0.71)[ab]	18.6 (0.53)[ab]		2.40 (0.06)	0.068 (0.003)		251.9 (--)	11.6 (--)
	0.422	60 (--)	0.145 (0.039)[b]	12.3 (1.89)[a]	16.9 (1.86)[a]	19 (1.41)[a]		2.26 (0.05)	0.061 (0.002)		221.8 (--)	10.2 (--)
Lake Guard Blue												
	0	100 (--)	0.263 (0.036)	10 (0)[c]	12.7 (0.52)[b]	19 (0)[b]		2.86 (0.09)	0.09 (0.004)		242.5 (--)	11.1 (--)
	0.089	100 (--)	0.225 (0.078)	10.3 (0.52)[c]	13.3 (0.82)[b]	19 (0)[ab]		2.61 (0.06)	0.078 (0.003)		245.7 (--)	11.3 (--)
	0.179	100 (--)	0.217 (0.048)	10.6 (0.79)[bc]	13.9 (1.07)[b]	18.6 (0.79)[ab]	0.98	2.60 (0.07)	0.077 (0.004)	8.6	246.3 (--)	11.3 (--)
	0.268	100 (--)	0.212 (0.062)	11 (1)[bc]	14.8 (1.98)[b]	19 (0.53)[ab]	(0.06)	2.54 (0.09)	0.074 (0.004)	(--)	245.9 (--)	11.3 (--)
	0.357	100 (--)	0.192 (0.062)	11.6 (0.89)[ab]	15 (1.4)[b]	19.2 (0.45)[ab]		2.46 (0.13)	0.071 (0.006)		257 (--)	11.8 (--)
	0.446	100 (--)	0.167 (0.047)	12.3 (0.46)[a]	18.3 (1.03)[a]	19.7 (0.58)[a]		2.30 (0.07)	0.063 (0.003)		154 (--)	7.0 (--)

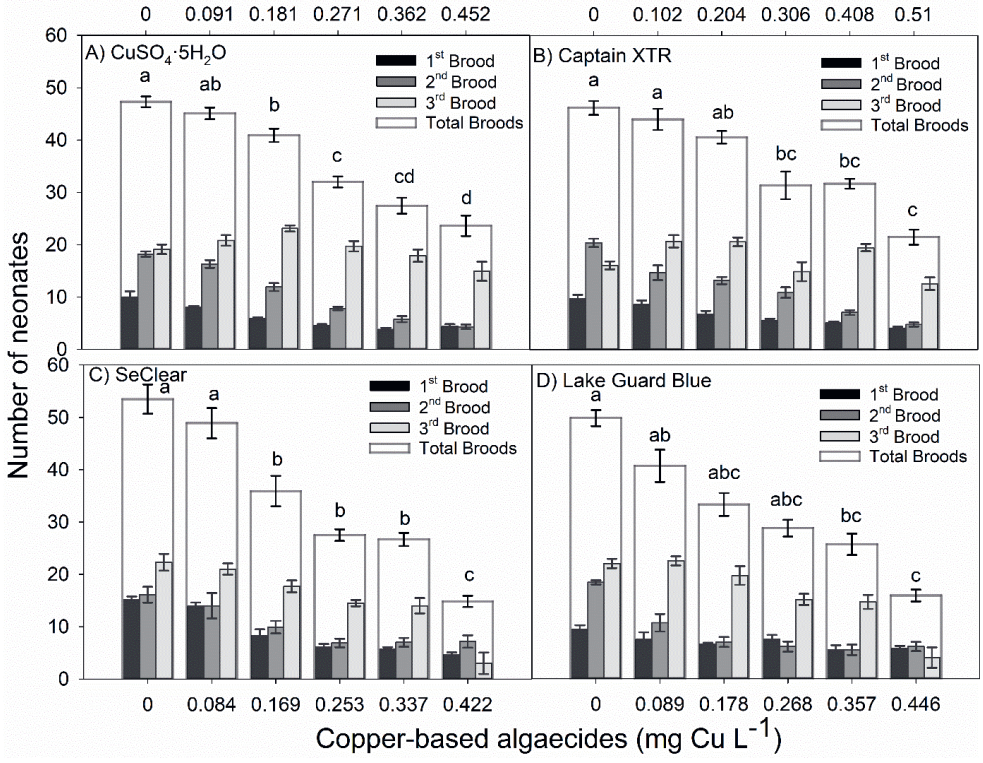

Fig. 3.1 Number of neonates per female *Daphnia magna* exposed to six copper concentrations of four algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue) for the first three consecutive broods. Different symbols (a, .., d) above total broods bars indicate significant differences at the 95% level (Tukey and Dunn's post hoc comparison tests). Error bars represent the standard deviation (n = 10)

Effects of the algaecides on the somatic growth

The *Daphnia magna* at the start of the experiment had a body length of 1.01 (± 0.08) mm and a body weight of 11.3 µg ($CuSO_4 \cdot 5H_2O$), 1.04 (± 0.07) mm and 11.1 µg (Captain XTR), 0.98 (± 0.02) mm and 7.3 µg (SeClear), and 0.98 (± 0.06) mm and 8.6 µg (Lake Guard Blue) (n =10 for body length, n = 20 for body weight). After 21 days, animals reached 2.95 (± 0.07) mm, 2.93 (± 0.07) mm, 2.88 (± 0.14) mm and 2.86 (± 0.09) mm in controls of the $CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue series, respectively (Fig. 3.2; Table 3.3). The animals' body length was reduced with increased copper concentrations in all algaecides treatments (Fig. 3.2; Table 3.3). Somatic growth rates, based on body length, were significantly lower at concentrations ≥ 0.181 mg Cu L^{-1} ($CuSO_4 \cdot 5H_2O$), ≥ 0.102 mg Cu L^{-1} (Captain XTR) ≥ 0.169 mg Cu L^{-1} (SeClear) and ≥ 0.089 mg Cu L^{-1} (Lake Guard Blue) (Fig. 3.2; Table 3.S4).

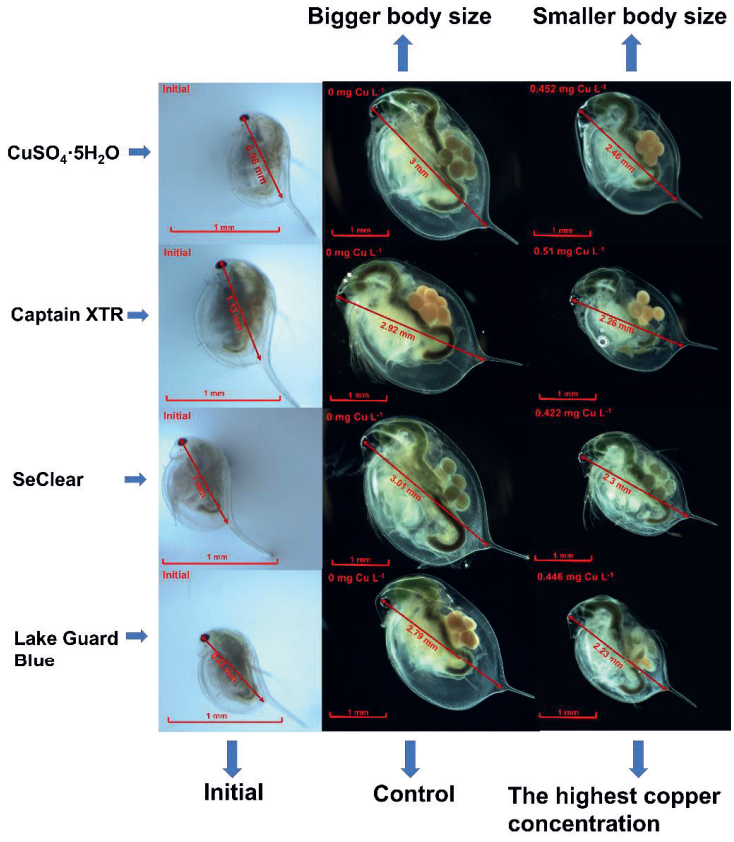

Fig. 3.2 Comparative body length among initial, control and the highest copper concentration for four copper algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue). Red lines on *Daphnia magna* represent the body length (the distance from the most posterior point on the eye to the base of the junction of the tail spine with the carapace)

The adult animals reached a body weight of 271.5, 264.2, 292.3 and 242.5 µg in the controls of the $CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue series, respectively (Table 3.3). In the copper sulphate series, the Captain XTR and the SeClear series, females were lighter in treatments with higher copper concentrations (Table 3.3). At the highest copper sulphate concentration, the animals became 25% lighter than the control (Table 3.3). In the Captain XTR treatments, the lightest females were found in the 0.408 mg Cu L^{-1} treatment (164 µg), which implies a body weight loss of 38% compared to controls. In the SeClear treatments, body weight had dropped by 24% from 292.3 µg in controls to 221.8 µg in the highest dose (Table 3.3). In Lake Guard Blue treatments, dry weights were similar (242.5 to 257 µg) in the treatments from 0 to 0.357 mg Cu L^{-1}, yet, dry weight was, compared to the control, reduced by 36.5% at the highest Cu concentration used (Table 3.3). SGR based on the dry weight were significantly

different between the different Cu concentration used for all the compounds except for Lake Guard Blue series (H_5 = 10.8; P = 0.055) (Fig. 3.3; Table 3.S4).

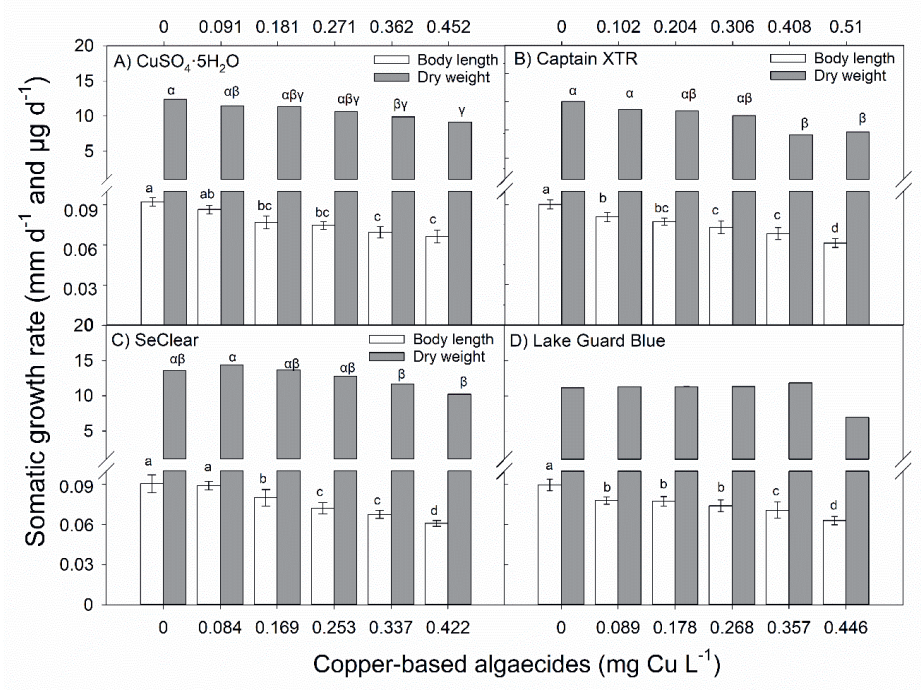

Fig. 3.3 Somatic growth rates of *Daphnia magna*, based on body length (white bars) and dry weight (grey bars), were exposed for 21 days to different concentrations of algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue). Error bars indicate standard deviation (n > 5). Different symbols indicate significant differences (a, ..., d for body length; α, β, γ for dry weight)

Chemical equilibrium modeling

The chemical equilibrium modeling indicated that most copper was chelated with EDTA present in the RT medium. When only copper which is the part of the composition of RT medium exists in the system, the concentrations of $Cu(EDTA)^{2-}$ was 0.035 mg L^{-1} and of $Cu(EDTA)(OH)^{3-}$ was 4.378E-06 mg L^{-1}. With the increased amount of copper added, the concentrations of $Cu(EDTA)^{2-}$ also increased (Fig. 3.S2). The regression between the copper concentration added-(Cu_{added}) and the concentrations of $Cu(EDTA)^{2-}$ yielded: $Cu(EDTA)^{2-} = 0.9995 \times Cu_{added} + 0.0354$ ($r^2 = 1.0$).

Discussion

Acute toxicity based on immobility of Daphnia magana

Daphnia magna belongs to the most sensitive genus of aquatic invertebrates towards copper and is a standard test species used in toxicity tests (Bishop et al., 2018; Mastin and Rodgers, 2000). The acute EC_{50} values for copper-based algaecides (0.501-0.599 mg Cu L^{-1}) were in agreement with values obtained by De Schamphelaere et al., (2002), who found 48 h-EC_{50} ranging from 0.0352 to 0.792 mg Cu L^{-1} in 19 natural European surface waters contaminated with copper. Yet, these acute toxicity values are higher than reported by others in the literature where for instance 48 h-LC_{50} of $CuSO_4$ for *D. magna* between 0.011 and 0.35 mg Cu L^{-1} has been found (Bishop et al., 2018; Mastin and Rodgers, 2000; Suedel et al., 1996), or even as low as 0.0065 mg Cu L^{-1} for unfed *D. magna* and 0.0185 mg Cu L^{-1} for fed *D. magna* (Dave, 1984). Differences between studies may be caused by using different clones that may vary in their sensitivity to copper (Chain et al., 2019), but also by water composition, such as hardness and presence of complexing compounds, that determines the speciation of copper (Andrew et al., 1977). Cationic copper forms (Cu^{2+}, $CuOH^{+}$, $Cu(OH)_2^{2+}$) were identified as toxic species, while complexed, chelated or precipitated Cu forms express less toxicity to *Daphnia* (Andrew et al., 1977). Thus, prior to each Cu application, tests on the effects of Cu compounds on the organisms being targeted should be done while considering the water chemistry.

In our study, chemical equilibrium modelling indicated that most copper was chelated with EDTA present in the RT medium. In another study, complexation of copper by EDTA resulted in significantly reduced toxicity to *Daphnia magna* (Sorvari and Sillanpää, 1996). Complexing compounds are omnipresent in aquatic ecosystems, and humic substances DOC may easily span concentrations from 1 to 22 mg L^{-1} in surface waters (Kramer et al., 2004). Strong relation has been found between reduced copper toxicity and DOC concentrations (Kramer et al., 2004), in which humic substances reduced both the acute and chronic toxicity of copper to *Daphnia* (Winner, 1985).

Chelators are used in novel copper algaecides to enhance uptake in algae implying that less copper needs to be applied (Calomeni et al., 2014) therewith reducing risks to non-target organisms. Some chelated copper formulations (e.g., Clearigate and Cutrine-Plus) were less toxic to cladocerans than was copper sulphate (Murray-Gulde et al., 2002), another chelated copper-based algaecide (e.g., Algimycin® PWF) had similar toxicity as copper sulphate (Johnson et al., 2008), or appeared even more toxic (Cutrine-Plus) (Mastin and Rodgers, 2000). In addition, other compounds present in formulations may contribute to observed toxicity,

which was evidenced in a study comparing Captain XTR, a chelated copper formulation (9.1% Cu) containing a surfactant, with a formulation without the surfactant (Captain) (Closson and Paul, 2014). The acute toxicity to brook trout and fathead minnows of Captain XTR was higher than that of copper sulphate, whereas the chelated formulation without surfactant was less toxic than copper sulphate (Closson and Paul, 2014).

Our study yielded that $CuSO_4 \cdot 5H_2O$ and Lake Guard Blue were more acutely toxic to *D. magna* based on immobility than Captain XTR and SeClear. Although 48 h-EC_{50} were close to each other (*see* Table 3.2), these results are not in line with our hypothesis.

Chronic toxicity of copper-based algaecides to Daphnia magna

The highest copper concentrations dosed in the chronic tests were based on the EC_{10} values obtained from the acute toxicity tests. In all treatments in the chronic tests, 100% survival of test animals was observed with the exception of the highest dose of SeClear that had 60% survival. All surviving *Daphnia* were observed actively swimming in the test tubes and hence this deviated from the dosed acute-EC_{10} in the highest concentrations. This is likely caused by the presence of algae lowering the copper concentration and therewith toxicity (Bishop et al., 2018). In addition, the green algal food used was grown in a medium containing vitamins that also may have contributed to less toxicity/more resistance in *D. magna* (Winner et al., 1977).

The chronic assay was based on the OECD (2012) standard 21-day reproduction test, but included several additional life-history parameters that have been proposed as more realistic in ecotoxicity testing (Van Leeuwen et al., 1985). AFR was significantly delayed with increased copper concentrations in all four algaecides, which is comparable to the reported delayed age at maturation in cladocerans exposed to copper (Sadeq and Beckerman, 2019). Reproduction was reduced with increasing concentrations of copper which is in line with the results of a meta-analysis on the effects of copper in cladocerans and *D. magna* (Sadeq and Beckerman, 2019). Delayed AFR and reduced number of offspring may have enabled females to allocate energetic resources to growth, maintenance/detoxification rather than reproduction (DeMille et al., 2016). However, copper exposure may also lead to reduced feeding activity in *Daphnia* (Agra et al., 2011) and therewith to fewer resources available for growth and reproduction. Copper exposed animals were smaller than non-exposed animals, which is in agreement with the results of a meta-analysis showing that with increasing copper concentrations somatic growth in *D. magna* decreased (Sadeq and Beckerman, 2019). In general, smaller *Daphnia* are most likely to produce smaller broods (Martins et al., 2017). Adult *Daphnia* mold after each brood has been released and subsequently grows in size (Anderson, 1932) until about the eleventh instar

(Anderson et al., 1937), which means that females shedding the third brood are larger than those producing the first brood and in healthy *Daphnia* brood sizes increase accordingly (Lürling and Van Donk, 1997). However, the number of newborns in third broods exposed to the highest doses of SeClear and Lake Guard Blue were lower or equal to those in the first and second broods, which is indicative of chronic toxicity.

The intrinsic rate of population increase (PGR) was least in the highest copper concentrations in each of four copper algaecides (Table 3.3 and Fig. 3.1), which is in agreement with literature findings (Agra et al., 2011). PGR was 30% lower in the highest copper sulphate and Captain XTR treatment compared to their corresponding controls, 37% in Lake Guard Blue and 50% lower in the highest SeClear dose compared to its control. Hence, we rejected our hypothesis that the novel-chelated copper algaecides would be less toxic to *Daphnia* than traditional copper sulphate that would have been expressed in higher population growth rates. PGR is more ecologically relevant than individual endpoints such as survival, or reproduction as it integrates effects at the population level (Forbes and Calow, 1999). Inasmuch as population growth rate and feeding rate may be correlated (Lürling and Van Donk, 1997), effects of copper-induced reduced feeding rates on PGR (Agra et al., 2011) cannot be excluded. Hence, follow up research on *Daphnia* feeding activities may be considered, which is of particular interest as these cladocerans are key organisms in lentic freshwater aquatic ecosystems capable of filtering considerable quantities of phytoplankton.

Implication for management

Copper-based algaecides are developed for the management of eutrophic systems that suffer from nuisance phytoplankton blooms, in particular cyanobacteria blooms (Bishop et al., 2014). Cyanobacteria may cause major problems for the use of the water and impair its ecosystem services. In addition, cyanobacteria may have strong negative effects on *Daphnia* resulting in distortion of the energy flow at the phyto-zooplankton interface causing severe ecosystem consequences (Ger et al., 2014). Hence, mitigating cyanobacterial nuisance and improving water quality is a key issue for water managers, and in cases where algaecides may be applied, these should preferably not exert irreversible negative effects on non-target organisms such as *Daphnia*. Both copper sulphate and three formulations of chelated copper caused a reduction in reproduction and PGR, but mostly at the higher doses of 0.4-0.5 mg Cu L^{-1}. Application of Cu-based compounds are not allowed in certain countries (Codd et al., 2005), however others permit copper application in surface water, for instance USA Environmental Protection Agency allows copper application up to 1 mg L^{-1}, which is twice as the EC_{50} found here (EPA Reg. No.

7364-09-8959). In the chronic exposure test, the water was renewed every week meaning three pulses of copper (at the start, after one week and after two weeks), which is likely more than a standard one-time application *in situ* in which copper will be bound rapidly to algae and transported to sediment reducing effects on nontarget *Daphnia* (Bishop et al., 2018; Willis and Bishop, 2016). Although the risk to non-target species, such as *Daphnia*, can be significant (Bishop et al., 2014), it can be reduced by choosing the copper formulation which has the strongest effect on the target algae using the effective dose (Bishop et al., 2018).

In decision-making, besides efficacy and side-effects, also costs and longevity of the intervention need to be included (Lürling et al., 2016). The SeClear formulation showed the strongest PGR reduction in *Daphnia* (50% lower in the highest SeClear dose compared to its control), but SeClear, according to the manufacturer, also contains a phosphate binder, and thus may reduce the regrowth of nuisance phytoplankton such as cyanobacteria considerably. As in general copper concentrations are reduced rapidly following an application (Willis and Bishop, 2016), up scaled *in situ* experiments with these chelated copper compounds can be considered that will provide more insight into efficacy, side effects and longevity. Given that catchment nutrient control measures are often still absent or insufficient, the use of copper-based algaecides remains an option as fast emergency control of nuisance phytoplankton.

Conclusion

- Acute toxicity based on immobilization was rather similar and 48 h-EC_{50}'s ranged from 0.50 mg Cu L^{-1} in Lake Guard Blue to 0.60 mg Cu L^{-1} in Captain XTR.
- Chronic toxicity based on PGR was reduced at the highest doses tested (0.4-0.5 mg Cu L^{-1}) in all four formulations tested. PGR was maximally reduced by 30% in copper sulphate and Captain XTR, 37% in Lake Guard Blue and 50% in SeClear.
- Most copper was chelated with EDTA present in the RT-medium.
- Novel chelated copper formulations (ethanolamine complexes) may not be necessarily less toxic to nontarget *Daphnia* than traditional copper sulphate.
- Up scaled *in situ* experiments with the chelated copper compounds will provide more insight into efficacy, side effects and longevity.

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Supplementary information

List:

- Table 3.S1 Recipe of RT medium*
- Table 3.S2 Recipe of WC medium
- Table 3.S3 Major ion composition of RT medium
- Table 3.S4 *F*-and *P*-values of one-way ANOVAs and *H*- and *P* values of Kruskal-Wallis One Way Analysis of Variance on Ranks when normality tests failed (Shapiro-Wilk) for total offspring per female, intrinsic rates of population increase (PGR, d^{-1}), age at first reproduction (AFR, d^{-1}), age at second reproduction (ASR, d^{-1}), age at third reproduction (ATR, d^{-1}), somatic growth rate (SGR) for body length (mm d^{-1}) and SGR for dry weight (µg d^{-1}) in four different copper-based algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue) after 21 days incubation
- Fig. 3.S1. The dose-response curves of the acute tests for all the four copper-based algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue)
- Fig. 3.S2. Different copper species concentration in function of copper added predicted from chemical equilibrium modelling in RT medium at pH 8

Table 3.S1 Recipe of RT medium*

Compositions	Concentration (mg L^{-1})
TES	85
$NaNO_3$	50
$MgSO_4 \cdot 7H_2O$	20
$CaCl_2 \cdot 2H_2O$	39
$Na_2SiO_3 \cdot 5H_2O$	10
KCl	10
$CaCO_3$	13
$Ca(OH)_2$	30
Trace elements	
Na_2-EDTA	5
H_3BO_3	5.72
$FeCl_3 \cdot 6H_2O$	1.94
$MnCl_2 \cdot 4H_2O$	0.72
LiCl	0.90
KBr	0.075
$Na_2MoO_4 \cdot 2H_2O$	0.125
$CuCl_2 \cdot H_2O$	0.065
$CoCl_2 \cdot 6H_2O$	0.2
KI	0.006
$Na_2SeO_3 \cdot 5H_2O$	0.002

*Adjust pH to 7.6 - 8.2 with 1 N HCl in the final.

Table 3.S2 Recipe of WC medium

Compositions	Concentration (mg L^{-1})
TES	85
K_2HPO_4	8.7
$NaNO_3$	85
$MgSO_4 \cdot 7H_2O$	37
$CaCl_2 \cdot 2H_2O$	36.8
$NaHCO_3$	12.6
$Na_2SiO_3 \cdot 9H_2O$	28.4
H_3BO_3	24
ATE	
Na_2-EDTA$\cdot 2H_2O$	4.36
$FeCl_3 \cdot 6H_2O$	1
$MnCl_2 \cdot 4H_2O$	0.18
$CuSO_4 \cdot 5H_2O$	0.001
$ZnSO_4 \cdot 7H_2O$	0.022
$CoCl_2 \cdot 6H_2O$	0.01
$NaMoO_4 \cdot 2H_2O$	0.022
H_2SeO_3	0.0016
Na_3VO_4	0.0018
VIM	
Thiamine HCl	0.0001
Biotin	0.0005
B_{12}	0.0005

Table 3.S3 Major ion composition of RT medium

Ions	Major composition (mg L^{-1})
Calcium (Ca^{2+})	32.1
Sodium (Na^{+})	16.4
Magnesium (Mg^{2+})	2.09
Potassium (K^{+})	5.26
Iron (Fe^{3+})	0.40
Manganese(Mn^{2+})	0.2
Lithium (Li^{+})	0.15
Cobalt (Co^{2+})	0.05
Copper (Cu^{2+})	0.04
Chloride (Cl^{-})	46.8
Molybdate (MO_4^{2-})	2649
carbonate(CO_3^{2-})	7.8
EDTA	4.3
Bromide (Br^{-})	0.05
Borate (BO_3^{3-})	5.4
Selenium oxide(SeO_3^{2-})	0.001
Silicate (SiO_3^{2-})	3.6
Iodide (I^{-})	0.005
Nitrate (NO_3^{-})	36.5
Sulphate (SO_4^{2-})	6.96
Total dissolved solids (TDS)	85

Table 3.S4 *F*-and *P*-values of one-way ANOVAs and *H*- and *P* values of Kruskal-Wallis One Way Analysis of Variance on Ranks when normality tests failed (Shapiro-Wilk) for total offspring per female, intrinsic rates of population increase (PGR, d^{-1}), age at first reproduction (AFR, d^{-1}), age at second reproduction (ASR, d^{-1}), age at third reproduction (ATR, d^{-1}), somatic growth rate (SGR) for body length (mm d^{-1}) and SGR for dry weight (µg d^{-1}) in four different copper-based algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue) after 21 days incubation.

Algaecides	First brood	Second brood	Third brood	Total offspring per female	PGR (d^{-1})	AFR (d)	ASR (d)	ATR (d)	SGR (d^{-1}) for body length	SGR (d^{-1}) for dry weight
$CuSO_4 \cdot 5H_2O$	$F_{5.54}$ = 20.43; P < 0.001	$F_{5.54}$ = 99.36; P < 0.001	$F_{5.54}$ = 5.88; P < 0.001	$F_{5,54}$ = 51.965; P < 0.001	$F_{5,54}$ = 2.56; P = 0.038	$F_{5.54}$ = 20.35; P < 0.001	$F_{5.54}$ = 10.85; P < 0.001	$F_{5,54}$ = 2.79; P = 0.026	H_5 = 49.19; $P \leq$ 0.001	H_5 = 26.79; $P \leq$ 0.001
Captain XTR	$F_{5.54}$ = 14.85; P < 0.001	$F_{5.54}$ = 42.44; P < 0.001	$F_{5.54}$ = 8.56; P < 0.001	H_5 = 43.1; $P \leq$ 0.001	$F_{5.54}$ = 2.63; P = 0.034	$F_{5.54}$ = 7.77; P < 0.001	$F_{5,54}$ = 1.84; P = 0.12	$F_{5,54}$ = 1.22; P = 0.314	$F_{5,54}$ = 71.16; P < 0.001	H_5 = 26.52; $P \leq$ 0.001
SeClear	$F_{5.48}$= 34.96; P < 0.001	$F_{5.48}$= 6.98; P < 0.001	$F_{5.48}$= 23.48; P < 0.001	H_5 = 43.1; $P \leq$ 0.001	$F_{5,48}$ = 5.32; P < 0.001	$F_{5.48}$ = 20.85; P < 0.001	$F_{5.48}$ = 14.35; P < 0.001	$F_{5,48}$ = 3.4; P = 0.01	$F_{5,48}$ = 73.81; P < 0.001	H_5 = 17.67; P = 0.003
Lake Guard Blue	$F_{5,34}$ = 4.83; P = 0.002	$F_{5.34}$= 26.85; P < 0.001	$F_{5.34}$= 24.19; P < 0.001	H_5 = 33.674; $P \leq$ 0.001	$F_{5,34}$ = 2.2; P =0.077	$F_{5.34}$ = 10.3; P < 0.001	$F_{5.34}$ = 18.01; P < 0.001	$F_{5,34}$ = 3.74; P = 0.008	$F_{5,34}$ = 26.36; P < 0.001	H_5 = 10.8; P = 0.055

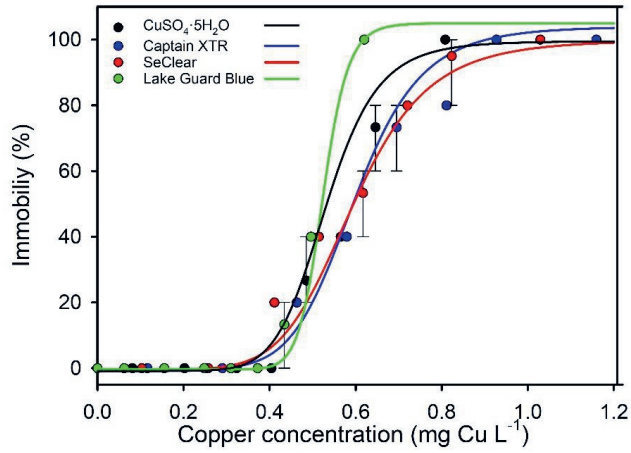

Fig. 3.S1. The dose-response curves of the acute tests for all the four copper-based algaecides ($CuSO_4 \cdot 5H_2O$, Captain XTR, SeClear and Lake Guard Blue)

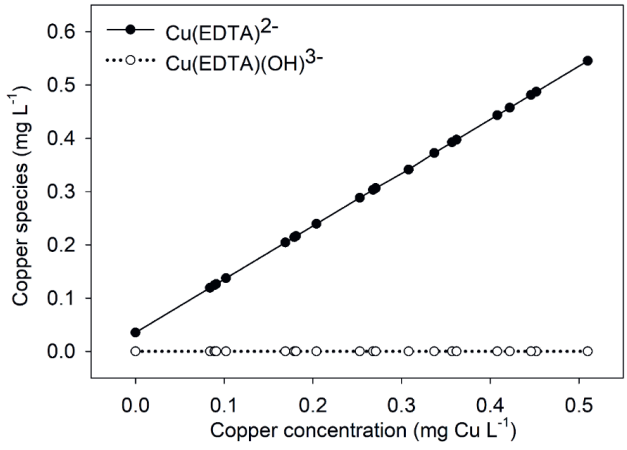

Fig. 3.S2. Different copper species concentration in function of copper added predicted from chemical equilibrium modelling in RT medium at pH 8

4

Influence of temperature and pH on phosphate removal efficiency of different sorbents used in lake restoration

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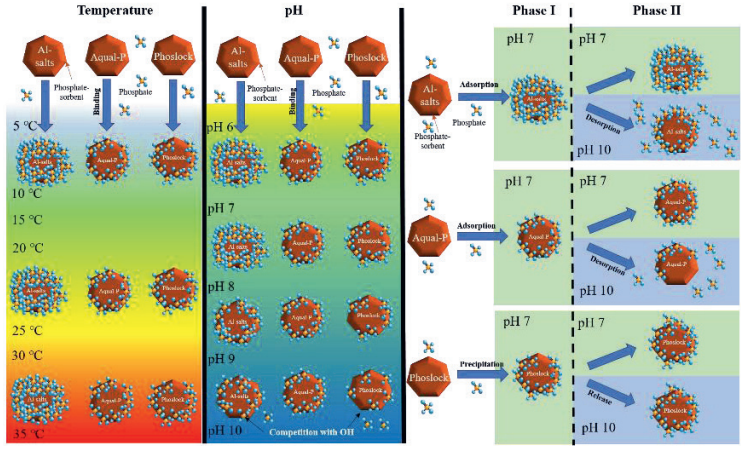


Abstract

Phosphorus sorbents (PS) are viewed as a powerful tool to manage eutrophication. Here, we tested three commercially available PS - lanthanum-modified bentonite (LMB), aluminium-modified zeolite (AMZ) and aluminium salts (Al) on their capacity to chemically inactivate soluble reactive phosphorus (SRP) at six different temperatures (5 to 35°C) and five pH values (6 to 10). We also evaluated if the SRP bound at a neutral pH would be released if pH increases to pH 10. Results showed that temperature affected the SRP binding behavior differently for each PS. For instance, the highest SRP binding capacities of LMB, AMZ and Al were 14.0, 29.9 and 251.1 mg P g^{-1} at 30°C, 35°C and 30°C, respectively; and the lowest was at 35°C for LMB, 25°C for AMZ and 20°C for Al (6.3, 4.0 and 205.2 mg P g^{-1}, respectively). The pH also affected the SRP binding differently. When pH increased from pH 6 to pH 10, LMB and Al decreased their binding capacity from 10.0 to 4.9 and from 571.7 mg P g^{-1} to 21.3 mg P g^{-1}, respectively. The SRP adsorption capacity of AMZ was similar at pH 7 and 10 (6.3 and 6.2 mg P g^{-1}). We observed that in high pH, LMB did not release the SRP precipitated. In contrast, AMZ and Al desorbed around 39%, and 71% of the SRP adsorbed when pH changed from 7 to 10. Abiotic factors such as pH should be considered when selecting the most promising material in lake restoration.

Introduction

Anthropogenic activities have led to significant water quality deterioration in lakes worldwide, in which eutrophication is the most prevalent water quality issue (OECD, 2017; Smith and Schindler, 2009). The most hazardous effect of eutrophication is the extensive biomass of phytoplankton, especially cyanobacterial blooms. These blooms increase water turbidity, shade submerged aquatic plants, smother aquatic animals due to nighttime hypoxia/anoxia, and present a danger to wildlife, pets and humans from the toxins several cyanobacteria species may produce (Paerl and Paul, 2012). Consequently, eutrophication impairs important ecosystem services. In Europe, 57% of the rivers and 44% of the lakes failed to meet the 2015 requirements set by the European Water Framework Directive (WFD) (Poikane et al., 2019). In the Netherlands, between 2011 and 2013, 60% of WFD water bodies were assessed as eutrophic (Fraters et al., 2017), and in a recent survey, still, 50% of the WFD waterbodies failed to meet the nitrogen (N) standard or the phosphorus (P) standard (van Galen et al., 2020). In China, 57.5% of freshwater lakes have become eutrophic or hypertrophic (Jin et al., 2005). Such widespread eutrophication affects important services that freshwater ecosystems provide to society, increasing the financial burden and causing substantial economic losses (Sanseverino et al., 2016). The estimated annual cost of freshwater eutrophication in OECD countries is approximately $1100 billion (OECD, 2017). Thus, it is key to manage eutrophication and mitigate its negative effects.

Generally, reducing external nutrient input is crucial to achieve sustainable restoration of eutrophic lakes and to prevent harmful cyanobacterial blooms (Hamilton et al., 2016; Paerl, 2014; Paerl et al., 2016). In most OECD countries, point source nutrient pollution has been tackled, while diffuse nutrient pollution remains a challenge (OECD, 2017). In addition, nutrient legacies from the past, accumulated and periodically recycled from the sediment, may hamper rapid recovery after external, point-source nutrient control (Fastner et al., 2016; Søndergaard et al., 2013). This internal nutrient load has prompted research into methods that effectively mitigate nutrient release from sediments and includes a plethora of techniques ranging from sediment removal, hypolimnetic water withdrawal, oxygenation and chemical treatments (Lürling et al., 2020a).

In particular, P-sorbents (PS) are promising candidates to reduce P, which is an important building block for cyanobacterial biomass (Douglas et al., 2016b; Lürling et al., 2016; Spears et al., 2013a). Reducing P sufficiently with PS may minimize the effects of eutrophication (Lürling and van Oosterhout, 2013). The use of PS to manipulate biogeochemical processes is

known as geo-engineering, which has been recognized as a powerful tool to manage eutrophication and control cyanobacteria blooms in water bodies where the internal load is high (Lürling et al., 2020a; Spears et al., 2013a). Those PS must be effective, easy to manufacture and use, relatively inexpensive and safe.

The most commonly used PS are aluminium salts, such as aluminium sulphate (alum), due to their wide application range, relatively low price and ubiquitous availability. Aluminium sulphate is a white crystalline solid and it is commonly composed of two atoms of aluminium, 3 sulfur and 12 atoms of oxygen (Dionisio et al., 2018). Once added, Al salts will form flocks ($Al(OH)_3$) adsorbing soluble reactive phosphorus (SRP) and, due to their coagulating properties will also instantaneously improve water transparency (Cooke et al., 2005; Gibbs and Hickey, 2018; Lürling et al., 2020a). Alum is often applied with a buffer such as sodium aluminate to avoid large pH changes. A ballast compound can also be added together with alum to enhance the settlement of Al-flocs, ensuring that flocs and cyanobacteria end up on the sediment. The ballast can be natural soils (Noyma et al., 2016) or a solid phase P sorbent (SPS), but SPS compounds can also be used alone (Lürling et al., 2020a). The most abundantly used SPS is Phoslock®, a lanthanum modified bentonite (LMB) (Copetti et al., 2016; Douglas, 2002) that can effectively precipitate SRP from the water column and intercept and capture SRP released from the sediments (Douglas et al., 2016b). Phoslock is manufactured via an ion exchange process whereby lanthanum ions displace sodium ions within the bentonite matrix (Fig. 4.S1). The bentonite structure contains two basic blocks, i.e. the aluminium octahedral sheets and silica tetrahedral sheets and due its high ions exchange capacity the cations (e.g. Na^{2+}) between the blocks can be easily changed by lanthanum (Hebbar et al., 2014; Ross and Shannon, 1926). The removal of SRP by LMB is attributed to 5% lanthanum (La), which binds phosphate molecules and forms rhabdophane ($LaPO_4 \cdot nH_2O$), a mineral with highly stable low solubility ($K_{sp} = 10^{-24.7}$ to $10^{-25.7}$ mol^2 L^{-2}) (Johannesson and Lyons, 1994). Thus, when LMB comes into contact with phosphate, the La in the clay can chemically binding with P through precipitation. Another promising SPS is Aqual-P™, an aluminium modified zeolite (AMZ). Zeolites are natural and synthetic silicoaluminates characterized by a microporous crystal structure which is based on two main structural units constituted by silicon or aluminium atoms or other elements bonded to four oxygen atoms, and oxygen being bonded to two tetrahedral atoms (Busca, 2014). AMZ is an aluminium-based P-inactivator that uses zeolite as an aluminium carrier and does not require buffering to avoid acidification of the lake water. AMZ differs from Al in both physical and chemical properties, with a higher density and a lower weight-specific P-binding capacity (Gibbs and Özkundakci, 2011). AMZ was tested and applied as a sediment

capping agent in Lake Okaro, New Zealand (Gibbs and Hickey, 2018) and so far it is the only known commercially available sediment capping agent that inactivates both P and N (Gibbs and Özkundakci, 2011). Hence, Al and AMZ chemically inactivate phosphorus via adsorption and LMB via precipitation (Fig. 4.S1).

The SRP binding efficacy of PS may depend on the prevailing conditions related to the binding mechanism, such as pH, redox state and temperature (Lürling et al., 2014b; Mucci et al., 2018; Noyma et al., 2016). For instance, increasing temperature improves the bond energy of phosphorus binding in the soil, increasing the rate of P transfer to strongly bound forms. Thus P retention ability is enhanced at a higher temperature (Reddy and DeLaune, 2008). It is common knowledge that water temperature changes temporarily and daily with the air temperature. As the average global temperature has risen by about 0.8°C since 1980, warming waters may also stimulate P release from the sediment, likely as a response to the lower oxygen concentrations and thus iron-bound P release increases from the sediment as well as enhanced mineralization of organic matter (Jeppesen et al., 2009). Similarly, pH may influence the efficacy of PS. The ligand exchange between SRP and OH^- can desorb SRP at high pH values because the ligands of OH^- in solution may replace the complexed HPO_4^{2-} or $H_2PO_4^-$ ions (Du et al., 2016). Each PS may act differently under different pH values. For instance, LMB showed excellent SRP removal when tested in the pH range of 6 to 9 (Li et al., 2019; Mucci et al., 2018; Ross et al., 2008). The maximum adsorption capacity of AMZ was highest at pH 9 (Mucci et al., 2018), while the optimum SRP removal of alum occurs between pH 5 to 7 (Georgantas and Grigoropoulou, 2007).

Although water bodies have different water temperatures within the season, which influence the system, none or a few studies have considered a realistic temperature range as a possible factor influencing PS binding efficiency (Georgantas and Grigoropoulou, 2007). Thus, here we determined the SRP binding capacity of LMB, AMZ and Al at six temperatures (from 5 to 35°C) to test the hypothesis that more SRP will be immobilized at warmer temperatures. Moreover, in a previous study (Mucci et al., 2018), both LMB and AMZ were tested in the pH range 6 to 9; however, depending on their acid-neutralizing capacity, pH values of 10 can be reached in shallow lakes (Kragh and Sand-Jensen, 2018). Hence, the present study elaborated on this, including pH 10. We tested the hypothesis that high pH will hamper the SRP binding capacity in all the PS tested due to competition with hydroxyl ions for binding sites (Li et al., 2019). In natural waters, pH can change within different seasons; however, little is known about SRP desorption caused by pH changes. Thus we also evaluated if a change in pH, from 7 to 10, would cause SRP release, testing the hypothesis that more SRP will be desorbed at a higher pH.

Methods

P sorbents

Three common P sorbents were selected: 1) La-modified bentonite Phoslock® (LMB), 2) Al-modified zeolite Aqual-P™ (AMZ), and 3) aluminium (Al) as combined aluminium sulphate (alum) and sodium aluminate. The La-modified bentonite Phoslock® (LMB), which size is 0.2~5 mm, was obtained from Phoslock® Europe GmbH (Zug, Switzerland). Before use, Phoslock was ground and sieved through a 0.5 mm mesh. Five different batches of LMB ("Open Luchtmuseum", and batches no. 20050922, no. 20050929, no. 20060727, no. 20060708) were destructed with a nitric acid/hydrochloric acid mixture (Aqua Regia) in the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University) and subsequently analyzed on Hg (AAS-cold vapour), Al, Fe, Mn, P, Zn (ICP-AES, IRIS Intrepid II; Thermo Electron Corporation, Franklin, MA, USA), and As, Cd, Cr, Cu, La, Pb (ICP-MS, Thermo Element 2; Thermo Fisher Scientific). LMB contains no detectable Hg (all below LOD <0.03 mg kg^{-1}), 49707 (1925) mg Al kg^{-1}, 43656 (1568) mg La kg^{-1},17354 (660) mg Fe kg^{-1}, 161 (30) mg Mn kg^{-1}, 211 (44) mg P kg^{-1}, 44 (3) mg Zn kg^{-1}, 1.3 (0.3) mg As kg^{-1}, 0.12 (0.01) mg Cd kg^{-1}, 12.1 (0.7) mg Cr kg^{-1}, 6.6 (0.6) mg Cu kg^{-1}, and 18 (2) mg Pb kg^{-1}. The Al-modified zeolite Aqual-P™ (AMZ), which size is <0.5 mm, was obtained from Blue Pacific Minerals (Tokoroa, New Zealand), AMZ composition was analyzed by ICP-OES (Thermo iCAP 6500 DV; Thermo Fisher Scientific) at the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University). Before using ICP-OES, AMZ were digested using a nitric acid/hydrochloric acid mixture (= Aqua Regia). AMZ contained 26458 mg Al kg^{-1}, 10131 mg Fe kg^{-1}, 15290 mg Na kg^{-1}, 8484 mg K kg^{-1}, 8200 mg Ca kg^{-1}, 2503 mg Mg kg^{-1}, 2295 mg S kg^{-1}, 1081 mg P kg^{-1}, 629 mg Mn kg^{-1}, 63 mg Zn kg^{-1} , 4 mg Cr kg^{-1}, 4 mg Cu kg^{-1}, and 1.9 mg Ni kg^{-1}. We combined two aluminium salts (Al), aluminium sulphate (alum) and sodium aluminate, as a buffer, which is a common procedure in lake restoration (e.g. Cooke et al., 2005; Smeltzer et al., 1999). Alum (aluminium sulphate, $Al_2(SO_4)_3 \cdot nH_2O$, sulfuric acid and aluminium salt 3:2, tetradecahydrate 80%-100%, size is below 5 mm) was obtained from Kemira (Helsinki, Finland), and sodium aluminate (Al_2O_3: 50-56%, Na_2O: 37-45%, size is below 0.5 mm) was acquired from Sigma-Aldrich (Darmstadt, Germany). Both alum and sodium aluminate were obtained as solid materials.

Effect of temperature on SRP binding capacity

Eight different phosphate (SRP) solutions (0, 5, 10, 20, 40, 80, 120 and 140 mg P L^{-1}) were

prepared by dissolving KH_2PO_4 in nanopure water. The SRP solution (50 mL) was transferred into 50 mL screw-cap centrifuge tubes and pH adjusted to pH 7 by adding HCl (0.1 M) or NaOH (0.1 M). Samples of the initial P solutions were stored at -20°C for further SRP analysis. To each of the eight different P solutions, 80 mg of AMZ or LMB were added. A stock solution from alum and sodium aluminate (both concentrations are 3158 mg Al L^{-1}) was prepared, and their addition was intercalated to avoid large pH change while adding the aluminium salts. pH was measured whenever a proportion of Al (alum or sodium aluminate) was added to the tubes. In the end, a total of 6.32 mg Al, half from alum and half from sodium aluminate, was added to each of the eight P solutions (Table 4.S1). Once the materials were added, the tubes were placed for 24 h in an incubator under different temperatures (5, 10, 15, 20, 25, 30 and 35°C) and continuously mixed (180 rpm) to keep the PS suspended. The experiment was run in triplicate. After 24 h, the tubes were centrifuged for 5 min at 2500 rpm (Heraeus sepatech centrifuge), and the supernatant was filtered through unit filters (Aqua 30/0.45 CA, Whatman, Germany). SRP concentrations of the filtrates and the initial solutions were determined using a Skalar SAN^+ segmented flow analyzer (Detection limit was 10 µg P L^{-1}), which follows the Dutch standard NEN 6663 (NNI, 1986). The final pH of the supernatant was also measured using a WTW pH meter equipped with a Sentix 61 electrode.

Effect of pH on SRP binding capacity

The maximum SRP binding capacity of the three PS was measured at pH 6, 7, 8, 9 and 10, following the same method described previously. The pH of the SRP solutions with LMB or AMZ was adjusted with HCl (0.1 M) or NaOH (0.1 M) to attain the desired pH. In the Al experiment, pH was adjusted by changing the alum and sodium aluminate ratio (Table 4.S1). After the pH adjustment and material addition, the tubes were shaken (180 rpm) for 24 h at 20°C. After 24 hours, SRP and pH were measured as previously described.

Effect of changes in pH on SRP release

A SRP binding experiment, as described previously, was conducted for each of the PS at pH 7 (Phase I). Per PS (LMB, AMZ and Al), 6 replicates were used for each of the eight SRP concentrations (0, 5, 10, 20, 40, 80, 120 and 140 mg P L^{-1}), yielding 48 experimental units per PS. The test tubes were incubated at 20°C and constantly shaken (180 rpm). After 24 hours, from each tube 15 mL solution was collected, filtered through unit filters and stored in the freezer for further SRP analysis, as described previously. Subsequently, for each compound, the six tubes per SRP concentration were split into two series of three replicates. In one series, the

pH was changed to pH 10 by adding NaOH (0.1 M), and the conductivity was adjusted by using NaCl to make sure all the incubations had similar conductivity, while the second series remained at pH 7 (Phase II). The tubes were incubated again, and after another 24 hours, the pH was measured, tubes were centrifuged, and 30 mL solution was collected and analyzed for SRP concentration as previously described. SRP release was calculated based on the difference between maximum SRP concentrations of Phase I and Phase II.

Data analysis

To calculate the maximum SRP binding capacity, the Langmuir and Freundlich models were used. The amount of SRP bound to PS at equilibrium Q_e (mg P g^{-1}) was calculated using equation (4-1) based on the initial concentration of SRP in solution C_0 (mg P L^{-1}), the SRP concentration at equilibrium C_e (mg P L^{-1}) from the adsorption/precipitation experiment, the volume of SRP solution V (L) and the weight of PS M (g) (Jalali and Peikam, 2013):

$$Q_e = \frac{(C_0 - C_e)V}{M} \tag{4-1}$$

The Langmuir isotherm was expressed in equations (2) and (3). Maximum binding capacity Q_m (mg P g^{-1}), the Langmuir adsorption/precipitation constant K_L (L mg^{-1}) was calculated using equation (4-2) based on plotting versus C_e (Langmuir, 1918; Mucci et al., 2018):

$$\frac{C_e}{Q_e} = \frac{1}{Q_m K_L} + \frac{C_e}{Q_m} \tag{4-2}$$

In order to evaluate the reaction rate and the binding affinity of phosphate of each PS, the Michaelis-Menten constant (K_m), which was defined as the SRP concentration at equilibrium at half of the maximum binding capacity (Q_m), was calculated by Michaelis-Menten kinetics equation (4-3) (Mosier and Ladisch, 2009):

$$Q_e = \frac{Q_m C_e}{K_m + C_e} \tag{4-3}$$

The Freundlich model is expressed in equation (4), K_F and n were the Freundlich constants, the Freundlich isotherm does not yield a maximum SRP binding capacity according to equation (4-4) (Qiu et al., 2012):

$$lnQ_e = \frac{lnC_e}{n} + lnK_F \tag{4-4}$$

To evaluate phosphate binding capacity onto the PS based on thermodynamics, enthalpy ΔH (kJ mol^{-1}) and entropy ΔS (kJ mol^{-1} K^{-1}) were calculated using the Van't Hoff equation (4-5), Plotting ln K_L versus $-\frac{1}{T}$, slope was $\frac{\Delta H}{R}$ and intercept was $\frac{\Delta S}{R}$ make it possible to calculate ΔH and ΔS, R was the gas constant (8.314 J mol^{-1} K^{-1}) and T was the water temperature (K) (Guerra et al., 2008; Gupta and Bhattacharyya, 2012; Huang et al., 2011):

$$lnK_L = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad (4\text{-}5)$$

The Gibbs free energy ΔG (kJ mol^{-1}) was calculated as equation (4-6):

$$\Delta G = \Delta H - T\Delta S \quad (4\text{-}6)$$

All data were expressed as the mean values of the three replicates. The maximum binding capacity at different temperatures, or pH, and the SRP desorption were compared for each product using one-way ANOVA or Kruskal–Wallis One-way Analysis of Variance on Ranks when the normality test (Shapiro–Wilk) or Equal Variance test (Brown–Forsythe) failed using the program SigmaPlot version 14.0. A Tukey or Dunn's post hoc test was performed to identify which means or medians were significantly different from each other (p = 0.05 level). A Wilcoxon Signed Rank Test was used to compare the difference between pH unchanged and changed in the SRP release part Phase II. SigmaPlot version 14.0 was used to fit isotherms of the phosphorus binding data using the Langmuir and Freundlich models.

Results

P sorbents binding capacity and thermodynamic parameters under different temperatures

All of the three materials (LMB, AMZ and Al) showed favorable SRP binding capacity under the six different temperatures (5, 15, 20, 25, 30 and 35°C) employed (Fig. 4.1). Isotherm models revealed that the r^2 from the Langmuir isotherms were higher than those obtained for the Freundlich isotherms for LMB and AMZ (Table 4.1). Therefore, the binding equilibrium of phosphate on LMB and AMZ can best be described with the Langmuir isotherm (Fig. 4.1 (a) and (b); Table 4.1). SRP binding capacity of LMB was significantly impacted by temperature (H_5 = 12.53; p = 0.028). The maximum SRP precipitation capacity of LMB varied between 6.3 mg P g^{-1} at 35°C and 14 mg P g^{-1} at 30°C. A Tukey post hoc comparison revealed that only the precipitation capacities at 30°C and 35°C were significantly different from each other (p = 0.02). Likewise, temperature affected the SRP sorption capacity of AMZ (H_5 = 18.22; p = 0.003) that varied between 4 mg P g^{-1} at 25°C and 29.9 mg P g^{-1} at 35°C (Table 4.1). A Tukey post hoc comparison showed that the adsorption capacities at 15°C and 25°C (p = 0.011), and 25°C and 35°C (p = 0.002) were significantly different from each other. Although LMB and AMZ had their maximum P binding capacity at 30°C and 35°C, respectively, the K_m values at these temperatures were higher than those at other temperatures, which indicates that the SRP binding affinity of LMB and AMZ was lower than at other temperatures. Also, for Al, the Langmuir model fitted better than the Freundlich isotherm, but the accuracy of fits was lower than those

for LMB and AMZ (Table 4.1). A Kruskal-Wallis one-way ANOVA on ranks indicated that the maximum SRP adsorption capacities in the Al treatments were not different from each other at the different temperatures tested (H_5 = 1.07; p = 0.96). Measured Q_m values ranged from 205 to 251 mg P g^{-1}, with a relatively large standard deviation varying between 11 and 16% (Table 4.1).

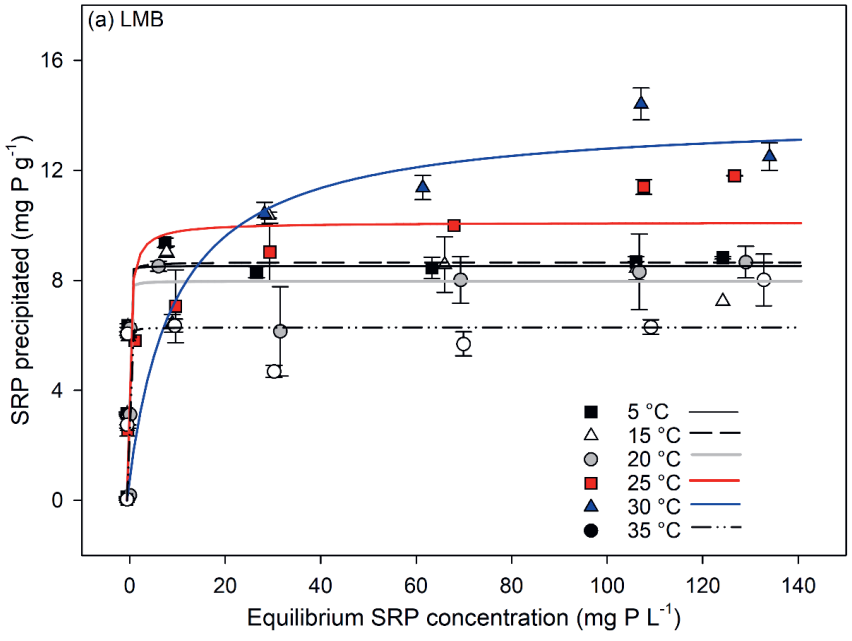
The pH in the LMB and Al treatments remained rather stable during the experiment and ranged between pH 6.47-7.91 in LMB treatments and between pH 6.94-7.51 in Al treatments (Table 4.S2). In contrast, in AMZ treatments pH had dropped from the initial pH 7 to pH 4.65-5.73.

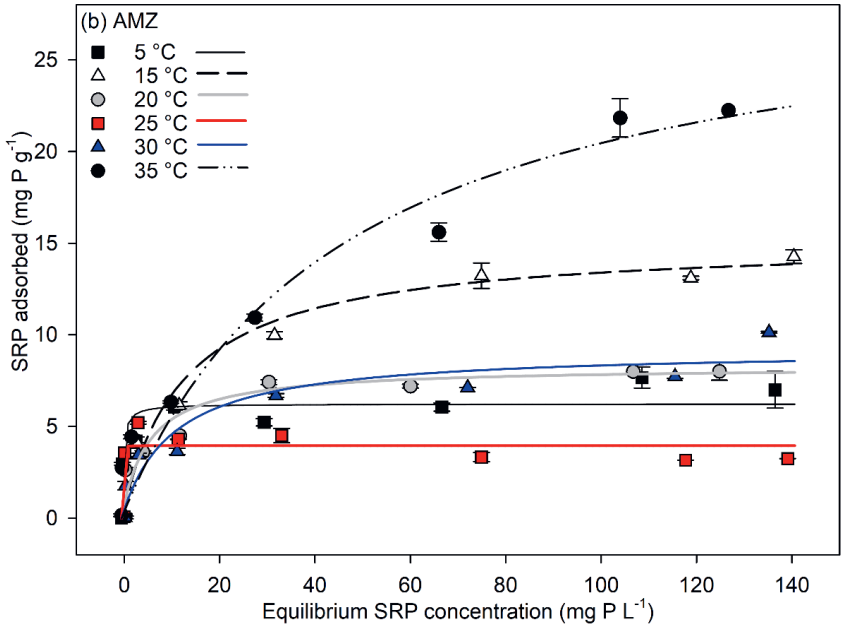
The ΔH values calculated were -81.27 (LMB), -45.58 (AMZ) and -19.92 (Al) kJ mol^{-1}, while ΔS were -0.26, -0.18 and -0.05 kJ mol^{-1} K^{-1}, for LMB, AMZ and Al, respectively (Table 4.1). The ΔG ranged from -9.69 to -1.97 kJ mol^{-1} in LMB, from -7.38 to -1.01 kJ mol^{-1} in AMZ and from -5.07 to -3.47 kJ mol^{-1} in Al when the temperature rose from 5°C to 35°C.

Table 4.1 Maximum SRP binding capacity (Q_m), estimated parameters for phosphorus binding at different temperatures of each PS using Langmuir isotherm and Freundlich adsorption isotherms

Material	Temperature		Langmuir isotherm						Freundlich isotherm			Thermodynamic		
			Q_m (mg P g^{-1})	r^2	*p*-value	t-value	K_L (mg P L^{-1})	K_m (mg P L^{-1})	r^2	K_F	n^{-1}	ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
LMB	5 ºC	278 K	8.5 (0.2)	0.96	<0.0001	53.07	50 (0.01)	0.02 (0.01)	0.69	1.80 (0.19)	0.10 (0.05)	-81.27	-0.26	-9.69
	15 ºC	288 K	8.7 (0.3)	0.91	<0.0001	35.29	20 (0.01)	0.05 (0.01)	0.65	1.78 (0.23)	0.09 (0.06)			-7.12
	20 ºC	293 K	8 (0.3)	0.85	<0.0001	26.88	33.33 (0.01)	0.03 (0.01)	0.72	1.75 (0.16)	0.08 (0.04)			-5.83
	25 ºC	298 K	10.1 (0.4)	0.86	<0.0001	24.13	2.63 (0.01)	0.38 (0.10)	0.61	1.59 (0.31)	0.17 (0.09)			-4.54
	30 ºC	303 K	14 (1.2)	0.75	<0.0001	11.54	0.10 (0.01)	9.98 (4.53)	0.63	1.65 (0.30)	0.19 (0.08)			-3.25
	35 ºC	308 K	6.3 (0.3)	0.78	<0.0001	21.31	33.33 (0.01)	0.03 (0.01)	0.53	1.56 (0.28)	0.07 (0.07)			-1.97
AMZ	5 ºC	278 K	6.2 (0.3)	0.83	<0.0001	25.03	3.23 (0.12)	0.31 (0.12)	0.84	1.31 (0.14)	0.13 (0.04)	-45.58	-0.18	-7.38
	15 ºC	288 K	15.2 (0.7)	0.95	<0.0001	22.98	0.07 (2.66)	13.98 (2.66)	0.80	1.38 (0.20)	0.25 (0.05)			-5.26
	20 ºC	293 K	8.0 (0.4)	0.88	<0.0001	20.23	0.17 (1.57)	5.98 (1.57)	0.61	1.18 (0.33)	0.19 (0.09)			-4.19
	25 ºC	298 K	4.0 (0.2)	0.77	<0.0001	23.45	106.38 (0.01)	0.01 (0.01)	0.55	1.41 (0.26)	-0.03 (0.07			-3.13
	30 ºC	303 K	9.2 (0.6)	0.88	<0.0001	16.28	0.09 (3.19)	11.29 (3.19)	0.73	0.08 (0.20)	0.47 (0.06)			-2.07
	35 ºC	308 K	29.9 (2.6)	0.95	<0.0001	11.71	0.02 (11.08)	49.04 (11.08)	0.78	1.46 (0.23)	0.30 (0.07)			-1.01
Al	5 ºC	278 K	239 (28.7)	0.37	<0.0001	8.35	7.84 (11.2)	0.13 (0.09)	0.14	103.02 (1.35)	0.17 (0.08)	-19.92	-0.05	-5.07
	15 ºC	288 K	244.5 (27.1)	0.44	<0.0001	9.03	6.49 (12.03)	0.15 (0.08)	0.15	94.15 (1.39)	0.20 (0.10)			-4.54
	20 ºC	293 K	205.2 (32.9)	0.19	<0.0001	6.24	8.46 (12.28)	0.11 (0.08)	0.16	130.11 (1.17)	0.11 (0.05)			-4.27
	25 ºC	298 K	223.5 (28.1)	0.33	<0.0001	7.95	10.6 (18.24)	0.09 (0.05)	0.14	93.13 (1.38)	0.19 (0.10)			-4.00
	30 ºC	303 K	251.1 (33.6)	0.29	<0.0001	7.47	3.50 (6.02)	0.28 (0.16)	0.20	85.71 (1.36)	0.21 (0.09)			-3.73
	35 ºC	308 K	232.4 (28.5)	0.34	<0.0001	8.14	10.4 (19.01)	0.09 (0.05)	0.18	97.38 (1.34)	0.19 (0.09)			-3.47

Note: Q_m is the P maximum binding capacity calculated via Langmuir isotherm; K_L is the Langmuir constant, K_m is Michaelis-Menten constant; K_F is Freundlich isotherm constant; n is an empirical constant; Values inside brackets are the standard error (SE); ΔH is apparent heat of sorption; ΔS is entropy change; ΔG is the change of Gibbs free energy.


(a) LMB
SRP precipitated (mg P g-1)
Equilibrium SRP concentration (mg P L-1)
5 °C
15 °C
20 °C
25 °C
30 °C
35 °C


(b) AMZ
SRP adsorbed (mg P g-1)
Equilibrium SRP concentration (mg P L-1)
5 °C
15 °C
20 °C
25 °C
30 °C
35 °C

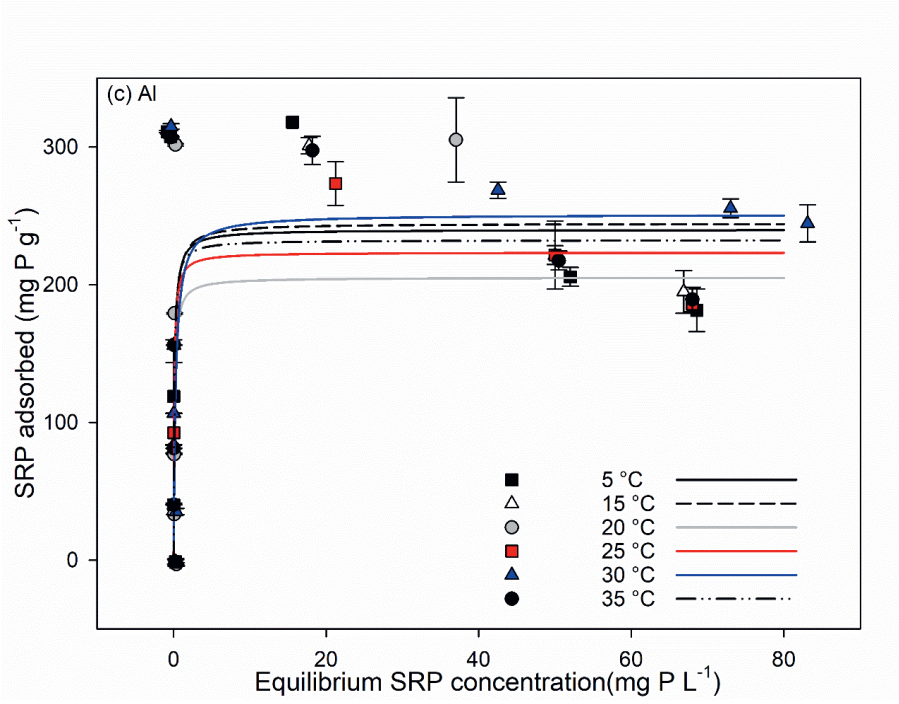


Fig. 4.1. SRP binding capacity for (a) LMB, (b) AMZ and (c) Al at different temperatures (5°C to 35°C). The colored lines indicate the Langmuir isotherms, error bars indicate standard deviation (n = 3)

Effect of pH on SRP binding capacity

The SRP binding capacities from LMB and Al were influenced by pH, while AMZ was not. For all the compounds, the highest maximum binding capacities were found at pH 6 (Table 4.2). The isotherm models revealed that the r^2 from the Langmuir isotherms were higher than those obtained for the Freundlich isotherms for LMB (Table 4.2). LMB had the highest binding capacity at pH 6 (10 mg P g^{-1}), the SRP binding capacity of LMB decreased gradually with increasing pH. The SRP precipitation capacity of LMB at pH 10 was about 2 times lower than at pH 6 (H_4 = 29.27; $p < 0.001$) (Fig. 4.2 (a); Table 4.2). Both the Langmuir isotherm and the Freundlich isotherm fitted well the SRP binding data for AMZ. The SRP binding capacities of AMZ were similar from pH 6 to pH 10 (H_4 = 9.98; $p > 0.05$), the maximum binding capacity reached up to 7.6 mg P g^{-1} at pH 6 (Fig. 4.2 (b); Table 4.2). Nonetheless, a decrease of 30% was observed at pH 9 (5.3 mg P g^{-1}) compared to pH 6.

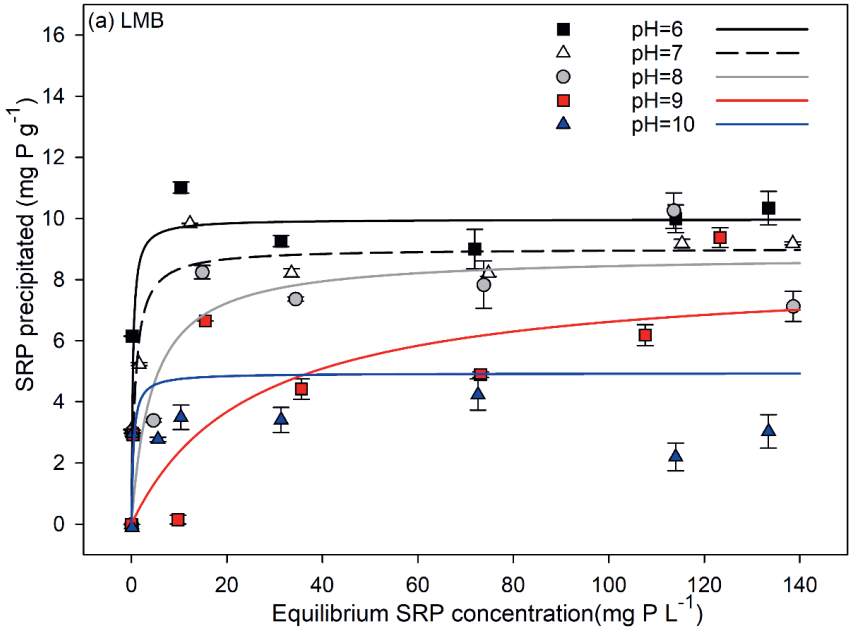
Similar to LMB, for Al, the r^2 from the Langmuir isotherms were higher than those from Freundlich isotherms, although the r^2 was still low (0.03 to 0.78). The highest binding capacity

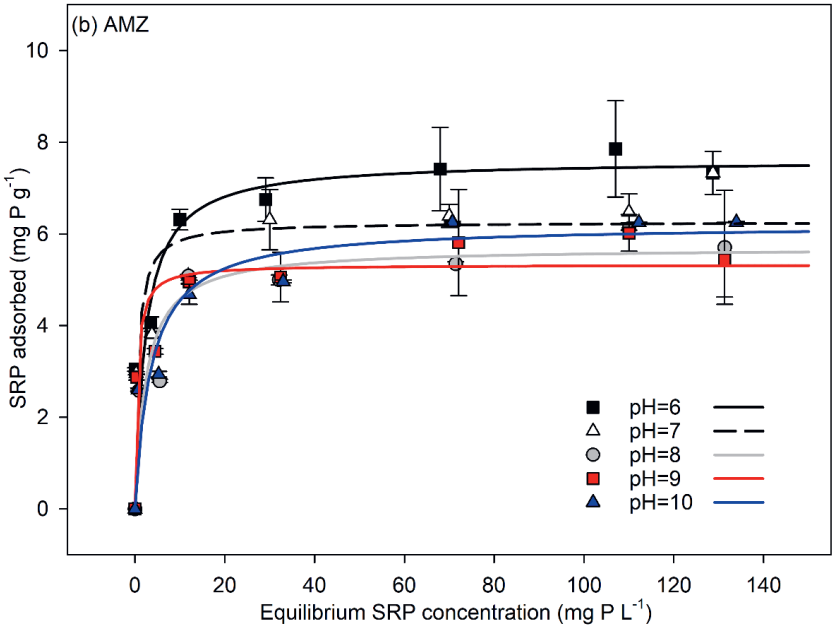
was found at pH 6 (572 mg P g^{-1}) (Table 4.2). The SRP binding capacity dropped sharply with increasing pH (Fig. 4.2 (c)). Kruskal-Wallis one-way ANOVA on Ranks indicated significant differences ($H_4 = 35.18$; $p < 0.001$) and a Tukey post hoc test revealed that the SRP binding capacity at pH 6 was significantly higher than the SRP binding at pH 10.

At the end of the experiment, the pH values measured in the LMB treatments ranged from pH 6.86 to pH 9.54 (Table 4.S3). In the AMZ treatments pH values were reduced compared to the initial pH values, and they ranged from pH 5.28 to pH 7.59, while pH values in the Al treatments had remained rather stable, ranging from pH 6.98 to pH 10.04 (Table 4.S3).

Table 4.2 Estimated parameters for SRP binding at different pH of each PS using Langmuir isotherm and Freundlich isotherms. Q_m is the SRP bind maximum in Langmuir isotherm; K_m is Michaelis-Menten constant; K_F is Freundlich isotherm constant; n is an empirical constant; Values inside brackets are the standard error (SE)

Material	pH	Langmuir isotherm						Freundlich isotherm		
		Q_m (mg P g^{-1})	r^2	*p*-value	t-value	K_L (mg P L^{-1})	K_m (mg P L^{-1})	r^2	K_F	n^{-1}
LMB	6	10.0 (0.4)	0.86	<0.0001	26.91	3.57 (14.93)	0.28 (0.07)	0.71	4.90 (0.05)	0.11 (0.02)
	7	9.0 (0.3)	0.80	<0.0001	23.11	1.27 (3.45)	0.79 (0.29)	0.001	3.82 (0.07)	0.02(0.02)
	8	8.8 (0.6)	078	<0.0001	15.71	0.23 (0.57)	4.28 (1.74)	0.001	3.32 (0.06)	0.01(0.02)
	9	8.3 (1.7)	0.47	<0.0001	5.01	0.04 (0.06)	23.1 (15.41)	0.031	3.25 (0.07)	0.03 (0.02)
	10	4.9 (0.7)	0.24	<0.0001	7.57	2.44 (2.44)	0.41 (0.41)	0.28	1.27 (0.26)	0.23 (0.07)
AMZ	6	7.6 (0.4)	0.82	<0.0001	19.64	0.43 (1.15)	2.34 (0.87)	0.89	4.06 (0.04)	0.14 (0.01)
	7	6.3 (0.2)	0.85	<0.0001	25.57	1.45 (4)	0.69 (0.25)	0.94	3.46 (0.03)	0.15 (0.01)
	8	5.7 (0.2)	0.87	<0.0001	23.59	0.41(1.45)	2.44 (0.69)	0.79	2.59 (0.07)	0.18 (0.02)
	9	5.3 (0.2)	0.84	<0.0001	25.91	2 (5.56)	0.50 (0.18)	0.81	3.19 (0.05)	0.13 (0.01)
	10	6.2 (0.2)	0.90	<0.0001	25.67	0.28 (1.16)	3.63 (0.86)	0.92	2.59 (0.04)	0.19 (0.01)
Al	6	571.7 (38.7)	0.78	<0.0001	14.78	2.13 (6.25)	0.47 (0.16)	0.001	484.54 (796.32)	8.33 (0.19)
	7	205.1 (33.2)	0.15	<0.0001	6.18	8.45 (12.5)	0.12 (0.08)	0.03	8000 (3.64E+6)	-0.39 (0.1)
	8	89.2 (28.8)	0.05	0005	3.10	2.14E+09 (12.5)	4.67E-10 (0.08)	0.001	197.95 (4.35)	-0.82 (0.88)
	9	38.8 (12.5)	0.03	0.005	3.10	8.75 (2.56)	0.11 (0.39)	0.001	43.38 (1.28)	-8.33 (8.33)
	10	21.3 (12.3)	0.044	0.1	1.73	9.42 (0.92)	0.11 (1.09)	0.001	43.82 (1.28)	3.45 (3.13)


(a) LMB
pH=6
pH=7
pH=8
pH=9
pH=10
SRP precipitated (mg P g-1)
Equilibrium SRP concentration(mg P L-1)


(b) AMZ
pH=6
pH=7
pH=8
pH=9
pH=10
SRP adsorbed (mg P g-1)
Equilibrium SRP concentration (mg P L-1)

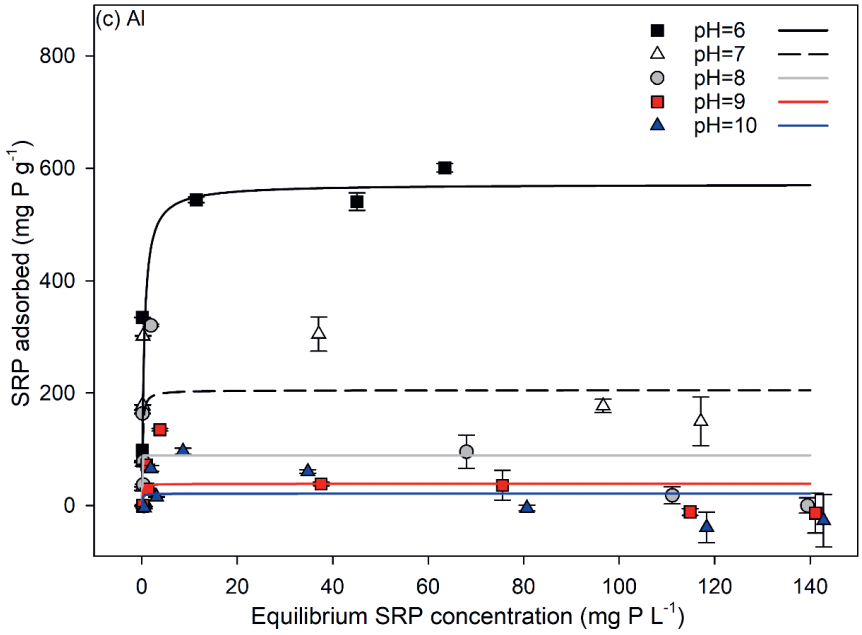


Fig. 4.2. The SRP maximum binding capacity of (a) LMB, (b) AMZ and (c) Al at different pH calculated by using Langmuir isotherms (fitted lines). Error bars indicate standard deviation (n =3)

Effect of pH on phosphate release

In Phase I, the Langmuir isotherm fitted slightly better than the Freundlich isotherm (Table 4.3). LMB was able to precipitate 9.4 mg P g^{-1} in Phase I at pH 7. In Phase II (release phase), LMB showed a slight increase in its binding capacity from 9.4 to 10.6 mg P g^{-1} in the series with unchanged pH 7, but this difference was not significant (H_2 = 0.05; p = 0.98). In the series in which pH was changed from pH 7 to pH 10, the performance of LMB increased less; from 9.4 mg P g^{-1} in Phase I to 9.7 mg P g^{-1} in Phase II (Table 4.3; Fig. 4.3 (a)). K_m was smallwst in Phase I and largest in Phase II in the series with elevated pH (Table 4.3). Wilcoxon Signed Rank Test revealed no difference between LMB binding capacity in Phase I and II (Z-Statistic (based on positive ranks) = -1.568; p = 0.121).

AMZ adsorbed 8.8 mg P g^{-1} in Phase I of the adsorption experiment at pH 7. In Phase II, in the series with unchanged pH (pH 7) the SRP adsorption capacity of AMZ was somewhat reduced to 7.7 mg P g^{-1} (Fig. 4.3 (b); Table 4.3). When pH was raised to pH 10, AMZ adsorption capacity significantly decreased to 5.4 mg P g^{-1} (H_2 = 6.36; p = 0.042) (Fig. 4.3 (b)). A Dunn's Method test revealed that the adsorption capacities significantly differed between phase I and II when pH was changed (p = 0.037). Similarly, the binding capacity between phase II changed pH and

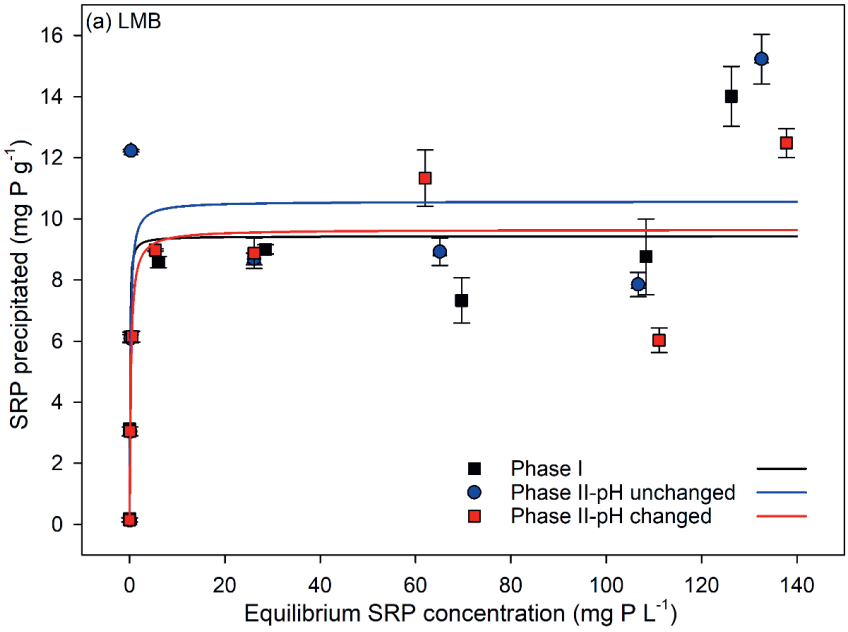
the unchanged pH was significantly different (Z-Statistic (based on positive ranks) = -4.015; $p \leq 0.001$).

Table 4.3 Estimated parameters for SRP binding capacity at different pH of each PS using Langmuir and Freundlich isotherms

Material	Phase Series		Langmuir isotherm						Freundlich isotherm		
			Q_m (mg P g^{-1})	r^2	*p*-value	t-value	K_m (mg SRP L^{-1})	SRP-release (µg P L^{-1})	r^2	K_F	n^{-1}
LMB	Phase II	Phase I	9.4 (0.4)	0.72	<0.0001	24.43	0.06 (0.03)	<LOD	0.75	6.05 (0.04)	0.11 (0.01)
		a (pH unchanged)	10.6 (1)	0.46	<0.0001	10.77	0.17 (0.07)	<LOD	0.30	1.22 (0.27)	0.27 (0.08)
		b (pH changed)	9.7 (0.5)	0.78	<0.0001	19.21	0.26 (0.10)	<LOD	0.67	1.71 (0.24)	0.13 0.07)
AMZ	Phase II	Phase I	8.8 (0.3)	0.85	<0.0001	25.69	6.42 (0.01)	<LOD	0.9	3.29 (0.04)	0.2 (0.01)
		a (pH unchanged)	7.7 (0.4)	0.82	<0.0001	7.02	4.21 (1.38)	1744	0.65	1.27 (0.26)	0.16 (0.07)
		b (pH changed)	5.4 (0.3)	0.79	<0.0001	18.74	1.99 (0.71)	5504	0.58	0.93 (0.36)	0.16 (0.10)
Al	Phase II	Phase I	238.3 (19.6)	0.34	<0.0001	12.17	0.11 (0.01)	<LOD	0.15	117.92 (0.20)	0.19 (0.06)
		a (pH unchanged)	245.6 (27.1)	0.36	<0.0001	9.05	0.25 (0.11)	<LOD	0.14	106.7 (0.29)	0.22 (0.10)
		b (pH changed)	68.3 (9.7)	0.04	<0.0001	7.04	0.07 (0.06)	21466.82	0.10	55.63 (1.11)	0.11 (0.03)

Note: Q_m is the maximum P binding capacity in Langmuir isotherm; K_m is Michaelis-Menten constant; SRP-release was calculated based on SRP concentrations difference between phase I and phase II series a or b; <LOD = below detected limit (10 µg SRP L^{-1}); K_F is Freundlich isotherm constant; n is an empirical constant; Values inside brackets are the standard error (SE)

Al was able to adsorb 238.3 mg P g^{-1} in Phase I at pH 7. In Phase II (release phase), Al showed a slight increase in its sorption capacity to 245.6 mg P g^{-1} in the series in which pH was not changed, but this difference was not significant (H_2 = 12.69; p = 1) (Table 4.3; Fig 3 (c)). However, when pH was raised to pH 10, Al binding capacity significantly decreased to 68.3 mg P g^{-1} (H_2 = 12.69; p = 0.002). The pH changed caused a desorption of 169.9 mg P g^{-1}, resulting in a SRP concentration of about 21.5 mg P L^{-1} desorbed (Table 4.3). A Signed Rank Test showed a significant difference in the binding capacity between pH unchanged and pH changed to 10 in Phase II from Al (Z-Statistic (based on positive ranks) = -3.657; $p \leq 0.001$).



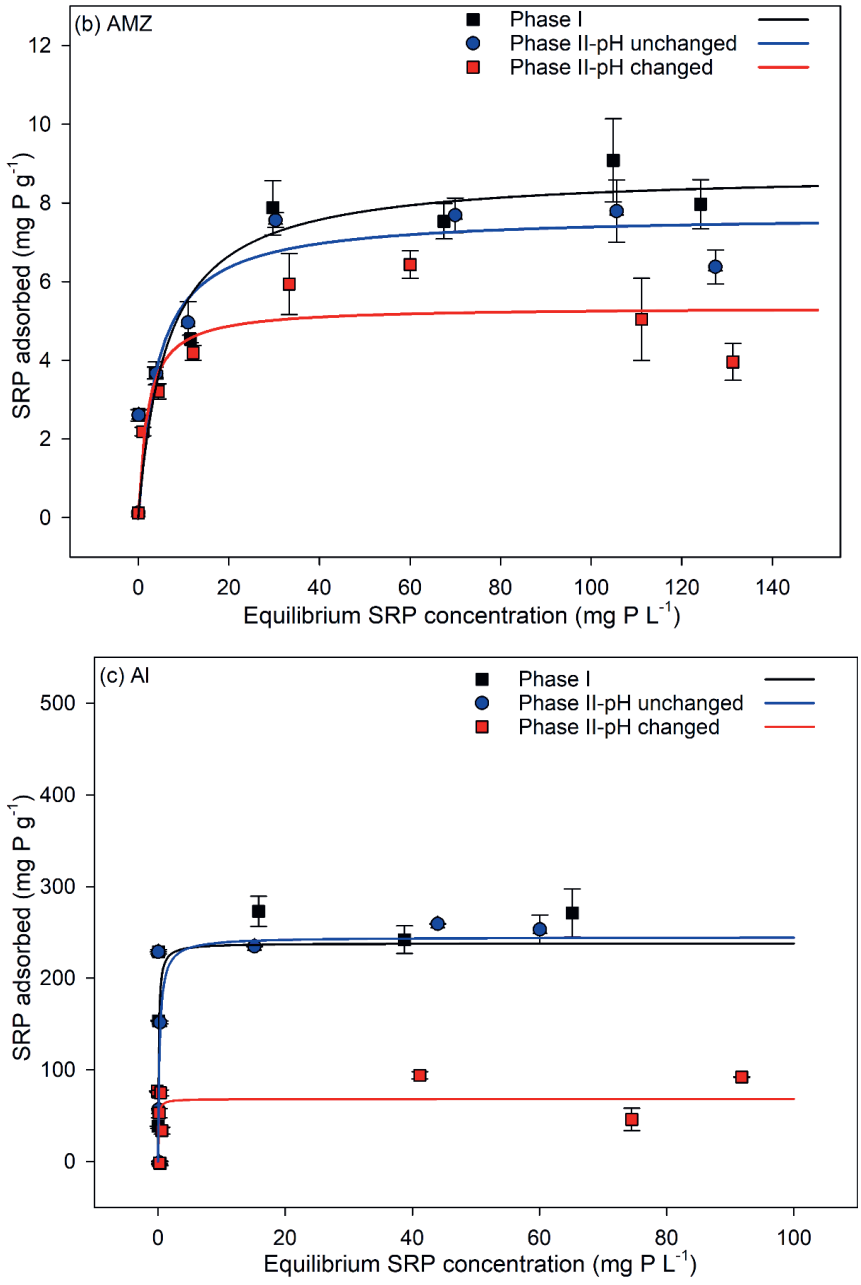


Fig. 4.3. Effect of changes in pH on the SRP release of (a) LMB, (b) AMZ and (c) Al. Phase I is the result at pH 7 after shaking 24 hours; Phase II-pH unchanged is the result for keeping pH=7 after shaking 48 hours; Phase II-pH changed is the result of adjusting pH to 10 after shaking 48 hours. Error bars indicate standard deviation (n =3).

Discussion

In this study, we evaluated the performance of three materials (LMB, AMZ and Al) on their SRP binding capacity at different temperatures and pH. We also compared Langmuir and Freundlich isotherms on their suitability to describe phosphorus binding capacity; and similar to other studies, we found that the Langmuir isothermal model was more suitable (Liu et al., 2016; Mucci et al., 2018; Xu et al., 2017; Spears et al., 2013c). Therefore, our maximum binding capacity calculated for LMB, AMZ and Al under different abiotic conditions were discussed using Langmuir results.

Several studies have determined the influence of environmental factors like pH, DOC, anoxic condition and salinity on SRP binding capacity of LMB and alum (Copetti et al., 2016; Li et al., 2019; Zamparas et al., 2015). Yet, temperature, a critical environmental factor, has been studied less. In contrast to our hypothesis, warmer temperatures did not lead to more SRP being immobilized by LMB. LMB precipitated SRP in all the 6 temperatures tested without a clear pattern among the temperatures, although the maximum binding capacity dropped significantly when the temperature exceeded 30°C (Table 4.1, Fig. 4.1 (a)). Contrary to our findings, He et al., 2017 showed that lanthanum-modified zeolite increased its P precipitation capacity from 14.8 mg g^{-1} to 17.2 mg g^{-1} when temperature increased from 20 to 40°C. Haghseresht et al., 2009 reported that the maximum SRP binding capacity of LMB (10.5 mg g^{-1}) was reached at 35°C, but with four runs (9.5 mg g^{-1} at 10°C, 9.5 and 10.2 mg g^{-1} at 23°C and 10.5 mg g^{-1} at 35°C) no conclusions on a temperature effect can be drawn from that study. It is known that when temperature increases, the kinetic energy increases, so the particles move faster which allows higher chances of interaction between PS and SRP. However, in our experiment, we used a shaker that already increased the chances of collision between SRP and the PS, thus, the shaker could have hampered the temperature effect. As for LMB, there are hardly any articles about the influence of temperature on the adsorption performance of AMZ. The efficiency of applying AMZ at a high temperature (35°C) was 654% higher than that at medium temperature (25°C) and was 375% higher than that of applying LMB at the same temperature (35°C). Although, at 35°C it seems that the equilibrium for AMZ was not completely achieved, thus it can be that the maximum adsorption capacity for this temperature was overestimated and might never be achieved under realistic SRP concentrations. High temperatures favor the formation of monomeric and small polymeric Al (Wang et al., 2003), and thus higher SRP adsorption could be expected at warmer temperatures. Georgantas and Grigoropoulou, (2007) observed increased SRP removal by Al-hydroxides with increasing temperature and attributed this to breaking of

$Al(OH)_3$ polymers into smaller particles creating a larger adsorption surface. In our study, no effect of temperature on the SRP adsorption capacity of alum was observed. The use of a shaking device may have overruled more subtle temperature effects that undoubtedly will play a role *in situ*, where, for instance, at 5°C the Al hydrolysis, polymerization, dispersion and floc settling will be lower than at 30°C (Georgantas and Grigoropoulou, 2007).

Most of the PS have been applied before the growing season (winter, autumn or early spring in temperate systems) when algae have not yet taken up the SRP released from the sediment. This is largely due to the mechanism by which the modified clays works, which, different from Al salts, are not a coagulant and thus only decreases phosphate and not particulate P. However, even for Al salts, the best P adsorption results are achieved when more phosphate is available in the system because alum flocs decrease its binding capacities over time if dosed when little or no phosphate is available (Berkowitz et al., 2006; De Vicente et al., 2008). This factor seems to be more important for efficiency than temperature; however, additional studies will be needed to develop a complete picture.

The ΔH during the SRP binding of three materials was -81.27 kJ mol^{-1}, -45.85 kJ mol^{-1} and -19.92 kJ mol^{-1} (Table 4.1), suggesting that the binding of phosphate onto PS was exothermic. Yet, there appeared to be no clear pattern in the SRP binding capacity of the three PS for temperature. It might be that also the use of a shaker in our study, overruled a temperature effect. As in our study, (Qian et al., 2017) did not find increased phosphate removal at warmer temperatures using boehmite (a γ-AlO(OH)) mineral) as PS. $\Delta S < 0$ showed that the randomness at the solid-liquid interface decreases during the binding process. The calculated enthalpy ΔH and entropy change ΔS on the three products were negative, which indicated that the binding process was enthalpy-driven rather than entropy-driven, since only the negative sign of ΔH (but not the negative sign of ΔS) leads to the reaction being favorable (Adamson and Gast, 1967). In addition, the ΔG for LMB (-9.69 ~ -1.97 kJ mol^{-1}), AMZ (-7.38 ~ -1.01 kJ mol^{-1}) and Al (-5.07~ -3.47 kJ mol^{-1}) were negative, which demonstrated a spontaneous process whereby the binding force was sufficient to pass the energy barrier required for the reaction between the adsorbent and the adsorbate (Liu et al., 2011).

In agreement with our hypothesis, we found that at the highest pH, LMB had a lower SRP binding capacity (Fig. 4.2 and Table 4.2). In several studies, LMB SRP binding efficiency has been tested under different pH values ranging from pH 4.6 to pH 9 (e.g., Gibbs et al., 2011; Mucci et al., 2018; Noyma et al., 2016; Zamparas et al., 2015). The LMB maximum binding capacity (9 mg P g^{-1}) at pH 7 in our study was higher than reported by Ross et al., 2008, who found 4.36 mg P g^{-1}, however, it was similar to values (9-12.3 mg P g^{-1}) reported in other studies

(Haghseresht et al., 2009; Mucci et al., 2018; Noyma et al., 2016; Spears et al., 2013c; Zamparas et al., 2015). Importantly, few studies have investigated the precipitation capacity at pH 10. In our study, LMB still could precipitate SRP at pH 10, although the maximum precipitation capacity was reduced by 50% compared to pH 6. The reduced removal capacity at pH 10 is a result of competition with hydroxyl ions for binding sites. Similar observations have been made for SRP removal by lanthanum-modified copper tailings (Jin et al., 2021). Li et al., 2019 also observed lower LMB efficiency at higher pH, with a 30% reduction in efficiency at pH 9 and a reduction of 80% at pH 10. It can, however, be expected that the hindrance by hydroxyl ions will only cause a delay in the formation of rhabdophane, but that over time most lanthanum will precipitate with phosphate. Such temporal hampering in SRP removal has also been observed in the presence of humic substances that delayed the formation of rhabdophane, but did not block it (Dithmer et al., 2016).

Similar to LMB, we found that the adsorption capacity of Al salts decreased when pH increased (Fig. 4.2), which is consistent with another study (Yang et al., 2006). When pH increased from 4.3 to 9.0, the P-adsorption capacity decreased from 3.5 to 0.7 mg P g^{-1} sludge (Yang et al., 2006). The addition of alum may cause the pH to drop in lakes with low alkalinity (Churchill et al., 2009), and at a low pH (below pH 5.5) and pH higher than 8, Al might occur predominantly as Al^{3+} and $Al(OH)_4^-$ (Georgantas and Grigoropoulou, 2007). These Al species are unable to adsorb phosphate, which might explain the relatively poor Langmuir fits in the experiment with pH higher than 8 ($r^2 < 0.04$). In addition, these Al species can be toxic to aquatic organisms (Yang et al., 2006). Therefore, Al salts should not be applied when the pH falls outside the safety range (6 to 8). For instance, Cooke et al., (2005) and Wagner et al., (2017b) have mentioned fish kill as a result of an aluminium treatment due to pH change. In field applications, buffers such as the one we used here (sodium aluminate) are commonly applied to avoid drastic pH change and adverse consequences as fish kills (Jacoby et al., 1994; Nogaro et al., 2013). Within the safe pH range (6 to 8), alum seems to perform better at pH 6, as shown in our experiment and also by Georgantas and Grigoropoulou, (2007).

Contrary to our hypothesis, AMZ did not reduce its efficiency under high pH. Our study revealed no significant difference in the SRP adsorption capacity of AMZ from the pH range pH 6 to pH 10, but the effect AMZ exerted on pH probably had a main impact on these results. The pH in our experiment had dropped from pH 9 and pH 10 at start to pH 7.2 and pH 7.6, respectively (*see* Table 4.S3), and thus the pH lowering effect of AMZ may likely have prevented competition with hydroxyl ions. Hence, AMZ has an effect on pH (lowering it by 1.8 and 2.4 units at start pH 9 and 10), and was also found to decrease pH in sediment by 0.3-0.5

pH units (Vopel et al., 2008). In our SRP release experiment pH was elevated after 24 hrs of incubation compensating for acidity released from AMZ and effects of elevated pH became prominent. Few other studies have investigated the influence of pH on the maximum SRP adsorption capacities of AMZ. For instance, one study reported an AMZ adsorption of 21.5 mg of P g^{-1} in lake water with pH 6 and 7, which was reduced to 11.6 mg P g^{-1} at pH 8.9 (Gibbs et al., 2011), while another study found adsorption capacities between 4.3-7.6 mg of P g^{-1} in the pH range from pH 6 to pH 9 without any clear relation between its adsorption capacity and pH (Mucci et al., 2018).

There is less information on the effects of environmental factors on AMZ ability to remove phosphate than for LMB and Al. AMZ has also been used only occasionally in field applications, except for some trials in New Zealand, AMZ has not yet been applied in whole lake restoration projects (Gibbs and Hickey, 2018; Tempero and Paul, 2015). Nonetheless, AMZ has great potential for phosphorus removal in the water column and in reducing the SRP release from the sediment (Li et al., 2017; Mucci et al., 2018) and in lowering ammonium release (Gibbs and Özkundakci, 2011), while no adeverse effects on freshwater fish, crayfish, or mussels have been found (Clearwater et al., 2014). However, more information about AMZ efficacy at high pH is required as well as potential the release of Al from the zeolite matrix under such conditions.

Contrary to our hypothesis not all of the products tested released P at higher pH (pH 10) (Fig. 4.3; Table 4.3). When pH changed from 7 to 10, AMZ desorbed approximately 39% of its adsorbed P, and alum desorbed approximately 71% of adsorbed P, while there was no release from LMB. Oppositely, LMB slightly improved its precipitation capacity when pH was changed to 10 (Phase II); however, this improvement was less than in incubations kept at pH 7, demonstrating ongoing SRP binding but also competition with hydroxyl ions as mentioned before. The higher SRP binding capacity in Phase II compared to Phase I at unchanged pH is caused by the contact time; in Phase I, LMB was in contact with SRP for 24 hours, while at the end of Phase II the contact time was 48 hours. Once the active ingredient of LMB, La precipitates with phosphate, it forms minerals with low solubility (i.e. rabdophane and monazite), which will not be solubilized at pH 10 (Firsching and Brune, 1991).

Differently from LMB, Al does not bind phosphate via precipitation, but via adsorption (Fig. 4.S1); thus the SRP release at pH 10 in AMZ and Al treatments is likely a result of the higher solubility and thus the dissolution of amorphous $Al(OH)_3$. Hence, Al salts and AMZ should be avoided in shallow lakes where pH can go up to pH 10. This has been mentioned by Reitzel et al., 2013a about aluminium salts, but for AMZ, such a study has never been done. AMZ differs from alum in physical and chemical properties, with higher density and lower weight-specific

P binding capacity (Clearwater et al., 2014). In AMZ the added aluminium is in the form of poly-aluminium chloride, which would suggest the phosphate removal mechanism to be similar to that of alum. Although our results indicated a relatively lower impact of elevated pH on AMZ than on Al, the earlier mentioned effect AMZ had on lowering pH seems a plausible confounding factor that warrants further investigation. In alum, the binding capacity not only decreases with pH, but also with the aging of the formed aluminium hydroxide flocs (Berkowitz et al., 2006; De Vicente et al., 2008). For instance, Berkowitz et al., 2006 found for alum added to Big Bear Lake water that the maximum sorption capacities decreased with increasing alum floc age, a maximum binding capacity of 30 mg P g^{-1} $Al(OH)_3$ for freshly-formed, amorphous material to about 15 mg P g^{-1} $Al(OH)_3$ after 180 days. If an aging effect will occur in AMZ needs to be determined.

The K_m value provides insights into the speed of the reaction between PS and SRP, while also considering the amount of saturation required (Mosier and Ladisch, 2009). A lower K_m value indicates that the PS requires only a small amount of phosphate to reach its maximum binding capacity, while a higher K_m value indicates that a higher concentration is required in a given time (Mucci et al., 2018). In this study, the overall K_m value of LMB and Al was lower than that of AMZ in all conditions, which means LMB and Al needed lower phosphate concentrations to achieve their maximum binding capacity than AMZ, suggesting better performance under realistic phosphate concentrations (Table 4.1 and 2). Although the binding capacity of LMB and AMZ was the largest at 30°C and 35°C, respectively, both of the reaction rates were the slowest, which means that a high concentration of phosphate was required to reach the maximum binding capacity at these temperatures.

In recent decades, the need for cost-effective and eco-friendly PS has increased to mitigate eutrophication nuisance via in-lake reduction of SRP and lowering the internal P load. These materials will be considered based on safety, costs, availability and efficacy (Douglas et al., 2016b; Lürling et al., 2020a; Lürling et al., 2016). All three compounds tested are capable of reducing the SRP concentration in water, yet only two of them (LMB and Al) have been applied widely in hundreds of water bodies up to now (e.g. Copetti et al., 2016; Huser et al., 2016). Inasmuch as AMZ will settle to the sediment rapidly, AMZ might be an alternative for sediment injection with Al salts (Schütz et al., 2017). From a safety perspective, a large number of studies have shown that LMB is not toxic to non-target organisms (Copetti et al., 2016; van Oosterhout and Lürling, 2011; Waajen et al., 2016). The active ingredient in LMB (lanthanum) was elevated after LMB applications in macrofauna and (cray) fish, but no detrimental effects were noted (van Oosterhout et al., 2014; Waajen et al., 2016). Hence, LMB is a powerful PS to be

used in lakes that suffer from internal load issues. Likewise, laboratory assays revealed that AMZ has no side effect on the survival or growth of crayfish, mussels or fish (Clearwater et al., 2014). Thus, AMZ may also be a good candidate for P control in surface water that experiences a strong impact from internal P load.

Given that AMZ is based on a zeolite, simultaneous reduction of SRP and ammonium release of sediments could be achieved (Gibbs and Özkundakci, 2011), which may attract the attention of water quality managers. These two solid phase P sorbents may be an alternative to aluminium salts in reducing sediment P release, mainly in places where aluminium addition is prohibited. However, Al salts also have coagulating properties, and therewith water column clearing potential besides being cheaper than AMZ and LMB (Lürling et al., 2020a). Overall, which compound or combination of compounds will be most suited will follow from a proper diagnosis of the problem, taking into account not only policy, cost, safety, availability, and efficacy, but also abiotic factors such as pH.

Conclusion

Lanthanum-modified bentonite (LMB), aluminium modified zeolite (AMZ) and aluminium salts (Al) all showed good SRP binding capacities at different temperatures and pH. Langmuir isotherm model provided better fits than Freundlich isotherm model; LMB reached its maximum binding capacity at relatively low phosphate concentrations compared to AMZ and Al; the adsorption of phosphate onto PS was exothermic. LMB and Al did not show any clear pattern in their binding capacity at different temperatures, AMZ seems to perform better at 35°C. All the three products performed better at pH 6, while AMZ efficiency was not affected by an increase in pH, LMB and Al decreased their efficiency under high pH. AMZ and Al desorbed P when pH changed to 10, while LMB was not affected. Therefore, pH and temperature have different effects on each PS material. Abiotic factors, specifical pH, should be taken into consideration while defining the most suitable PS.

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Supplementary information

List:

- Table 4.S1 The ratio of alum and aluminate (stocks both concentrations are 3158 mg Al L^{-1}) at different pH and different P concentration
- Table 4.S2 pH after binding at different materials, SRP concentration and temperatures
- Table 4.S3 pH after binding at different materials, SRP concentration and initial pH
- Fig. 4.S1 Size, active ingredient, appearance and removing P mechanism of three PS

Table 4.S1 The ratio of alum and aluminate (stocks both concentrations are 3158 mg Al L^{-1}) at different pH and different P concentration

Initial pH	P concentration (mg P L^{-1})	Alum stock (ml)	Aluminate stock (ml)	Final volume (ml)	Final concentration (mg Al L^{-1})	Final pH
6	0	3.85	4.15	200	126.32	6
	5	3.9	4.1	200	126.32	6.07
	10	3.9	4.1	200	126.32	6.00
	20	4	4	200	126.32	6.02
	40	4	4	200	126.32	6.18
	80	4.45	3.55	200	126.32	5.99
	120	4.55	3.45	200	126.32	5.99
	140	4.5	3.5	200	126.32	6.19
7	0	4	4	200	126.32	6.96
	5	3.525	4.475	200	126.32	7.01
	10	3.6	4.4	200	126.32	7.05
	20	3.5	4.5	200	126.32	7.1
	40	5.2	4.8	250	126.32	6.97
	80	4.1	3.9	200	126.32	6.94
	120	3.55	4.45	200	126.32	7.05
	140	3.55	4.45	200	126.32	7.08
8	0	3.35	4.65	200	126.32	8.06
	5	3.375	4.625	200	126.32	8.04
	10	3.4	4.6	200	126.32	7.99
	20	3.5	4.5	200	126.32	8.15
	40	3.7	4.1	195	126.32	8.1
	80	3	5	200	126.32	8.1
	120	4.5	5.5	250	126.32	8.06
	140	2.8	5.2	200	126.32	7.99
9	0	2.75	5.25	200	125.32	8.99
	5	2.75	5.25	200	126.32	9.01
	10	2.82	5.18	200	126.32	9.03
	20	3.2	4.8	200	126.32	9.04
	40	3.2	4.8	200	126.32	9.05
	80	2.9	5.1	200	126.32	9.07
	120	2.35	5.65	200	126.32	9.05
	160	2.05	5.95	200	126.32	9.05
10	0	1	7	200	126.32	9.96
	5	0.85	7.15	200	126.32	9.97
	10	0.35	7.65	200	126.32	9.99
	20	0.24	7.76	200	126.32	9.99
	40	0.09	7.91	200	126.32	9.98
	80	0	8	200	126.32	9.98
	120	0	8	200	126.32	9.88
	140	0	8	200	126.32	9.83

Table 4.S2 pH after binding at different materials, SRP concentration and temperatures

Material	Temperature	Initial pH	Final pH								
			0 mg P L^{-1}	5 mg P L^{-1}	10 mg P L^{-1}	20 mg P L^{-1}	40 mg P L^{-1}	80 mg P L^{-1}	120 mg P L^{-1}	140 mg P L^{-1}	Average
LMB	5°C	7	6.64	6.87	6.66	6.93	6.35	6.29	6.08	5.98	6.47
	15°C	7	7.29	7.28	7.17	7.27	7.15	7.02	7.12	7.26	7.19
	20°C	7	8.91	9.25	8.40	7.75	7.45	7.39	7.15	7.20	7.94
	25°C	7	7.15	7.22	7.22	7.30	7.17	7.08	7.05	6.97	7.14
	30°C	7	7.12	7.20	7.25	7.27	7.20	7.03	7.07	6.92	7.13
	35°C	7	7.25	7.26	7.32	7.25	7.19	7.09	7.13	6.99	7.19
AMZ	5°C	7	5.15	5.75	4.63	5.36	5.49	5.07	5.66	5.74	4.65
	15°C	7	4.58	4.57	4.70	4.91	4.90	4.94	4.85	4.91	4.80
	20°C	7	4.51	4.88	5.02	5.58	6.13	6.25	6.78	6.72	5.73
	25°C	7	4.50	4.45	4.52	4.63	4.73	4.78	4.78	4.79	4.65
	30°C	7	4.47	4.33	4.64	4.57	4.95	4.96	4.78	5.23	4.74
	35°C	7	5.24	4.55	4.75	4.69	4.87	4.89	4.89	4.94	4.85
Al	5°C	7	7.16	7.17	7.14	7.02	6.88	7.23	7.19	7.14	7.12
	15°C	7	7.74	7.57	7.50	7.41	7.85	7.26	7.21	7.15	7.46
	20°C	7	6.69	6.80	6.81	6.78	6.76	7.22	7.37	7.37	7.38
	25°C	7	8.27	7.88	7.73	7.58	7.10	7.22	7.19	7.10	7.51
	30°C	7	7.29	7.04	7.02	6.93	6.75	6.74	6.89	6.89	6.94
	35°C	7	8.14	7.75	7.47	7.26	6.92	7.07	7.09	7.15	7.35

Table 4.S3 pH after binding at different materials, SRP concentration and initial pH

Material	Initial pH	Final pH								
		0 mg P L^{-1}	5 mg P L^{-1}	10 mg P L^{-1}	20 mg P L^{-1}	40 mg P L^{-1}	80 mg P L^{-1}	120 mg P L^{-1}	140 mg P L^{-1}	Average
LMB	6	6.57	7.41	7.41	7.19	6.83	6.63	6.47	6.41	6.86
	7	7.12	8.35	8.21	7.67	7.32	7.17	7.09	7.05	7.50
	8	7.88	9.49	9.21	8.62	8.23	8.07	8.03	8.00	8.44
	9	8.65	9.67	9.50	9.09	8.71	8.65	8.70	8.68	8.96
	10	9.33	9.88	9.82	9.49	9.40	9.30	9.25	9.30	9.47
AMZ	6	5.00	4.73	4.82	5.01	5.43	5.53	5.86	5.83	5.28
	7	5.01	4.99	5.54	6.45	6.72	6.83	6.89	6.82	6.16
	8	4.97	5.91	6.82	7.18	7.50	7.61	7.75	7.86	6.95
	9	5.05	5.93	7.02	7.57	7.85	8.07	8.09	8.10	7.21
	10	5.33	6.59	7.51	7.82	8.00	8.33	8.60	8.50	7.59
Al	6	8.02	7.59	7.31	7.05	6.75	6.43	6.19	6.27	7.31
	7	6.69	6.80	6.81	6.78	6.76	7.22	7.39	7.38	6.98
	8	8.39	8.13	8.06	8.22	8.13	8.26	8.18	8.17	8.19
	9	9.98	9.41	9.29	9.29	9.27	9.21	9.09	9.14	9.33
	10	11.15	10.04	10.04	10.06	10.12	10.08	10.02	9.98	10.04

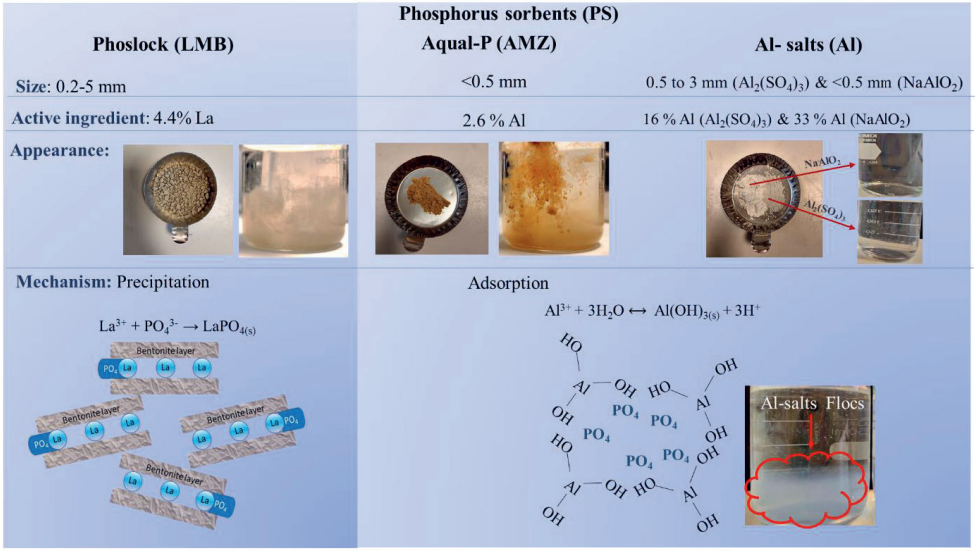

Fig. 4.S1 Size, active ingredient, appearance and removing P mechanism of three PS

5

Comparison of dredging, lanthanum-modified bentonite, aluminium-modified zeolite, and $FeCl_2$ in controlling internal nutrient loading

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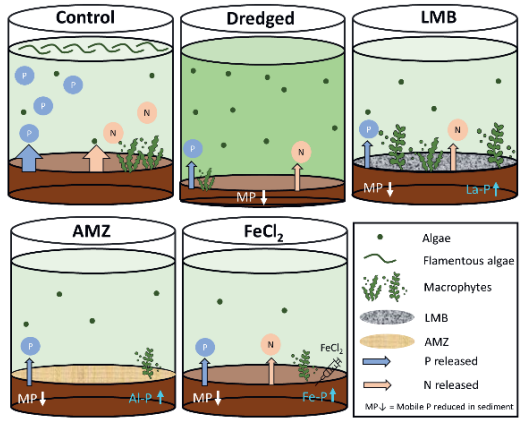

Abstract

The eutrophic Bouvigne pond (Breda, The Netherlands) regularly suffers from cyanobacterial blooms. To improve the water quality, the external nutrient loading and the nutrient release from the pond sediment have to be reduced. An enclosure experiment was performed in the pond between March 9 and July 29, 2020 to compare the efficiency of dredging, addition of the lanthanum-modified bentonite clay Phoslock® (LMB), the aluminium-modified zeolite Aqual-P™ (AMZ) and $FeCl_2$ to mitigate nutrient release from the sediment. The treatments improved water quality. Mean total phosphorus (TP) concentrations in water were 0.091, 0.058, 0.032, 0.031, and 0.030 mg P L^{-1} in controls, dredged, $FeCl_2$, LMB and AMZ treated enclosures, respectively. Mean filterable P (FP) concentrations were 0.056, 0.010, 0.009, 0.005, and 0.005 mg P L^{-1} in controls, dredged, $FeCl_2$, LMB and AMZ treatments, respectively. Total nitrogen (TN) and dissolved inorganic nitrogen (DIN) were similar among treatments; lanthanum was elevated in LMB treatments, Fe and Cl in $FeCl_2$ treatments, and Al and Cl in AMZ treatments. After 112 days, sediment was collected from each enclosure, and subsequent sequential P extraction revealed that the mobile P pool in the sediments had reduced by 71.4%, 60.2%, 38%, and 5.2% in dredged, AMZ, LMB, and $FeCl_2$ treatments compared to the controls. A sediment core incubation laboratory experiment conducted simultaneously with the enclosure experiment revealed that FP fluxes were positive in controls and cores from the dredged area, while negative in LMB, AMZ and $FeCl_2$ treated cores. Dissolved inorganic nitrogen (DIN) release rate in LMB treated cores was 3.6 times higher than in controls. Overall, the applied in-lake treatments improved water quality in the enclosures. Based on this study, from effectiveness, application, stakeholders engagement, costs and environmental safety, LMB treatment would be the preferred option to reduce the internal nutrient loading of the Bouvigne pond, but additional arguments also have to be considered when preparing a restoration.

Introduction

Eutrophication is the most prevalent water quality problem worldwide (Downing, 2014; OECD, 2017; Smith and Schindler, 2009). This nutrient pollution, caused primarily by excessive input of nitrogen (N) and phosphorus (P), often results in an overgrowth of harmful algae, toxic cyanobacteria and other aquatic plants. The negative consequences for aquatic ecosystem health, services and goods delivered by the aquatic ecosystems urged authorities to minimize eutrophication impacts. The European Union Water Framework Directive (WFD; 2000/60/EC) has been implemented to improve the ecological and chemical status of water bodies, and as such the WFD is important in combatting eutrophication. However, diffuse nutrient pollution from primarily agricultural sources confronts water authorities with a 'wicked problem' to minimize eutrophication impacts and to realize the WFD goals (Wiering et al., 2020). Likewise, in the Netherlands, nutrient pollution affected 65% of surface water bodies in which the most significant pressures come from diffuse agricultural sources (EuropeanUnion, 2019).

Diffuse nutrient pollution and legacy sources in catchments and lake sediments due to past nutrient loads are viewed as the main reason why water quality remains impaired, i.e. not recovering from eutrophication, even when point source nutrient pollution has been managed adequately. Therefore, the importance of in-lake measures is growing in which measures are aimed at reducing the impacts of eutrophication symptoms (Lürling and Mucci, 2020).

There are several in-lake measures that target the internal nutrient pool in the sediment. For instance, sediment dredging is a straightforward but relatively expensive measure to tackle internal nutrient release (Peterson, 1982; Welch and Cooke, 2005). Further, dredging may negatively impact benthic invertebrate communities, cause water column oxygen depletion, release pollutants, or result in the resuspension of sediments increasing turbidity (Knott et al., 2009; Manap and Voulvoulis, 2015; Peterson, 1982). To avoid such environmental impacts, geo-engineering techniques have been introduced that may effectively immobilize nutrients (primarily P) in the sediment (Douglas et al., 2016b).

Metal salts based on aluminium (Al) find wide applications in mitigating eutrophication (Cooke et al., 2005). These salts are commonly added to the water where they may adsorb phosphate and/or form $Al(OH)_3$ flocs that entrap cyanobacterial cells. Iron (Fe) salts (Fe^{2+}/Fe^{3+}) are used far less in eutrophication control, despite their common use in wastewater treatment plants as efficient, simple, and cost-effective P-flocculants (Azam et al., 2019; Douglas et al., 2016a). The main reason is the redox sensitivity of iron which may impair its phosphate adsorption capacity under low redox conditions that may occur in or near the sediment (Cooke et al., 2005).

Nonetheless, several studies have reported that Fe dosing in natural systems controlled internal P-cycling via decreased sediment P-release rates (Azam et al., 2019; Bakker et al., 2016; Boers et al., 1992; Gächter and Müller, 2003; Immers et al., 2015; Kleeberg et al., 2013; Nriagu, 1972; Rothe et al., 2015; Smolders et al., 2001; Smolders et al., 1995). In this study, we seek to elaborate on these findings and test the injection of ferrous iron ($FeCl_2$) directly into the sediment. The rationale for using ferrous iron is that under reductive, organic matter rich conditions, such as in sediment, the stable paramagnetic Fe-P mineral vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) may be formed (Rothe et al., 2014). Vivianite may potentially contribute to long-term P burial (Heinrich et al., 2022) but has also gained attention as a potential recovery phase for Fe and P.

As an alternative to liquid metal salts, solid-phase P-sorbents (SPS) have gained interest as they can effectively strip dissolved P from the water column while settling down on the sediment reducing sediment P release (Gibbs et al., 2011; Ross et al., 2008). These SPS are mainly modified clays and zeolites enriched with metals (aluminium, lanthanum (La), iron, or calcium (Ca)) (Gibbs and Hickey, 2018; Gibbs et al., 2011; Haghseresht et al., 2009; Zamparas et al., 2012). One of the most commonly used SPS in lake restoration is the lanthanum-modified bentonite (LMB), known commercially as Phoslock®, which has been used in hundreds of lakes and reservoirs worldwide (Copetti et al., 2016). The lanthanum in the clay matrix of LMB can precipitate with phosphate forming an extremely stable mineral, rhabdophane ($LaPO_4 \bullet nH_2O$) LMB is effective in permanently immobilizing phosphate over a wide pH and temperature range (Kang et al., 2022b; Mucci et al., 2018). Another SPS, the aluminium-modified zeolite (AMZ), known commercially as Aqual-P™, may reduce both P and N release from sediments (Gibbs et al., 2011). Although promising and tested under laboratory conditions (Kang et al., 2022b) rather little information is available on the performance of Aqual-P under semi-natural conditions.

To get more insight in the efficacy of different in-lake methods to counteract eutrophication by tackling sediment nutrient release, two experiments were conducted, one at field-scale in the form of an enclosure experiment and the other one at lab-scale in the form of a sediment core incubation experiment. The measures tested were 1) dredging, 2) application of LMB, 3) application of AMZ and 4) sediment injection with $FeCl_2$. It was hypothesized that dredging and AMZ addition would reduce both N and P release from sediment, and that LMB addition and $FeCl_2$ injection would reduce only P release from sediment. This was tested by incubating sediment cores for four weeks, taken from a dredged and a non-dredged area in the shallow

eutrophic Bouvigne pond. The cores from the non-dredged area remained either untreated (controls) or were amended with AMZ, LMB, or $FeCl_2$. The effects of the treatments on water quality variables in the enclosures were monitored for 112 days to test the hypothesis that all sediment nutrient release abatement measures would improve water quality compared to untreated controls. The hypothesis that the different measures would result in a shift in various sediment P forms was tested by collecting sediment from each enclosure at the end of the experiment and determining the different P fractions via sequential P extraction. For the water manager of the experimental site, the results are used to determine the restoration measures for the eutrophic pond.

Methods

Enclosure site

The enclosure experiment was carried out from the 9th of March until the 29th of July 2020 in Bouvigne pond (51°33' N, 4°46' E), Breda, the Netherlands. This period was chosen to ensure the sediments had been treated before they normally start to release nutrients, which in temperate regions in northwestern Europe is around May (Søndergaard et al., 2013). The pond has an open water surface area of 1.43 ha and an average water depth of 1.08 m, ranging from 0.85 m to 1.4 m. The pond is surrounded by a park with gardens that are open to the public. Ongoing diffuse pollution via leaf litter, groundwater, run-off and precipitation was estimated at 0.2 g P m^{-2} $year^{-1}$ (Haasler, 2020). The internal P load was estimated between 0.3 and 0.6 g P m^{-2} $year^{-1}$, the critical P load based on PCLake from clear water to a turbid state at 1.5 g P m^{-2} $year^{-1}$, and the critical P load from turbid water to clear water at 0.6 g P m^{-2} $year^{-1}$ (Haasler, 2020). Most of the submerged macrophytes present (primarily *Elodea nuttallii*) disappeared during 2013 and did not return on a large scale. Since a major reconstruction of the pond in 2010, cyanobacterial blooms (primarily *Microcystis sp.*) were recorded in 2013, 2014, 2016, 2018 and 2019 (data from Water Authority Brabantse Delta). In April 2020, in the pond itself chlorophyll-*a* (Chl *a*) concentrations were 29.1 µg L^{-1}, transparency was similar to the water depth of 90 cm, total suspended solid was 13.6 mg L^{-1}, pH was 7.65, conductivity was 328 µS cm^{-1}, oxygen saturation was 90%, total phosphorus (TP) 0.06 mg P L^{-1}, filterable P (FP) 12.4 µg P L^{-1}, total nitrogen (TN) 0.33 mg N L^{-1}, ammonium (AN) 0.02 mg N L^{-1}, NO_2-N and NO_3-N 0.01 mg N L^{-1}. During the course of the experiment the mean surface water temperature was 14.6 (± 0.6)°C in April, 20.0 (± 1.2)°C in May, 20.8 (± 2.4)°C in June and 18.7 (± 1.1)°C in July.

Enclosure set-up

Before setting up the enclosures, four sediment cores (59.6 cm long, 5.9 cm in diameter) of the upper sediment were collected with a UWITEC core sampler in the middle of Bouvigne pond on October 3rd 2019. These cores were used to determine the mobile P pool, which is important to define the dose of the P binders used in the experiment. The top 9 cm of each sediment core was sliced into 3 cm thick slices using a UWITEC core slicer and subjected to a sequential P extraction method (Psenner, 1988). In short, loosely bound P (H_2O-P), redox-sensitive P (Bicarbonate dithionite (BD-P)), organic P in microorganisms, organic P in detritus, P bound in humic compounds (NaOH-NRP, non-reactive phosphorus), metal oxide-bound P (NaOH-SRP, soluble reactive phosphorus), P bound to carbonates and apatite-P (HCl-P) and residual organic and other refractory P after H_2SO_4 digestion (refractory-P (Ref-P)) were determined. The mobile P that can become available under anoxia or after organic matter degradation was determined by the sum of the H_2O-P, BD-P and the NaOH-NRP fractions (Hupfer et al., 1995). The mobile-P pool varied between 2.11 and 3.78 mg P g^{-1} DW for the first 9 cm of sediment and was on average 3.20 ± 0.47 mg P g^{-1} DW, in which average H_2O-P content was 0.52 ± 0.13 mg P g^{-1} DW, average BD-P was 1.84 ± 0.46 mg P g^{-1} DW, and average NaOH-NRP was 0.84 ± 0.67 mg P g^{-1} DW.

The enclosure experiment consisted of twenty Perspex cylinders (1.05 m in diameter, 1.3 m in height) that were placed in two rows 2-5 m offshore where water depth showed a gradient from ~ 0.8 m to ~1 m (Fig. 5.S1). Using an excavator 1-2 hours before placing the enclosures at the shallowest part, approximately 20 cm of upper soft and dark muddy sediment was removed down to the grey sandy substrate. Four enclosures were placed in this dredged area, pushed into the sediment to allow sediment water interaction, and were further labeled as dredging treatment. The other 16 enclosures were placed to the west of the dredged area to which four were treated with LMB (Phoslock®, Water Solutions Limited, Australia), four with AMZ (Aqual-P™, Blue Pacific Minerals, New Zealand), four with $FeCl_2$ (Iron(II) Chloride tetrahydrate, CAS-Nr.: 13478-10-9, Honeywell) and four left untreated as control. The chemical amendments were assigned randomly to these enclosures (Fig. 5.S1). Approximately 779 L of water was enclosed in each enclosure. The enclosures were placed on March 9th 2020 and allowed to stabilize for a few weeks. On April 8th 2020 (day 0), LMB, AMZ and Fe treatments were applied. The doses of LMB and AMZ were based on the water column TP concentration measured just before the enclosures were placed (~0.08 mg L^{-1}), the mobile P pool in the top 6 cm of the sediment, and a sorption capacity of 11.4 and 8.9 mg P g^{-1} product for LMB and AMZ, respectively (Mucci et

al., 2018). A slurry of 563 g LMB or 725 g AMZ was made with water from the corresponding enclosures on site that was added to the water surface and allowed to settle on the sediment. The $FeCl_2$ dose was based on the mobile P in the upper 6 cm of the sediment. A 2 L $FeCl_2$ solution was prepared by dissolving 375 g $FeCl_2$ in HA/Ac buffered water with a pH = 4.2 to prevent quick Fe oxidation as a result of higher pH during the addition and subsequent dilution in each Fe(II) enclosure. The $FeCl_2$ solution was injected directly into the upper ~ 6 cm of the sediment at 10 different spots randomly chosen from a 16 squared grid that was placed on top of each enclosure.

Over four months, all enclosures and the pond were monitored on physicochemical water quality variables every two weeks. The initial measurements were taken shortly before the treatment on the 8th of April 2020 (day 0), the last sampling day was on the 29th of July 2020 (day 112). Secchi-disk depth (SD), pH, electrical conductivity (EC), dissolved oxygen concentration and saturation (DO), and temperature (Temp) were measured *in situ* in the middle of the water column in each enclosure. pH, EC and Temp were measured using a WTW pH/Cond 3320 multimeter. DO was measured using an OxyGuard Handy Polaris 2. A two L whole water column integrated water sample was taken at the center of each enclosure using a sampling tube. Water samples were transported to the laboratory for further analysis of water quality variables. Finally, SD was measured by a 30 cm diameter black/white Secchi-disk.

In the laboratory, turbidity was measured in unfiltered water samples with a Hach 2100 turbidity meter (Hach, Tiel, The Netherlands). The Chl *a* concentrations were determined with a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany). Total suspended solids concentrations (TSS) were determined after filtration of a known volume of unfiltered water over Whatman GF/C glass fiber filters (Whatman GF/C, VWR International B.V., Amsterdam, The Netherlands) that had been dried at 105°C. Total nitrogen (TN) and total phosphorus (TP) concentrations were measured using a Skalar SAN^+ segmented flow analyzer following Dutch standard protocols (NNI, 1986, 1990). Filtered (Whatman GF/C) water samples were stored in 50 mL PE bottles at -20°C upon further analysis.

In filtered water samples, chloride (Cl) concentrations were measured with a Thermo Scientific Orion 720Aplus pH/ISE Meter equipped with a Cl^- ion specific electrode (Thermo Fisher Scientific, Waltham, MA USA). Dissolved inorganic nitrogen (DIN) concentrations consisting of NH_4-N, NO_2-N and NO_3-N, and filterable phosphorus (FP) were measured using a Skalar SAN^+ segmented flow analyzer following the Dutch standards NEN 6663 (NNI, 1986) and NEN-EN-ISO 13395 (NEN, 1997). In samples taken at days 0, 28, 56 and 84 filterable metals (Al, Fe, Mn, and S) were determined by ICP-OES (Thermo Electron Corporation, Franklin,

MA, USA) and La was measured by ICP-MS (Thermo Element 2; Thermo Fisher Scientific). Additionally, sediment from each enclosure and from the pond was collected using a core sampler on July 29th 2020. These sediment cores were sliced into 3 cm slices (0-3 cm, 3-6 cm and 6-9 cm) and each slice was subjected to a sequential P-fractionation analysis (Psenner, 1988). Sediment from two Fe(II)-treated enclosures and one control enclosure was analyzed for vivianite by Mössbauer spectroscopy. The ^{57}Fe Mössbauer absorption spectra were collected at 300 K with a conventional constant-acceleration spectrometer using a ^{57}Co (Rh) source. The velocity calibration was carried out using an α-Fe foil while the fitting of the spectra was performed using the software Mosswin 4.0. The standard vivianite samples are based on two different Fe ion donors: Mohr's salt (S1 Viv.) and $FeCl_2$ (S4 Viv.).

Core incubation set-up

Twenty sediment cores were taken from the pond on April 8th 2020. Four cores were taken at the dredged area in the pond, whereas the rest of the cores were taken close to the placement of the non-dredged enclosures. In the laboratory, four cores were dosed with LMB, four others with AMZ, four cores were treated with $FeCl_2$ injected in the sediment, while four cores remained untreated (controls) as were the four cores from the dredged area. To test the efficiency of four materials on sediment nutrients release under anoxic conditions, before applying materials to the cores, the overstanding water in each core was bubbled gently with N_2 until oxygen saturation was less than 1%. Subsequently, a water sample was taken from each core (day 0). The doses of LMB, AMZ and $FeCl_2$ were the same as in the enclosures, 1.827 g dry LMB and 2.354 g dry AMZ were mixed into a slurry with overlying water, respectively, while $FeCl_2$ was injected at one point into the sediment as 20 mL of HA/Ac buffered 0.48 M $FeCl_2$-solution. To prohibit oxygen production by photosynthesis, all cores were incubated at 7°C in the dark from the 8th of April (day 0) to 6th of May (day 28).

Initially (day 0), and subsequently once every week pH, DO, and EC were measured, and water samples were collected from each core and analyzed on NH_4-N, NO_2-N+NO_3-N and FP concentrations as well as on filterable metal concentrations (Al, Fe, Mn and La) and S using the same methods as given previously described. Nutrient fluxes (FP in mg P m^{-2} d^{-1} and DIN in mg N m^{-2} d^{-1}) were calculated based on the differences in FP and DIN concentrations between day 0 and day 28.

Statistics

Data visualization and statistical analysis were performed using SigmaPlot 14, OriginPro 2021

software (Originlab, Northampton, MA, USA) and IBM SPSS 19.0 (IBM, New York, USA). The time points data of the water quality variables were analyzed by repeated-measures analysis of variance (rmANOVA) and simple effect analysis. Homogeneity of variance for the obtained data was tested and significant levels were reported at $p < 0.05$ (*) and $p < 0.01$ (**). For further intuitive understanding of the linkages between the materials applications and environmental variables, a structural equation model (SEM) was built to develop prediction equations and path analyses and to analyze multivariate hypotheses using SPSS AMOS 26. The experimental data from control were inputted to compare with the data from the other four treatments. The model parameters, such as Chi-square (χ^2), χ^2/df, root mean square residual (RMR), and goodness fit index (GFI) were used to determine the fit of the model. In this study, the parameters of four SEM models were: (A) dredged: $\chi^2 = 3.13$, χ^2/df = 1.04, RMR <0.0001, GFI = 0.98; B) LMB: $\chi^2 = 5.03$, χ^2/df = 1.68, $p = 0.17$, RMR= 0.001, GFI = 0.97; C) AMZ: $\chi^2 = 2.4$, χ^2/df = 0.8, $p = 0.49$, RMR = 0.001, GFI = 0.99; D) $FeCl_2$: $\chi^2 = 3.23$, df = 3, χ^2/df = 1.08, $p = 0.38$, RMR = 0.001, GFI = 0.98. All these parameters indicated our SEM model was fitting well. In the enclosure experiment, changes in treatments and water quality variables through time were assessed using principal response curves (PRC) (Van den Brink and Braak, 1999). Here, the pond itself and control were taken as the reference, respectively. More specifically, we used the pond itself as one reference and the control group as another reference. All data (except pH) were log-transformed before analysis. The multivariate analysis was performed using the CANOCO software package (version 4.5, Wageningen University, Wageningen, The Netherlands) (Ter Braak and Smilauer, 2002).

Results

Physicochemical water quality variables

Water temperature showed similar courses in the enclosures and in the pond ranging from on average 15.7°C in spring to 20.2°C in summer (Fig. 5.1A). Differences in DO concentration (Fig. 5.1B) and saturation (Fig. 5.1C) were more pronounced; DO was also higher in the enclosures than in the pond (Figs. 1B, 1C). The highest values were recorded in June (Figs. 1B, 1C). The rmANOVA's yielded strong evidence that DO concentration and saturation were different between treatments (Table 5.S1), which was supported by the post-hoc comparison (Table 5.2). In general, DO concentration and saturation were highest in controls and AMZ treatments, and lowest in dredged and $FeCl_2$ treated enclosures (Figs. 1B, 1C). Also, pH differed over time and between treatments and it was a little higher in enclosures than in the pond (Fig.

5.1D). Compared to the control, the pH in LMB, and treated enclosures was significantly higher, whereas pH in $FeCl_2$ treated enclosures was lower (Table 5.S1, 2), albeit those differences became most pronounced during the second part of the experiment (Fig. 5.1D). Likewise, EC differed between treatments (Fig. 5.1E), where EC in control and LMB treatments were similar, EC was significantly elevated in dredged and AMZ treatments, and the highest in $FeCl_2$ treated enclosures (Fig. 5.1E; Table 5.S1, 2). EC in the latter was also significantly higher than in the pond (Fig. 5.1E, Table 5.S1). Turbidity was significantly elevated in the first weeks after $FeCl_2$ addition, but returned to similar levels as in the other enclosures which were comparable to turbidity in the pond (Fig. 5.1F, Table 5.S1, 2). The course of TSS concentrations was similar in all treatments and comparable to the pond (Fig. 5.1G). TSS concentrations differed in time, but not among treatments (Table 5.S1, 2). Secchi-depth (SD) showed the opposite pattern to turbidity, the visibility of the water column decreased initially after the addition of LMB, AMZ and $FeCl_2$ (Fig. 5.1H). The $FeCl_2$ treated enclosures had initially the lowest SD, after which the SD of the water column was recovered, SD's were similar to SD in the pond (Fig. 5.1H).

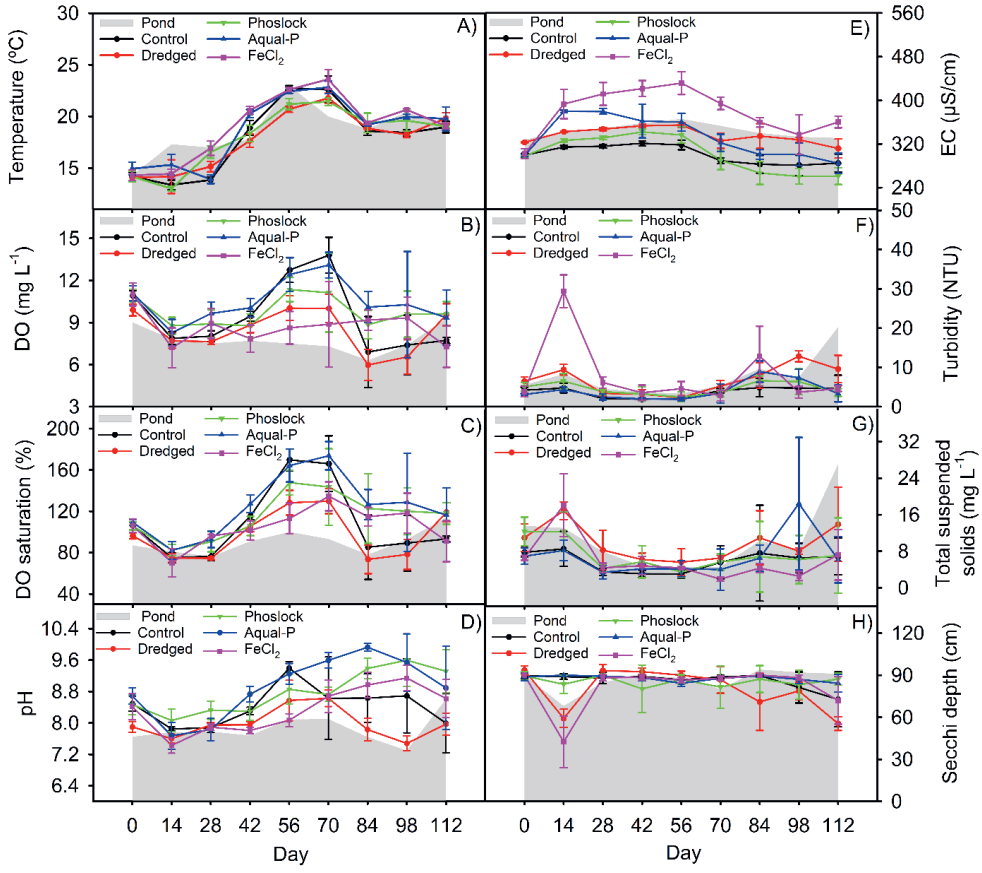

Fig. 5.1. Course of the environmental variables in non-treated (Control), LMB treated (Phoslock), AMZ treated (Aqual-P), $FeCl_2$ treated ($FeCl_2$), and dredged (Dredged) enclosures during the 112 d experimental period in 2020. A) Temperature (°C); B) dissolved oxygen concentration (DO, mg L^{-1}); C) dissolved oxygen saturation (DO, %); D) pH; E) electric conductivity (EC, µS cm^{-1}); F) Turbidity (NTU); G) Total suspended solids concentration (TSS, mg L^{-1}); H) Secchi disk depth (SD, cm). The grey area represents the measurements done in the pond. Error bars indicate one standard deviation (n = 4)

Nutrients and Chlorophyll a

TP concentrations showed an increase towards the end of the experiment, particularly in the controls (Fig. 5.2A). TP concentrations were significantly different between treatments (Table 5.S1) and the LMB, AMZ and $FeCl_2$ treated enclosures differed significantly from the control (Table 5.S1). Over the entire experimental period, TP concentrations were on average 0.091 mg P L^{-1} in controls, 0.058 mg P L^{-1} in dredged enclosures, and 0.032, 0.031, 0.030 mg P L^{-1} in $FeCl_2$, LMB and AMZ treatments, respectively (Fig. 5.2A). Also, FP concentrations were significantly different between treatments (Table 5.S1), and the highest in the controls (Table 5.2; Fig. 5.2B). Mean FP concentrations were 0.056, 0.010, 0.009, 0.005, 0.005 mg P L^{-1} in control, dredged, $FeCl_2$, AMZ and LMB treated enclosures, respectively. The course of TN concentrations was comparable in all treatments (Fig. 5.2C). Although a bit higher in dredged enclosures during the first part of the experiment (before June 17th), TN concentrations were not different between treatments (Table 5.S1 and 2). Likewise, DIN concentrations were similar among treatments (Fig 2D; Table 5.S1 and 2).

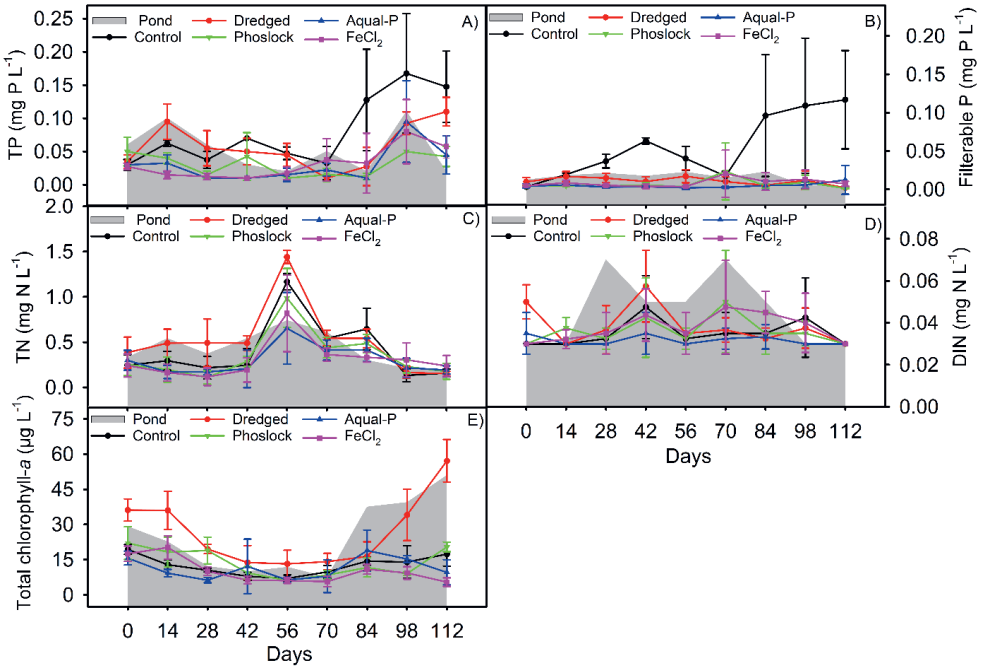

Fig. 5.2 Total and filterable nutrients (TP, FP, TN and DIN) and total chlorophyll-*a* in non-treated (Control), LMB treated (Phoslock), AMZ treated (Aqual-P), $FeCl_2$ treated ($FeCl_2$), and dredged (Dredged) enclosures during the 112 d experimental period in 2020. A) TP (mg P L^{-1}); B) FP (mg P L^{-1}); C) TN (mg N L^{-1}); D) DIN (mg N L^{-1}); E) total chlorophyll-*a* (µg L^{-1}). The

grey area represents the measurements done in the pond. Error bars indicate one standard deviation (n = 4)

The course of total chlorophyll-*a* (Chl *a*) concentrations differed per treatment and over time (Table 5.S1; Fig. 5.2E). Chl *a* concentrations in the pond and dredged enclosures showed a comparable pattern with lowest values in the middle of the experiment and a stark increase towards the end (Fig. 5.2E). Median Chl *a* concentrations were 22.6 µg L^{-1} in the pond, 21.8 µg L^{-1} in the dredged enclosures, 11.6 µg L^{-1} in LMB treatments, 11.1 µg L^{-1} in controls, 8.6 µg L^{-1} in $FeCl_2$ treatments and 8.4 µg L^{-1} in the AMZ treated enclosures (Fig. 5.2E).
Moreover, the information about filterable metals, S and Cl concentrations, and macrophytes have been shown in supplementary materials (Fig. 5.S2 and Table 5.S3).

Relationship between water quality and materials applications

In the pond itself, TP had a strong correlation with turbidity and TSS, whereas TN was negatively correlated with them (Fig. 5.3A). In controls, TP was positively correlated with FP (Fig. 5.3B). In dredged enclosures, TP was correlated with FP, whereas DIN had a strongly positive correlation with AN (Fig. 5.3C). In LMB treated enclosures, Cl concentrations had a negative relationship with TSS, Chl *a*, TP, while Fe concentrations were positively correlated among N-nutrients (Fig. 5.3D). In AMZ treatments, TSS and TN were positively correlated, as were Fe and pH, DIN and DO (Fig. 5.3E). In $FeCl_2$ treatments, Chl *a* was positively correlated with TSS and TN with FP (Fig. 5.3E). When combining all data, the PRC curves indicated a clear enclosure effect when the pond was used as reference (Fig. 5.S3A), while there was not a clear pattern when the control was used as reference (Fig. 5.S3B).

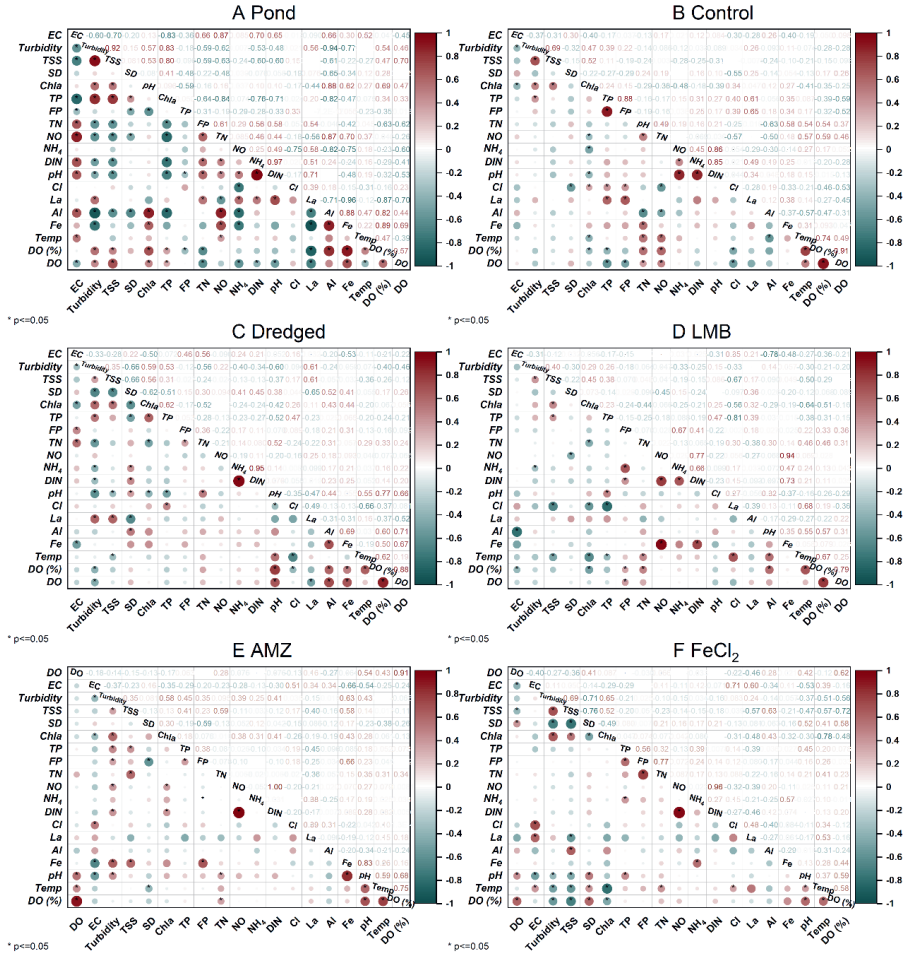

Fig. 5.3 Heatmap of correlation of physicochemical parameters of water during the 112 days of the experiment. A) Pond; B) Control; C) Dredged; D) Phoslock; E) Aqual-P; F) $FeCl_2$. The Pearson correlation coefficient varied from -1 to 1

SEM analysis was performed to outline the direct or indirect influence of the addition of materials on selected variables (turbidity, TSS, and Chl *a*) and on nutrients (TP, TN, and DIN) during the 112 days enclosure experiment. Selected variables were derived from the confirmatory factor analysis (CFA) and exploration factor analysis (EFA) in SPSS, and included turbidity, TSS and Chl *a*. Dredging had a strong positive impact on selected variables (turbidity, TSS, Chl *a*) and explained 29% of the variations in turbidity, TSS, and Chl *a*. TP was positively impacted by selected variables (turbidity, TSS, Chl *a*) and negatively affected by dredging, they together explained 19% of the variations in TP (Fig. 5.4A). TN was negatively affected by the selected variables (turbidity, TSS, Chl *a*) and positively affected by dredging, they together

explained 18% of the variations in TN (Fig. 5.4A). TP was strong negatively affected by LMB, positively affected by the variables (turbidity, TSS, Chl *a*), LMB and selected variables (turbidity, TSS, Chl *a*) together explained 28% of variance in TP (Fig. 5.4B). AMZ had a significant negative relationship with TP, while selected variables (turbidity, TSS, Chl *a*) had strong negative relationship with TN (Fig. 5.4C). Selected variables (turbidity, TSS, Chl *a*) and $FeCl_2$ also had the positive and negative pathways on TP, respectively, both of them explained 11% of variance in TP (Fig. 5.4D).

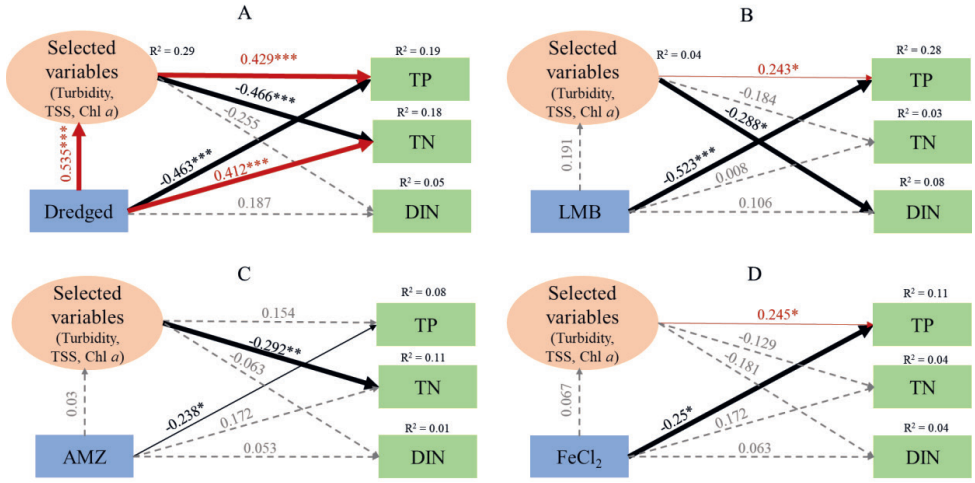

Fig. 5.4 Structural equation model exploring the relationships among materials, selected variables (turbidity, TSS, Chl *a*) and water nutrients (TP, TN, DIN). (A) dredged: $\chi^2 = 3.13$, df = 3, $\chi^2/df = 1.04$, $p = 0.37$, GFI = 0.98; B) LMB: $\chi^2 = 5.03$, df = 3, $\chi^2/df = 1.68$, $p = 0.17$, GFI = 0.97; C) AMZ: $\chi^2 = 2.4$, df = 3, $\chi^2/df = 0.8$, $p = 0.49$, GFI = 0.99; D) $FeCl_2$: $\chi^2 = 3.23$, df = 3, $\chi^2/df = 1.08$, $p = 0.38$, GFI = 0.98. Solid and dashed arrows indicate significant ($p < 0.05$) and nonsignificant ($p > 0.05$) relationships, respectively; The black and red lines represent positive and negative pathways, respectively; The arrow thickness is proportional to the strength of the relationship

Phosphorus fractions in sediment

At the end of the experiment, differences in the P fractions at three different sediment depths (0-3, 3-6 and 6-9 cm) were observed (Fig. 5.5). In the 0-3 cm layer, sediment TP was highest in the pond (Fig. 5.5). The sediment TP content in the first 3 cm of the different enclosures were significantly different ($F_{4,15} = 15.5$; $p < 0.001$). The 0-3 cm sediment TP content in control was the highest (383.78 ± 84.62 mg kg^{-1}) and in dredged it was the lowest (98.96 ± 26 mg kg^{-1}). TP content did not show a difference between AMZ and $FeCl_2$ (Tukey Test, $p = 0.997$). The sediment TP in the sediment depth of 3 to 6 cm among enclosures was significantly different ($F_{4, 15} = 15.5$; $p < 0.001$). Sediment TP content in $FeCl_2$ (190.18 ±16.38 mg kg^{-1}) in the sediment

depth of 3 to 6 cm was higher than in other enclosures ($p < 0.001$). The sediment TP concentration in the sediment depth of 6-9 cm did not indicate a difference among the treatments ($F_{4, 15} = 15.5$; $p = 0.373$). According to statistical output, mobile-P concentrations differed significantly among enclosure in all the sediment depth analyzed, 0-3 cm ($F_{4, 15} = 27.4$; $p < 0.001$), 3-6 cm ($F_{4, 15} = 200.5$; $p < 0.001$), 6-9 cm ($F_{4, 15} = 13.3$; $p < 0.001$). In comparison with controls, the content of mobile P in the first 9 cm of sediment was reduced by 71.4%, 60.2%, 38% and 5.2% in dredged, AMZ and LMB and $FeCl_2$, respectively. In the first sediment layer (0-3 cm), mobile P fractions such as organic-P and Fe/Mn-P were dominating the overall sediment P pool in controls and $FeCl_2$, contributing on average 26.6% and 50% to the sediment TP, respectively (Fig. 5.5). In LMB and AMZ groups, immobile-P fractions comprised most of the sediment P in the 0-3 cm layer, where for instance the Ca-P fraction (a fraction that also includes La-P) contributed on average 41.7% to the sediment TP in LMB treated sediment and 31.7% to the sediment TP in AMZ treatments (Fig. 5.5).

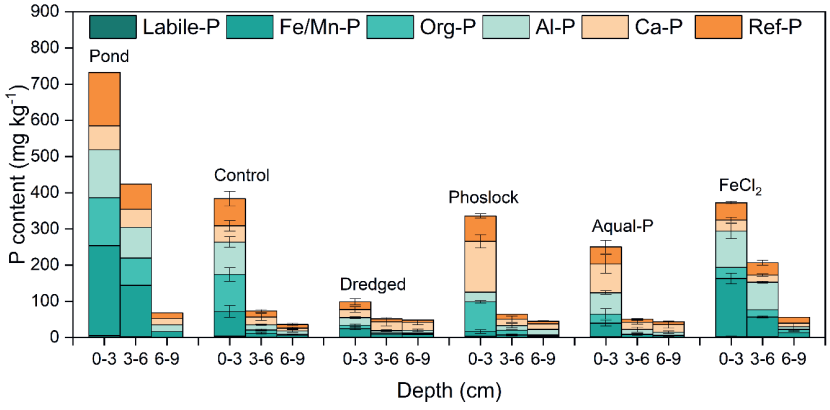

Fig. 5.5 Characteristics of sediments P fractions (0-3 cm, 3-6 cm and 6-9 cm) in the pond and each enclosure at the end of the experiment. Error bars indicate one standard deviation (n = 4)

Mössbauer Spectroscopy

The obtained Mössbauer spectra (Table 5.S4; Fig. 5.S4) did not detect vivianite in response to the $FeCl_2$-injection or in the control sediment (Fig. 5.S4). None of the Mössbauer spectra of the sediment samples present the characteristic doublets of vivianite (Doublet 1: Isomer Shift (IS) = 1.2±0.1 mm/s, Quadrupole Splitting (QS) = 2.4±0.1 mm/s and Doublet 2: IS = 1.25±0.1 mm/s, QS = 3.0±0.1 mm/s (McCammon and Burns, 1980) indicating the absence of this mineral in our samples. Moreover, Fe^{3+} accounts for 65-90% of the total iron (Table 5.S4).

Sediment core incubation

The pH had increased by 0.5 unit in all treatments on day 7 and then remained stable within pH 7.0 - 7.4 during the subsequent experiment duration (Fig. 5.S5). Addition of LMB, AMZ or $FeCl_2$ significantly increased EC (Table 5.S5), EC showed the strongest increase in AMZ treatments (Fig. 5.S5). All sediment cores had higher initial DO and DO saturation, from 4.33 (±3.15) mg L^{-1} in dredged to 7.63 (±1.23) mg L^{-1} in AMZ and from 39 (±27.8)% in dredged to 69.5 (±12.4)% in AMZ. Afterwards, DO concentration decreased due to the N_2 bubbling at each sample timepoint (Fig. 5.S5). In the untreated cores (controls) and cores from the dredged area, FP concentrations increased over time. In contrast, in the LMB, AMZ or $FeCl_2$ treated cores, FP reduced to below detection limits (4 µg P L^{-1}) and remained low throughout the experiment (Fig. 5.S5). A rmANOVA revealed a statistically significant difference in FP between the treatments over time ($F_{16, 60} = 24.142$; $p < 0.001$). FP concentrations after LMB, AMZ and $FeCl_2$ treatment reduced strongly (Fig. 5.S5). A post-hoc comparison based on FP concentrations revealed two homogeneous groups: 1) controls and dredged; 2) LMB, AMZ and $FeCl_2$ treated cores. The rmANOVAs showed statistically significant differences in NH_4^+-N (Ammonium Nitrogen, AN), and NO_2-N + NO_3-N concentrations (Table 5.S5). NO_2-N + NO_3-N concentrations in all treatments gradually decreased (Fig. 5.S5). AN concentrations gradually increased in control, dredged and LMB treated cores, which was the strongest in the LMB treatments. In contrast, AN concentrations in AMZ and $FeCl_2$ treated cores remained similar during the experiment, but overall AN concentrations in the AMZ treatments were the lowest (Fig. 5.S5). Positive FP fluxes were found in controls and dredged cores, while negative fluxes were measured in LMB, AMZ and $FeCl_2$ treated cores (Fig. 5.6). DIN release rate in LMB was much higher than in controls, while in AMZ and $FeCl_2$ treated cores the DIN release was inhibited (Fig. 5.6).

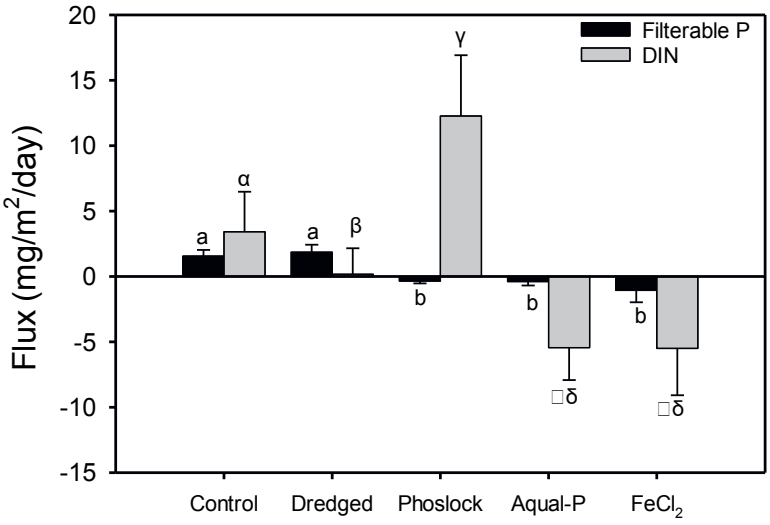

Fig. 5.6 Flux of the Filterable P and DIN of Control, Dredged, Phoslock, Aqual-P and $FeCl_2$ during 28 days (mg m^{-2} d^{-1}). Error bars indicate one standard deviation (n = 4). Different symbols per column (a, b; α, β, γ, δ) indicate groups that are significantly different

LMB application increased filterable La content within the first 7 days, after that it gradually reduced over time ($F_{4.954,\ 18.576}$ = 15.713; p = 0.003) (Fig. 5.S5). Based on the calculation, the average filterable La flux during 28 days yielded 0.26 mg La m^{-2} day^{-1}. Filterable Al, Fe, and Mn did not differ among treatments (Table 5.S5). Filterable S concentration seemed to be slightly higher in the cores treated with Aqual-P: A Greenhouse-Geisser corrected rmANOVA followed by a Tukey post hoc test revealed a significant difference between controls and Aqual-P treatments over time ($F_{10.727,\ 40.228}$ = 2.129; p = 0.003) (Fig. 5.S5).

Discussion

This enclosure study tested the hypothesis that the four sediment nutrient release abatement measures (dredging, LMB addition, AMZ addition, and Fe(II) injection in the sediment) would improve water quality compared to untreated controls. Clear differences were observed between treatments and controls where for instance compared to controls water column TP was reduced by ~ 36% in the dredged enclosure and ~ 66% in LMB, AMZ and $FeCl_2$ treated ones.

Nutrient concentrations remained higher in the dredged enclosure than in other treatments and chlorophyll-*a* concentrations were equal to the pond and even higher than in control enclosures. Variable effects of dredging in lake restoration have been attributed to limited and insufficient sediment removal, no external load control (Peterson, 1982), or uncovering of organic matter and nutrient-rich layers (Geurts et al., 2010). Although the excavator used removed ~20 cm of

sediment from a site larger than where enclosures were placed and the presence of some remaining sediment cannot be excluded, the course of the phosphate concentrations in each enclosure does not point towards such an effect as a pattern comparable to controls would have occurred (Lürling and Faassen, 2012). The external load was assumed to be equal for all enclosures, while the fresh accumulated nutrient rich layer seems also not to have played a role as the sedimentary P-content in the dredged enclosures was strongly reduced compared to non-dredged enclosures (*see* Fig. 5.5). Despite this strongly reduced P content, TP in the water column was not that strongly reduced as in other treatments and the sediment core incubation revealed the P flux was not reduced at all (*see* Fig. 5.6), which is opposing findings of others (Oldenborg and Steinman, 2019; Yin et al., 2021; Yu et al., 2017). Possibly a rapid mobilization of releasable P combined with the relatively short duration of the experiment (4 weeks) resulted in a similar P flux as in the controls. The N flux was, however, sharply reduced in line with the removal of nutrient-rich sediment.

In other enclosure studies, dredging strongly improved water quality compared to controls, reduced phytoplankton biomass (Lürling and Faassen, 2012; Zhan et al., 2022) and increased transparency (Lürling et al., 2017; Zhan et al., 2022). The somewhat deviating results in this study may be a result of indirect effects caused by dredging. A short-term response to sediment disturbance due to denitrification and N_2O production were stimulated shortly after dredging (Salk et al., 2018). In the dredged enclosures, macrophytes were either absent or present in lower abundance compared to the other enclosures and potentially a large part of the seed bank got removed allowing phytoplankton to take up nutrients. Inasmuch as clear water with submerged macrophytes is the desired state in shallow waters (Scheffer et al., 1993), in the case when a large part of the seed bank has been removed, the introduction of macrophytes after dredging may be considered to improve water quality (Waajen et al., 2016), even if the re-establishment of these communities takes time (Hassett and Steinman, 2022).

While dredging only partly met the expectations of improved water quality, the results of LMB, AMZ and $FeCl_2$ treatments were more in line with expectations. The sediment core incubation experiment revealed a negative P flux for LMB, AMZ and $FeCl_2$ treated cores, which implies P was effectively removed from the water and kept in the sediment. These findings are in line with previous studies(e.g. (de Magalhães et al., 2019; Gibbs et al., 2011; Zeller and Alperin, 2021; Zhan et al., 2021)). The active ingredient lanthanum in LMB precipitates with phosphate, forming a stable mineral that is not affected by natural pH and redox fluctuations, unlike other aluminium (Al) or iron (Fe)-based phosphate binders (Copetti et al., 2016; Dithmer et al., 2016). SEM modeling indicated a negative impact of LMB on TP and the sediment core experiment

showed a negative P flux. Moreover, the HCl extractable P pool was significantly larger in the LMB amended sediment, which is also the fraction in which most of the La-P is found (Reitzel et al., 2013b; Yin et al., 2021).

The sediment core experiment revealed a strongly increased DIN flux in LMB treated cores, which was attributed to NH_4^+-N. Similar observations have been made in other studies, where adding LMB increased NH_4-N concentrations (e.g. (Reitzel et al., 2013b; Zeller and Alperin, 2021)), which might be caused by NH_4^+-N leaching from the product (van Oosterhout and Lürling, 2013) and concentrated in a rather small volume of overstanding water. LMB did not change the bacterial community composition or *Proteobacteria* responsible for N-cycling (Yin et al., 2021). In the enclosures, no increase of DIN or TN in LMB treatments was observed, which is in line with other enclosure studies (Lürling and Faassen, 2012; Zhan et al., 2022). Hence, no impact from the leached NH_4^+-N is expected. Filterable lanthanum concentrations were elevated in the LMB treated enclosures and showed a tendency to decline gradually over time, which is in line with other studies of whole lake LMB treatments (Spears et al., 2013b; van Oosterhout et al., 2020). LMB has been tested extensively and no harmful effects on aquatic life have been found (Copetti et al., 2016; van Oosterhout et al., 2020).

AMZ, formerly known as Z2G1, was highly effective in hindering the P release in sediment cores collected in Lake Okaro, New Zealand (Gibbs and Özkundakci, 2011), and in Lake Rotorua, New Zealand (Gibbs et al., 2011).

AMZ is designed as a sediment capping agent and besides the modification with poly-aluminium chloride, which gives it its P sorption capacity, the zeolite carrier has a natural affinity for NH_4-N (Gibbs and Hickey, 2018). The sediment core experiment revealed a negative DIN flux indicating the capacity of AMZ to bring DIN to the sediment and prevent release. A reduced NH_4-N release was also observed in sediment cores from Lake Okaro (Gibbs et al., 2011) and in another study, AMZ was estimated to absorb NH_4-N ~4.5 mg NH_4-N g^{-1} AMZ (Gibbs and Özkundakci, 2011). AMZ resulted in elevated Al and Cl^- concentrations, but these were within an acceptable range and AMZ is not expected to cause toxic effects in aquatic biota (Gibbs and Hickey, 2018).

In contrast to LMB, experiments with $FeCl_2$ are relatively rare. Smolders et al., 2001 found that the addition of $FeCl_2$ strongly decreased the phosphate concentrations in sediment pore water. Likewise, in enclosures, $FeCl_2$ treatment caused very low porewater sulfide and phosphate concentrations, low water column phosphate and low turbidity (Smolders et al., 1995). Phosphate was likely precipitated with oxidized Fe(III) formed in the enclosures, a similar explanation was given for the effectiveness of $FeSO_4$ addition to the inlet water of De Grote

Rug reservoir, The Netherlands (Oskam, 1983). Probably the same occurred in our $FeCl_2$ treated enclosures. Despite $FeCl_2$ being injected in the sediment, it diffused out of the sediment causing a reddish turbid water after two weeks and gradually increased filterable Fe concentrations and caused elevated Cl concentrations in the overstanding enclosure water.

The formation of ferric iron oxi/hydroxides may explain the low P concentrations in the enclosures. In the sediment core experiment, cores were made anoxic prior to injecting $FeCl_2$ in the sediment and the low oxygen concentrations during the experiment likely prevented oxidation of introduced iron. Nonetheless, P flux was negative implying P was moved from the overstanding water into the sediment. The main reason for injecting an overdose of $FeCl_2$ directly in the sediment was to scavenge sulfide in porewater and therewith facilitate the formation of solid-phase reduced iron-phosphate compounds, such as reduced-iron-oxi/hydroxide-phosphate complexes and vivianite (Roden and Edmonds, 1997). Vivianite precipitation is favored by high concentrations of ferrous iron in pore water (Rothe et al., 2014). In addition, iron-sulfides may form a protective layer around ferric iron-oxi/hydroxide particles (Davison and Dickson, 1984), but the abundance of ferrous iron may also have favored nitrate-dependent Fe^{2+} oxidation (Weber et al., 2006) that may subsequently have led to production of ferric iron (hydr)oxides such as goethite (Senko et al., 2005) which may adsorb phosphate (Parfitt and Atkinson, 1976) and subsequently settle down to the sediment. The increased reductive labile P pool in $FeCl_2$ amended sediments may point to this process, while the larger pool of NaOH extractable SRP (Al-P) may point to Fe(II)-phosphate minerals such as vivianite (Rothe et al., 2015). Mössbauer spectroscopy did not yield evidence for presence of (detectable amounts of) vivianite. Mössbauer spectroscopy indicated the vast majority of iron being present as Fe^{3+} and thus precipitation of diverse Fe-oxides (Fe(III)(oxyhydr)oxides) is favored. That vivianite was not detected in our study could therefore be due to insufficient Fe^{2+}-availability for authigenic vivianite formation. In addition, the mineral's purity and crystallinity may have played a role as the vivianite crystal structure is highly sensitive to oxidation. An advanced oxidation status makes vivianite amorphous, consequently, hampering its detection and analysis (Grodzicki and Amthauer, 2000; McCammon and Burns, 1980; Rouzies and Millet, 1993; Wilfert et al., 2016). We did, however, not measure the oxygen state in sediment. The direct mineral detection via Mössbauer may also have been not sensitive enough for the sediment samples analysed. Mössbauer spectroscopy has a precision of 3%, yet Fe-P mineral phases only make up a very small fraction of the total sediment matrix. In the sediment of the pond the Fe-concentration was < 1% of the DW (6.5 mg g DW^{-1}).

Consequently, adjustments to improve the sensitivity of the detection methods could be

considered. To improve vivianite detection via Mössbauer in sediments, Rothe et al., 2016 suggested high-density heavy-liquid separation during sample processing to enrich possibly formed vivianite in the high-density fraction of the sediment.

pH showed considerable variability and could reach maximum values of pH 9.1 and pH 9.5 in dredged and $FeCl_2$ treatments, pH 10 in LMB and controls or even pH 10.3 in AMZ treated enclosures. Such pH may have an effect on P binding and ligand exchange may promote the release of phosphate from iron and aluminium complexes (Boers, 1991). The elevated pH was a result of photosynthetic activity by plants and filamentous algae growing in the enclosures that may indicate a potential risk of using pH-sensitive P binders such as AMZ, aluminium and iron-based products. At pH 10 AMZ desorbed ~39% of its adsorbed P, alum ~71% of its adsorbed P, while LMB did not desorb any of the P precipitated (Kang et al., 2022b). Hence, in shallow surface waters in which clear water and submerged macrophytes are the preferred states, sediment P mobilization by photosynthetically driven high pH (Welch and Cooke, 2005) may limit the options of sediment P release to dredging or use of LMB.

A clear enclosure effect was observed causing the controls to deviate from the pond. Enclosing a small volume of water creates some artifacts such as the limitation of external nutrient loading and the exclusion of wind and fish effects, whereas a relatively large wall may support a community of otherwise far less abundant organisms. The experiment provides valuable insights into sediment nutrient release and highlights the promotion of submerged macrophyte growth with reduced turbulence. Stable weather conditions in spring may contribute to macrophyte development, while more turbulent conditions may favor a phytoplankton-dominated state. The presence of cyanobacterial blooms during turbid conditions was a key factor in conducting the enclosure experiment.. This enclosure experiment revealed that all four treatments could improve the water quality of the Bouvigne pond. Treating this pond of 14,344 m^2 with LMB as dosed in the enclosures (650 g m^{-2}) would imply a treatment cost of around € 30,000, for AMZ (837 g m^{-2}) it would be ~€40,000, and removing 20 cm from the entire pond by dredging would come to ~€ 130,000. Despite the product price of $FeCl_2$ being a few times less per ton than LMB or AMZ, it is difficult to make an estimate of treatment cost as $FeCl_2$ injection into the sediment requires specific equipment (Schütz et al., 2017; Wiśniewski et al., 2010). A system analysis of the pond including the results of this study, insight in the external nutrient loading and the required loading reductions for water quality improvement can guide the water manager to an effective set of executable restoration measures. Based on the results and estimated costs, LMB treatment would appear the first choice to reduce the internal loading

of the pond. The water manager decided to dredge the pond as part of maintenance and restoration of the water system, including the realization of a desired increase in water depth. After the restoration of the pond, monitoring of water quality development is essential and if the improvement falls short of expectations, the introduction of macrophytes and an additional polishing step with LMB can be considered.

Conclusion

- The addition of AMZ, $FeCl_2$ or LMB resulted in a stronger improved water quality than dredging did in an enclosure experiment lasting 112 days.
- A strong enclosure effect was noted promoting clear water and submerged macrophytes in controls, but no macrophytes were observed in dredged enclosures.
- The amount of total P as well as potentially releasable P was strongly reduced by dredging. In LMB amendments the "Ca-P" pool was enlarged reflecting $LaPO_4$ formation, in AMZ treated sediment the "Al-P" (NaOH-SRP) pool was enlarged indicating more metal-oxide adsorbed P, while in $FeCl_2$ treatments the reductive labile P pool was enlarged reflecting more iron-oxi/hydroxide adsorbed P.
- In the sediment core experiment, P fluxes were negative for AMZ, $FeCl_2$ and LMB treatments, but positive for controls and cores from the dredged area. Negative DIN fluxes were found in AMZ and $FeCl_2$ treated cores, while LMB caused a positive DIN flux. The latter was not observed in the enclosures and probably is due to leaching from the product itself.
- AMZ and $FeCl_2$ increased chloride concentrations and therewith conductivity, LMB led to higher filterable lanthanum concentrations, but all at acceptable levels. The $FeCl_2$ injection did not keep the material in the sediment leading to temporarily turbid water (ferric iron oxi/hydroxide complexes).
- In restoring the Bouvigne pond, a combination of dredging followed by a polishing step with LMB treatments and planting of selected macrophytes could be considered as a meaningful follow-up of the intended deepening of the pond in case water quality improvements are insufficient.

Acknowledgments

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Supplementary information

List:

- S1: Fe-dosage calculations
- S2:Vivianite detection: Mössbauer spectroscopy
- S3: Macrophyte
- S4: Filterable metals, S and Cl concentrations
- S5: Figures
- S6: Tables
- S7: References

S1: Fe-dosage calculations

The following Fe-dosage calculation, slightly modified after (Kleeberg et al., 2013), was applied for both, lab and field experiments, and considers bioavailable P in the first 6 cm of the sediment:

$$[Fe]dosage = 1.5 \times [P]releasable + [Fe]overdose + [Fe - S] + [Fe - OC] - [Fe]sed$$

1. 1.5 * $[P]_{releasable}$ = amount of Fe needed for stochiometric vivianite formation ($Fe:P_{vivianite}$ = 1.5 (Rothe et al., 2015)), relative to the total amount of sediment to be treated
2. $[Fe]_{overdose}$ = overdose amount of Fe^{2+}, equal to the first term but multiplied by a factor depending on the sedimentary composition and mass balance calculations, factor 1 was chosen in this study
3. [Fe-S] = equivalent for Fe^{2+} theoretically lost to precipitation with S present in the sediment
4. [Fe-OC] = equivalent for Fe^{2+} theoretically lost to OC-complexation = 0.2 * 0.5 $OM_{sediment}$ (Kleeberg et al., 2013) suggest that around 20% of the available Fe is complexed by OC and $OC_{sediment}$ = 0.5 $OM_{sediment}$)
5. $[Fe]_{sed}$ = amount of Fe already present in the sediment

S2: Filterable metals, S and Cl concentrations

Filterable La concentrations in LMB treated enclosures and filterable Al concentrations in AMZ treated enclosures were significantly higher than the concentrations measured in the control and the other treatments (Table S1 and S2; Figs. S2A, S2B). Filterable Fe showed a tendency to increase over time in $FeCl_2$ treatments (Fig. S2C; Table S2). Filterable Mn (Fig. S2D) and filterable S (Fig. S2E) were similar among treatments (Table S1 and S2).

The Cl concentrations increased significantly after the addition of AMZ or $FeCl_2$ (Table S1 and 2; Fig. S2F). Moreover, Cl levels after dredging were very close to the concentrations measured in the pond and LMB treatments showed Cl contents very close to the controls (Fig. S2F). The mean Cl concentrations in $FeCl_2$ treatments were 200% higher than that found in the pond, while Cl concentrations in AMZ treatments were 36% higher than in the pond (Fig. S2F).

S3: Macrophyte

Macrophyte observation revealed a distinct pattern at different enclosure cores over time (Table S3). Low abundance or even no macrophytes were found in dredged, AMZ and $FeCl_2$ treated enclosures, while higher macrophytes abundances were found in control and LMB treated

enclosures. Only two species of macrophytes were found (most likely *Elodea nuttallii* and *Potamogeton pectinatus*). In two control enclosures filamentous algae were abundantly present hampering reliable observation of macrophytes (Table S3).

S4: Vivianite detection: Mössbauer spectroscopy

Fresh sediment was dried under dark, atmospheric conditions (24 hours at 25°C) and analyzed for vivianite occurrence using Mössbauer spectroscopy. The maximum amount of dry sediment for one measurement was used (~0.5 g) due to the relatively low Fe-concentration (6.5 mg g DW^{-1}; < 5% of the DW). The dry sediment was transferred in plastic rings (2 cm in diameter, 0.3 cm in height) and wrapped in a γ-ray transparent foil. Transmission ^{57}Fe Mössbauer spectra were collected at 300 K (26.85°C) with conventional constant-acceleration and sinusoidal velocity spectrometers using a ^{57}Co(Rh) source. The obtained Mössbauer was fitted using Mosswinn 4.0.

S5: Figures

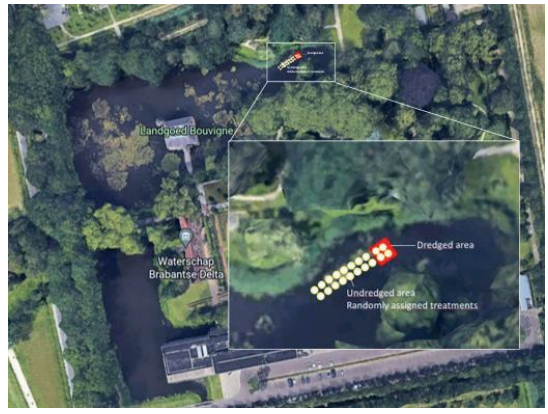


Fig. 5.S1 Enclosure cores set-up

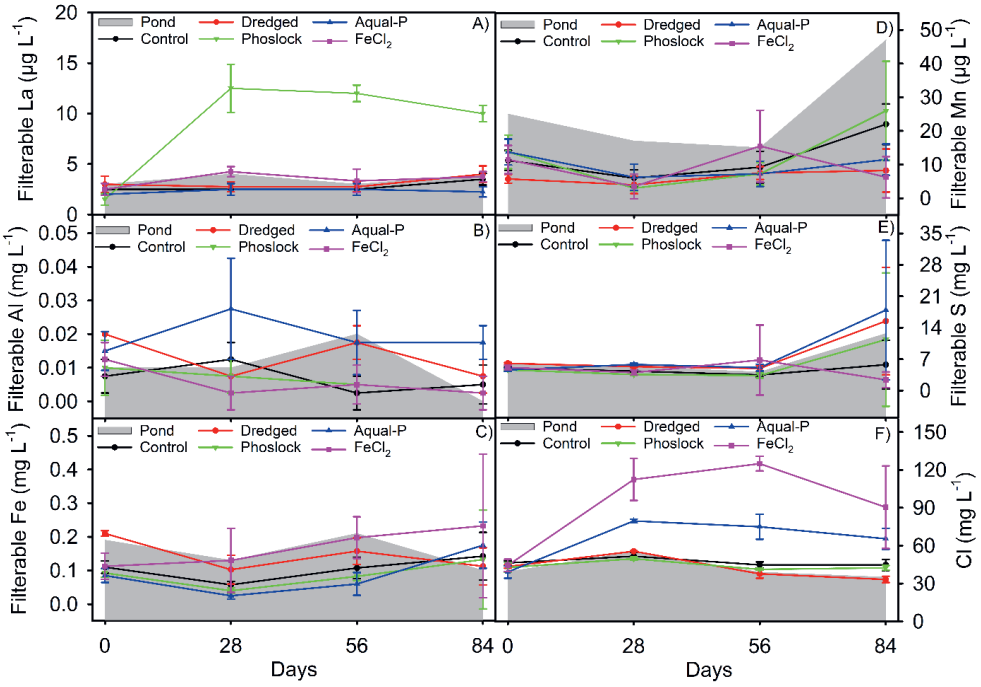


Fig. 5.S2 Filterable metal (La, Al, Fe, Mn), S and Cl concentrations in non-treated (Control), LMB treated (Phoslock), AMZ treated (Aqual-P), $FeCl_2$ treated ($FeCl_2$), and dredged (Dredged) enclosures during the 112 d experimental period in 2020. A) Filterable La (µg L^{-1}); B) Filterable Al (mg L^{-1}); C) Filterable Fe (mg L^{-1}); D) Filterable Mn (µg L^{-1}); E) Filterable S (mg L^{-1}); F) Cl (mg L^{-1}). The grey area represents the measurements done in the pond. Error bars indicate one standard deviation (n = 4)

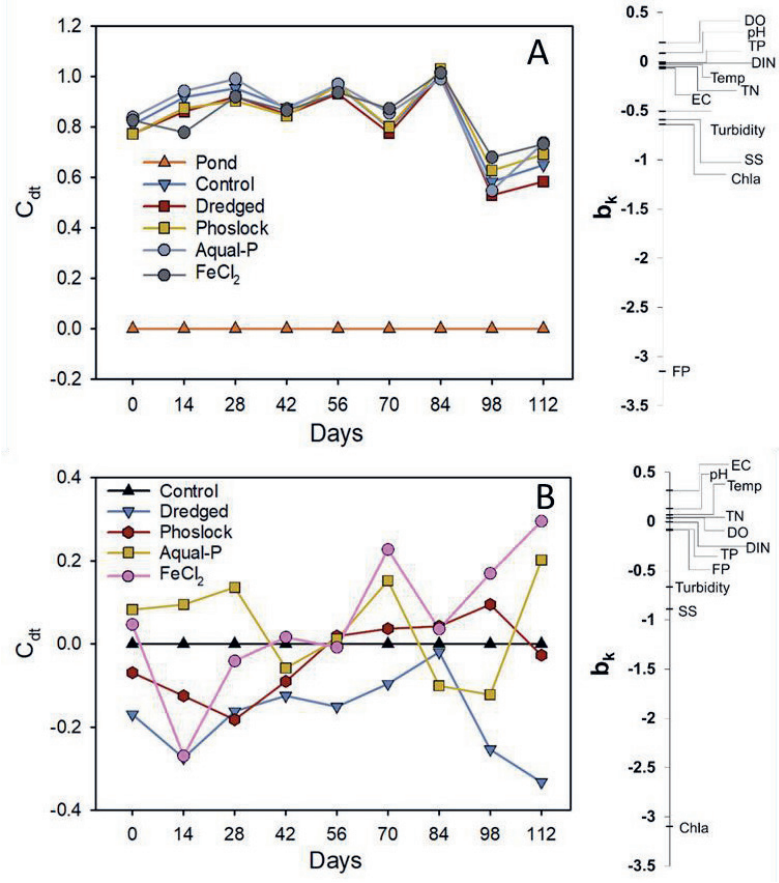


Fig. 5.S3 Principal response curves for all water quality variables, where differences between treatments (enclosures) and the reference (A: the pond; B: the control) are given as the regression coefficients (C_{dt}) of the PRC model. The variable's weight (b_k) in the right vertical diagram indicates the position of the variable on the first PRC axis, in which a negative value indicates a treatment-related increase and a positive value a treatment-related decrease

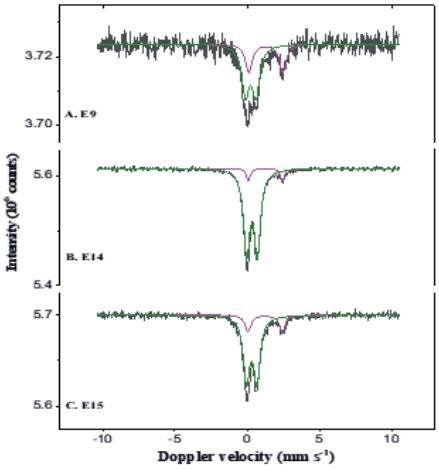
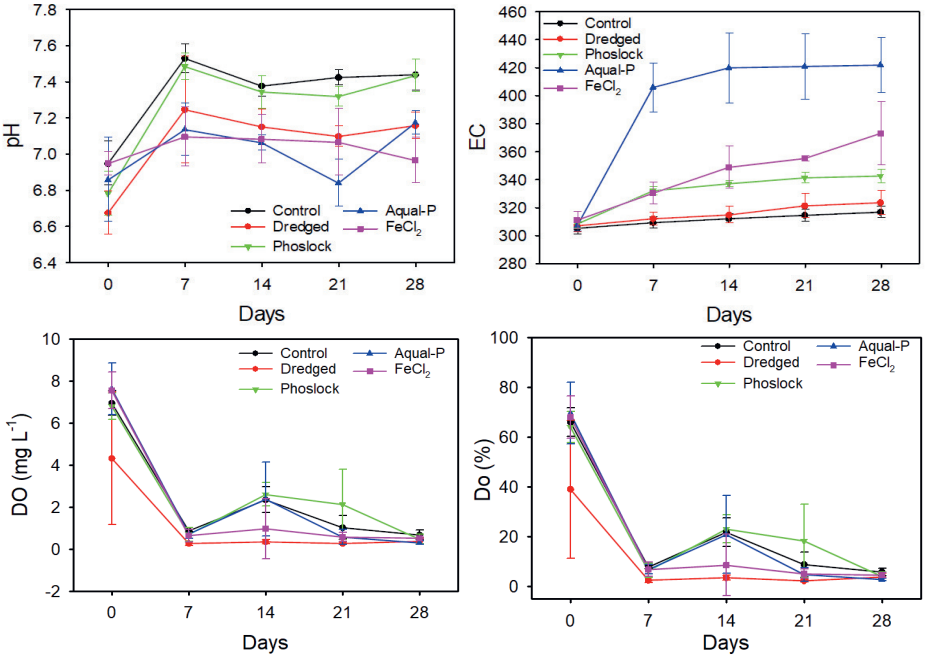


Fig. 5.S4 Mössbauer spectra for enclosures (E9/14/15). Both of E9 and E14 were Fe (II)-treated enclosures, E15 was control enclosure. The black curve is the total signal, the green one is the Fe^{3+} (Viv. I+II) contribution and the pink is the Fe^{2+} (Viv. I+II). Vivianite has two Fe^{2+}-sites: The doublet 1 (Fe^{2+} (Viv. I) and doublet 2 (Fe^{2+} (Viv. II) (Grodzicki and Amthauer, 2000). Fe-oxidation takes preferably place at the B site, but also at doublet 1 when doublet 2 is oxidized (Grodzicki and Amthauer, 2000; McCammon and Burns, 1980). Fe^{3+} (Viv. I+II) represents the fraction of oxidized Fe at both sites but does not distinguish other Fe^{3+}-species. Mössbauer theoretical parameters for vivianite: doublet 1: Isomer shift (IS) = 1.2 ± 0.1 mm s^{-1}, quadrupole splitting (QS) = 2.4 ± 0.1 mm s^{-1}; doublet 2: IS = 1.25 ± 0.1, QS = 3.0 ± 0.1 mm s^{-1}



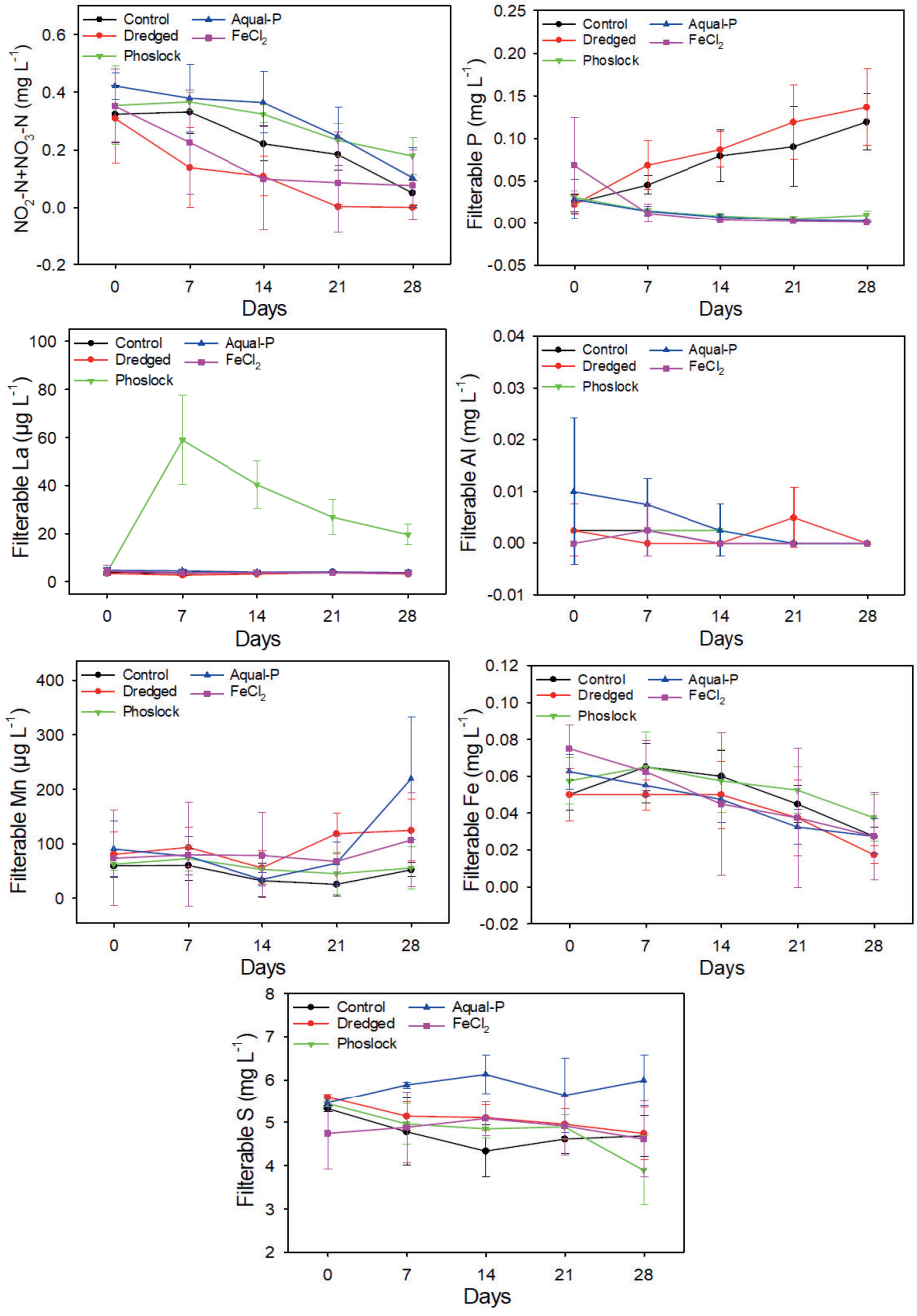


Fig. 5.S5 Physicochemical parameters of water in sediment incubation experiment (28 days)

S6: Tables

Table 5.S1. Results of repeated-measures analysis of variance (rmANOVA) for water quality variables, nutrients and Chl *a*, dissolved metals (La, Al, Fe and Mn), S and Cl during the experiment ($^{**}p < 0.01$; $^{*}p < 0.05$; n.s. means no significance)

Water quality variables																	
	df	Temp		DO (mg L^{-1})		DO (%)		pH		EC		Turbidity		TSS		Secchi depth	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Groups	4	19.1	n.s.	78.7	**	9.3	*	21.1	**	19.9	**	21.9	**	1	n.s.	4.5	*
Time (T)	8	482	**	67.5	**	46	**	41.7	**	194.3	**	37	**	57.1	**	8.9	*
T×Control	8	175	**	7.1	**	18.8	**	13.9	**	5.4	*	2.5	n.s.	4.2	*	0.1	n.s.
T×Dredged	8	64.3	**	64.1	**	9.9	*	3.0	n.s.	5	*	2.5	n.s.	4.6	*	8.6	*
T×LMB	8	70	**	22.3	**	6.7	*	3.5	**	20.6	**	2.5	n.s.	38.6	**	1.4	n.s.
T×AMZ	8	126.4	**	8.6	**	12.8	*	37.2	**	59.2	**	5.1	*	16.6	**	0.1	n.s.
T× $FeCl_2$	8	106.8	**	9.2	**	13.7	*	6.9	**	248.5	**	71.2	**	13.3	*	11.7	n.s.

Nutrients and Chl *a*													
	df	TP		FP		TN		NO_2-N and NO_3-N		AN		Chl *a*	
		F	p	F	p	F	p	F	p	F	p	F	p
Groups	4	11.9	**	10.3	**	1.1	n.s.	1.3	n.s.	2.7	n.s.	184.5	**
Time (T)	8	21.58	**	14.3	*	22.1	**	3.1	n.s.	5.2	*	67.5	**
T×Control	8	10.3	*	47.5	**	6.4	*	1.1	n.s.	0.6	n.s.	7.1	**
T×Dredged	8	10.6	*	4.37	*	10.8	*	0.2	n.s.	8.1	*	64.1	**
T×LMB	8	8.4	*	0.7	n.s.	5.1	*	4.6	*	1	n.s.	22.3	**
T×AMZ	8	4.9	*	0.8	n.s.	1.3	n.s.	0.6	n.s.	0.05	n.s.	8.6	**
T×$FeCl_2$	8	1.3	n.s.	1.4	n.s.	5.1	*	1.9	n.s.	2.4	n.s.	9.2	**

Metals										Others			
	df	La		Al		Fe		Mn		S		Cl	
		F	p	F	p	F	p	F	p	F	p	F	p
Groups	4	150.2	**	7.3	*	2.6	n.s.	1.1	**	7.0	**	16.6	**
Time (T)	3	45	**	4.7	*	11.4	*	8.9	**	3.9	*	135.5	**

T×Control	3	2.1	n.s.	2.4	n.s.	2.4	n.s.	0.9	n.s.	8.2	**	7.7	*
T×Dredged	3	2.9	n.s.	7.2	*	12.8	*	3.1	n.s.	3.4	*	17.1	**
T×LMB	3	153.4	**	2.3	n.s.	2.6	n.s.	0.5	n.s.	5.2	*	13.7	*
T×AMZ	3	0.4	n.s.	2.4	n.s.	4.3	*	12.2	**	4	*	42.5	**
T×$FeCl_2$	3	1.9	n.s.	3.5	*	5.7	*	1.6	n.s.	1.2	n.s.	148.9	**

Table 5.S2. The results of non-parametric Kruskal–Wallis tests for water quality variables, nutrients and Chl *a*, dissolved metals (La, Al, Fe and Mn), S and Cl. Different symbols per column (a, b, c) indicate groups that are significantly different (Tukey post-hoc comparison, $p < 0.05$)

Water quality variables								
Groups	Temp	DO (mg L^{-1})	DO (%)	pH	EC	Turbidity	TSS	Secchi depth
Control	a	bc	a	a	c	a	a	a
Dredged	a	a	a	c	b	a	a	a
Phoslock	a	b	b	b	c	a	a	b
Aqual-P	b	c	b	b	b	a	a	b
$FeCl_2$	b	c	a	a	a	b	a	b

Nutrients and Chl *a*						
Groups	TP	FP	TN	NO_2-N and NO_3-N	AN	Chl *a*
Control	a	a	a	a	a	bc
Dredged	b	b	a	a	a	a
Phoslock	bc	b	a	a	a	b
Aqual-P	bc	b	a	a	a	c
$FeCl_2$	bc	b	a	a	a	c

Metals					Others	
Groups	La	Al	Fe	Mn	S	Cl
Control	b	b	ab	a	a	c
Dredged	b	b	a	a	a	c
Phoslock	a	b	b	a	a	c
Aqual-P	b	a	b	a	a	b
$FeCl_2$	b	b	a	a	a	a

Table 5.S3 Macrophytes observation in enclosure cores at day 70, day 84, day 98 and day 112. Not visible indicated that filamentous algae cover the surface. 2 species were most probably *Elodea nuttallii* and *Potamogeton pectinatus*

Date	17/06/2020	01/07/2020	15/07/2020	29/07/2020
Day	Day 70	Day 84	Day 98	Day 112
Control 1	Yes (low abundance)	Yes (low abundance at bottom)	Yes (low abundance at bottom)	Yes (high abundance)
Control 2	Yes (low abundance)	Yes (high abundance)	Yes (high abundance)	Yes (high abundance)
Control 3	Yes (very low abundance)	Not visible	Not visible	Not visible
Control 4	No	Not visible	Not visible	Not visible
Dredged 1	No	No	No	No
Dredged 2	No	No	No	No
Dredged 3	Yes (low abundance)	Yes (low abundance)	Yes (low abundance)	Yes (low abundance)
Dredged 4	No	Yes (low abundance)	Yes (low abundance)	Yes (low abundance)
LMB 1	Yes (low abundance)	Not visible	Not visible	Not visible
LMB 2	Yes (high abundance, reach surface)	Yes (high abundance, 2 species)	Yes (high abundance, 2 species)	Yes (high abundance, 2 species)
LMB 3	Yes (high abundance)	Yes (high abundance, 2 species)	Yes (high abundance, 2 species)	Yes (high abundance)
LMB 4	Yes	Yes (high abundance)	Yes (high abundance)	Yes (high abundance)
AMZ 1	Yes (high abundance)	Yes (high abundance)	Yes (high abundance)	Yes (high abundance)
AMZ 2	No	Yes (low abundance)	Yes (low abundance)	Not visible
AMZ 3	No	No	No	Yes (low abundance)
AMZ 4	Yes	Yes (low abundance)	Yes (low abundance)	Yes (low abundance)
$FeCl_2$ 1	Yes (high abundance, reach surface)	Yes (high abundance)	Yes (high abundance)	Yes (high abundance)
$FeCl_2$ 2	Yes (low abundance)	Yes (low abundance, very clear)	Yes (low abundance, very clear)	Yes (low abundance)
$FeCl_2$ 3	No	No (very clear water)	Yes (low abundance)	Yes (low abundance)
$FeCl_2$ 4	No	Yes (low abundance)	Yes (low abundance)	Yes (low abundance)

Table 5.S4 The Mössbauer fitted parameters of enclosure field samples*. Both of E9 and E14 wereFe (II)-treated enclosures, E15 was control enclosure

Sample	IS ($mm \cdot s^{-1}$)	QS ($mm \cdot s^{-1}$)	Γ ($mm \cdot s^{-1}$)	Phase	Spectral contribution (%)
E9 (Fe)	0.25	0.72	0.63	Fe^{3+}/Fe^{II}	66
	1.29	2.37	0.56	Fe^{2+}	34
E14 (Fe)	0.34	0.74	0.52	Fe^{3+}/Fe^{II}	91
	1.27	2.37	0.34	Fe^{2+}	9
E15 (Control)	0.32	0.72	0.54	Fe^{3+}/Fe^{II}	81
	1.25	2.37	0.49	Fe^{2+}	19

*Experimental uncertainties: Isomer shift: I.S. ± 0.01 mm s^{-1}; Quadrupole splitting: Q.S. ± 0.01 mm s^{-1}; Line width: Γ ± 0.01 mm s^{-1}; Spectral contribution: ± 3%.

Table 5.S5 Results of repeated measures analysis of variance (rmANOVA) for sediment core incubation experiment ($^{**}p < 0.01$; $^{*}p < 0.05$; n.s means no significance)

Water quality variables									
	df	pH		EC		DO (mg L^{-1})		DO (%)	
		F	p	F	p	F	p	F	p
Groups	4	12.8	**	55.3	**	78.7	**	9.3	**
T	4	51.8	**	97.6	**	25.2	**	46.0	**
T×Control	4	10.3	**	1.1	n.s.	7.1	**	18.8	**
T×Dredged	4	15.1	n.s.	2.7	n.s.	64.1	**	9.9	**
T×LMB	4	15.1	**	12.4	**	22.3	**	6.7	**
T×AMZ	4	23	**	198.5	**	8.6	**	12.8	*
T×$FeCl_2$	4	2.2	n.s.	30.8	**	9.2	**	13.7	**

Nutrients							
	df	FP		NO_2-N and NO_3-N		AN	
		F	p	F	p	F	p
Groups	4	16.8	**	4.5	*	7.2	*
T	4	8.6	**	30.3	**	46.7	*
T×Control	4	17.3	**	8.4	**	12.9	**
T×Dredged	4	14.1	*	6.7	**	16.9	**
T×LMB	4	0.5	n.s.	5.4	*	39.1	**
T×AMZ	4	0.5	n.s.	15.3	**	0.23	n.s.
T×$FeCl_2$	4	4	*	3.3	*	0.7	n.s.

Metals								Others			
	df	La		Al		Fe		Mn		S	
		F	p	F	p	F	p	F	p	F	p
Groups	4	2166.9	**	1.4	n.s.	0.58	n.s.	1.1	**	7.0	**
T	4	133.4	**	4.6	**	74.6	**	8.9	**	3.9	*
T×Control	4	0.026	n.s.	3	n.s.	19.8	**	0.9	n.s.	8.2	**
T×Dredged	4	0.068	n.s.	0.2	n.s.	18.7	**	3.1	n.s.	3.4	*
T×LMB	4	645.3	**	1.9	n.s.	9.7	**	0.5	n.s.	5.2	*
T×AMZ	4	0.3	n.s.	4.6	*	13.2	**	12.2	**	4	*
T×$FeCl_2$	4	0.2	n.s.	0.5	n.s.	20.3	**	1.6	n.s.	1.2	n.s.

6

Controlling eutrophication by lanthanum-modified bentonite in the large, shallow lake Kralingse Plas, the Netherlands

Abstract

Lake Kralingse Plas (Rotterdam, the Netherlands), surface area 115 ha and 2.3 m average depth, regularly suffered from cyanobacterial blooms. To improve the water quality and meet the requirements of The European Union Water Framework Directive (WFD), the responsible authorities, after a system analysis, applied 1100 tons of lanthanum-modified bentonite (LMB, commercial name Phoslock®) into the whole lake in December, 2021. Water quality was monitored by the water authorities every month. At pre-LMB application, post-3 months, and post-15 months the LMB application, sediment phosphorus (P) fractionation and Lanthanum (La) fate in the sediment were estimated. Also, sediment nutrients released under anaerobic condition in cores taken from five locations in the lake was assessed. Post-application, the sediment La content was significantly higher in the top 4 cm of the sediment layers compared to pre-application conditions. "Ca-P (HCl-P)" pool was increased after LMB application reflecting $LaPO_4$ formation, from 53.7% of total P at pre-application, 59.5% of total P at post-3 months application, and 62.6% of total P at post-15 months application. This suggested that LMB reduced sediment P release by increasing the non-bioavailable P in the sediment. The average filterable P (FP) fluxes in the sediment cores under anaerobic conditions were 6.27 (±4.4), 0.29 (±0.21) and -4.65 (±3.1) mg P m^{-2} day^{-1} in cores at pre-application, post-3 months and post-15 months application, respectively. In addition, to address the LMB efficacy on nutrients release at different pH (pH 7 and pH 10), a 256 days sediment core incubation was conducted. P release rate following LMB application under pH 7 and pH 10 did not show a difference. This study provided new insight for applying P-capping materials in moderately large shallow lakes.

Introduction

Eutrophication is an ecological process in which water bodies are enriched with nutrients such as nitrogen (N) and phosphorus (P) (Downing, 2014; Smith and Schindler, 2009),. Eutrophication may lead to phytoplankton blooms, often comprised of toxic cyanobacteria, and subsequent anoxic conditions in the deeper water layers near the sediment as a result of the decay of detritus, or in shallow waters to nocturnal oxygen deficiency causing fish kills (Bhagowati and Ahamad, 2019; Schindler et al., 2008). Toxic cyanobacterial blooms, ecosystem alterations and fish kills impair aquatic ecosystem services, and pose a threat to human and animal health, which implies authorities must take action to minimize these negative effects of eutrophication.

Eutrophication causes water quality issues worldwide (Chislock et al., 2013) and there is growing evidence that the frequency and severity of cyanobacterial blooms are also increasing globally (Huisman et al., 2018). Even in countries, such as the Netherlands, that put high effort into reducing point source nutrient pollution, eutrophication and cyanobacterial blooms are a re-occurring, widespread phenomenon (Lürling and Mucci, 2020). One of the waterbodies that experienced eutrophication-driven cyanobacterial blooms is the shallow lake Kralingse Plas.

Lake Kralingse Plas is located in the city of Rotterdam, the Netherlands. It is a moderately large (115 ha), shallow (mean depth is 2.3 m) lake that originated from peat excavation in the 17th century and currently fulfills an important recreational function with over 3.5 million visitors per year. Due to anthropogenic activities about a third of the lake's sediment in the southern part got polluted with lead. The first recordings of cyanobacteria date back to 1971, from 1992 till 1996 the lake was dominated by the cyanobacterium *Planktothrix*, whereas *Microcystis* blooms developed in the early 2000s (information Water Authority Schieland and Krimpenerwaard, HHSK). In 2000 also a massive fish kill occurred and the overall Water Framework Directive (WFD) classification was bad. HHSK initiated together with the municipality of Rotterdam a restoration program that included the diversion of a nutrient-rich seepage ditch, dephosphatizing inlet water, dredging of the polluted sediment (~30000 m^3), sand capping of newly exposed peat and reconstruction of the banks (make them less steep). The intervention took place between 2009 and 2010, but due to the inlet of nutrient-rich water and the use of nutrient-rich sediment to reconstruct the banks, in 2011 a massive bloom of *Gloeotrichia* developed during summer which was succeeded by a mixed bloom of *Dolichospermum* and *Microcystis*. In subsequent years, canopy-forming submerged macrophytes developed and regular summer outbreaks of cyanobacterial blooms occurred hampering the recreational use of

the lake.

The municipality and the water authority HHSK performed a system analysis that provided insight into several additional measures needed to improve water quality to meet the demands. Besides a reconstruction of a sluice gate and improved inlet water dephosphatization, it included the addition of 1100 tons of lanthanum-modified bentonite (LMB) to prevent sediment P release in periods of photosynthesis driven high pH. The latter was based on extensive research that proved LMB to be suited to control sediment P release and keep it low under elevated pH (Poelen and Smolders, 2021).

In this study, to evaluate the effectiveness of LMB in reducing sediment nutrient release, we conducted core incubation experiments before the whole-lake LMB application, and then again after 3 and 15 months of the application. We also analyzed La fate and changes in the P fraction in the sediment. Since Kralingse Plas is a high-pH lake (up to 10), we conducted an additional experiment to mimic the LMB application and changed the pH to determine if the LMB would perform as expected at higher pH. We hypothesize that: 1) LMB will change the P fraction in the sediment, decreasing bioavailable P and increasing more stable P fraction; 2) LMB will reduce sediment P release after 3 and 15 months of the intervention in Kralingse Plas; 3) LMB efficiency will be decreased at a higher pH (10).

Methods

LMB dosage calculation

LMB dose was calculated based on 420 intact sediment cores sampled in December 2021, the first 10 cm of the sediment was used to analyze the bioavailable P (i.e. the sum of redox-sensitive P, organic P and Al-P). The dose was based on the bioavailable P in the sediment, TP mass in the water column and a ratio (w/w) of 100:1 (LMB and P) (Yasseri and Mucci, 2021).

Sediment P fractions, lanthanum concentrations, and water quality variables

We sampled three intact sediment cores using a UWITEC core sampler in the middle of the lake at three sampling times, pre-LMB application (November 2021), post-3 months LMB application (February 2022), and post-15 months LMB application (February 2023).

The sediment cores were brought to the laboratory and were sliced every 2 cm. The corresponding 2 cm depth samples from each of the three cores were pooled in a plastic Ziplock bag before being stored in a fridge at 7°C. Within one week, they were determined the potential releasable P based on a sequential P extraction method (Psenner, 1988) and Lanthanum (La) contents, respectively. In short, loosely bound P (labile-P), redox-sensitive P (Fe-P), organic

bound P (Org-P), metal oxide-bound P (Al-P), acid labile bound P, e.g. P bound to calcium and La (HCl-P) and refractory P (Ref-P) were determined. Extracted P was analyzed using a Skalar SAN^+ segmented flow analyzer following the Dutch standards NEN 6663 (NNI, 1986).

La contents were determined in each 2 cm sediment slice. To this end, 2.5 g of air-dried sediment was transferred into a 50 ml tube, to which 25 ml of 0.43 M HNO_3 (Sigma-Aldrich, Darmstadt, Germany) was added, whereafter the tubes were shaken (180 rpm) for 4 hours at room temperature. Subsequently, these tubes were centrifuged for 5 min at 2500 rpm (Heraeus sepatech centrifuge) and the supernatant was filtered through unit filters (Aqua 30/0.45 CA, Whatman, Germany). The La concentration was determined by ICP-MS (Thermo Fisher Scientific, Waltham, USA) in the Chemical Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University & Research).

From 26/01/2021 to 21/06/2023, we used the public water quality data from the middle of the lake Kralingse plas from the water authority (HHSK) (https://www.schielandendekrimpenerwaard.nl/kaart/ActueleMetingen/), including total phosphorus (TP), filterable phosphorus (FP), total nitrogen (TN), ammonium (AN, NH_4-N), nitrite and nitrate (NN, NO_2-N+NO_3-N), total Chl *a*, La, filterable La.

Sediment nutrients release

To test the filterable nutrients (FP, AN and NN) release rate from the sediment under anoxia, 15 sediment cores (59.6 cm long, 5.9 cm in diameter) were collected with a UWITEC core sampler in 5 locations in the Kralingse Plas at the three sampling times (Fig. 6.S1). At each location, 3 sediment cores were taken. In the laboratory, all sediment cores were incubated at 7°C in the dark for 28 days. The overstanding water in each core was bubbled continuously with N_2 until the oxygen saturation was less than 1%. Initially (day 0) and subsequently once a week, pH, EC, DO (mg L^{-1}) and DO (%) were measured as described previously. Weekly for 28 days, a 30 mL water sample was taken from each core filtered through unit filters (Aqua 30/0.45 CA, Whatman, Germany), and 30 mL Milli-Q water was refilled in the cores. The water samples was analyzed on FP, AN and NN concentrations using a Skalar SAN^+ segmented flow analyzer following the Dutch standards NEN 6663 (NNI, 1986) and NEN-EN-ISO 13395 (NEN, 1997). Nutrient fluxes (FP in mg P $m^{-2}d^{-1}$, AN, NN and DIN in mg N $m^{-2}d^{-1}$) were calculated based on the differences in FP, AN, NN, and DIN (i.e. the sum of AN and NN) concentrations between day 0 and day 28, respectively.

Effect of changes in pH on sediment nutrient release

Before the whole lake LMB application started (November 2021), 16 sediment cores were taken from the center of the lake (Fig. S1C) using a UWITEC core sampler and transported to the laboratory. Here, 8 cores were left untreated (control) into 4 cores as control-series 1 and the other 4 cores as control-series 2. The remained cores were treated with 2.4 g LMB into 4 cores as LMB-series 1 and 4 cores as LMB-series 2). The sediment core LMB dosage is corresponding to the applied dosage in the specific section where the cores were taken Kralingse Plas (8.8 tons/ha). All 16 sediment cores were incubated under anaerobic conditions at 7°C in the dark without pH changes for 182 days (Phase I). On day 182, the pH was changed to pH 10 by adding NaOH (0.1 M) in control-series 2 and LMB-series 2, while in control-series 1 and LMB-series 1 pH was left unchanged. From day 182 to day 265 (Phase II), pH was checked on control and LMB-series 2 cores, if the pH was not 10, then it was adjusted to pH 10 by adding NaOH (0.1 M), while for series 1 pH was unchanged. A 30 mL of water sample from each core was taken and filtered through unit filters (Aqua 30/0.45 CA, Whatman, Germany) before the application and subsequently sampling times. pH, DO, EC, FP, AN and NN concentrations using the same methods as given in previously described. Nutrient fluxes (FP in mg P $m^{-2}d^{-1}$, AN, NN and DIN in mg N m^{-2} d^{-1}) were calculated based on the differences in FP, AN, NN and DIN concentrations between day 0 and day 182 (Phase I) and between day 182 and day 265 (Phase II), separately.

LMB P adsorption in KH_2PO_4 solution under different pH

The effect of pH on FP adsorption by LMB was assessed over 49 days in a laboratory experiment. Hereto, a 2 mg P L^{-1} solution was prepared by dissolving KH_2PO_4 in nanopure water. Twenty Scott Duran glass bottles with 250 mL of P solution were used. Ten bottles remained untreated, 5 of them were used as control at pH 7 (control-series 1), and the 5 other bottles were as control but at pH 10 (control-series 2). The remaining ten bottles were treated with 50 mg LMB, 5 were kept pH of 7 (LMB-series 1) and 5 were kept at pH 10 (LMB-series 2). The pH of FP solutions was adjusted by adding HCl (0.1 M) or NaOH (0.1 M) before LMB addition and adjusted throughout the experiment if needed. 10 ml water sample was collected by a syringe and filtered using a unit filter (Aqua 30/0.45 CA, Whatman, Germany) from each bottle before LMB application and after 2, 4, 8, 14, 21, 28, 35, 42 and 49 days to analyze FP concentration. The FP concentration was measured colorimetrically by a UV/Visible spectrophotometer (DU 730, Beckman Coulter). pH was measured at each sampling time as

mentioned before.

Statistical analysis

Graphs were created using Sigmaplot, version 14.0. All statistical analyses were performed in R (Team, 2013). In section "Sediment nutrients released under different pH", we compared the nutrients data before 182 days (Phase I) and after 182 days (Phase II); the normality and heteroscedasticity of the residuals were tested by the Shapiro-Wilk test, if data did not pass the normality test, data transformations were applied (logarithm transformation or square root transformation). When data showed a normal distribution, repeated measures ANOVA (RM-ANOVA) with Mauchly's Test for Sphericity or Greenhouse-Geisser correction was applied followed by a post hoc test. In case the data were not normally distributed, the non-parametric Friedman test was executed, followed by a Nemenyi test. We used one-way ANOVA to compare the nutrient fluxes. The packages "rstatix" (Kassambara, 2020) and "PMCMRplus" (Pohlert and package PMCMRplus, 2020) were used.

Results

Temporal water quality

Before LMB application, both TP and FP in the lake were between 0.15~0.2 mg P L^{-1}, but dropped sharply during LMB application to < 0.05 and < 0.02 mg P L^{-1}, respectively TP and FP remained low (average TP concentration 0.026 mg P L^{-1}, average FP concentration 0.009 mg P L^{-1}) until the end of the measuring period in 2023 (Fig. 6.1). There was no significant difference between the post-3 months and 15 months LMB, with TP being 0.033 mg P L^{-1} and FP being 0.02 mg P L^{-1} (Fig. 6.1). TN, AN and NN concentrations in the lake were unaffected by the LMB application (Fig. 6.1). Average Chl *a* concentrations remained low after LMB treatment until the end of winter 2023, where a peak in total Chl *a* concentration of 42 µg L^{-1} was measured. This peak was composed mostly of diatoms (PHYTO-PAM: 51.77 µg L^{-1}) and declined rapidly (Fig. 6.1). Total and filterable La were low before the LMB application, total La concentrations peaked during application, subsequently decreased gradually, yet peaked again after 3 months application to around 170 µg L^{-1} (Fig. 6.1). Filterable La remained low throughout the study, ranging from 0.22 to 10 µg L^{-1} (Fig. 6.1).

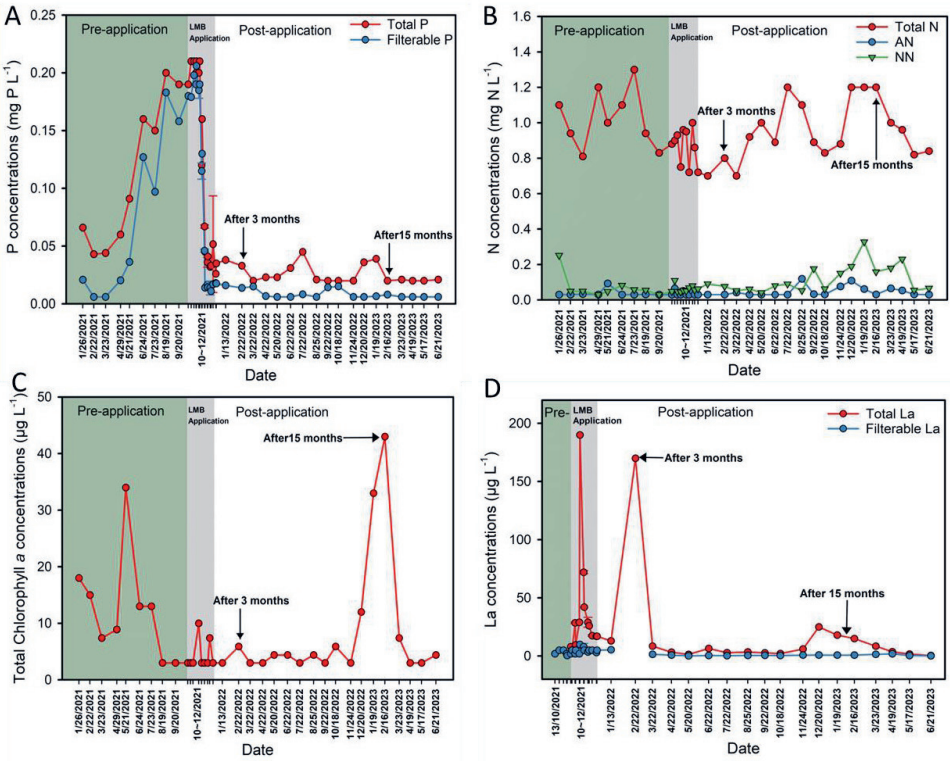


Fig. 6.1 Temporal P (panel A), N (panel B), Chl *a* (panel C) and La (panel D) concentration in lake Kralingse Plas. Data from HHSK (https://www.schielandendekrimpenerwaard.nl/kaart/ActueleMetingen/)

Sediment P fractions

Total P concentrations in the first 10 cm of sediments in samples from pre-application, post-3 months application and post-15 months application were 1357, 2718 and 2009 mg P kg^{-1}, respectively (Figs. 6.2 and 6.3). Mobile P (i.e., labile-P + Fe-P + Organic-P) concentrations in the first 10 cm sediment layers from pre-application, post-3 months application and post-15 months application samples were 292, 365, and 252 mg P kg^{-1}, respectively, which accounted for 21.5%, 13.4% and 12.5% of TP concentrations. Labile-P fraction had the lowest values in samples collected at the three sampling times, which only accounts for 0.05 ~ 0.2% of total P in the top 10 cm sediment layers. The content of organic-P fractions as the second lowest P fraction in sediment was in the range of 27 mg P kg^{-1} at pre-application to 63 mg P kg^{-1} at post-3 months application to 52 mg P kg^{-1} at post-15 months application. The remaining three P fractions, Al-P, HCl-P and Ref-P as the immobile-P fraction accounted for 78.5%, 86.6% and 87.5% of the TP in the first 10 cm sediments layers at pre-application, post-3 months application

and post 15 months application, respectively. During the three phases, in the first 2 cm of sediment layers, the content of "HCl-P" fraction was 212, 760 and 628 mg P kg^{-1} at pre-application, post-3 months application and post-15 months application, respectively. The concentrations of "HCl-P" fraction in the first 10 cm of sediment layers are 728.7 mg P kg^{-1} (53.7% of total P) in pre-application, 1617.5 mg P kg^{-1} (59.5% of total P) in post 3 months application and 1258.7 mg P kg^{-1} (62.6% of total P) in post 15 months application (Figs. 6.2 and 3).

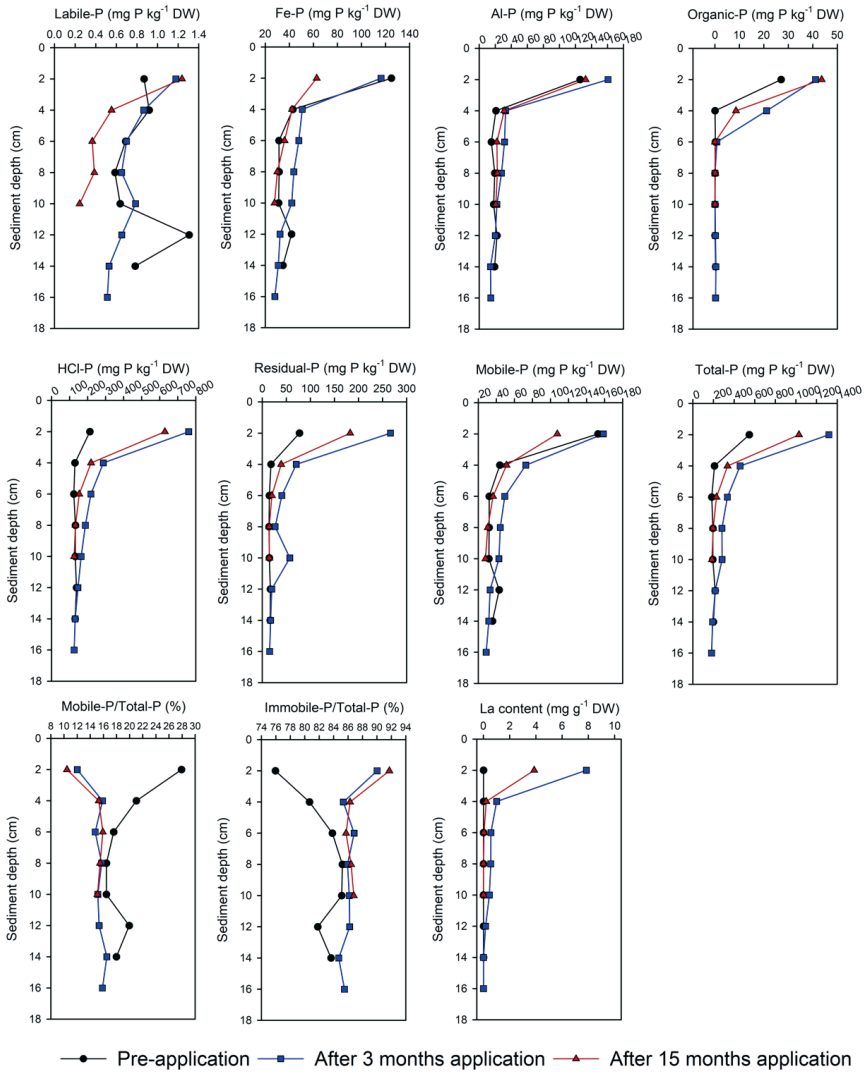


Fig. 6.2 All P fractions and La content in sediment depth profiles at pre-application, post-3 months application and post-15 months application

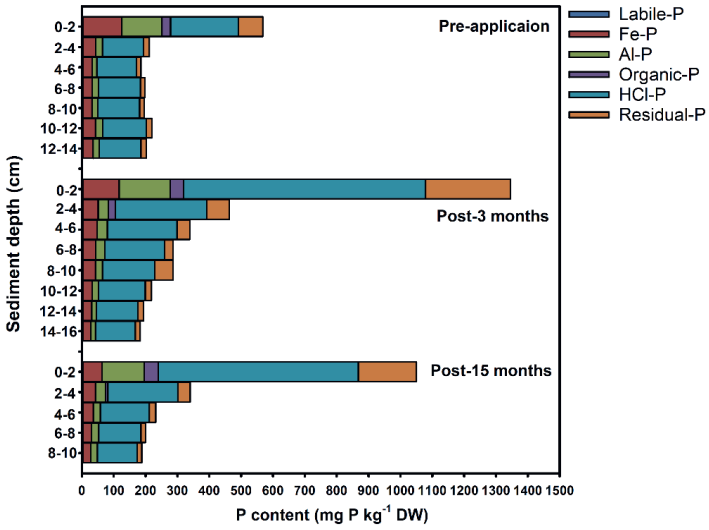


Fig. 6.3 P contents at pre-application, post-3 months application and post-15 months application

Sediment lanthanum content

Before the LMB application, the average La content in the sediment was 0.0015 (±0.0006) mg g^{-1} DW (Fig. 6.2). Three months after the LMB application, the La content in the first 2 cm sediment layer was 7.84 mg g^{-1} and clearly higher than in the other sediment layers. Fifteen months after the LMB application, most of La was still recorded in the top 2 cm sediment layer (3.87 mg g^{-1}), but the La content was less than three months after LMB addition (Fig. 6.2).

Sediment nutrients released under anaerobic condition

Sediment cores sampled before LMB application showed a positive P release (Fig. 6.4). However, the sediment P release was rather very low (< 0.39 mg P m^{-2} day^{-1} after 3 months and -6.3 mg P m^{-2} day^{-1} after 15 months) in five locations. The average FP fluxes in the cores from Kralingse Plas were 6.27 (±4.4), 0.29 (±0.21) and -4.65 (±3.1) mg P m^{-2} day^{-1} in cores taken before LMB application, cores taken 3 months after LMB application and cores taken 15 months after application, respectively. Fifteen months after the LMB application, negative FP fluxes were determined in sediment cores taken at the five locations in Kralingse Plas that ranged from -6.3 to -1.8 mg P m^{-2} day^{-1} (Fig. 6.4). It is noteworthy that the recorded initial FP concentration in the cores was much higher than the FP value from water authority due to the boat transportation, leading to a negative FP value. Negative ammonium (AN) fluxes were determined in cores taken before the LMB application, while positive fluxes were measured

after the LMB application. The average AN fluxes were -4.4, 1.3 and 1.0 mg N m^{-2} day^{-1} in cores taken before, 3 months after, and 15 months after the LMB application, respectively (Fig. 6.4). The nitrite and nitrate (NN) flux was higher in cores taken before the LMB application (4.6 mg N m^{-2} day^{-1}) than in cores taken three months (0.12 mg N m^{-2} day^{-1}) and 15 months after the application (-1.4 mg N m^{-2} day^{-1}) (Fig. 6.4). Table 6.S1 showed pH, EC, DO (mg L^{-1}) and DO (%) under the anoxic condition in sediment cores incubation at pre-application, post-3 months application and post-15 months application during 28 days.

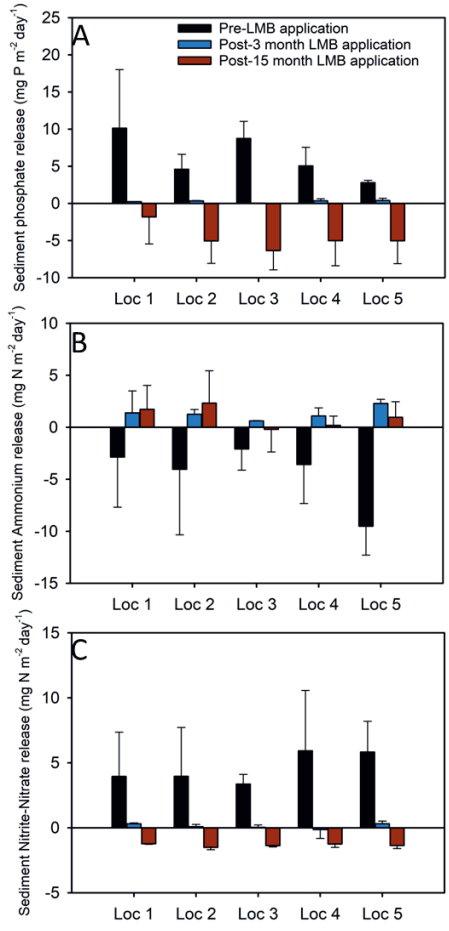


Fig. 6.4 Spatial-temporal nutrient fluxes in Kralingse Plas under anaerobic conditions (Panel A: filterable Phosphorus release, Panel B: ammonium release, Panel C: Nitrite-Nitrate release)

Sediment nutrients released under different pH

At the start of the experiment, the pH was 8.69 (±0.32) in the 16 sediment cores used (Fig. S1). During the first phase of the experiment (182 days, Phase I, pH was unchanged), the average

pH was 7.93 (±0.15),7.63 (±0.13), 7.80 (±0.12) and 7.72 (±0.16) in control- series 1, -series 2, LMB-series1 and -series2, respectively (Fig. 6.S2A). The result of the repeated measures ANOVA (RM-ANOVA) and Greenhouse-Geisser correction showed that LMB addition slightly decreased pH compared to the control($F_{1.17;9.39}$ = 6.62; p = 0.026; η_g^2 = 0.06), however, Bonferroni-corrected post hoc comparisons test did not show a statistical difference between control and LMB series. In the second phase of the experiment, from day 182 to day 265 (Phase II), we changed the pH to pH 10 in 8 sediment cores (control and LMB series 2), the average pH was 8.39 (±0.3), 8.23 (±0.17), 9.58 (±0.1) and 9.6 (±0.17) in control- series 1, -series 2, LMB-series1 and -series2, respectively (Friedman test: χ^2 (3) = 25.56; p < 0.0001). The addition of LMB did not significantly increase EC levels (Fig. 6.S2B). In Phase I, the average EC (±1 SD) was 713.7 (±11.5), 741.1 (±26.8), 756.3 (±30) and 738.4 (±24.1) µS cm^{-1} in control-series 1, -series 2, LMB-series 1 and -series 2, respectively (Friedman test: χ^2 (3) = 19.8; p < 0.001). In Phase II, EC slightly decreased in control-series 1 and LMB-series 1, and increased in control-series 2 and LMB-series 2 (Fig. 6.S2B) (RM-ANOVA: $F_{1.2;10.79}$ = 50.21; p < 0.0001; η_g^2 = 0.79). All sediment cores kept low DO concentrations, during Phase II DO was 0.5 ~ 1.2 mg L^{-1} and DO saturation was 4.4 ~ 10.3% (Fig. 6.S2C and D).

During Phase I, the FP concentrations increased over time in control-series 1 and -series 2 from 0.12 (±0.04) mg L^{-1} to 0.63 (±0.27) mg P L^{-1}, in contrast, FP concentrations remained low in LMB treatments, with on average 0.09 (±0.03) and 0.06 (±0.07) mg P L^{-1} in LMB-series 1 and -series 2, respectively (Friedman test: χ^2 (3) = 24.6; p < 0.0001) (Fig. 6.5). During phase II, the FP concentrations were on average 0.82 (± 0.11), 0.85 (± 0.17), 0.08 (± 0.07) and 0.09 (± 0.09) mg P L^{-1} in control-series 1, -series 2, LMB-series 1 and -series 2, respectively (Friedman test: χ^2 (3) = 24.12; p < 0.0001). FP concentrations increased in the control series during phase II from 0.77 (± 0.12) to 0.87 (± 0.09) mg P L^{-1} in control-series 1 and from 0.54 (± 0.16) to 1.0 (± 0.21) mg P L^{-1} in control-series 2 (Fig. 6.5A). FP fluxes were calculated from the difference in FP concentrations between day 182 and day 0 (Phase I), and between day 265 and day 182 (Phase II). In Phase I, FP fluxes in controls were on average 1.23 (± 0.68) mg P m^{-2} d^{-1} for series 1 and 0.83 (± 0.25) mg P m^{-2} d^{-1} for series 2, while fluxes in LMB treatments were on average 0.04 (± 0.17) mg P m^{-2} d^{-1} for LMB series 1 and -0.15 (± 0.09) mg P m^{-2} d^{-1} for LMB series 2 (Fig. 6.5B). The one-way ANOVA revealed the LMB significantly decreased the FP fluxes during Phase I ($F_{3,12}$ = 24.39; p < 0.0001). When comparing the P fluxes in phase II, FP fluxes were significantly higher in control-series 2 (pH 10) compared to control-series 1 (pH 7), and the FP fluxes in LMB-series 1 and -series 2 remained low with no significant difference (F (3, 11) = 10.44; p < 0.001) (Fig. 6.5B).

Ammonium (AN) concentrations in control-series 1 and LMB-series 2 over 256 days do not have clear patterns (Fig. 6.5C). The AN concentrations increased in LMB-series 1 before day 182, and then decreased after day 182. The AN increased and had the highest positive flux in the second stage in control-series 2 (Fig. 6.5D). Nitrite and nitrate (NN) concentrations in the control-series showed negative values after 182 days (Fig. 6.5E). The control two series have negative NN fluxes after day 182, while the LMB two series have positive NN fluxes (Fig. 6.5F). The DIN concentrations increased at the first stage and decreased in control-series 2 and LMB-series 1. The DIN fluxes in both series 2 (pH 10) were positive, while in both series 1 (pH 7) were negative (Fig. 6.5G).

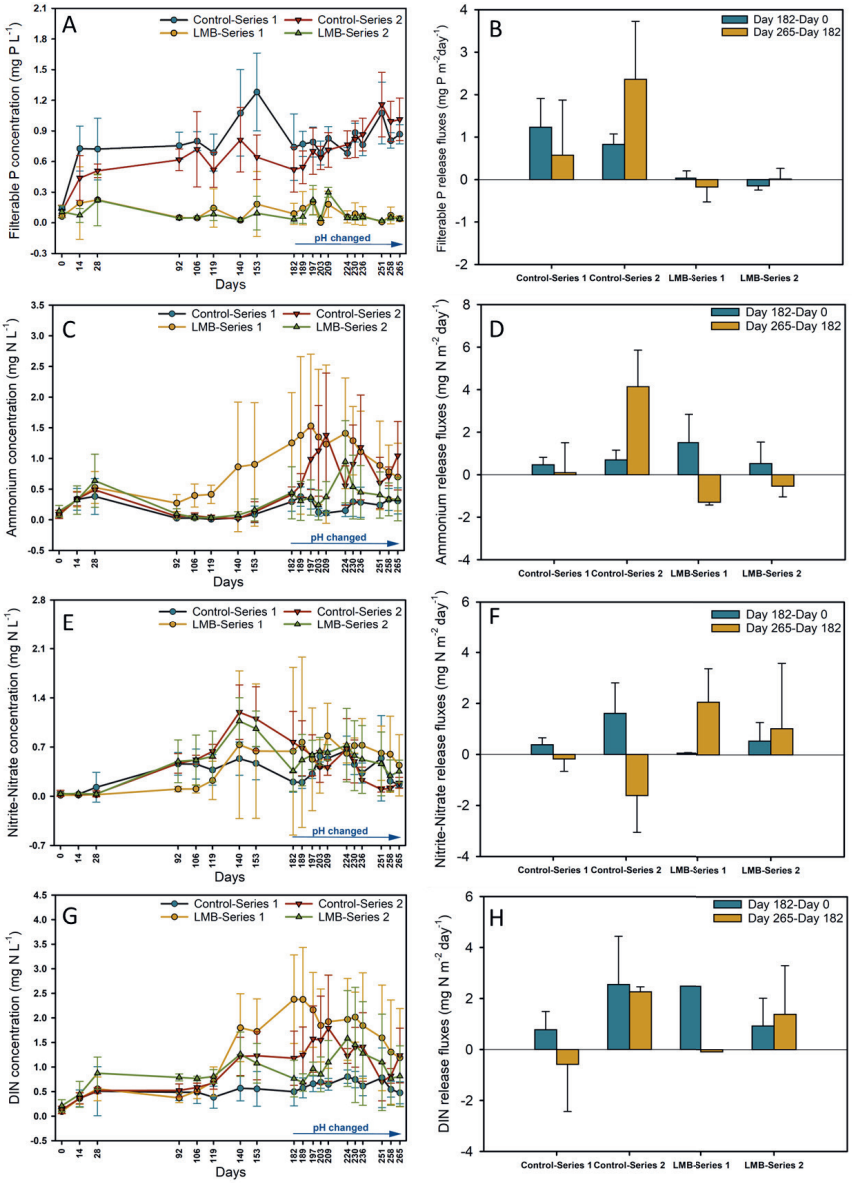


Fig. 6.5 Filterable nutrients (FP, AN, NN and DIN) concentrations over time and fluxes under different treatments series and pH (Panel A: filterable P concentration; Panel B: filterable P release fluxes; Panel C: ammonium concentration; Panel D: ammonium release fluxes, Panel E: Nitrite-Nitrate concentration; Panel F: Nitrite-Nitrate release fluxes; Panel G: DIN concentration; Panel H: DIN release fluxes)

P adsorption under different pH

FP concentrations remained similar around 2.2 mg P L^{-1} in the control-series 1 (pH 7) and -series 2 (pH 10) over the 49-day duration of the experiment (Fig. 6.6). In contrast, FP concentrations decreased over time in both the LMB-series 1 (pH 7) and -series 2 (pH 10). The decline in LMB series 1 (pH 7) seemed faster than in LMB series 2 (pH 10) (Fig. 6). FP concentrations in LMB treatments remained stable around 1.2 mg P L^{-1} from day 28 in both series. Average pH values over time were 6.88 (0.17), 9.76 (0.18), 6.94 (0.18) and 9.88 (0.20) for control-series 1, -series 2, LMB-series 1 and -series 2, respectively (Table 6.S2).

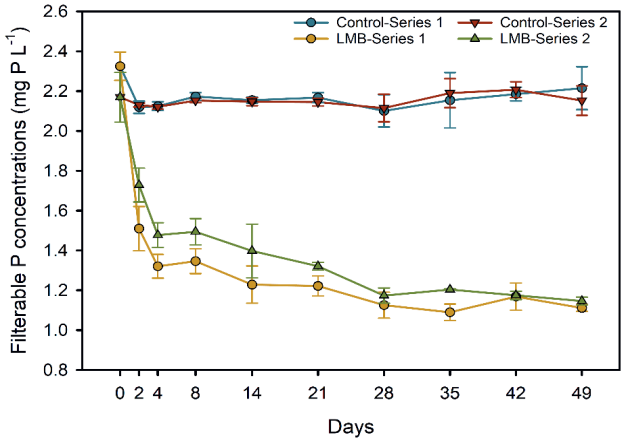


Fig. 6.6 Filterable P adsorption under different pH

Discussion

In the scientific literature, 139 documents (till March 2023) on Phoslock can be found. The vast majority of these deal with small-scale or laboratory studies, some reflect on whole lake applications albeit mostly in relatively small-sized lakes. For example, Spears et al., 2016 present a meta-analysis of 18 LMB-treated lakes that ranged between 0.9 and 64 ha. Although moderately large lakes have been treated, such as Lake Pampulha (250 ha, Brazil) and Goldap (150 ha, Poland), scientific reports on LMB applications in large lakes are limited, and to date, no insight into the efficacy of LMB in moderate large shallow lakes exists. To address the knowledge gap, we tested the hypothesis that LMB would improve water quality in Lake Kralingse Plas (115 ha). Monitoring data of HHSK are in favor of the hypothesis as not only P concentrations were reduced but until present no cyanobacteria bloom has reoccurred, whereas before the LMB application, cyanobacteria nuisance was recorded often. This study explored the effect of LMB on sediment P fraction, La content, sediment nutrient release and the possible

impact of elevated pH.

P from "mobile-P" pool to "HCl-P" pool after LMB application

Sediment mobile P content (sum up loosely bound P (labile-P), redox-sensitive P (Fe-P) and P bound in humic compounds (Org-P)) increased 73 mg P kg-1 in top 10 cm of sediment layers at post-3 months application compared to pre-application (Fig 6.2 and 6.3). This could reflect external nutrient input from human activities nearby in Lake Kralingse Plas, a redistribution of sediment, but also be caused by sediment heterogeneity (Yao et al., 2016). "Org-P" fraction increased by 132% in sediment collected three months after the LMB application (Fig. 6.2), indicating P settling and accumulation in the top 6 cm sediment. This is likely due to the settling of phytoplankton and/or material from decomposing macrophytes causing a significant increase in the mass of P stored in the "organic P" fraction (Meis et al., 2013). It is in line with the observation of macrophytes presence in the shallow lake Kralingse Plas at post-3 months applications. Nonetheless, the redistribution of sediment due to the wind or bioturbation cannot be excluded.

Sediment P partitioning changed after LMB application, the "HCl-P" fraction increased by 121% and 73% at 3 and 15 months post-LMB application, respectively, compared to the pre-application (Fig. 6.3). The increase in this fraction is likely caused by lanthanum-bound P (La-P) as has been demonstrated in a lab study (Meis et al., 2012). The mass of P present in the HCl-P fraction increased gradually over time in our study suggesting that P in sediment from redox-sensitive P-fraction is transferred into the more refractory fraction, which is in agreement with previous studies (Meis et al., 2012; Meis et al., 2013; Reitzel et al., 2013b; Yin et al., 2016). La in LMB could precipitate with phosphate to form the stable mineral rhabdophane (Copetti et al., 2016a). La-bound P is no longer bioavailable and a reduction in the mobile P pool from 22% before LMB application to 13% of TP after LMB addition underpins that the sediment P release was reduced accordingly. Hence, we accepted the first hypothesis that LMB can effectively decrease 14% of mobile P and increase by 65% of immobile P in the top 10 cm of sediment.

In our study, sediment La contents were significantly higher in the top 4 cm of sediment at post-LMB application (Fig. 6.2 and 3). Vertical sediment La distribution showed that La was subjected to a vertical sediment transport process, possibly influenced by wind-induced sediment resuspension (Hilton et al., 1986) and bioturbation (Fisher et al., 1980; Reitzel et al., 2013b).

Sediment nutrients released under anaerobic condition

The optimal dosages of LMB in controlling sediment nutrients release are affected by the physicochemical properties of water bodies, pH, alkalinity, and salinity (Kang et al., 2022b; Lürling et al., 2016; Meis et al., 2012; Meis et al., 2013; Mucci et al., 2020a; Mucci et al., 2018; Reitzel et al., 2013a; Ross et al., 2008). In sediment, due to the consumption of oxygen, and reduction of nitrate, iron and sulfate, microbial breakdown of organic matter can result in a strong decrease in the sediment P binding capacity (Smolders et al., 2006). This further leads to the formation of FeS_x, which has fewer sorption sites to bind P than iron(hydr)oxides (FeOOH) (Smolders et al., 2006). In addition, Fe will be mainly reduced under anoxic conditions and the P bounded released. Hence, using of Fe-based materials in lake restoration is limited as its redox-sensitive, whereas La in LMB is not redox-sensitive and forms either rare-earth-metal phosphates (Firsching and Brune, 1991) or carbonates (Firsching and Mohammadzadei, 1986). The predominantly P would be assumed to release from the mobile P fraction in the sediment into the water column under anaerobic conditions, therefore, it is necessary to investigate whether LMB dose to Lake Kralingse Plas was sufficient to control sediment P release under anoxic conditions.

Although La is not redox-sensitive, our previous laboratory study had shown that the P adsorption capacity of LMB from 11.4 to 5.3 mg P g^{-1} under anoxia (Mucci et al., 2018). This might point to redox-sensitive P adsorption too (Meis et al., 2013). In Kralingse Plas, LMB effectively decreases the FP flux in the anoxic sediment cores at 5 locations after 3 months and 15 months LMB application compared to pre-application (Fig. 6.4). Thus, we accept the second hypothesis, which suggests that the dose of LMB should be sufficient to control sediment P release under anoxia in lake Kralingse Plas. This finding is consistent with previous research that LMB decreases iron-rich sediment-water P flux when bottom waters are anoxic (Gibbs et al., 2011). The efficacy of LMB increased with time by comparing the FP fluxes between post 3 months and 15 months application, indicating further exploring the efficacy of LMB as time and then exploring whether top-up doses are needed.

Some studies have indicated that LMB can act as a source of ammonium when leached with ultrapure water (Reitzel et al., 2013b; van Oosterhout and Lürling, 2013; Zeller and Alperin, 2021) and lake water (Zeller and Alperin, 2021). Based on the field data, AN was not high in the lake after application. In the enclosures in Bouvigne-pond (**Chapter 5**, this thesis), in pond Heesch (Lürling and Faassen, 2012) and in canal Geetruidenberg (Zhan et al., 2022), no increase of DIN or TN in LMB treatments was observed. Our study showed that the LMB

application caused a rise in ammonium (AN) concentrations and a decrease in nitrite (NN) concentrations (Fig. 6.4). This increase in AN and decrease in NN can be attributed to I) nitrogen leached from Phoslock (van Oosterhout and Lürling, 2013); II) decreases in the ability of sediment to remove excess AN by retarding nitrification/denitrification processes (Zeller and Alperin, 2021); III) decreases the oxygen penetration depth in sediments and increase porewater AN concentrations (Song et al., 2020; Vopel et al., 2008); IV) reduces the abundance of archaeal ammonia-oxidizers (Lin et al., 2017). Yet, our study did not quantify N_2 and explore the relevant N cycle microorganisms. Further research about quantifying nitrification/denitrification rates is needed to better understand the possible reduction of nitrification from LMB.

pH effect

After day 182, elevated pH in control-series 2 (pH 10) led to the release of FP, as the competition between hydroxyl ions (OH^-) and phosphate ions (PO_4^{3-}) for anion adsorption sites occurred (Kang et al., 2022b; Mucci et al., 2018; Reitzel et al., 2013a; Ross et al., 2008). As our prior best knowledge, high pH weakened the P binding capacity of LMB in the short-term, because La would be formed as $La(OH)_3$ rather than $LaPO_4 \bullet nH_2O$ at higher pH levels (Haghseresht et al., 2009; Ross et al., 2008), but in a long-term, La will be bound to PO_4 based on chemical equilibrium modeling, in this study, P adsorption capacity under pH 7 and pH 10 were similar after LMB application (Fig. 6.6). We had to reject the third hypothesis, as the effectiveness of LMB was not affected by pH 10 and the FP fluxes results were similar under pH 7 and pH 10 in our sediment cores (Fig. 6.5A and B). The decrease in pH levels before day 182 was likely due to the buildup of dissolved total CO_2 as a result of an anaerobic process might as the consumption of O_2 (Fig. 6.S2). After day 182, when pH increased, the excess OH^- reacted with the carbonic acid (H_2CO_3) to form bicarbonate ions (HCO_3^-) and eventually carbonate ions (CO_3^{2-}). This indicated the main effect of pH is stimulated by an increase CO_3^{2-} rather than by hydroxylation of La. Thus, alkaline should be taken into account in future research in order to gain a better understanding of the relationship between pH, alkalinity and LMB efficacy.

Regarding our results from day 182, the change in pH seems to not have an effect on NN, but it does affect the AN in control and LMB sediment cores (Fig. 6.5B). AN concentrations increased in control-series 2, indicating that the nitrogen (N_2) oxidation process was promoted, and N_2 is more readily oxidized to nitrite and nitrate and AN under alkaline conditions. Additionally, the high pH also affects microbial metabolism, leading to increased rates of nitrogen transformation and subsequent accumulation of DIN.

Lower EC values in control-series 1 and LMB-series 1 are a result of both reduced inorganic

compounds and the decrease in dissolved salts under anoxic conditions (Fig. 6.S2). In contrast, in control-series 2 EC was higher than in LMB-series 2 when Na^+ was added into pH 10 cores, which presents an increase of EC values, Na^+ addition was not able to completely counteract nutrients removal from LMB under anoxic conditions (Fig. 6.S3).

Conclusion

This is the first study investigating Phoslock® (LMB) efficacy on nutrient control in a large-scale and shallow lake in the Netherlands. The results of this study indicated that:

- LMB application resulted in a significant increase in the immobile "HCl-P" fraction, with the partitioning process more apparent after 15 months LMB application.
- An anaerobic core incubation experiment showed that the dosage of LMB was likely to be sufficient to control sediment P release and more effective in controlling P with the time effect.
- LMB demonstrated its ability to reduce sediment P release not only at neutral pH but also at pH 10.
- The application of LMB led to an increase in ammonium levels in sediment cores, but no such increase was observed in the lake itself.

Acknowledgements

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Supplementary information

List:

- Fig. 6.S1 Study site
- Fig. 6.S2 pH, EC and DO change over time in the experiment
- Table 6.S1 pH, EC, DO (mg L^{-1}) and DO (%) under the anoxic condition in sediment cores incubation at pre-application, post-3 months application and post-15 months application
- Table 6.S2 pH after binding at the control and LMB under different pH. Values inside brackets are the standard deviation (SD)

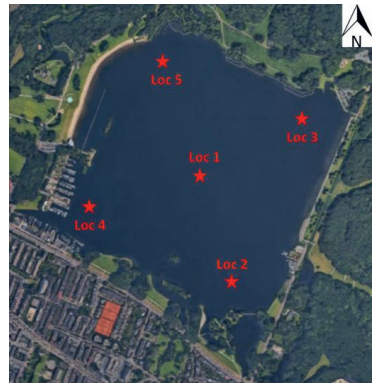

Fig. 6.S1 Study site

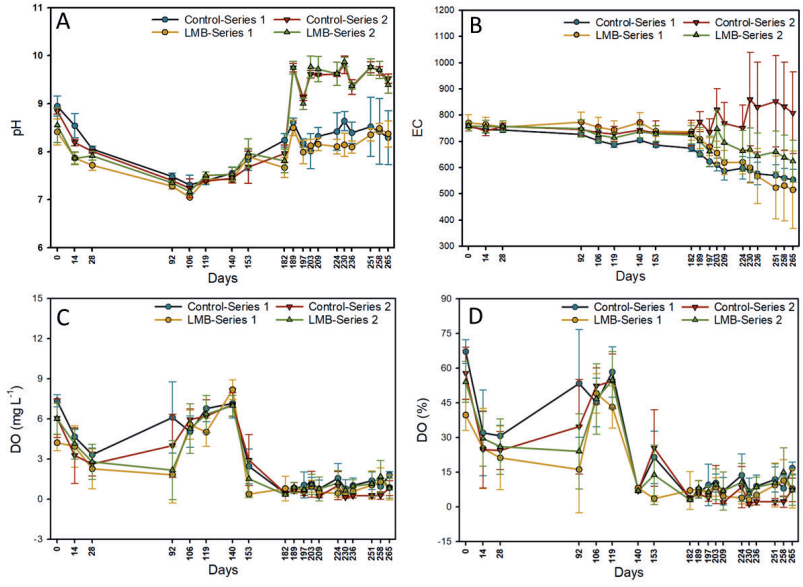

Fig. 6.S2 pH, EC and DO change over time in the experiment

Table 6.S1 pH, EC, DO (mg L^{-1}) and DO (%) under the anoxic condition in sediment cores incubation at pre-application, post-3 months application and post-15 months application

Site	Location 1			Location 2			Location 3			Location 4			Location 5		
Time	Pre-application	Post 3 months	Post 15 months	Pre-application	Post-3 months	Post-15 months	Pre-application	Post-3 months	Post-15 months	Pre-application	Post-3 months	Post-15 months	Pre-application	Post-3 months	Post-15 months
pH															
Day 0	8.69 (0.49)	7.85 (0.15)	7.79	8.76 (0 72)	7.88 (0.13)	7.79	8.55 (0.52)	7.80 (0.08)	7.79	8.58 (0.34)	8.04 (0.03)	7.79	missed	7.78 (0.04)	7.79
Day 14	8.4 (0.17)	7.30 (0.15)	8.42 (0.03)	8.31 (0.54)	7.52 (0.04)	8.42 (0.05)	7.84 (0.2)	7.55 (0.01)	8.33 (0.2)	8.14 (0.29)	7.60 (0.1)	8.08 (0.11)	8.45 (0.05)	7.48 (0.01)	8.16 (0.22)
Day 28	7.46 (0.39)	7.29 (0.15)	8.31 (0.09)	8.04 (0.12)	7.35 (0.08)	8.42 (0.07)	7.9 (0.05)	7.46 (0.04)	8.25 (0.28)	8.03 (0.06)	7.45 (0.09)	8.23 (0.17)	8.23 (0.12)	7.43 (0.04)	8.51 (0.1)
EC															
Day 0	767 (37)	770 (24)	766	758 (26)	738 (22)	766	761 (28)	745 (11)	766	733 (3)	729 (17)	766	missed	752 (12)	766
Day 14	736 (41)	765 (31)	700 (19)	732 (23)	764 (17)	715 (8)	742 (12)	743 (16)	719 (10)	716 (8)	736 (5)	693 (22)	723 (9)	759 (6)	740 ()
Day 28	753 (19)	741 (35)	677 (18)	741 (6)	754 (53)	703 (8)	737 (9)	703 (34)	700 (16)	713 (10)	706 (4)	674 (24)	727 (5)	714 (11)	703 (23)
DO (mg L^{-1})															
Day 0	6.7 (2)	6.1 (1.4)	11.2	6.1 (2.3)	2.6 (4)	11.2	5.9 (2.8)	5.4 (1.6)	11.2	5.6 (1.9)	7.9 (1.2)	11.2	missed	5.0 (3.5)	11.2
Day 14	5 (1.5)	3.3 (2.6)	0.19 (0.03)	3.5 (0.9)	3.9 (2)	0.18 (0.03)	4.4 (0.3)	3 (4.3)	0.25 (0.15)	0.7 (0.3)	5.9 (1)	0.18 (0.03)	3.8 (1.1)	5.9 (0.8)	0.28 (0.1)
Day 28	2.7 (1)	3.1 (1.5)	0.14 (0.04)	3.0 (1.3)	3.1 (1.5)	0.15 (0.12)	3.4 (1.1)	2.9 (0.9)	0.08 (0.05)	2.9 (0.5)	2.6 (0.5)	0.06 (0.02)	2.9 (1.8)	2.4 (0.5)	0.12 (0.11)
DO (%)															
Day 0	68 (22)	57 (14)	98	64 (23)	26 (42)	98	58 (30)	53 (15)	98	58 (20)	78 (11.7)	98	missed	48.3 (33.9)	98
Day 14	41 (10)	28 (22)	1.8 (0.4)	30 (8)	33 (18)	1.5 (0.3)	38 (3)	26 (36)	2.2 (1.5)	5.9 (2.8)	50 (8.7)	1.9 (0 .6)	33.5 (10.6)	50.9 (6.4)	2.3 (1)
Day 28	25 (9)	34 (15)	1.3 (0.3)	26 (12)	34 (13)	1.27 (1)	30 (10)	32 (7)	0.7 (0.5)	26.3 (5)	29 (5.5)	0.5 (0.2)	32 (20)	27 (5.5)	0.5 (0.05)

Table 6.S2 pH after binding at the control and LMB under different pH. Values inside brackets are the standard deviation (SD)

	Control-Series 1	Control-Series 2	LMB-Series 1	LMB-Series 2
0	7 (0.05)	10 (0.07)	7 (0.06)	10.1 (0.06)
2	6.7 (0.06)	9.6 (0.06)	6.6 (0.05)	9.8 (0.06)
4	6.9 (0.02)	9.8 (0.05)	7 (0.08)	9.6 (0.08)
8	6.6 (0.06)	9.5 (0.08)	6.8 (0.06)	9.6 (0.07)
14	7.2 (0.05)	10 (0.02)	6.9 (0.02)	10.1 (0.05)
21	6.8 (0.05)	9.9 (0.05)	7.1 (0.06)	10 (0.08)
28	7 (0.02)	9.9 (0.05)	6.8 (0.02)	10 (0.06)
35	6.9 (0.05)	9.6 (0.02)	6.9 (0.02)	10.1 (0.02)
42	6.8 (0.02)	9.7 (0.01)	7.2 (0.01)	9.8 (0.05)
49	6.9 (0.01)	9.6 (0.05)	7.1 (0.06)	9.7 (0.02)

7

General reflection and synthesis

"*Much of the necessary knowledge is now available but we do not use it. We train ecologists in our universities and even employ them in our governmental agencies but we seldom take their advice. We allow the chemical death rain to fall as though there were no alternative, whereas in fact there are many, and our ingenuity could soon discover many more if given opportunity. Have we fallen into a mesmerized state that makes us accept as inevitable that which is inferior or detrimental, as though having lost the will or the vision to demand that which is good*?"- *Silent Spring-Rachel Carson*

I was always puzzled by the transformation of a clear and shallow creek in my hometown. It was relatively pristine, women washed their lovely family's clothes on the creek shore planted with vegetables. Not a long time passed, and the creek became smelly and green, then shallow and shallow over time until this creek disappeared. Unfortunately, this is not a solo incident, anthropogenic activities have accelerated the eutrophication process and toxic cyanobacterial blooms, which are likely to worsen with global population growth and environmentally unfriendly lifestyle. Thus, it is of high importance to find effective ways to restore impaired water systems. The research objective of this thesis is "to explore the effectiveness of potential techniques/materials on managing eutrophication and mitigating cyanobacteria blooms". In this final chapter, I will discuss current water body (lake) restoration techniques in four steps. First, I will extend the objective and the main findings as introduced in **Chapter 1**. Next, I will reflect on the current research and limitations of in-lake interventions. Third, I will point out the development and future of these techniques/materials. Finally, I will give the outlook on restoring water ecosystems.

Overview

Eutrophication and harmful cyanobacteria have been studied for almost one century (Naumann, 1919), however, tackling the cyanobacterial blooms still remained a huge challenge. As **Chapter 1** indicated that the key to controlling eutrophication and cyanobacterial is to reduce the external and internal nutrients inputs as much as possible. However, controlling external nutrient loading is not always feasible for most countries (Huser et al., 2016). Also, legacies accumulated in lake beds and ongoing diffuse pollution further aggravate eutrophication and cyanobacteria blooms (OECD, 2014, 2017). Controlling both external and internal nutrient load as a two-pronged approach would be more sensible.

In-lake measures contain "acute" and "chronic" applications. The application of "acute" measures has been well developed to direct target cyanobacteria in the water body. Generally, algaecides are quite effective in controlling algal blooms, but a downside is that toxins, such as microcystins

(MCs), can be decreased, and the longevity of the positive effect might be short. Some of the algaecides in lake restoration have been extensively studied, such as hydrogen peroxide (H_2O_2) (Huang and Zimba, 2020; Matthijs et al., 2016) and copper sulphate ($CuSO_4 \cdot 5H_2O$) (Jančula and Maršálek, 2011; Willis and Bishop, 2016). $CuSO_4 \cdot 5H_2O$ has already been applied for managing the blue algae more than a century (Buley et al., 2021; Jančula and Maršálek, 2011), yet, a considerable number of novel algaecides have not been studied or have not been intensively studied, such as some novel copper-based algaecides studied in the thesis (SeClear, Captain® XTR, and Lake Guard Blue). Another useful and "acute" tool to mitigate the effects of eutrophication is using low doses of flocculants (e.g., chitosan, aluminium salts (aluminium sulphate and sodium aluminate, Al-salts), or iron chloride ($FeCl_2$)) followed by the addition of soils or clays, have gained attention, named "Flock & Sink" technique (Cooke et al., 2005; Noyma et al., 2016; Noyma et al., 2017; Waajen et al., 2016). It can remove cyanobacteria from the water column whilst blocking sediment P efflux.

Several in-lake interventions, also "chronic", achieve indirect goals by reducing internal loads, which are usually more expensive than "acute" measures. For example, solid-phase phosphate (P) sorbent (SPS), Phoslock® (LMB), has been applied successfully in hundreds of lakes and reservoirs worldwide by controlling the internal P loading (Copetti et al., 2016; Lürling et al., 2016; Lürling and Mucci, 2020). The Aqual-P™ (AMZ) as another SPS has few scientific studies. In addition, Al-salts and $FeCl_2$) as P binders could be as chronic in-lake measures (Cooke et al., 2005). The source-oriented dredging measure has been applied commonly, but is difficult to find in the scientific literature results from before and after application in lake restoration due to economic concerns and ecological consequences (Lürling et al., 2020b).

This thesis provided a series of valuable views of current in-lake measures to manage eutrophication and cyanobacteria across different scales from laboratory to field observation. To address the impact of current techniques on aquatic ecosystems, four scientific questions and knowledge gaps have emerged from our current understanding: (1) how effective are the selected in-lake measures when directly targeting algae? (**Chapter 2**) (2) how novel copper-based algaecides affect a non-target organism? (**Chapter 3**) (3) what are the impacts of environmental factors such as pH and temperature on P-binder' performance? (**Chapter 4**) (4) is it feasible to use P binders to suppress sediment nutrients released? (**Chapters 5 and 6**).

The different research chapters were carried out to address these four research questions by investigating the influence of materials across different scales. The outline of the main findings is presented in Fig. 7.1.

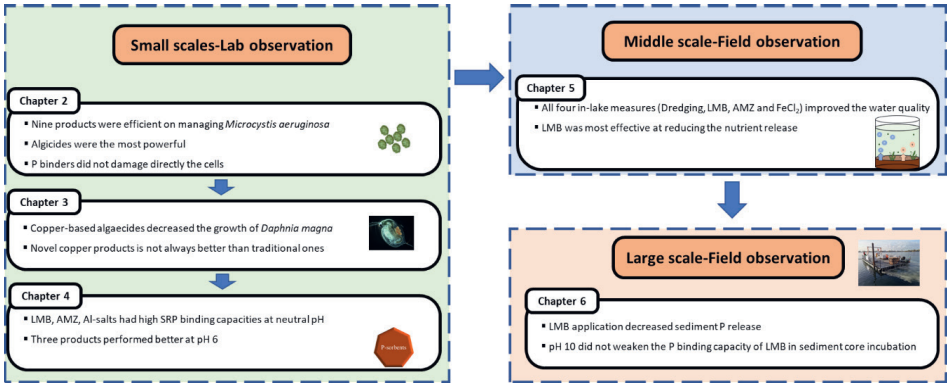

Fig. 7.1 The outline of the main findings of this thesis

The main findings of the individual chapters are:

♦ **Chapter 2:** The effectiveness of the LMB, AMZ, chitosan, Al-salts, SeClear, Captain® XTR and $CuSO_4 \cdot 5H_2O$, Streptomycin and H_2O_2 on a bloom of *Microcystis aeruginosa* and cyanotoxin (microcystins) releases had not been explored intensively. Thus, a 7-day lab-observation experiment with an initial chlorophyll *a* (Chl *a*) concentration of 100 µg L^{-1} was conducted (Fig. 7.1). The results can be classified by three aspects: (1) From the cyanobacteria biomass aspect, the algaecides were the most powerful one in decreasing *Microcystis aeruginosa* biomass, while SPS such as LMB and AMZ evoked a milder response; (2) From the growth rates aspect, growth rates were reduced more strongly as the chemicals' dosages increased, except for Al-salts; (3) From MCs aspect, algaecides induced release of MCs and SPS did not damage algae cells. I proposed the possibility of delaying cyanobacterial regrowth and prolonging the period of low cyanobacteria abundance by combining selected algaecides that kill most of the cells with P binders that reduce the nutrients available.

♦ **Chapter 3:** I learned from **Chapter 2** that copper-based algaecides had excellent performance in suppressing *Microcystis aeruginosa*, and copper exerts toxicity to phytoplankton due to its negative effect on photosystem II efficiency (Brix et al., 2001). Yet they may also be toxic to the non-target organisms, such as *Daphnia magna* (*D. magna*), which play an important role in the water ecosystem. Novel copper-based algaecides have been developed to reduce toxicity towards non-target organisms whilst increasing effectiveness against target organisms (algae). Hence, I evaluated the toxicity of three novel copper-based algaecides (Captain XTR, SeClear and Lake Guard Blue) to the model organism-*D. magna* and compared it with the traditional $CuSO_4 \cdot 5H_2O$ in 48 h acute and 21 d chronic tests. The results indicated that the four copper-based algaecides decreased the growth and fecundity of *D. magna*. Moreover, the novel chelated copper formulations may not be necessarily less toxic to nontarget *D. magna* than traditional copper

sulphate.

♦ **Chapter 4:** In terms of **Chapter 2**, I understood that P sorbents (PS) LMB, AMZ and Al-salts did not damage directly *Microcystis aeruginosa* cells, but they have good efficiency in reducing phosphate concentration. It is known that abiotic factors might affect their P binding efficiency, like pH, DOC, anoxic condition and salinity (Copetti et al., 2016; Mucci et al., 2018; Zamparas et al., 2015). Yet, few studies have evaluated the effects of temperature and a broad pH range. Although water bodies have different water temperatures within the season, only a few studies have considered a realistic temperature range (Georgantas and Grigoropoulou, 2007). Therefore, I explored the efficiency of the three PS on SRP removal under six different temperatures (5 to 35 ºC) and a wide pH range (5 to 10). I found that (1) LMB and Al did not show any clear pattern in their binding capacity at different temperatures, while AMZ seems to perform better at 35°C; (2) all PS bound P and they performed better at pH 6; (3) AMZ and Al desorbed P when pH changed to 10, while LMB was not affected. Here, I noticed pH should be taken into consideration while defining the most suitable PS.

♦ **Chapter 5:** After a series of small-scale research, I was interested in the performance of these in-lake measures on mitigating nutrients released from sediment in a more realistic condition. Even though in-lake measures have been developed and widely applied for the restoration of water systems, a few studies have compared them together under natural conditions. Hence, I conducted an enclosure experiment and a sediment cores experiment. I compared the efficiency of dredging, LMB, AMZ and $FeCl_2$ to improve water quality and control nutrient release from the sediment in enclosures placed in a eutrophic pond in the south of the Netherlands. After 112 days, all four in-lake measures improved the water quality, while LMB was most effective in reducing the nutrient release; Dredging reduced total P and mobile P most in sediment, LMB enlarged the "Ca-P" pool reflecting $LaPO_4$ formation; AMZ enlarged the "Al-P" pool indicating more metal-oxide adsorbed P; $FeCl_2$ enlarged the "BD-P" pool reflecting more iron-oxi/hydroxide adsorbed P.

♦ **Chapter 6:** According to **Chapter 5**, I learned LMB was the most effective in reducing the nutrient release in enclosure cores. Following study was enlarged to a large-scale 114 ha of Lake Kralingse Plas, where regularly suffered from cyanobacterial blooms. Sediment P fractionation and nutrients release rate under anoxic conditions were assessed at pre-LMB application, post-3 months LMB application and post-15 months LMB application. I found that "Ca-P" pool was increased after LMB application reflecting $LaPO_4$ formation; In addition, to address the LMB efficacy on nutrient release at different pH (pH 7 and pH 10) during 256 days, a sediment core incubation was conducted. Results revealed that pH 10 did not weaken the P binding capacity of

LMB.

In-lake interventions

Due to growing requirements on environmental policies and safety in water quality all over the world, the demand and standard for materials against harmful cyanobacteria bloom and/or suppressing bioavailable phosphorus into insoluble forms has increased. Today, there is an endless supply of products on the market (*see* Lürling et al., 2016). However, these new techniques usually have not been explored deeply in the following aspects: 1) effectiveness; 2) the level of difficulty of the application; 3) stakeholder engagement; 4) economics; 5) environmental safety. I will discuss the in-lake interventions studied in this thesis from solid-phase P sorbents, Copper-based algaecides and flocculant agents below.

Solid-phase P sorbents-LMB, AMZ

As many studies have indicated that solid-phase P sorbents (SPS) LMB and AMZ are created to reduce internal nutrients load, rather than as quick fix agents eradicating cyanobacterial blooms (Copetti et al., 2016; Gibbs and Hickey, 2018; Lürling and Mucci, 2020; Mucci et al., 2018). LMB has recently been (mis)used as a palliative measure to control cyanobacterial blooms in Guandu Lagoon, Brazil (Bacha et al., 2021). This misuse and the following conclusion were responded too (Lürling et al., 2022), and my results (**Chapter 2**) underpin that SPS like LMB and AMZ cannot direct kill algae cells as "emergence management", also, it was not developed as, nor should be considered a panacea for the management of cyanobacteria nuisance (**Chapter 2**). The effectiveness of LMB on SRP removal under environmental factors, pH and temperature, has been explored. I observed a lower LMB efficiency occurs at higher pH (**Chapter 4**), due to the competition with hydroxyl ions for binding sites. On the other hand, this competition is just a temporal hamper in SRP removal, given enough time most of the lanthanum will precipitate with phosphate over time. Alkaline conditions only slow down the rate of SRP adsorption by LMB. Thus, it is essential to further investigate the duration of how long P can fully occupy the binding site. In theory, the elevated temperature would increase the chance of interaction between SPS and SRP, but this could not be concluded from **Chapter 4**. Likely, the shaker I used hampered the temperature effect. Thus, it would be crucial to not use the shaker to simulate adsorption experiments testing the temperature effect. LMB can effectively precipitate the SRP from the water column and prevent SRP released from the sediments, which binds phosphate molecules and forms rhabdophane ($LaPO_4 \cdot nH_2O$), and is buried in sediment permanently (**Chapter 5 and 6**). AMZ has been studied less and has not yet been applied in whole lake

restoration projects widely (Gibbs and Hickey, 2018). In this thesis, I investigated its efficiency on SRP across different scales. More information on the release of Al from the zeolite matrix under such conditions needs to be analyzed. Furthermore, with limited information about long-term monitoring of the efficiency of SPS in realistic conditions, a deeper study of AMZ is needed. I recommend that the impact and effectiveness of the proposed interventions/materials are necessary to be clarified by controlled experiments prior to *in situ* application, as was done in **Chapters 2** and **4**.

Copper-based algaecides - old vs new

To put it bluntly, controlling algae is currently caught in a scientific vicious circle. People nowadays are fascinated to develop new techniques or strategies to solve this old problem, which however, may have detrimental environmental impacts without testing/assessment materials before the application. Copper is probably the most known and extensively used algaecides, with an increasing number of copper-based products being developed for the management of eutrophication and the proliferation of phytoplankton (**Chapter 3**). Copper is commonly applied in low concentrations, which usually has to be repeated as its concentrations rapidly decline. Hence, up scaled *in situ* experiments with these novel copper-based algaecides with chelated copper compounds can be considered that will provide more insight into efficacy, side effects and longevity (**Chapter 3**) (Willis and Bishop, 2016). Copper can cause cell lysis and release of MCs (**Chapter 2**), can be accumulated in sediment, and might exhibit toxicity towards non-target organisms (**Chapter 3**). For example, the toxicity of copper-based algaecides towards *D. magna* is similar (24h EC_{50} = 0.3-0.45 mg Cu L^{-1}) to that towards *Microcystis aeruginosa* (24h EC_{50} = 0.27-0.50 mg Cu L^{-1}) (**Chapters 2** and **3**). In spite of this, copper is still one of the most used algaecides. Application of copper-based compounds is not allowed in certain countries (Jančula and Maršálek, 2011), such as the Netherlands, Sweden, Czech Republic, etc., while others permit copper application in surface water, such as USA, Canada, China, and so on. These countries allowing the use of copper products need to establish a better risk management strategy. Furthermore, according to manufacturers, those new products were invented to increase effectiveness against algae blooms and to reduce the toxicity towards non-target organisms, like zooplankton, but obviously, further research is needed to verify their claims. As shown in **Chapter 3**, novel products like SeClear and Lake Guard Blue had more adverse effects on the reproduction and body length of *D. magna*. Moreover, SeClear contains a phosphate binder based on the manufacturer's description and thus may reduce the phosphate concentrations in the system, but **Chapter 2** gave the opposite results. Thus, the controlled experiments are essential to

understand more about materials used to mitigate eutrophication and cyanobacterial blooms. Given the persisting lack or inadequacy of nutrient control measures in catchment areas, the use of copper-based algaecides remains as a rapid and emergency option for the control of harmful algae considering the attractive cost of copper-based algaecides.

Flocculant agents - chitosan, Al-salts, $FeCl_2$

Flocculant agents can also be effective in fastly improving water transparency. In the thesis, I used three flocculants as "eco-friendly" chitosan, as well as metal-based flocculants such as Al-salts. Chitosan damaged *M. aeruginosa* cells causing cell lysis and cyanotoxins release, which might have unwanted side effects and as an "eco-friendly" material (**Chapter 2**). Besides its negative effect in releasing toxins, chitosan flocculation efficiency is hampered by high pH and alkalinity (Lürling et al., 2017). Thus, using chitosan may not be a favorable option compared to metal-based coagulants. Additionally, in the decision-making process, it is crucial to consider the affordability of materials, and the price of chitosan may pose a drawback (Mucci, 2018). On the other hand, Al-salts, being significantly cheaper, were found to adsorb phosphate effectively (**Chapter 2 and 4**). However, they also exhibited the drawback of potentially releasing toxins. In terms of $FeCl_2$, when injected into the sediment, it resulted in an enlargement of the reductive labile P pool, indicating increased adsorption of P by iron-oxi/hydroxides (**Chapter 5**). While $FeCl_2$ showed good performance in P removal, its application *in situ* might be challenging due to the need for specific equipment such as a syringe for injection, or on larger scale underwater injection systems. Hence, in addition to being effective, the affordability and practicality of the materials should also be considered.

Developments and Future of in-lake measures

Given the extensive water ecosystem damage caused by humans over approximately 200 years, the selection of materials or techniques requires a thorough approach. As earlier mentioned, most of the materials have not been explored deeply. An in-depth study of the effectiveness, affordability and practicality of these materials or technologies as well as the safety is essential. However, their impact goes beyond these aspects and extends to social, economic and environmental aspects, which must be carefully considered to obtain a comprehensive solution.
From a social perspective, engaging stakeholders in decision-making processes is crucial. By involving local communities, environmental organizations and/or relevant authorities the inclusiveness and effectiveness of measures can be enhanced. Moreover, fostering public awareness through education about water quality can further strengthen the success and

acceptance of in-lake measures.

Considering the economic aspect, it is not sufficient to merely evaluate the initial cost of implementing in-lake measures. For instance, the compound H_2O_2 is unlikely to stay in the water system for long and decays to water and oxygen within a few hours to a few days (Häkkinen et al., 2004; Matthijs et al., 2012). The frequency and whether repeated treatments should be considered, which is related to the economic investment. Yet, on the other hand, a comprehensive assessment of their long-term cost-effectiveness is vital. For example, these benefits can include increased opportunities for recreation, enhanced ecosystem services, improved public health outcomes, and even job creation within the local economy. By quantifying and valuing these benefits, decision-makers can better understand the return on investment and make informed choices that prioritize both environmental restoration and economic sustainability.

From an environmental point of view, rigorous risk management is necessary for the selection of materials during water restoration. Building on previous knowledge, such as the findings in **Chapters 2** and **3**, it is important to recognize that even new products introduced by companies may not have the ability on removing P concentration and may have ecotoxicological risks to other non-target organisms. To address this issue, independent third-party testing should be conducted to assess the potential risks associated with these materials. Assessing their effectiveness in inhibition of the cyanobacterial and potential effects on non-target species, as well as their bioaccumulation potential, is critical to ensure the overall safety and effectiveness of the chosen approach. In cases where materials do not pass rigorous testing for lake (water) restoration applications, alerts should be raised and it may be more prudent to explore alternatives or consider a conservative "do nothing" approach until a suitable solution is available.

Outlook

There is no single way to manage eutrophication and cyanobacterial blooms, each individual water body has its temper. In short, as Figure. 7.2 showed that whole system analysis is a critical and initial step to assess internal and external P loading. The next step is to determine whether external and internal nutrients are high, and then select the potential and appropriate compounds. Then, testing and monitoring these compounds across different scales and conditions, such as was done in this thesis (**Chapters 2, 3, 4, 5).** After small and medium scales, if water quality meets standards, a whole water restoration application could be considered, such as **Chapter 6**. If not, then re-estimating the system analysis is required.

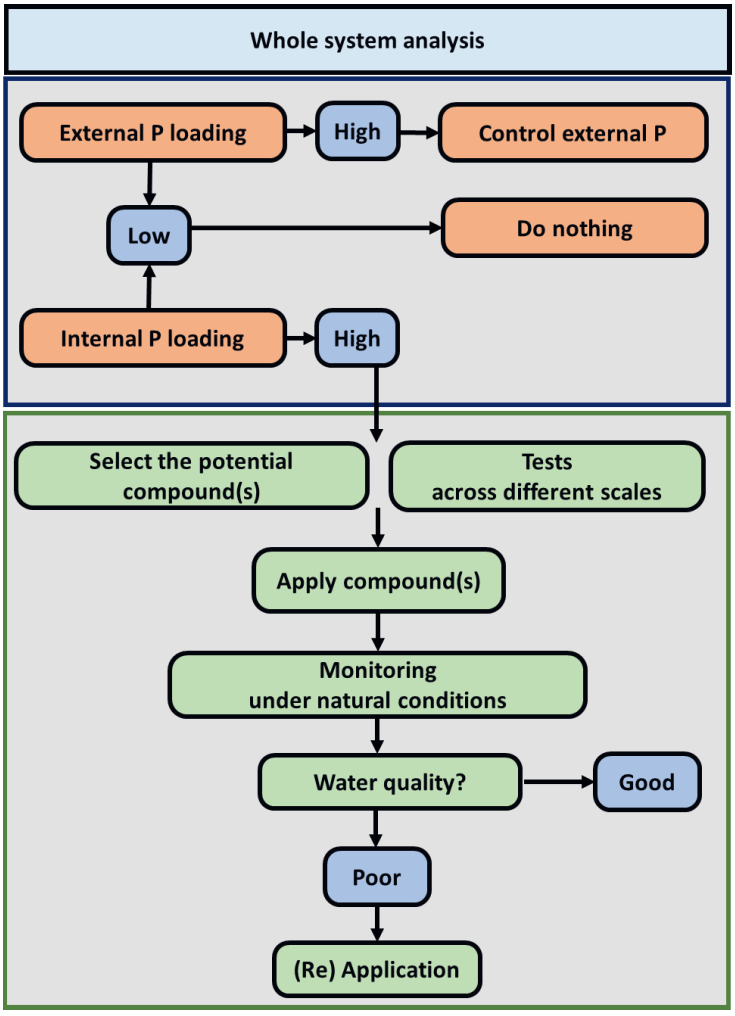

Fig. 7.2 A conceptual model for managing eutrophication and cyanobacterial blooms

This PhD work is expected to provide a deeper understanding of current eutrophication and cyanobacterial blooms management. However, we are currently in an ongoing process of learning as we go. From PhD stage, I propose the following potential areas for future research in terms of "eutrophication and cyanobacterial management".

- Investigating the efficacy of compounds and materials for managing other bloom-forming cyanobacteria, beyond *Microcystis aeruginosa*, and mixed field algae to provide a broader understanding of cyanobacterial management.
- Conducting ecological studies to assess the long-term effects of in-lake measures, including phytoplankton diversity, zooplankton dynamics and other trophic interactions.

- Evaluating the effectiveness and behavior of in-lake measures in coastal areas.
- Exploring the intricate relationship between sediment nutrient dynamics and cyanobacterial blooms, deepening the understanding of how nutrients released from sediments contribute to bloom persistence.
- Studying the effectiveness of integrating multiple management techniques, such as combining compounds with other nutrient reduction strategies or biological controls.

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Summary

Managing eutrophication and mitigating cyanobacteria blooms has been a hot topic for over 100 years. Tackling cyanobacteria blooms remains a challenge. Over the years, numerous compounds aiming to manage the issues have been developed and applied in lakes, rivers and ponds over the world. I studied potential compounds including solid-phase phosphorus sorbent (SPS) and algaecides to manage eutrophication and control the nuisance of cyanobacterial blooms and associated scum across different scales, ranging from laboratory to field observations.

This thesis began by evaluating the effectiveness of nine different compounds in controlling bloom-forming and world-wide occurring cyanobacterium, *Microcystis aeruginosa*. Algaecides rapidly killed *M. aeruginosa* cells, leading to the release of their toxins, microcystins (MCs), while SPS, such as Lanthanum (La) modified bentonite (LMB), commercially called Phoslock, and Aluminium modified zeolite (AMZ), commercially called Aqual-P, were able to adsorb P and decrease the nutrients availability (**Chapter 2**).

Copper-based algaecides have been developed for fast emergency control of nuisance phytoplankton bloom. These should preferably not exert irreversible negative effects on non-target organisms such as *Daphnia magna*. In **Chapter 3**, we conducted an ecotoxicological study comparing the toxicity of traditional $CuSO_4 \cdot 5H_2O$ and novel copper-based algaecides (Captain XTR, SeClear and Lake Guard Blue) on the model organism *D. magna* in acute (48 h) and chronic tests (21 days). All copper-based algaecides negatively affected the reproduction and body length of *D. magna*. Most copper was chelated with EDTA and the novel chelated copper formulations may not be less toxic to *D. magna* than traditional one. We strongly suggested considering water chemistry and the effects of copper compounds on non-target organisms before their application prior to copper application.

P sorbents (PS) have been used in lake restoration widely. **In Chapter 4**, we tested the effect of LMB, AMZ and Al-salts on P removal under different pH and temperatures. The results showed that all three materials had good performance on P removal under neutral pH. Compared to soluble reactive phosphorus (SRP) binding at pH 6 at elevated pH 10 the SRP binding had dropped more than 95% for Al salt and 50% for LMB, but remained unaffected for AMZ. Desorption of bound P was 70% for Al-salt, 30% for AMZ, and 0% for LMB when the pH was increased form pH 7 to pH 10. No clear pattern emerged in the SRP binding capacity of the three PS at temperatures varying from 5 to 35°C. Thus, abiotic factors in particular pH should be considered when selecting the most promising material in lake restoration, especially when there is a risk of elevated pH.

In **Chapter 5**, we conducted a mesosystem enclosure experiment, to investigate the performance of four in-lake interventions, namely dredging, addition of LMB, AMZ and $FeCl_2$ in mitigating nutrients released from sediment during 112 days. All four strategies improved the water quality, with LMB being the most effective in reducing P in the water column and controlling sediment nutrient release. Meanwhile, we recommend that besides effectiveness, the cost, whether easy to apply, social, economic (benefit) and environmental safety aspects should be considered when selecting materials for lake restoration.

In **Chapter 6**, we presented field results from a whole-lake treatment with LMB in the large (115 ha), shallow lake Kralingse Plas. After applying LMB, the filterable P decreased in the water column and LMB controlled sediment P release by shifting mobile P to immobile P. In addition, we found pH 10 did not weaken the P binding capacity of LMB in sediment core incubation. Long-term monitoring of the whole lake needs still to be performed to shed light on the possible limitations of this technique. Furthermore, post-restoration monitoring of water quality development is essential, and regardless of whether the improvements has achieved the expected results, a comprehensive system analysis should be conducted again.

In **Chapter 7**, I proposed a conceptual model and provided a new perspective on lake restoration. Each lake possesses unique characteristics, and each in-lake intervention has its limitations. Therefore, conducting a system analysis of individual lakes is the initial step in managing eutrophication and cyanobacterial blooms. Controlled experiments under realistic conditions across different scales are needed before a field application can be carried out for a successful restoration plan. An independent institute testing materials and techniques for in-lake interventions could be useful to fill the tool-box with effective and safe measures.

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About the author

Li Kang was born on the 17th of January 1995 in Shanxi, China. She developed a strong interest in ecology due to the increasing environmental concerns after finishing her bachelor. Driven by her passion, she embarked on a new academic path, pursuing a master's program in Ecology at Chongqing University in China. During her master's study from 2016 to 2019, Li engaged in the project to explore the world of algae. Her work involved conducting various experiments, including monthly monitoring of algal toxins, risk assessment, and studying algal physiology.

After graduating, Li received financial support from the China Scholarship Council grant, which enabled her to continue her academic journey as a PhD candidate at Wageningen University & Research in the Netherlands starting in 2019. Her doctoral research centers on finding solutions to address eutrophication and cyanobacteria issues. After completing her PhD project, she looks forward to embrace new challenges and questions.

List of Publications

Miquel Lürling, **Li Kang**, Maíra Mucci, Frank van Oosterhout, Natalia Pessoa Noyma, Marcela Miranda, Vera L.M. Huszar, Guido Waajen, Marcelo Manzi Marinho. Coagulation and precipitation of cyanobacterial blooms. *Ecological Engineering* (2020). https://doi.org/10.1016/j.ecoleng.2020.106032

Li Kang, Maíra Mucci, Miquel Lürling. Influence of temperature and pH on phosphate removal efficiency of different sorbents used in lake restoration. *Science of the Total Environment* (2022). https://doi.org/10.1016/j.scitotenv.2021.151489

Li Kang, Maíra Mucci, Miquel Lürling. Compounds to mitigate cyanobacterial blooms affect growth and toxicity of *Microcystis aeruginosa*. *Harmful Algae* (2022). https://doi.org/10.1016/j.hal.2022.102311

Li Kang, Maíra Mucci, Jingyi Fang, Miquel Lürling. New is not always better: Toxicity of novel copper based algaecides to *Daphnia magna*. *Ecotoxicology and Environmental Safety* (2022). https://doi.org/10.1016/j.ecoenv.2022.113817

Li Kang, Sina Haasler, Maíra Mucci, Leon Korving, Achim Iulian Dugulan, Thomas Prot, Guido Waajen, Miquel Lürling. Comparison of dredging, lanthanum-modified bentonite, aluminium-modified zeolite, and $FeCl_2$ in controlling internal nutrient loading. *Water Research* (2023). https://doi.org/10.1016/j.watres.2023.120391

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- Posters and Pitching, Wageningen Graduate Schools (2022)
- Scientific Integrity, Wageningen Graduate Schools (2022)
- Multivariate Analysis, PE&RC Graduate School (2022)
- Internal Phosphorus Loading and Cyanobacteria, North America Lake Management Society, USA (2022)

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- Assisting Practical of the MSc course 'Aquatic Ecology and water Quality' (2021-2022)

Oral Presentations

- *Influence of temperature and pH on Phosphorus removal of selected P sorbents.* 12th Symposium for European Freshwater Sciences, 25-30 July 2021, Online
- *Comparing four different in-lake treatments to control sediment nutrient release in an enclosure study.* 36th Congress of the International Society of Limnology, 7-10 August 2022, Berlin, Germany
- *Comparing four different in-lake treatments to control sediment nutrient release in an enclosure study.* North America Lake Management Society, 14-17 November 2022, Minneapolis, USA

SENSE coordinator PhD education

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